Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

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Abstract:	Soil System Budgets (SSB) of nutrients are generally performed annually over arable land to infer their use efficiency and water pollution risk in highly exploited agricultural watersheds. They are seldom partitioned into seasonal budgets and matched with seasonal nutrient transport in adjacent river reaches. We calculated seasonal soil nitrogen (N) budgets in a Mincio River sub-basin (Italy), and we analyzed the dissolved inorganic N net export in the river reach draining such sub-basin. Our results show seasonal differences of SSB with N excess in winter and even more in spring, equilibrium among sources and sinks during autumn and N deficit during summer. Seasonal inorganic N loads transported by the river were not correlated with SSB as they peaked in late summer and were at their minimum in early spring. Fertilization uncoupled to significant uptake supports N excess in winter and spring, whereas crop uptake uncoupled to N inputs supports summer N deficit. Nitrification cannot explain nitrate accumulation in the river reach, suggesting alternative dynamics driving the local hydrology. Flood irrigation results in large soil nitrate solubilization, transport and in upward migration of the groundwater piezometric head during spring and summer periods. River water is likely replaced by nitrate-rich groundwater when the groundwater recharge exceeds a certain threshold coinciding with late summer. Irrigation is then interrupted and the piezometric head, together with nitrate exchange, decreases. This work suggests that a deep understanding of N dynamics in agricultural watersheds with flooding irrigation on permeable soils needs the reconstruction of the vertical pathways of nitrate and of river-groundwater interactions. Moreover, the partitioning of annual into seasonal N budgets and their combination with irrigation practices allows the identification of hot moments in N cycling. Agricultural practices minimizing nitrate excess, its mobility and the risk of surface and groundwater pollution are suggested for this area.
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Response to Reviewers:	

Dear Editor

We submit a revised version of our manuscript “Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges”. The revised version incorporates all the suggestions provided by the two reviewers. In particular, we improved the Introduction and Material & Methods sections.
We have found comments constructive and useful to improve our manuscript; we thank the two reviewers for the time spent analyzing our manuscript and you for giving us the opportunity to revise our work.
Please find below detailed replies to the comments received, explaining how we considered each suggestion and where changes were made (line numbers refer to the "Track Changes" Microsoft Word version of the manuscript).

Best regards,
Monica Pinardi and co-authors

Manuscript Number: AGWAT-D-22-01024

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Answers to reviewer #1

Reviewer #1: In this study, an input-output model was used to assess nitrogen balance in an agricultural watershed, and combined with flow velocity data to estimate nitrogen load. The research design of this paper is scientific and reasonable, the data is accurate and credible, and the writing is concise and comprehensive. The overall performance of the article is excellent, reaching the level of publication in this journal.

General: We thank the reviewer for appreciating our effort to assess the seasonal soil nitrogen budgets in an agricultural watershed, and to combine such budgets with riverine nitrogen loads.

There are still a few minor issues, which are briefly described below.
1. There are too many keywords, need to be simplified.

Answer: done, we removed the keyword “watershed”

2. For the title, this paper only studies nitrogen budget, not much about N exchange.

Answer: We partially agree with this comment. Our study analysed seasonal nitrogen budgets in agricultural soil and N loads transported by the river reach draining such agricultural sub-basin. We discussed how N dynamics in this system are regulated by the summer aquifer/river water interaction and the associated N load exchange. We explain the mismatch between seasonal soil N budgets and riverine N loads in terms of N transfer from the soil to the groundwater and then N exchange between the groundwater and river water. This is the reason of the choice of the title. The literature reporting similar investigations commonly reports the terms “N exchange” when referring to surface-groundwater or river-groundwater interaction. If the reviewer and the Editor are not convinced by the present title, we suggest as alternative option “River-groundwater interactions couple seasonal N budgets in agricultural land with riverine N loads”.

We provide below a few examples of studies using the term “exchange”:

Li, G., Li, H., Wang, X., Qu, W., Zhang, Y., Xiao, K., ... & Zheng, C. (2018). Groundwater-surface water exchanges and associated nutrient fluxes in Dan'ao Estuary, Daya Bay, China. Continental Shelf Research, 166, 83-91.

Brunke, M., & Gonser, T. O. M. (1997). The ecological significance of exchange processes between rivers and groundwater. Freshwater biology, 37(1), 1-33.

3. The introduction part does not provide clearly research goals of this investigation, only a few research hypotheses are given.

*Answer: thanks for the suggestion. At the end of Introduction paragraph (lines 102-104), we added a sentence explaining the research goal: "*In this context, the main aim of the present study was to contrast seasonal N soil budget in an agricultural area drained by a river stretch with seasonal N loads transported by the same draining river stretch, to assess riverine N dynamics in relation to agricultural practices."

4. For all budget calculation, the data is from the year of 2015, while the water sampling and analyses is conducted in 2016 and 2017?

Answer: thanks for the comment. The soil N budget was estimated by employing agricultural census data referring to the agricultural year 2015-2016. The agricultural year overlaps the vegetative cropping cycle, covering two consecutive years, i.e., from November of the first year to October of the following one. The N budget calculated for the agricultural year 2015-2016 is relevant in the present day because, in the last decade, only minor variations occurred in crop surfaces and livestock densities of the study area and fertilization rates did not change appreciably. We specified this better in the text (see lines 171-175). As agriculture is the main land use in the studied area, changes in agricultural practices could result in possible changes in riverine N loads and surface-groundwater exchanges. Changes in agricultural practices over the last decade were checked through agricultural survey data from multiple regional (Agricultural Information System of Lombardy Region) and national sources (National Veterinary Information System of Livestock Registry, Annals of Agrarian Statistics published yearly by the National Institute of Statistics). Anthropogenic pressures in the studied area, i.e., the main input data for the calculation of the soil N budget (livestock density and synthetic fertilizers application) did not show significant changes over the last decade and specifically in the 3-year period 2015-2017. Arable land has remained substantially stable over time and the breakdown in the various crop typologies has not changed markedly, as demonstrated by the application of synthetic fertilizers averaging at 90 (±15%) kg N ha-1 of agricultural land. This is consistent with the budget outcomes reported in Supplementary material B (Table B.1). In the 3-year period 2015-2017, the study area hosted a stable livestock density, i.e., 150,000 (±2%) pigs and 28,000 (±4%) cows. Therefore, input and output N terms were reasonably comparable between consecutive years. Moreover, the uncertainties calculated with the Monte Carlo analyses can include the variation of the input and output data between two consecutive years. For all the reasons discussed above, we are confident that the comparison done in our study is reliable and consistent.

Reviewer #2: The assessment of soil nutrient budget of cultivated land is based on the unit of year, and the influence of seasonality of agricultural activities on nitrogen dynamics is ignored. The seasonal changes of human agricultural activities affect the dynamic changes of nitrogen and hydrological processes, and have a great impact on agricultural soil nitrogen budget and river nitrogen transport. The main assumption of this study is that River groundwater interaction affects nitrogen transport in specific river areas and changes seasonally due to irrigation measures and excess inorganic nitrogen in soil. At the same time, the seasonal dynamics of the interaction of these variables can be obtained by comparing and analyzing the seasonal nitrogen budget of agricultural soil and seasonal river nitrogen transport. This is of great significance for understanding the transformation mechanism of nitrogen on the land water path and improving agricultural measures to improve nitrogen utilization efficiency and reduce nitrogen pollution.

The topic is interesting, but the article needs to be improved before it can be considered for publication.

General: We thank the reviewer for considering the topic novel and of general interest and for the useful suggestions.

1. Can the dynamic change of nitrogen in four large urban rivers be represented by only setting up two sampling points in the upstream and downstream of the river? Because the sources and biological processes of nitrogen are complex and changeable, and the river flows through four cities, the upstream point of the river should be located in the city 1, and the downstream point of the river should be located in the city 4.

Answer: thanks for the comment. It is likely that the study area section does not provide a clear description of the Mincio river segment under investigation and of the 4 municipalities that are drained by such segment. A municipality is an administrative unit within a province. According to the official EU division for regional statistics elaborated by EUROSTAT (2015, https://ec.europa.eu/eurostat/web/products-manuals-and-guidelines/-/KS-GQ-14-006), Italian municipalities correspond to the LAU-2 territorial level, Italian provinces to the NUTS-3 territorial level, and Italian regions to the NUTS-2 territorial level. Municipality is the smallest administrative unit at which official agricultural and demographic statistics are usually available for the whole national territory. Agricultural land is the main (>75%) land use of the four municipalities considered in the present study (Marmirolo, Goito, Valeggio sul Mincio, and Volta Mantovana). The river reach under investigation is about 8.1 km long, and it does not cross the urban area of 4 cities (there are no cities in the study area) and not even the urban area of the 4 municipalities. It simply drains their agricultural land. The downstream sampling point is located upstream the Goito village (resident population about 10,000) and is not influenced by factors as WWTP or other N sources associated to the village.

When we speak about the 4 municipalities, we refer to the whole surface of each municipality (see the borders in Fig.1). This is due to the need of analysing the soil system budget of each municipality at agricultural level, that is the Utilized Agricultural Area (UAA). The surfaces of the municipalities are cut on the border of the Mincio river watershed (See also our previous work, Pinardi et al., 2018 - Biogeochemistry, 140(2), 175-197.). Moreover, the river reach under investigation drains the portion of the Mincio watershed included in the 4 municipalities identified. We improved the study area section to better explain these issues (lines 126-130).

2. How to obtain the calculation data of four kinds of nitrogen input and output?

Answer: We described in the Material and methods section the general budget calculation procedure at the annual scale and all the sources of statistic data and coefficients. We also specified that all the equations and specific agronomic coefficients employed to obtain the seasonal breakdown are reported in Supplementary material A (Lines 189-190).

3. Nitrate pollution may come from point source pollution or non-point source pollution. Please supplement the distribution of industry and agriculture in the overview of the study area.

Answer: thank for the suggestion. The Mincio River watershed is mainly agricultural, and the land use map shows that the urban, infrastructural and industrial areas cover only 5% of the total surface of the four *municipalities (see Fig. 1). We clarified this in the manuscript (lines 132-133). Moreover, previous studies have demonstrated that N load produced by urban areas accounted for less than 2% of the total N input by diffuse sources (lines 191-195).*

4. Soil nitrogen budget has obvious seasonal changes, with seasonal changes, including nitrogen deficiency period (summer), balance period (Autumn), moderate period (winter) or large excess period (spring). These differences are caused by the seasonal balance between different agricultural measures, such as large amount of fertilization in spring and the fertilization of synthetic fertilizers are not coupled with crop absorption, moderate fertilization in winter is little or no absorption, or crops absorb too much nitrogen input in summer. The absorption and utilization of nitrogen in different crops are different, which needs to be supplemented in materials and methods.

Answer: we agree with the reviewer, and the calculations we have made consider all crop-specific N uptake rates. We would say that one of the elements of strength of the manuscript is the reliability of the N soil system budget, that is built on a detailed dataset of statistical data and on an accurate set of species-specific coefficients taken from the literature in the field. We clarified this in the manuscript (lines 166-168). Moreover, we better explained in the text that all the procedures for the calculation of the seasonal budget is reported in Supplementary material A: "Details about annual budget data, equations, seasonal breakdown, and sources of census data and species-specific agronomic coefficients are presented in Supplementary Material A."

In fact, in Suppl. Mater. A we report different tables which include:

- *Table A.1. Livestock categories, live weights, and N excretion rates.*
- *Table A.2. Seasonal distribution of N fertilizers. The timing of N fertilization along the crop growth stages allowed to estimate the N amount distributed seasonally to croplands.*
- *Table A.3. Seasonal cut events and yield for alfalfa and permanent grasslands.*
- *Table A.4. Crop parameters used in the calculation of N seasonal budget. Formula: harvest (or stock) = area*yield*uptake rate (or straw uptake rate).*
- *Table A.5. Nitrogen-fixing crop parameters used in the calculation of N seasonal budget. Formula: harvest (or stock) = area*yield*uptake rate (or aboveground uptake rate).*

5. In this study, the net output of dissolved inorganic nitrogen in the river section of the basin is analyzed. Why not analyze total nitrogen?

Answer: thanks for the question. There are two main reasons for focusing on inorganic nitrogen. The first is that from our database and from those of national monitoring agencies in the Mincio river nearly 80% of the total N load is made of inorganic N (DIN, and within the DIN pool >90% is nitrate). The second is that from the same dataset nitrate represents more than 95% of the total dissolved N in groundwater. Due to a) the dominance of the inorganic form in surface and groundwater, b) our main study aim (to highlight the seasonality of river-groundwater nitrate exchange) and c) the still unsolved Nitrate Directive related issues, we focused on inorganic N dissolved forms. We acknowledge that future studies should include all forms of N, as the reviewer suggest. We have clarified this in the revised version of the paper (lines 220-223).

6. In the season of river transportation, inorganic matter reaches its peak at the end of summer and reaches its lowest in early spring. This may be due to the lag of nitrogen application to the soil in agricultural cultivated land.

Answer: Our evidences from this and previous investigations in rivers draining similar agricultural areas, with flood-based irrigation techniques and crossing the spring belt zone suggest a different interpretation (see Racchetti et al., 2019 - Water, 11(11), 2304). If we have understood the comment, the reviewer suggests that riverine DIN loads, peaking in Summer (august) and decreasing in spring (April), could be *explained by a lag in their transport from N fertilizers applied in spring to the soil to surface water, which is reached during summer (Aug). Our opinion, which is supported by different studies carried out in the same geographical area (Pinardi et al., 2018- Biogeochemistry, 140(2), 175-197., Rotiroti et al., 2019 - Science of the Total Environment, 672, 342-356., Racchetti et al., 2019 - Water, 11(11), 2304; Balestrini et al., 2021 - Science of The Total Environment, 753, 141995.) is that the flood irrigation over permeable soil with large N excess determines rapid vertical transfer of nitrate to the groundwater. This mechanism starts in spring, together with the irrigation period. Months of flood irrigation recharge the groundwater and determine the vertical rise, by up to 4/5 m, of the aquifer. Such rise peaks in late summer (August) leads to river-groundwater interaction and exchange of nitrate polluted groundwater with river water. In our opinion this is the mechanism that explain the seasonality of riverine N loads.*

Highlights

- Seasonal budgets of soil nitrogen (N) were calculated in an agricultural watershed
- Seasonal nitrate export from the river draining such watershed was also quantified
- Soil N budgets and river export were temporally uncoupled
- Flood irrigation and subsurface water flow drove excess nitrogen transport
- Best agricultural practices should consider the seasonality of budgets and hydrology

Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

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Abstract

Soil System Budgets (SSB) of nutrients are generally performed annually over arable land to infer their use efficiency and water pollution risk in highly exploited agricultural watersheds. They are seldom partitioned into seasonal budgets and matched with seasonal nutrient transport in adjacent river reaches. We calculated seasonal soil nitrogen (N) budgets in a Mincio River sub-basin (Italy), and we analyzed the dissolved inorganic N net export in the river reach draining such sub-basin. Our results show seasonal differences of SSB with N excess in winter and even more in spring, equilibrium among sources and sinks during autumn and N deficit during summer. Seasonal inorganic N loads transported by the river were not correlated with SSB as they peaked in late summer and were at their minimum in early spring. Fertilization uncoupled to significant uptake supports N excess in winter and spring, whereas crop uptake uncoupled to N inputs supports summer N deficit. Nitrification cannot explain nitrate accumulation in the river reach, suggesting alternative dynamics driving the local hydrology. Flood irrigation results in large soil nitrate solubilization, transport and in upward migration of the groundwater piezometric head during spring and summer periods. River water is likely replaced by nitrate-rich groundwater when the groundwater recharge exceeds a certain threshold coinciding with late summer. Irrigation is then interrupted and the piezometric head, together with nitrate exchange, decreases. This work suggests that a deep understanding of N dynamics in agricultural watersheds with flooding irrigation on permeable soils needs the reconstruction of the vertical pathways of nitrate and of river-groundwater interactions. Moreover, the partitioning of annual into seasonal N budgets and their combination with irrigation practices allows the identification of hot moments in N cycling. Agricultural practices minimizing nitrate excess, its mobility and the risk of surface and groundwater pollution are suggested for this area.

Keywords: nitrogen; retention; transport; loads; watershed; irrigation; river-groundwater interaction

1. Introduction

The dramatic increase of anthropogenic reactive nitrogen (N) inputs in watersheds with intensive agriculture and animal farming has demonstrated negative effects for inland water and groundwater chemical and biological quality, drinking water supplies, ecosystem integrity and functioning and human health (Van Grinsven et al., 2006; Galloway et al., 2008; Rivett et al., 2008; Schlesinger, 2009; Sobota et al., 2015; Huang et al., 2017). Such negative effects are amplified by the human-derived alteration of the hydrological cycle at the watershed scale and by climate change (Galloway et al., 2008; Overeem et al., 2013; Woolway and Merchant, 2019; Woolway et al., 2020). Among the underlying mechanisms are water abstraction for irrigation or industrial purposes or climate change-related drought reducing river discharge and its capacity to dilute and process N loads (Palmer et al., 2008). Low discharge promotes also hyporheic anoxia and ammonium recycling from sediments (Hlaváčová et al., 2005). Hydrological extremes include also short-term, heavy precipitations resulting in high discharge events transferring large N loads from cultivated areas saturating riverine denitrification capacity (Viaroli et al., 2018; Magri et al., 2019).

Nitrogen budgets calculated for agricultural soils within a river basin allow to evaluate the potential risk of diffuse N pollution (Oenema et al., 2003; Soana et al., 2011). In agricultural soils, N inputs associated with organic or synthetic fertilizers, atmospheric deposition or biological fixation can be either temporarily retained in crops or released to the atmosphere as gaseous losses. Nitrogen inputs in excess to temporary retention or permanent loss can be transferred via runoff to adjacent aquatic ecosystems (Howarth et al., 1996; Seitzinger et al., 2006; Pinardi et al., 2018, 2020; Kwon et al., 2022). If soil system budgets in arable land produce reliable snapshots of N pools and fluxes in cultivated areas, the detailed reconstruction and partitioning of N pools and fluxes within watersheds is a challenging objective. For example, seasonally variable water inputs to agricultural soils via precipitation and irrigation affect soil N leaching, horizontal and vertical transport and transformation, N use efficiency as well as river-groundwater interactions and associated N exchange (Schaefer and Alber, 2007; Chae et al., 2009; Howarth et al., 2012; Sinha and Michalak, 2016). Moreover, in intensively cultivated floodplains the hydrological cycle has been regulated by the realization of infrastructures as dams and networks of canals that help buffering climatic anomalies and ensure water availability for crops. In Italy for example, the Alpine sector of the Po River basin hosts large dams that regulate the release of water from deep subalpine lakes (Maggiore, Como, Iseo, Idro and Garda Lakes) to their emissaries (Ticino, Adda, Oglio, Chiese and Mincio Rivers). Winter water retention in subalpine lakes occurs at the cost and drawbacks of reduced water discharge and contributes to the downward vertical migration of groundwater, often resulting in downwelling river-groundwater interactions (i.e. the river feeds the groundwater) (Rotiroti et al., 2019; Severini et al., 2021). On the contrary summer irrigation, besides representing a vehicle for N transport, produces opposite effects, often reversing the direction of river-groundwater interactions (i.e. upwelling, the groundwater feeds the river). These practices, that characterize anthropogenic, intensively cultivated, and hydraulically regulated watersheds with permeable soil, introduce marked seasonality in N budgets (Lin et al., 2019; Racchetti et al., 2019).

Many authors reported a significant correlation between annual N input to croplands and river N export (Neff et al., 2003; Yan et al., 2010; Xu et al., 2013; Strokal et al., 2014; Tong et al., 2017), but they did rarely account for the seasonality of N input and export (McCrackin et al., 2014; Chen et al., 2019). Studies targeting N budgets in agricultural watersheds are generally conceived at the annual scale for mainly practical reasons, as agricultural census data are collected and published by national statistical institutions with annual frequency. Such an approach from one side allows to calculate N use efficiency in cropland and potential N loss, but from the other side, it misses temporal resolution and precludes the understanding of seasonal variations of the array of N-related processes, potentially regulated also by climate change. For example, human activities (e.g., crop production) and altered hydrology may influence the seasonality of N river export (Basu et al., 2010; Compton et al., 2020), together with the seasonal evolution of temperature that influences N losses, retention and removal processes (e.g., denitrification) (McCrackin et al., 2014). Understanding how seasonal variations in human activities and hydrology influence N budgets in agricultural soils and N transport by rivers is important to better understand the mechanisms underlying N transformations along the terrestrial-aquatic path, improve agricultural practices to increase N use efficiency and decrease N pollution, and eventually forecast how climate change will affect N dynamics (Mas-Pla and Menció, 2019). This important set of objectives is a difficult target at the scale of whole watersheds due to scarce resolution of available data and spatial heterogeneity (e.g. pedology, land use, etc). Smaller scales of analysis, targeting specific and homogeneous river and watershed sectors, seem much more promising (McCrackin et al., 2014; Chen et al., 2019; Compton et al., 2020).

Different studies carried out at large temporal and spatial scales (Soana et al., 2011; Pinardi et al., 2018; Viaroli et al., 2018; Lassaletta et al., 2021) have highlighted the presence of hot-spots within watersheds that represent outliers in N budgets (e.g., with very large N excess or very low N use efficiency). They also emphasized the presence of hot-moments within watersheds, that are specific periods during which N mass transfer peaks as a combination of decreased uptake, increased runoff or variation of the water table level, resulting in the reactivation of river-

groundwater interaction (Rosenzweig et al., 2008; Preisendanz et al., 2020; Taherisoudejani et al., 2018). The analysis of N hot-spots and hot-moments in watersheds require specific studies, focusing on small spatial and temporal scales.

In Northern Italy, the Po River valley is an alluvial plain heavily exploited by human activities such as agriculture, animal farming, industry, and tourism. Land use change and hydrological alterations determined high pressure on both surface and groundwater (May, 2013; Pérez-Martín et al., 2014; Lasagna and De Luca, 2019) and a wide portion of the plain is classified as vulnerable to nitrate pollution (Martinelli et al., 2018). The main aim of this study is to analyze the seasonal evolution of dissolved inorganic N loads in a fluvial segment of the Mincio River, a tributary of the Po River, characterized by natural banks, gravel bottom with submerged vegetation, and regulated discharge. This segment crosses a transitional area between permeable and non-permeable soils, characterized by springs and classified as an area of river-groundwater interactions (Balestrini et al., 2021). Due to its hydrogeological features and the large water availability, the considered sub basin is a hotspot of intensive agriculture and animal farming and represents a key study area to analyze if and how the seasonality of agricultural practices affects N dynamics.

In this sector of the Po River, groundwater in the phreatic and shallow aquifer has a short residence time as compared to semiconfined or confined deeper aquifers. This is supported by fast (few days) surface-groundwater dynamics of micro-pollutants (Balderacchi et al., 2016) and low concentrations of total dissolved solids (Martinelli et al., 2018). Results of Balderacchi et al. (2016) suggest also fast response of shallow aquifers to changing conditions; as such they allow to trace agricultural practices (e.g., use of herbicides or fertilization) and they respond quickly to hydrologic variations (e.g., drought, precipitations, irrigation). It can be assumed that macrocontaminants as nitrates undergo the same fast transfer mechanisms, also due to their elevated solubility and absence of interaction with soil and sediment.

The main hypotheses of this work are that river-groundwater interactions affect N transport in specific river sectors and vary seasonally due to combination of irrigation practices and inorganic nitrogen excess in soil. We also hypothesized that the seasonal dynamics of such variable interactions can be captured analyzing comparatively seasonal N budget in agricultural soils and seasonal riverine N transport.

2. Study area

The Mincio River (~75 km) originates from the Lake Garda, the largest Italian Lake, and is a tributary of the Po River (Fig. 1). The hydrological regime of the Mincio River is regulated upstream by a dam, which controls the water discharge from the Lake Garda. Along the river course, a series of dams and weirs feeds a network of canals for irrigation and industrial purposes and controls discharge variations to avoid the flooding of cities and villages. Water management for Lake Garda recreational activities and for agricultural purposes results in marked flow variations. Indeed, since the establishment of the river regulation in the 60's of the last century, the Mincio River discharge averaged ~80 and ~30 $m^3 s^{-1}$ during the irrigation (May to September) and outside the irrigation periods, respectively (Lombardy Region, 2006). More recently, projections of decreasing water availability resulted in a further reduction of the Mincio River discharge to ~14 $m^3 s^{-1}$ (www.laghi.net) in autumn and winter to keep water in the Lake Garda and guarantee water availability for irrigation and tourism in the summer season. During winter, the flow reduction and absence of irrigation result in a decreased aquifer recharge, a phenomenon described by different authors in this geographical area at regional (Rotiroti et al., 2019) and local scales (Severini et al., 2021) and, consequently, in a lowering of the phreatic surface. This water transfer dynamic results in a decrease of groundwater upwelling in winter and early spring (Balderacchi et al., 2016).

A wide segment of the Mincio River, including the portion investigated in this study, flows in a flood plain characterized by a multilayered aquifer system with a cyclic facies architecture mainly made of fluvial-channel (gravel and sand) and floodplain (clay) deposits (Amorosi et al., 2008). As a result, the northern part of the plain (high plain) is locally characterized by shallow phreatic aquifers, while in the southern part (low plain) the floodplain facies act as aquitards or aquicludes, resulting in confined and semi-confined aquifers (Chelli et al., 2018). The river reach investigated, from S1 to S2 (length 8.1 km, mean depth ~1 m, mean water velocity ~1.0 $m s^{-1}$) is in the high-medium plain of the Mincio watershed and includes four municipalities (1- Valeggio sul Mincio, 2- Volta Mantovana, 3- Goito, and 4- Marmirolo) for a total surface of 184 km^2 (Fig. 1). Since 2006, these municipalities are classified as Nitrate Vulnerable Zones (NVZs) according to the European Nitrate Directive (91/676/CEE). The study area is characterized by fertile soils due to calcareous gravel deposits and is intensively exploited by agriculture (Utilized Agricultural Area - UAA covers 76% of the study area; Fig. 1) and animal farming (1.2 and 0.6 t of live weight per hectare for cattle and pigs, respectively). The S1-S2 river segment flows into natural banks, has a mainly gravel bottom, and has transparent waters. The main primary producers are submerged vegetation (e.g., *Vallisneria spiralis*) with associated epiphytes, benthic biofilms and different emergent macrophytes growing along the river banks or forming islands (Pinardi et al., 2009, 2014). The linear development of irrigation canals in S1-S2 river reach sub basin sums ~560 km (Fig. 1). The surface covered by the other aquatic environments, such as quarry lakes is ~0.62 km^2.

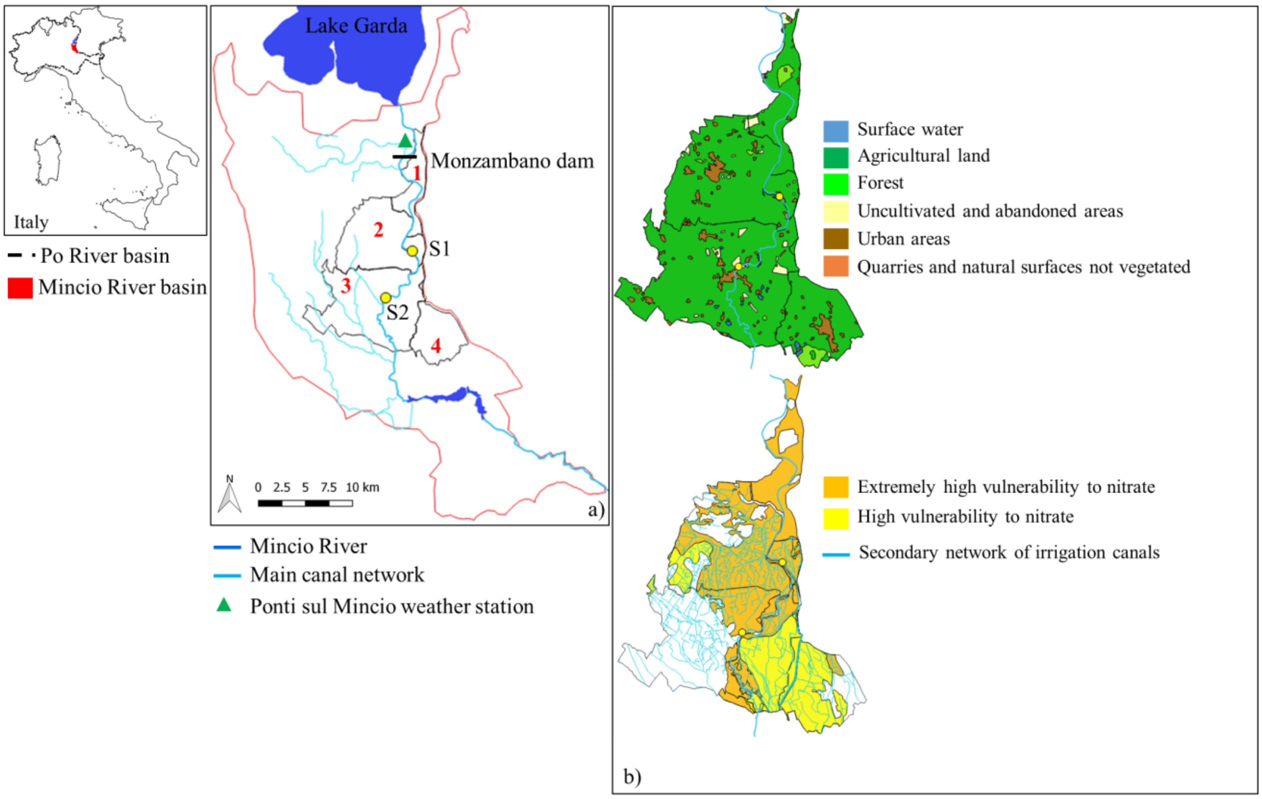

Figure 1. Maps of the study area: the Mincio River segment from Pozzolo dam (S1) to Goito village (S2) (yellow points = water sampling stations). a) Municipalities where the nitrogen mass budget was performed are reported (1 - Valeggio sul Mincio, 2 - Volta Mantovana, 3 – Goito, 4 - Marmirolo). b) Land use and maps of soil vulnerability to nitrate are reported for the four municipalities under study.

3. Material and methods

3.1. Nitrogen budgets and water inputs

A comprehensive input–output N budget across the Utilized Agricultural Area (UAA) was compiled by using locally-derived data on farming activity, agronomic coefficients and atmospheric deposition. Nitrogen budget was first calculated at the municipal scale, i.e., the administrative level at which official agricultural statistics are available, then weighted for the percentage of each municipality surface included within the study area, and finally summed up. Census data were integrated in a nutrient budgeting approach proposed by Oenema et al. (2003), recently reviewed by Zhang et al. (2020), and formerly applied to the whole Mincio River basin (Pinardi et al., 2018). Four inputs of N to the UAA were considered (land application of livestock manure, land application of synthetic fertilizers, atmospheric deposition, and biological fixation by crops), together with four outputs of N from UAA (crop harvest, crop stock, ammonia volatilization and denitrification in soils). The difference between N inputs and outputs results in a net, which represents a condition of equilibrium, surplus or deficit of N across the UAA.

The Soil System Budget (SSB) was calculated as follow:

$SSB\ N = N_{Man} + N_{Fert} + N_{Fix} + N_{Dep} - N_{Harv} - N_{stock} - N_{Vol} - N_{Den}$

where:

N_{Man} = N in livestock manure applied to agricultural soils

N_{Fert} = N in synthetic fertilizer applied to agricultural soils

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

N_{Dep} = atmospheric N deposition on agricultural land

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

N_{stock} = organic N in crop's standing stock

N_{Vol} = NH_3 volatilization in agricultural soils

N_{Den} = denitrification in agricultural soils

All budget terms were expressed in unit of mass per time (t N y^{-1}), and on a per-area basis, after normalization for the UAA (kg N ha^{-1} y^{-1}). The calculation was based on agriculture and farming data for the year 2015 reported by the Agricultural Information System of Lombardy Region (SIARL, www.siarl.regione.lombardia.it) and by the Annals of Agrarian Statistics, published yearly by the National Institute of Statistics (ISTAT, http://agri.istat.it/). SIARL databases, retrieved from the Open Data portal of the Lombardy Region (https://dati.lombardia.it/), provided data for livestock density and agricultural areas at the municipality level, whereas the database of the Annals of Agrarian Statistics provided data for crop yield and fertilizer application (http://dati.istat.it/) at the provincial level. Inputs and outputs were initially calculated for each municipality and then aggregated at the study area level.
Uncertainty in N budget calculations was assessed by a Monte Carlo analysis using Excel and R software (R Core Team 2019). All coefficients used to convert census data into N amounts were assumed to vary stochastically and

independently around the average value with a normal probability distribution. For each simulation, a set of coefficients was randomly generated from probability distribution functions and a total of 1000 simulations were run. Budget calculation was conducted both at the annual and at the seasonal scales and compared with seasonal in-stream N loads. Details about annual budget equations, seasonal calculations and sources of census data and agronomic coefficients are presented in Supplementary Material A.

The N loads produced by the urban areas were not included in the calculation because more than 95% of the sewers in the study area are connected to wastewater treatment plants (WWTP). Nearly 75% of the N inputs to WWTP is removed via denitrification in tertiary treatment (Lombardy Region, 2017). Indeed, the calculation of the urban load produced by the resident population, obtained by the conversion of equivalent inhabitant in kg of N per day, resulted in less than 2% of the total N input by diffuse sources (Pinardi et al., 2018).

The daily precipitation data were downloaded from the ARPA Lombardy website (https://www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx) at Ponti sul Mincio station (Fig. 1) for the period from 2010 to 2017. The mean annual, seasonal (irrigation and non-irrigation period) and monthly precipitation data were calculated. Irrigation data at the municipality level was obtained from the 6th Agricultural Census (National Institute of Statistics, 2010, http://dati-censimentoagricoltura.istat.it) and then aggregated at the study area level.

3.2. Water sampling and analyses

Two stations located at the extremes of the identified river reach (S1 and S2; Fig. 1) were sampled for water analyses. The two stations were selected as they were located upstream and downstream the area where the Mincio River can be considered as a gaining river in groundwater-surface water interaction, that is the river is fed by groundwater (Racchetti et al., 2019). Given the constant discharge between S1 and S2, the identified river reach was more recently characterized as a flow-through reach (Severini et. al., 2022), with groundwater feeding the river in the western bank and being fed by the river in the eastern bank. Field campaigns were carried out seasonally with a series of daily cycles of repeated samplings carried out on 12-13 August and 15-16 November 2016, 14-15 February, 12-13 April and 13-14 June 2017. Water samples were taken in three replicates every 4 hours for a 24-hour period. An aliquot was transferred into a 12 mL exetainer (Labco, UK), added with 100 µL of $HgCl_2$, and analyzed for dissolved inorganic carbon (DIC) with Gran titration (0.1 N HCl) within 24 hours from sampling. DIC was measured as it may trace differences between surface and groundwater chemistry. Water aliquots were filtered (GF/F glass fiber filters) and transferred to plastic vials for nitrate (NO_3-N), nitrite (NO_2-N), and ammonium (NH_4-N) determination by spectrophotometric methods (Rodier, 1978; APHA, AWWA, WPCF, 1999). Hourly or daily water flow data were obtained by the Interregional Agency for the Po River (AIPO), and by the Mincio Consortium for Pozzolo and Goito sites.

The Mann-Whitney Rank Sum Test was used to test the difference between upstream and downstream values of water flow, NO_3-N, NO_2-N, NH_4-N and DIC concentrations. The R software package (R Development Core Team, 2019) was used to perform all statistical tests.

3.3. Dissolved inorganic nitrogen and carbon daily loads

For each sampling date, daily NO_3-N, NO_2-N, NH_4-N and DIC riverine loads transported at S1 and S2 (kg d^{-1}) were calculated multiplying concentrations by river discharge. The difference between loads at S2 and S1 was calculated according to the following equation:

$$\Delta NO_3\text{-N (or } NO_2\text{-N, } NH_4\text{-N, DIC)} = \sum [C_t \times \Delta t \times Q]_{S2} - \sum [C_t \times \Delta t \times Q]_{S1} \qquad (1)$$

where: Ct = concentration of NO_3-N (or NO_2-N, NH_4-N, DIC) at time t downstream (S2) or upstream (S1) (mg m^{-3}); Δt = time interval between samplings (h); Q = water flow (m^3 h^{-1}). Such difference can be null, suggesting equilibrium between inputs and outputs, negative, suggesting net retention or dissipation (e.g., uptake or denitrification), or positive, suggesting the occurrence of production or additional inputs along the stretch (e.g., nitrification or point and diffuse inputs). A standard deviation was associated to NO_3-N, NO_2-N, NH_4-N and DIC measurements made in replicates. The errors conveyed through the mathematical description were calculated with classical error propagation equations.

4. Results

4.1. Nitrogen budgets and water inputs

In the four municipalities under study, the total N inputs to arable land (6010±498 t y^{-1}) were mainly due to livestock manure (59%). Nitrogen outputs (5078±494 t y^{-1}) accounted for 84% of the N inputs and were mainly due to crop harvest (50% of the total N outputs). The main cultivated crop is maize (63% of arable land) followed by permanent grassland. The difference between N inputs and outputs denoted a N soil surplus (932±702 t y^{-1}). The mean areal N surplus was 67±50 kg ha^{-1} y^{-1} with values calculated for the different municipalities ranging between -172 and 204 kg ha^{-1} y^{-1}. All these results are reported in Supplementary material B, Table B.1 and Figure B.1.

The seasonal SSB is reported in Figure 2. Manure N fertilization was higher in spring (1590±268 t) and summer (883±149 t), and similar in autumn and winter (530±89 t). Synthetic N fertilization was higher in spring (769±190 t) and winter (257±63 t). Biological N fixation was higher in summer (559±259 t) and spring (350±162 t) whereas atmospheric N depositions were concentrated mainly in summer and autumn (32±4 and 38±4 t, respectively). In spring, N associated to the crop's standing stock (487±98 t) was higher than N in the crop harvest (398±80 t). With respect to N outputs, crop harvest was highest in summer (1492±301 t) and autumn (637±128 t). Ammonia volatilization and soil denitrification were quantitatively important in spring (324±233 t and 210±102 t, respectively). Coupling the seasonal input and output data, a transition from N deficit to N surplus is evident moving from summer (-971±472 t) to spring (1317±464 t).

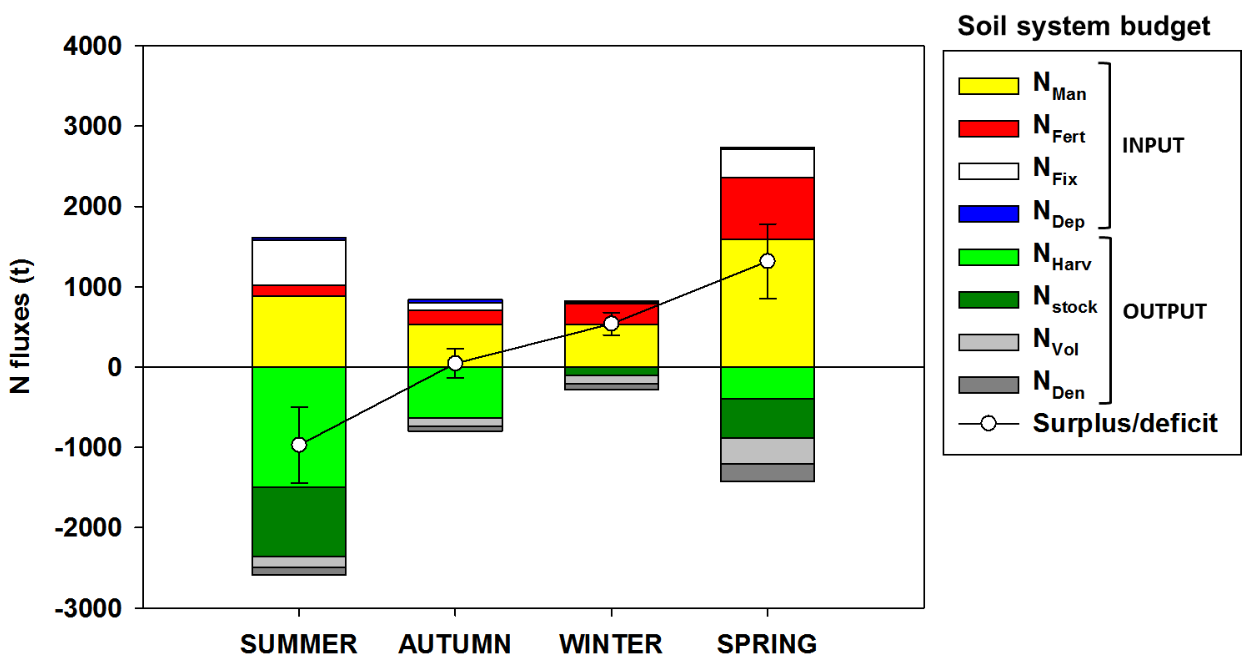

Figure 2. Components of the Soil System Budget (SSB) of nitrogen (N) for the study area within the Mincio River watershed for all seasons. N_{Man} = N in livestock manure applied to agricultural soils; N_{Fert} = N in synthetic fertilizer applied to agricultural soils; N_{Fix} = agricultural N_2 fixation associated with N fixing crops; N_{Dep} = atmospheric N deposition on agricultural land; N_{Harv} = N exported from agricultural soils with crop harvest; N_{stock} = organic N in

crop's standing stock; N_{Vol} = NH_3 volatilization in agricultural soils; N_{Den} = denitrification in agricultural soils. White dots represent seasonal N budgets (Σ INPUTS - Σ OUTPUTS); positive values suggest surplus whereas negative values suggest deficit.

During the 2010-2017 period the mean annual precipitation in the study area was 910 ± 197 mm y^{-1}, of which about 43 ± 6% occurred during the irrigation period (Fig. 3).

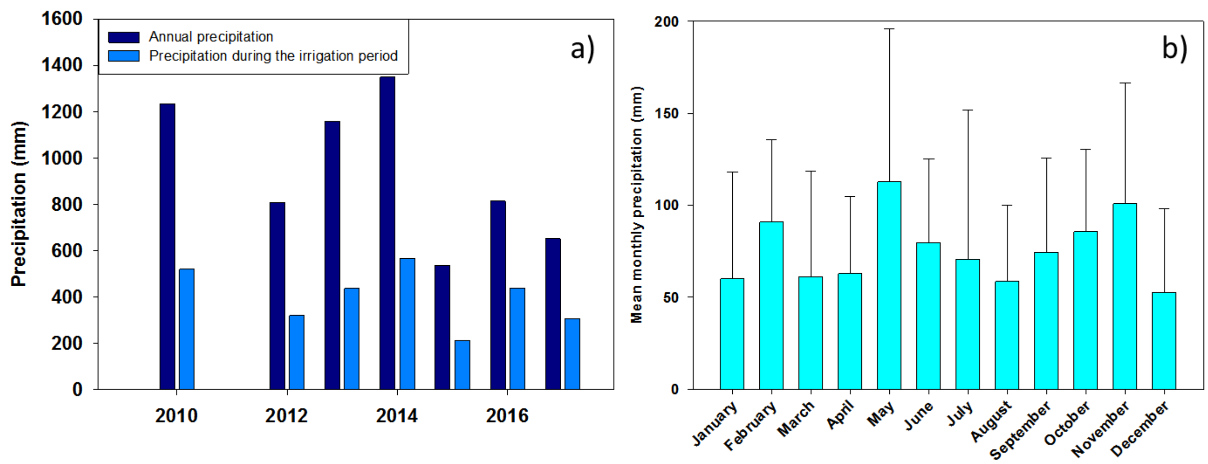

Figure 3. Annual precipitation and the fraction of precipitation during the irrigation period (from May to September) (a) and mean monthly precipitation (± standard deviation) (b) in the period from 2010 to 2017 at Ponti sul Mincio meteorological station (see Fig. 1 for the localization) (2011 was not considered due to missing data for August and September).

During the May-September period a water volume of 53.3 $x10^6$ m^3 was used to irrigate 13,513 ha of UAA, which represent 75% of the total arable land. Flooding and sprinkler were the main irrigation typologies (72% and 27% of the irrigated surface, respectively) (data from the National Institute of Statistics).

4.2. Water physico-chemical features of sampling sites

Nitrate and DIC concentrations were significantly higher at the downstream site for all sampling dates (Mann-Whitney Rank Sum Test, $p < 0.001$, n=68 for each parameter; Fig. 4). On the contrary, the concentrations of the other dissolved inorganic forms of nitrogen (NO2-N and NH4-N) were significantly higher at the upstream site (p <

0.001, n=68 for each parameter; Fig. 4). The highest NO3-N and DIC concentrations were measured in June 2017 at both sampling sites (1.6 and 3.0 mg NO3-N L^{-1}, and 33 and 41 mg DIC L^{-1} at S1 and S2, respectively). The highest values of NO2-N were recorded in summer at S1 and S2 (up to 101 and 31 µg L^{-1}, respectively), whereas NH4-N concentrations peaked in August 2016 at S1 (up to 122 µg L^{-1}) and were high at both sites in February 2017. Nitrate was always the main form of inorganic nitrogen, accounting on average for 88% and 98% of the total N at S1 and S2, respectively.

The mean annual water flow was 10.3±2.5 $m^3 s^{-1}$ in the S1-S2 river reach (whole dataset 2016-2017, n=140). During the irrigation period the water flow was not significantly different upstream and downstream (11.7 ± 2.0 $m^3 s^{-1}$ at S1 and 13.0 ± 3.9 $m^3 s^{-1}$ at S2; $p > 0.05$). No significant differences were also found between water flow during the irrigation and not-irrigation periods ($p > 0.05$, n=34). The water discharge measured during the experimental activities fell within the annual range of flow variation.

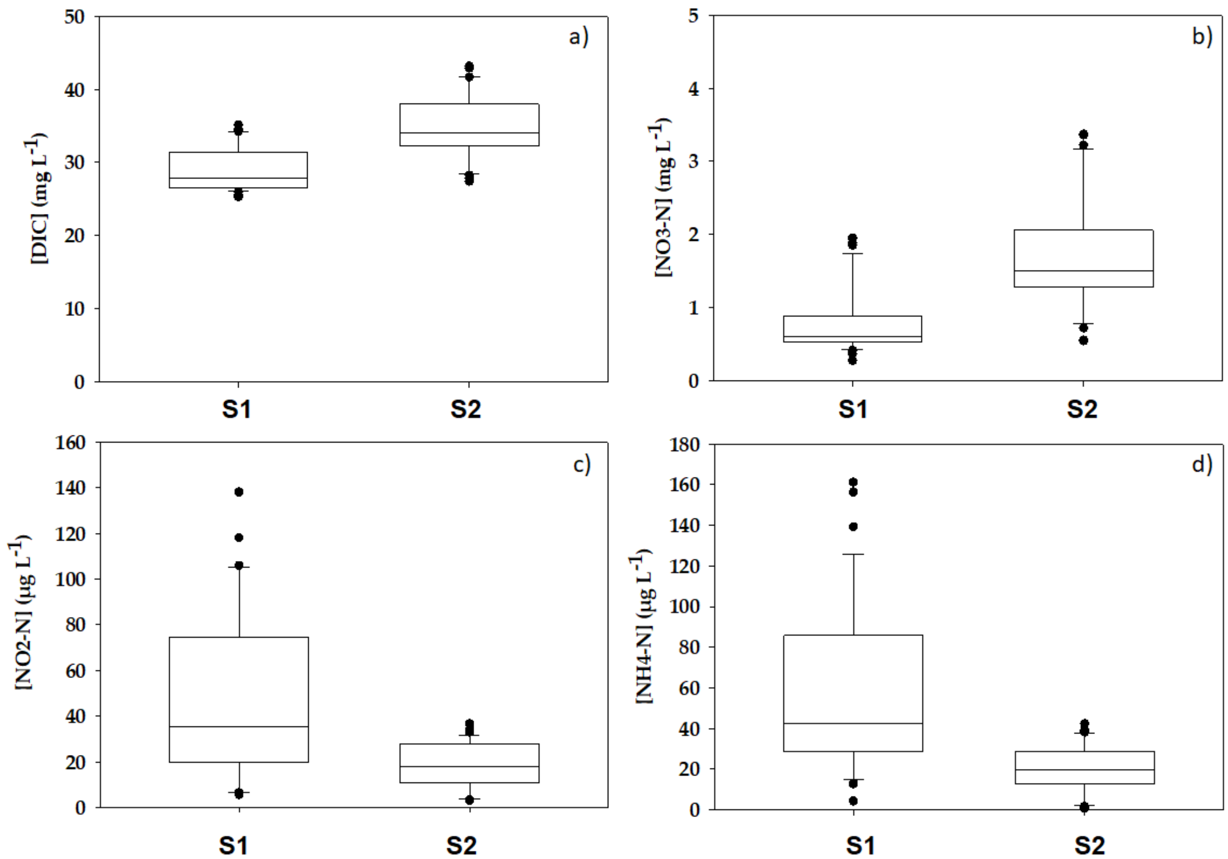

Figure 4. Box plot showing the concentrations of dissolved inorganic carbon (DIC; a), and of nitric (NO3-N; b),

nitrous (NO2-N; c) and ammonium (NH4-N; d) nitrogen measured seasonally from August 2016 to June 2017 at S1 and S2. Note different concentration units.

4.3. Dissolved inorganic nitrogen and carbon daily loads

In all seasons, NO3-N and DIC transported loads were higher at S2, whereas NO2-N and NH4-N loads were higher at S1, but by much lower extent (Fig. 5). A positive correlation was found between NO3-N and DIC concentrations (Pearson's correlation coefficient r = 0.899, p < 0.001, n=68 for each parameter) supporting the possibility of the same origin for the two solutes. A similar seasonal trend was detected for DIC and NO3-N accumulation along the analyzed river reach (Fig. 5). The maximum increase of transported loads (nearly 11,000 and 1500 kg d^{-1} for DIC and NO3-N, respectively) was measured in August 2016, whereas the minimum increase (nearly 2,000 and 200 kg d^{-1} for DIC and NO3-N, respectively) was measured in April 2017 (Fig. 5). The highest NO2-N and NH4-N loads reduction along the stretch were recorded during summer months (Fig. 5).

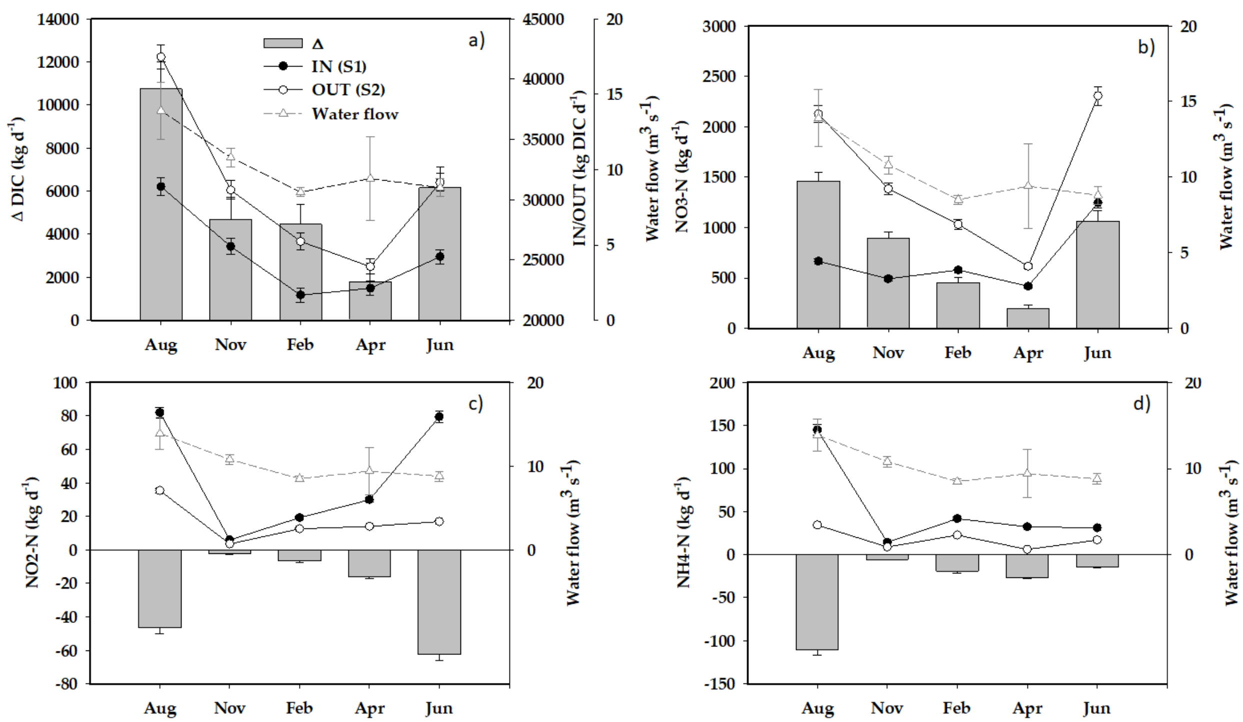

Figure 5. Daily loads of inorganic carbon (DIC; a), nitric nitrogen (NO3-N; b), nitrous nitrogen (NO2-N; c), and ammonium nitrogen (NH4-N; d) transported at the extremes of the studied river reach and their differences (Δ=S2-

S1) in the period August 2016 to June 2017. Water flow is also reported. Mean values are given, with error bars corresponding to ±1 standard deviation.

5. Discussion

5.1. The seasonality of soil N budgets

Previous studies carried out in the Po River basin and in other geographical areas characterized by intensive agriculture and animal farming suggest a generalized N surplus and inefficient N use, leading to large N losses to surface and groundwater (Soana et al., 2011; Hou et al., 2015; Özbek et al., 2015; Viaroli et al., 2018; Häußermann et al., 2020). In the Po River plain, to our knowledge, only a few sub-basins (e.g., Ticino and Po di Volano) represent exceptions to this rule due to very limited animal farming and synthetic fertilizers inputs balanced with crop needs (Racchetti et al., 2019; Soana et al., 2021). All those studies were carried out on an annual temporal scale, that potentially masked marked seasonal differences. Our calculations suggest a clear seasonal variation in N soil budgets, changing along seasons with periods with deficit (summer), equilibrium (autumn), moderate (winter) or large (spring) excess. These differences arise from variable seasonal balance among agricultural practices, such as large spring manure and synthetic fertilizer spreading uncoupled to crop uptake, moderate spread of fertilizers during winter with little to no uptake or large summer crop uptake in excess to N inputs (Chen et al., 2019). The N assimilation term is calculated as the product of the standing stock by the biomass-specific uptake rates and peaks in summer. Indeed, the biomass-specific uptake tends to decrease along with the crop's growth, but the crop's standing stock is much smaller in spring than in summer. Large spring N inputs are therefore coupled to relatively low uptake, resulting in a maximum surplus, exceeding that calculated for winter, when uptake is minimum. Large summer uptake on the contrary exceeds inputs and results in a seasonal deficit of N in the soil system budget. High fertilization is probably driven by the N need of the main cultivated crop, i.e., maize, which is a water and N-demanding species (FAO, 2006). These results on intra-annual variations in N mass budgets support the relevance of seasonal studies in highlighting critical moments in terms of potential water pollution (Lin et al., 2019; Compton et al., 2020). As nitrate water pollution is correlated with N excess in soils, our outcomes indicate a maximum nitrate pollution risk in the spring, a minimum risk in the summer and something intermediate in winter and autumn. Results from seasonal river N transport suggest something different as the highest N accumulation along the stretch was measured in summer and the lowest in spring. Taken together, these apparently contrasting results indicate that more factors are involved in the horizontal transfer of soil N excess to surface water and that such factors determine a temporal lag. In the perspective of efficient N use at the soil level, our results stress that the spring is a critical season that requires a thorough re-thinking of practices by better balancing crops needs with fertilizers inputs. During spring, organic and synthetic N inputs need to be better balanced with N uptake, as the crops have high potential growth but low biomass, which results in an insufficient uptake of the large N inputs (Robertson and Vitousek, 2009).

5.2. The seasonality of inorganic N loads in the Mincio River

Results from this work add to a few seasonal studies coupling land mass budgets of N and net river N export-retention (e.g., Chen et al., 2019; Lin et al., 2019; Compton et al., 2020). For the portion of the Mincio River considered in this study, Fig 6 reports the monthly water inputs, either from precipitation or irrigation, the seasonal soil system N budget and the nitrate accumulation between S1 and S2, which is the difference of the nitrate loads transported past the two river sections.

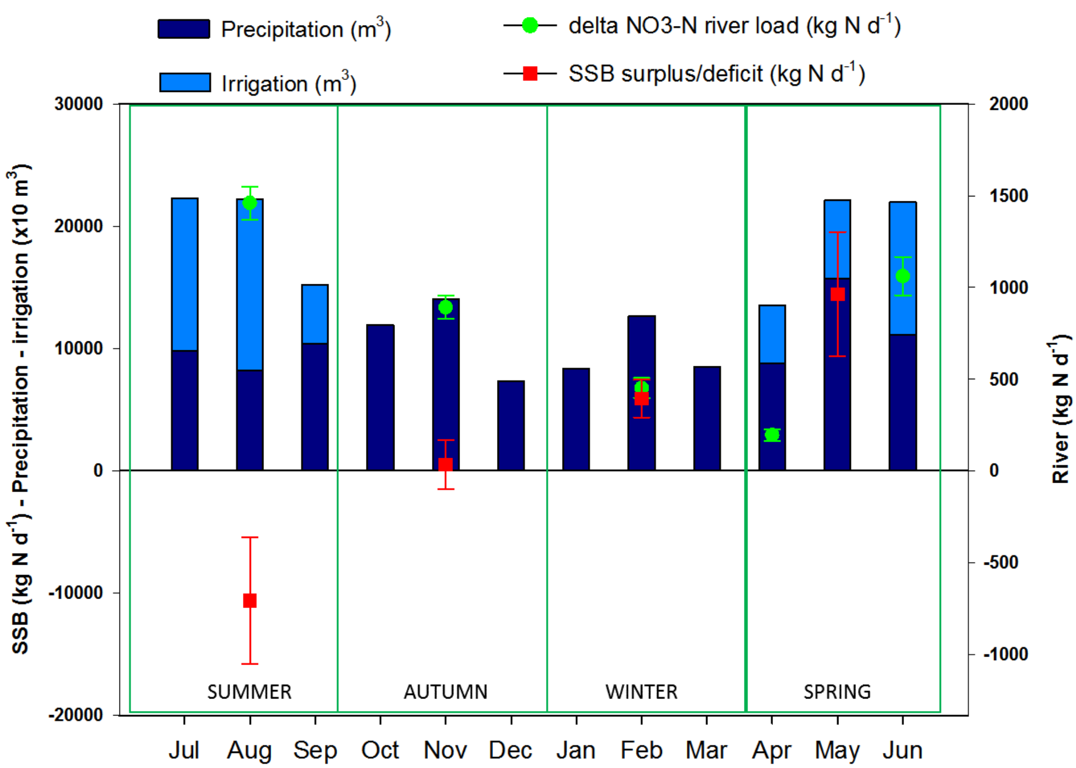

Figure 6. Histograms report monthly water input due to precipitation and irrigation in the municipalities of the

Mincio basin under study. Green dots show the delta nitrate loads of the river reach (delta NO3-N river load) in the five sampling dates and red squares show seasonal N budget of agricultural soils (SSB – Soil system budget – surplus/deficit).

In the regulated river reach under study, which is characterized by gravel bottom and colonized by submerged macrophytes, ammonium and nitrite loads were higher upstream and suggested net retention in all seasons, peaking in summer when the primary producers' activity is maximum. Differently, dissolved inorganic carbon and nitrate loads evidenced a significant increase from upstream to downstream in all the investigated seasons. Nitrate and inorganic carbon net export decreased from August to April, with August as the central month for irrigation and April the last month before the start of the irrigation period. From these results, we calculated the annual net export of inorganic carbon and nitrate multiplying the daily values by the number of days between consecutive samplings, and then integrating the results over one year. Despite 6-8 repeated water samplings during the 24 hours, these calculations are based on measurements carried out in single days along different seasons. However, the nitrate concentrations measured in this study are consistent with the seasonal NO3-N concentrations measured by ARPA Lombardy (the authority that manages water monitoring for the WFD) in the period 2009-2017 and by the Laboratory of Aquatic Ecology of the University of Parma in the period 2011-2017 (Fig. C.1 in Supplementary material C).

It was estimated that 2153±174 t DIC y^{-1} and 317±12 t NO3-N y^{-1} were net exported from the 8 km long reach between S1 and S2. These amounts can be explained by element transformation (e.g., respiration or nitrification), and lateral or vertical inputs. As the loads of the other two inorganic N forms were negligible as compared to nitrate, this calculation was done only for the latter. Using dissolved oxygen budgets (not reported in this work) we converted dark river respiration rates (night oxygen uptake along this stretch; from -1.3 to -5.7 mm O_2 m^{-2} h^{-1} in winter and summer, respectively) into potential nitrification rates (~75 t N y^{-1}) according to nitrification stoichiometry. To this purpose, we assumed that 100% of the oxygen consumed was used to oxidize ammonium to nitrate. Results suggest that microbes-mediated processes as nitrification can explain at most 23% of the nitrate accumulation in the river reach in all seasons. Such percentage is a large overestimation of the real value as it was obtained neglecting all other oxygen-consuming processes, including macrophytes, fish, macroinvertebrates, and the whole heterotrophic microbial community respiration. A comparable nitrate production (~80 t N y^{-1}) was obtained using the nitrification rate set by Taherisoudejani et al. (2018) in the QUAL2Kw model applied to the Oglio River, a nearby Po River tributary with similar hydrological characteristics. Pinardi et al. (2014) found that the processes in the hyporheic zone or the microbial metabolism of carbonate dissolution could explain up to 15% of the DIC increase, including the role of macrophytes in modulating dissolved CO_2 saturation values and fixation of C.

If biological processes cannot explain inorganic carbon and nitrate increase, also point pollution sources can be excluded, due to low discharge of small tributaries along this stretch with water chemistry comparable to that of the Mincio River. Another potential source of N and DIC is groundwater, via seasonally variable river-groundwater interactions. Indeed, the level of the phreatic surface increases and interacts with the river due to precipitation, flooding and sprinkler irrigation during the spring-summer period (Racchetti et al., 2019; Severini et al., 2020) (Fig. 7). In a period before and after fertilization (from March to May 2021), Severini et al. (2022) measured in the same area of the Mincio River significantly higher HCO_3^- concentrations in groundwater than in surface water (Appelo and Postma, 2005). Hence, the DIC increase in the investigated river stretch can be associated to the groundwater feeding the Mincio River. Considering the abundant use of organic fertilizers in the area, the higher DIC in groundwater can be related to the presence of calcite ($CaCO_3$) in the mineralogical composition of the aquifer and to the oxidation of the organic matter, which promotes a higher DIC concentration in groundwater. Future investigations should include the mineralogical composition of the aquifer.

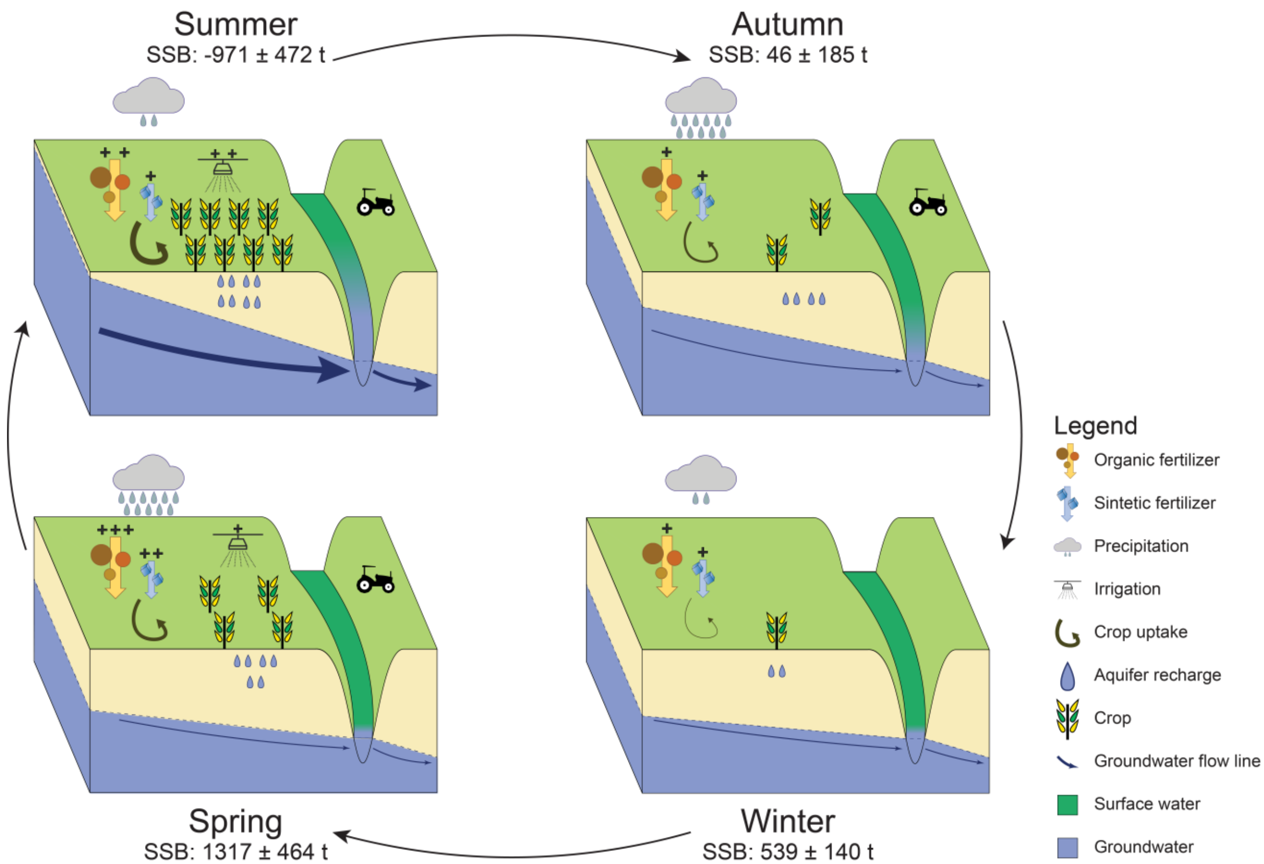

Figure 7. Synthesis of the seasonal nitrogen budgets at agricultural land and at river section. The relation with the groundwater is also reported. SSB = Soil System Budget.

5.3. Linking soil N budgets and N riverine export: the key role of the aquifer

The main period of N fertilization is in spring when there is the highest N soil surplus and surface and groundwater pollution risk due to limited crop uptake. The crop harvest occurs mainly in summer and autumn when the N soil budget is in deficit or close to equilibrium, respectively. These data are reflected by an increasing N export by the river reach from spring to summer, favored by large volumes of nitrate-enriched water displaced through the irrigation across the aquifer-river continuum (Isidoro et al., 2006). Using SiO_2 as tracer, Severini et al. (2022) typified the investigated river stretch as a flow-through system, where groundwater feeds the Mincio River in its west bank and it is fed from the river's east bank (Fig. 7). As a result, N-rich groundwater can displace N-poor water from the Mincio River without a significant modification of the river flow (Fig. 7). Our data are consistent with this hydrogeological conceptual model, since the higher NO3-N delta loads were found in summer, when the groundwater level are the highest and there is the maximum groundwater seeping to the Mincio River, highlighting the deep effects of the recharge given by irrigation. On the contrary, some differences were found during the rest of the year, characterized by a less anthropic recharge of the aquifer. These differences are more related to the dissimilar distribution of precipitation and percolation of water and N to groundwater, which fosters the migration of N to the Mincio River. In fact, as we move away from the end of the irrigation period (September), the lower groundwater heads reported in Severini et al. (2022) could result in a lower groundwater seepage to the river (Fig. 7). Having less nutrient enriched water available guarantees a minor nitrate surplus in the river reach, even if N fertilization starts again in winter, resulting in another period with N soil surplus (Figs. 6, 7).

Considering that the mean annual N surplus on the agricultural land in the study area averaged 67 kg ha^{-1} y^{-1}, it is possible to speculate that the agricultural land surface that can potentially generate the NO3-N river export (317±12 t NO3-N y^{-1}) is equivalent to ~4700 ha^{-1}. In addition, dividing this surface by the length of the river reach investigated, it is possible to estimate the width from the river, which is about 2.9 km for each side, that might be involved in the N surplus production. These data allow to speculate that the N surplus was generated in the 25% of the surface of municipalities under study, giving useful information to better address arable land management.

Our results on N input and output trends in agricultural soils and into the river reach at annual and seasonal basis allow to better understand N patterns from land to river and the potential nitrate pollution to surface and groundwater. This information at seasonal resolution can help policy-makers in developing effective plans to improve N management at the macroscale. In fact, this combination of information can guide the identification of proper spatial-temporal management strategies to reduce N pollution and river export to avoid eutrophication processes of water bodies. For example, our results suggest that more nitrate was delivered downstream in summer because of spring soil N excess coupled to flood irrigation over permeable soils. Hence, it is important to focus on agricultural sources (manure and synthetic fertilizers in particular) to better balance N inputs and output by crop harvest (or stock). Our approach was applied in a pilot study at the sub basin level, but it is exportable to the whole basin and to other rivers. It becomes very important to have local information and basin-specific data to perform seasonal analysis on N patterns (Lassaletta et al., 2021).

5.4. Possible remediation strategies in the context of climate change and the regulation of river discharge

Temporal disconnections between N fertilization, transport and uptake in agricultural land can result in low N use efficiency (Robertson and Vitousek, 2009). Specific monitoring of crop growth, nutrient demand and soil availability is useful to obtain a more synchronous nutrient supply in response to crop needs (Quemada et al., 2013). Alternative practices arranged to implement nutrient management directly in the field include actions such as variable rate or split of fertilizer applications matched to crop growth demand, improvements in efficiency of irrigation practices and use of nitrification inhibitors (Lacey and Armstrong, 2015; Fernández et al., 2016). The nutrient best management practices, for N, should be designed in view of seasonal N leaching losses and hydrologic export to properly depict crop growth dynamics and N demand, soil conditions and hydrology (Lin et al., 2019).

It is during summer that the investigated reach experienced the highest water nitrate accumulation. An action useful to buffer the N export is the implementation of riparian buffer strips that can promote N retention during the spring-summer irrigation period or the use of cover crops in winter (Dabney et al., 2010; Cole et al., 2020). During winter, our calculations suggest N soil excess in a period where uptake is minimum, and denitrification is likely limited by low temperatures and by the thick unsaturated soil. The latter follows the downward winter migration of the water table, previously discussed. For this reason, the adoption of practices that can favor water retention to increase soil humidity in the cold season and the presence of water in canals, commonly dry in autumn and winter, might be a solution that can favor denitrification process. As an example, the construction of artificial ponds or wetlands can be useful to intercept N runoff from agricultural lands (Nõges et al., 2003; Carstensen et al., 2020).

In the geographical area of our case study, the Alps host a series of large lakes regulated by dams that feed rivers among which the Mincio River. The dams regulate the lake water level and the river discharge with rules that aims to accumulate water in winter and release it during the irrigation period, adapting also to local meteorology and water inputs to lakes. This regulation practice guarantees sufficient summer level in lakes for tourism and navigation purposes and large water availability for irrigation and electricity production in the downstream river section. Irrigation is supported by several water abstraction infrastructures that facilitated agriculture and animal farming activities. Irrigation practices, supported mainly by flooding irrigation in the sector of the Po River plain including our study area are carried out over permeable soils, and favor the recharge of the aquifer mainly in summer (Rotiroti et al., 2019). Therefore, water retention upstream during non-irrigation periods and flood irrigation with large water volumes during summer are probably the main drivers of the groundwater head variation, which is subject to strong seasonal differences (Taherisoudejani et al., 2018). Under the current climate change scenario, also in this geographical area, the rapid changes of global warming are manifested with lower precipitation, dry winters, heatwaves and storms events, and an increasing number of consecutive days with high temperatures (Cifrodelli et al., 2015; Pedro-Monzonís et al., 2016; Lassaletta et al., 2021; Ranasinghe et al., 2021). The response to these global trends could increase or decrease river nitrate concentration depending on regional or site-specific linkage between N concentration and discharge (Stelzer et al., 2020). For this reason, the geographical sector under study seems extremely vulnerable to climate change as the system is depicted and managed for large water availability (i.e., large lakes regulation, high water demanding crops, and flooding as main irrigation practice) and therefore a discussion on water management at political level is urgent. Predicting scenarios on the fate of the N excess with different water availability is difficult, we can hypothesize a reduction of water discharge from rivers and consequently from irrigation that will not recharge sufficiently the groundwater due to its deep level (Taherisoudejani et al., 2018). This condition will lead to more thick vadose zones, fostering a higher nitrification rate and nitrate accumulation in soil during winter, whereas denitrification, the main process that removes N permanently from the system, is favored in water saturated soils with high organic matter (Ascott et al., 2017). In soil and rivers close to N saturation, it is expected a lower nitrate retention efficiency and therefore an increase in N availability and vertical and horizontal transfer (Stelzer et al., 2020). Moreover, for the future it can be expected a delay in the river feeding by groundwater with hot-moments of N mass transfer. In fact, we can expect that with the increment of unsaturated zone, the rate of soil denitrification will be reduced and conversely the nitrification process will be favored supplying a short N mass-transfer as soon as the first rainfall or flooding irrigation will occur, carrying water with high N concentration to surface or groundwater. A possible solution to limit this hot-moment can be the improvement of irrigation practices with less water consumption and a more widespread use of precision farming supported for example with remote sensing technique (Nutini et al., 2021). Such a new vision on the irrigation practices can allow a lower winter water retention in the Lake Garda, that can be partially used in the non-irrigation period to guarantee a minimal vital flow in a certain number of drainage canals as well as in the Mincio River favoring denitrification process also in autumn and winter, although with lower rates driven by lower temperatures.

6. Conclusions

Published soil system budgets in agricultural areas generally reveal net N excess on an annual basis, whereas the present study reveals seasonally variable inventories of inputs and outputs, resulting in periods of large N excess and periods of pronounced deficit. The export of N excess via the river draining the investigated area has a temporal lag that depends on irrigation, vertical migration of the water table and subsurface water flow. Flood irrigation first fills the unsaturated zone and then favors river-groundwater interactions. Subsurface water flow replaces N-poor river water with N-rich groundwater. Seasonal soil N budget and the mechanisms of N transfer described in this study should foster more efficient agricultural practices, minimizing N losses and improving N use. Results from this work should also be carefully considered in future planning of agricultural and irrigation activities, in a scenario of climate change and variable availability of water. Winter retention of water in lakes, upstream the agricultural areas, has serious drawbacks as it will increase the volume of the unsaturated soil and the production of nitrate via organic N ammonification and nitrification. Adaptive strategies based on precision farming, new material to retain soil humidity, irrigation techniques alternative to flooding and a management of the canal network targeting the restoration of biogeochemical services (e.g., N-uptake and denitrification) seem effective and sustainable options.

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Declarations

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

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Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

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Abstract

Soil System Budgets (SSB) of nutrients are generally performed annually over arable land to infer their use efficiency and water pollution risk in highly exploited agricultural watersheds. They are seldom partitioned into seasonal budgets and matched with seasonal nutrient transport in adjacent river reaches. We calculated seasonal soil nitrogen (N) budgets in a Mincio River sub-basin (Italy), and we analyzed the dissolved inorganic N net export in the river reach draining such sub-basin. Our results show seasonal differences of SSB with N excess in winter and even more in spring, equilibrium among sources and sinks during autumn and N deficit during summer. Seasonal inorganic N loads transported by the river were not correlated with SSB as they peaked in late summer and were at their minimum in early spring. Fertilization uncoupled to significant uptake supports N excess in winter and spring, whereas crop uptake uncoupled to N inputs supports summer N deficit. Nitrification cannot explain nitrate accumulation in the river reach, suggesting alternative dynamics driving the local hydrology. Flood irrigation results in large soil nitrate solubilization, transport and in upward migration of the groundwater piezometric head during spring and summer periods. River water is likely replaced by nitrate-rich groundwater when the groundwater recharge exceeds a certain threshold coinciding with late summer. Irrigation is then interrupted and the piezometric head, together with nitrate exchange, decreases. This work suggests that a deep understanding of N dynamics in agricultural watersheds with flooding irrigation on permeable soils needs the reconstruction of the vertical pathways of nitrate and of river-groundwater interactions. Moreover, the partitioning of annual into seasonal N budgets and their combination with irrigation practices allows the identification of hot moments in N cycling. Agricultural practices minimizing nitrate excess, its mobility and the risk of surface and groundwater pollution are suggested for this area.

Keywords: nitrogen; retention; transport; loads; ~~watershed;~~ irrigation; river-groundwater interaction

1. Introduction

The dramatic increase of anthropogenic reactive nitrogen (N) inputs in watersheds with intensive agriculture and animal farming has demonstrated negative effects for inland water and groundwater chemical and biological quality, drinking water supplies, ecosystem integrity and functioning and human health (Van Grinsven et al., 2006; Galloway et al., 2008; Rivett et al., 2008; Schlesinger, 2009; Sobota et al., 2015; Huang et al., 2017). Such negative effects are amplified by the human-derived alteration of the hydrological cycle at the watershed scale and by climate change (Galloway et al., 2008; Overeem et al., 2013; Woolway and Merchant, 2019; Woolway et al., 2020). Among the underlying mechanisms are water abstraction for irrigation or industrial purposes or climate change-related drought reducing river discharge and its capacity to dilute and process N loads (Palmer et al., 2008). Low discharge promotes also hyporheic anoxia and ammonium recycling from sediments (Hlaváčová et al., 2005). Hydrological extremes include also short-term, heavy precipitations resulting in high discharge events transferring large N loads from cultivated areas saturating riverine denitrification capacity (Viaroli et al., 2018; Magri et al., 2019).

Nitrogen budgets calculated for agricultural soils within a river basin allow to evaluate the potential risk of diffuse N pollution (Oenema et al., 2003; Soana et al., 2011). In agricultural soils, N inputs associated with organic or synthetic fertilizers, atmospheric deposition or biological fixation can be either temporarily retained in crops or released to the atmosphere as gaseous losses. Nitrogen inputs in excess to temporary retention or permanent loss can be transferred via runoff to adjacent aquatic ecosystems (Howarth et al., 1996; Seitzinger et al., 2006; Pinardi et al., 2018, 2020; Kwon et al., 2022). If soil system budgets in arable land produce reliable snapshots of N pools and fluxes in cultivated areas, the detailed reconstruction and partitioning of N pools and fluxes within watersheds is a challenging objective. For example, seasonally variable water inputs to agricultural soils via precipitation and irrigation affect soil N leaching, horizontal and vertical transport and transformation, N use efficiency as well as river-groundwater interactions and associated N exchange (Schaefer and Alber, 2007; Chae et al., 2009; Howarth et al., 2012; Sinha and Michalak, 2016). Moreover, in intensively cultivated floodplains the hydrological cycle has been regulated by the realization of infrastructures as dams and networks of canals that help buffering climatic anomalies and ensure water availability for crops. In Italy for example, the Alpine sector of the Po River basin hosts large dams that regulate the release of water from deep subalpine lakes (Maggiore, Como, Iseo, Idro and Garda Lakes) to their emissaries (Ticino, Adda, Oglio, Chiese and Mincio Rivers). Winter water retention in subalpine lakes occurs at the cost and drawbacks of reduced water discharge and contributes to the downward vertical migration of groundwater, often resulting in downwelling river-groundwater interactions (i.e. the river feeds the groundwater) (Rotiroti et al., 2019; Severini et al., 2021). On the contrary summer irrigation, besides representing a vehicle for N transport, produces opposite effects, often reversing the direction of river-groundwater interactions (i.e. upwelling, the groundwater feeds the river). These practices, that characterize anthropogenic, intensively cultivated, and hydraulically regulated watersheds with permeable soil, introduce marked seasonality in N budgets (Lin et al., 2019; Racchetti et al., 2019).

Many authors reported a significant correlation between annual N input to croplands and river N export (Neff et al., 2003; Yan et al., 2010; Xu et al., 2013; Strokal et al., 2014; Tong et al., 2017), but they did rarely account for the seasonality of N input and export (McCrackin et al., 2014; Chen et al., 2019). Studies targeting N budgets in agricultural watersheds are generally conceived at the annual scale for mainly practical reasons, as agricultural census data are collected and published by national statistical institutions with annual frequency. Such an approach from one side allows to calculate N use efficiency in cropland and potential N loss, but from the other side, it misses temporal resolution and precludes the understanding of seasonal variations of the array of N-related processes, potentially regulated also by climate change. For example, human activities (e.g., crop production) and altered hydrology may influence the seasonality of N river export (Basu et al., 2010; Compton et al., 2020), together with the seasonal evolution of temperature that influences N losses, retention and removal processes (e.g., denitrification) (McCrackin et al., 2014). Understanding how seasonal variations in human activities and hydrology influence N budgets in agricultural soils and N transport by rivers is important to better understand the mechanisms underlying N transformations along the terrestrial-aquatic path, improve agricultural practices to increase N use efficiency and decrease N pollution, and eventually forecast how climate change will affect N dynamics (Mas-Pla and Menció, 2019). This important set of objectives is a difficult target at the scale of whole watersheds due to scarce resolution of available data and spatial heterogeneity (e.g. pedology, land use, etc). Smaller scales of analysis, targeting specific and homogeneous river and watershed sectors, seem much more promising (McCrackin et al., 2014; Chen et al., 2019; Compton et al., 2020).

Different studies carried out at large temporal and spatial scales (Soana et al., 2011; Pinardi et al., 2018; Viaroli et al., 2018; Lassaletta et al., 2021) have highlighted the presence of hot-spots within watersheds that represent outliers in N budgets (e.g., with very large N excess or very low N use efficiency). They also emphasized the presence of hot-moments within watersheds, that are specific periods during which N mass transfer peaks as a combination of decreased uptake, increased runoff or variation of the water table level, resulting in the reactivation of river-

groundwater interaction (Rosenzweig et al., 2008; Preisendanz et al., 2020; Taherisoudejani et al., 2018). The analysis of N hot-spots and hot-moments in watersheds require specific studies, focusing on small spatial and temporal scales.

In Northern Italy, the Po River valley is an alluvial plain heavily exploited by human activities such as agriculture, animal farming, industry, and tourism. Land use change and hydrological alterations determined high pressure on both surface and groundwater (May, 2013; Pérez-Martín et al., 2014; Lasagna and De Luca, 2019) and a wide portion of the plain is classified as vulnerable to nitrate pollution (Martinelli et al., 2018). The main aim of this study is to analyze the seasonal evolution of dissolved inorganic N loads in a fluvial segment of the Mincio River, a tributary of the Po River, characterized by natural banks, gravel bottom with submerged vegetation, and regulated discharge. This segment crosses a transitional area between permeable and non-permeable soils, characterized by springs and classified as an area of river-groundwater interactions (Balestrini et al., 2021). Due to its hydrogeological features and the large water availability, the considered sub basin is a hotspot of intensive agriculture and animal farming and represents a key study area to analyze if and how the seasonality of agricultural practices affects N dynamics.

In this sector of the Po River, groundwater in the phreatic and shallow aquifer has a short residence time as compared to semiconfined or confined deeper aquifers. This is supported by fast (few days) surface-groundwater dynamics of micro-pollutants (Balderacchi et al., 2016) and low concentrations of total dissolved solids (Martinelli et al., 2018). Results of Balderacchi et al. (2016) suggest also fast response of shallow aquifers to changing conditions; as such they allow to trace agricultural practices (e.g., use of herbicides or fertilization) and they respond quickly to hydrologic variations (e.g., drought, precipitations, irrigation). It can be assumed that macrocontaminants as nitrates undergo the same fast transfer mechanisms, also due to their elevated solubility and absence of interaction with soil and sediment.

The main hypotheses of this work are that river-groundwater interactions affect N transport in specific river sectors and vary seasonally due to combination of irrigation practices and inorganic nitrogen excess in soil. We also hypothesized that the seasonal dynamics of such variable interactions can be captured analyzing comparatively seasonal N budget in agricultural soils and seasonal riverine N transport. In this context, the main aim of the present study was to contrast seasonal N soil budget in an agricultural area drained by a river stretch with seasonal N loads transported by the same draining river stretch, to assess riverine N dynamics in relation to agricultural practices.

2. Study area

The Mincio River (~75 km) originates from the Lake Garda, the largest Italian Lake, and is a tributary of the Po River (Fig. 1). The hydrological regime of the Mincio River is regulated upstream by a dam, which controls the water discharge from the Lake Garda. Along the river course, a series of dams and weirs feeds a network of canals for irrigation and industrial purposes and controls discharge variations to avoid the flooding of cities and villages. Water management for Lake Garda recreational activities and for agricultural purposes results in marked flow variations. Indeed, since the establishment of the river regulation in the 60's of the last century, the Mincio River discharge averaged ~80 and ~30 m^3 s^{-1} during the irrigation (May to September) and outside the irrigation periods, respectively (Lombardy Region, 2006). More recently, projections of decreasing water availability resulted in a further reduction of the Mincio River discharge to ~14 m^3 s^{-1} (www.laghi.net) in autumn and winter to keep water in the Lake Garda and guarantee water availability for irrigation and tourism in the summer season. During winter, the flow reduction and absence of irrigation result in a decreased aquifer recharge, a phenomenon described by different authors in this geographical area at regional (Rotiroti et al., 2019) and local scales (Severini et al., 2021) and, consequently, in a lowering of the phreatic surface. This water transfer dynamic results in a decrease of groundwater upwelling in winter and early spring (Balderacchi et al., 2016).

A wide segment of the Mincio River, including the portion investigated in this study, flows in a flood plain characterized by a multilayered aquifer system with a cyclic facies architecture mainly made of fluvial-channel (gravel and sand) and floodplain (clay) deposits (Amorosi et al., 2008). As a result, the northern part of the plain (high plain) is locally characterized by shallow phreatic aquifers, while in the southern part (low plain) the floodplain facies act as aquitards or aquicludes, resulting in confined and semi-confined aquifers (Chelli et al., 2018). The river reach investigated, from S1 to S2 (length 8.1 km, mean depth ~1 m, mean water velocity ~1.0 m s^{-1}) is in the high-medium plain of the Mincio watershed. This river segment drains the surface ~~and includes~~ of four municipalities (1- Valeggio sul Mincio, 2- Volta Mantovana, 3- Goito, and 4- Marmirolo; total area 184 km^2) ~~for a total surface of 184 km^2~~ (Fig. 1). Since 2006, these municipalities are classified as Nitrate Vulnerable Zones (NVZs) according to the European Nitrate Directive (91/676/CEE). The ~~study~~ area of the four municipalities investigated in mainly classified as agricultural land with~~is characterized by~~ fertile soils due to calcareous gravel deposits with favor ~~and is~~ intensively exploitation~~ed~~ by agriculture (Utilized Agricultural Area - UAA covers 76% of the study area; Fig. 1) and animal farming (1.2 and 0.6 t of live weight per hectare for cattle and pigs, respectively). The urban, infrastructural and industrial areas cover only 5% of the total surface ~~of the four municipalities~~ (Fig. 1). The S1-S2 river segment flows into natural banks, has a mainly gravel bottom, and has transparent waters. The main primary producers are submerged vegetation (e.g., *Vallisneria spiralis*) with

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associated epiphytes, benthic biofilms and different emergent macrophytes growing along the river banks or forming islands (Pinardi et al., 2009, 2014). The linear development of irrigation canals in S1-S2 river reach sub basin sums ~560 km (Fig. 1). The surface covered by the other aquatic environments, such as quarry lakes is ~0.62 km^2.

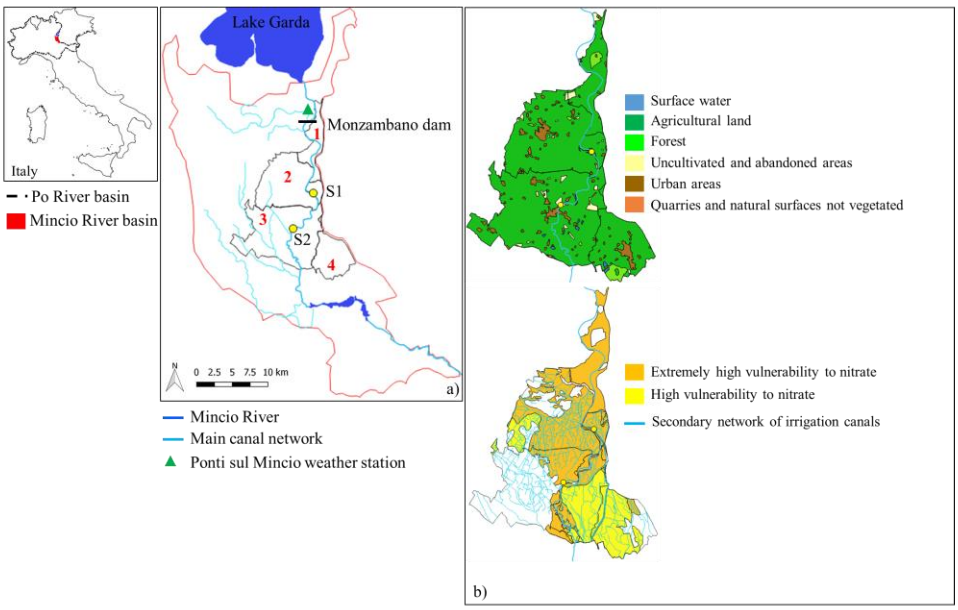

Figure 1. Maps of the study area: the Mincio River segment from Pozzolo dam (S1) to Goito village (S2) (yellow points = water sampling stations). a) Municipalities where the nitrogen mass budget was performed are reported (1 - Valeggio sul Mincio, 2 - Volta Mantovana, 3 – Goito, 4 - Marmirolo). b) Land use and maps of soil vulnerability to nitrate are reported for the four municipalities under study.

3. Material and methods

3.1. Nitrogen budgets and water inputs

A comprehensive input–output N budget across the Utilized Agricultural Area (UAA) was compiled by using locally-derived data on farming activity, agronomic ~~coefficients~~coefficients, and atmospheric deposition. One of the elements of strength of the present work is the reliability of the soil N budget, that is built on a detailed dataset of statistical data and on an accurate set of species-specific coefficients taken from the literature in the field. Nitrogen budget was first calculated at the municipal scale, i.e., the administrative level at which official agricultural statistics are available, then weighted for the percentage of each municipality surface included within the study area, and finally summed up. Census data were integrated in a nutrient budgeting approach proposed by Oenema et al. (2003), recently reviewed by Zhang et al. (2020), and formerly applied to the whole Mincio River basin (Pinardi et al., 2018). Four inputs of N to the UAA were considered (land application of livestock manure, land application of synthetic fertilizers, atmospheric deposition, and biological fixation by crops), together with four outputs of N from UAA (crop harvest, crop stock, ammonia volatilization and denitrification in soils). The difference between N inputs and outputs results in a net, which represents a condition of equilibrium, surplus or deficit of N across the UAA.

The Soil System Budget (SSB) was calculated as follow:

$$SSB\ N = N_{Man} + N_{Fert} + N_{Fix} + N_{Dep} - N_{Harv} - N_{stock} - N_{Vol} - N_{Den}$$

where:

N_{Man} = N in livestock manure applied to agricultural soils

N_{Fert} = N in synthetic fertilizer applied to agricultural soils

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

N_{Dep} = atmospheric N deposition on agricultural land

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

N_{stock} = organic N in crop's standing stock

N_{Vol} = NH_3 volatilization in agricultural soils

N_{Den} = denitrification in agricultural soils

All budget terms were expressed in unit of mass per time (t N y^{-1}), and on a per-area basis, after normalization for the UAA (kg N ha^{-1} y^{-1}). The soil N budget was estimated by employing agricultural census data referring to the agricultural year 2015-2016. The agricultural year overlaps the vegetative cropping cycle, covering two consecutive years, i.e., from November of the first year to October of the following one. The N budget calculated for the agricultural year 2015-2016 is relevant in the present day because, in the last decade, only minor variations occurred in crop surfaces and livestock densities of the study area and fertilization rates did not change appreciably.

The calculation was based on agriculture and farming data ~~for the year 2015~~ reported by the Agricultural Information System of Lombardy Region (SIARL, www.siarl.regione.lombardia.it) and by the Annals of Agrarian Statistics, published yearly by the National Institute of Statistics (ISTAT, http://agri.istat.it/). SIARL databases, retrieved from the Open Data portal of the Lombardy Region (https://dati.lombardia.it/), provided data for livestock density and agricultural areas at the municipality level, whereas the database of the Annals of Agrarian Statistics provided data for crop yield and fertilizer application (http://dati.istat.it/) at the provincial level. Inputs and outputs were initially calculated for each municipality and then aggregated at the study area level.

Uncertainty in N budget calculations was assessed by a Monte Carlo analysis using Excel and R software (R Core Team 2019). All coefficients used to convert census data into N amounts were assumed to vary stochastically and independently around the average value with a normal probability distribution. For each simulation, a set of coefficients was randomly generated from probability distribution functions and a total of 1000 simulations were run. Budget calculation was conducted both at the annual and at the seasonal scales and compared with seasonal in-stream N loads. Details about annual budget data, equations, seasonal breakdown,~~seasonal calculations~~ and sources of census data and agronomic coefficients are presented in Supplementary Material A.

The N loads produced by the urban areas were not included in the calculation because more than 95% of the sewers in the study area are connected to wastewater treatment plants (WWTP). Nearly 75% of the N inputs to WWTP is removed via denitrification in tertiary treatment (Lombardy Region, 2017). Indeed, the calculation of the urban load produced by the resident population, obtained by the conversion of equivalent inhabitant in kg of N per day, resulted in less than 2% of the total N input by diffuse sources (Pinardi et al., 2018).

The daily precipitation data were downloaded from the ARPA Lombardy website (https://www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx) at Ponti sul Mincio station (Fig. 1) for the period from 2010 to 2017. The mean annual, seasonal (irrigation and non-irrigation period) and monthly precipitation data were calculated. Irrigation data at the municipality level was obtained from the 6th Agricultural Census (National Institute of Statistics, 2010, http://dati-censimentoagricoltura.istat.it) and then aggregated at the study area level.

3.2. Water sampling and analyses

Two stations located at the extremes of the identified river reach (S1 and S2; Fig. 1) were sampled for water analyses. The two stations were selected as they were located upstream and downstream the area where the Mincio River can be considered as a gaining river in groundwater-surface water interaction, that is the river is fed by groundwater (Racchetti et al., 2019). Given the constant discharge between S1 and S2, the identified river reach was more recently characterized as a flow-through reach (Severini et. al., 2022), with groundwater feeding the river in the western bank and being fed by the river in the eastern bank. Field campaigns were carried out seasonally with a series of daily cycles of repeated samplings carried out on 12-13 August and 15-16 November 2016, 14-15 February, 12-13 April and 13-14 June 2017. Water samples were taken in three replicates every 4 hours for a 24-hour period. An aliquot was transferred into a 12 mL exetainer (Labco, UK), added with 100 µL of $HgCl_2$, and analyzed for dissolved inorganic carbon (DIC) with Gran titration (0.1 N HCl) within 24 hours from sampling. DIC was measured as it may trace differences between surface and groundwater chemistry. Water aliquots were filtered (GF/F glass fiber filters) and transferred to plastic vials for nitrate (NO3-N), nitrite (NO2-N), and ammonium (NH4-N) determination by spectrophotometric methods (Rodier, 1978; APHA, AWWA, WPCF, 1999). There are two main reasons for focusing on inorganic nitrogen. The first is that from our database and from those of national monitoring agencies in the Mincio river nearly 80% of the total N load is made of inorganic N (DIN, and within the DIN pool >90% is nitrate). The second is that from the same dataset nitrate represents more than 95% of the total dissolved N in groundwater.

Hourly or daily water flow data were obtained by the Interregional Agency for the Po River (AIPO), and by the Mincio Consortium for Pozzolo and Goito sites.

The Mann-Whitney Rank Sum Test was used to test the difference between upstream and downstream values of water flow, NO3-N, NO2-N, NH4-N and DIC concentrations. The R software package (R Development Core Team, 2019) was used to perform all statistical tests.

3.3. Dissolved inorganic nitrogen and carbon daily loads

For each sampling date, daily NO3-N, NO2-N, NH4-N and DIC riverine loads transported at S1 and S2 (kg d^{-1}) were calculated multiplying concentrations by river discharge. The difference between loads at S2 and S1 was calculated according to the following equation:

$$\Delta\text{NO3-N (or NO2-N, NH4-N, DIC)} = \sum [\text{Ct x } \Delta\text{t x Q}]_{S2} - \sum [\text{Ct x } \Delta\text{t x Q}]_{S1} \qquad (1)$$

where: Ct = concentration of NO3-N (or NO2-N, NH4-N, DIC) at time t downstream (S2) or upstream (S1) (mg m^{-3});

Δt = time interval between samplings (h); Q = water flow (m^3 h^{-1}). Such difference can be null, suggesting equilibrium between inputs and outputs, negative, suggesting net retention or dissipation (e.g., uptake or denitrification), or positive, suggesting the occurrence of production or additional inputs along the stretch (e.g., nitrification or point and diffuse inputs). A standard deviation was associated to NO3-N, NO2-N, NH4-N and DIC measurements made in replicates. The errors conveyed through the mathematical description were calculated with classical error propagation equations.

4. Results

4.1. Nitrogen budgets and water inputs

In the four municipalities under study, the total N inputs to arable land (6010±498 t y^{-1}) were mainly due to livestock manure (59%). Nitrogen outputs (5078±494 t y^{-1}) accounted for 84% of the N inputs and were mainly due to crop harvest (50% of the total N outputs). The main cultivated crop is maize (63% of arable land) followed by permanent grassland. The difference between N inputs and outputs denoted a N soil surplus (932±702 t y^{-1}). The mean areal N surplus was 67±50 kg ha^{-1} y^{-1} with values calculated for the different municipalities ranging between -172 and 204 kg ha^{-1} y^{-1}. All these results are reported in Supplementary material B, Table B.1 and Figure B.1.

The seasonal SSB is reported in Figure 2. Manure N fertilization was higher in spring (1590±268 t) and summer (883±149 t), and similar in autumn and winter (530±89 t). Synthetic N fertilization was higher in spring (769±190 t) and winter (257±63 t). Biological N fixation was higher in summer (559±259 t) and spring (350±162 t) whereas atmospheric N depositions were concentrated mainly in summer and autumn (32±4 and 38±4 t, respectively). In spring, N associated to the crop's standing stock (487±98 t) was higher than N in the crop harvest (398±80 t). With respect to N outputs, crop harvest was highest in summer (1492±301 t) and autumn (637±128 t). Ammonia volatilization and soil denitrification were quantitatively important in spring (324±233 t and 210±102 t, respectively). Coupling the seasonal input and output data, a transition from N deficit to N surplus is evident moving from summer (-971±472 t) to spring (1317±464 t).

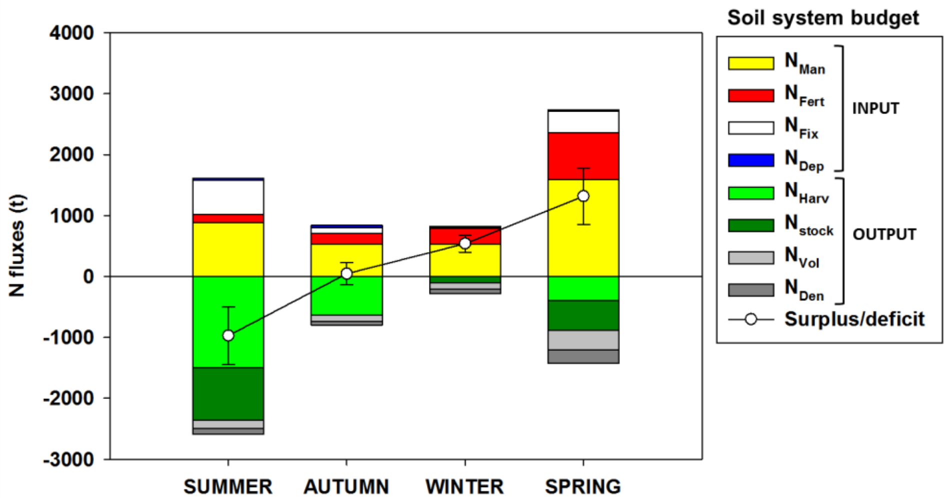

Figure 2. Components of the Soil System Budget (SSB) of nitrogen (N) for the study area within the Mincio River watershed for all seasons. N_{Man} = N in livestock manure applied to agricultural soils; N_{Fert} = N in synthetic fertilizer applied to agricultural soils; N_{Fix} = agricultural N_2 fixation associated with N fixing crops; N_{Dep} = atmospheric N deposition on agricultural land; N_{Harv} = N exported from agricultural soils with crop harvest; N_{stock} = organic N in crop's standing stock; N_{Vol} = NH_3 volatilization in agricultural soils; N_{Den} = denitrification in agricultural soils. White dots represent seasonal N budgets (Σ INPUTS - Σ OUTPUTS); positive values suggest surplus whereas negative values suggest deficit.

During the 2010-2017 period the mean annual precipitation in the study area was 910 ± 197 mm y^{-1}, of which about 43 ± 6% occurred during the irrigation period (Fig. 3).

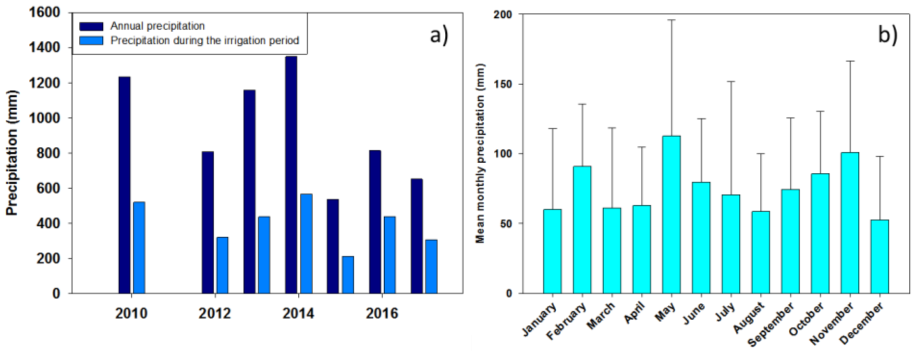

Figure 3. Annual precipitation and the fraction of precipitation during the irrigation period (from May to September) (a) and mean monthly precipitation (± standard deviation) (b) in the period from 2010 to 2017 at Ponti sul Mincio meteorological station (see Fig. 1 for the localization) (2011 was not considered due to missing data for August and September).

During the May-September period a water volume of 53.3 $x10^6$ m^3 was used to irrigate 13,513 ha of UAA, which represent 75% of the total arable land. Flooding and sprinkler were the main irrigation typologies (72% and 27% of the irrigated surface, respectively) (data from the National Institute of Statistics).

4.2. Water physico-chemical features of sampling sites

Nitrate and DIC concentrations were significantly higher at the downstream site for all sampling dates (Mann-Whitney Rank Sum Test, $p < 0.001$, n=68 for each parameter; Fig. 4). On the contrary, the concentrations of the other dissolved inorganic forms of nitrogen (NO2-N and NH4-N) were significantly higher at the upstream site ($p < 0.001$, n=68 for each parameter; Fig. 4). The highest NO3-N and DIC concentrations were measured in June 2017 at both sampling sites (1.6 and 3.0 mg NO3-N L^{-1}, and 33 and 41 mg DIC L^{-1} at S1 and S2, respectively). The highest values of NO2-N were recorded in summer at S1 and S2 (up to 101 and 31 µg L^{-1}, respectively), whereas NH4-N concentrations peaked in August 2016 at S1 (up to 122 µg L^{-1}) and were high at both sites in February 2017. Nitrate was always the main form of inorganic nitrogen, accounting on average for 88% and 98% of the total N at S1 and S2, respectively.

The mean annual water flow was 10.3±2.5 m^3 s^{-1} in the S1-S2 river reach (whole dataset 2016-2017, n=140). During the irrigation period the water flow was not significantly different upstream and downstream ($11.7 \pm 2.0\ m^3\ s^{-1}$ at S1 and $13.0 \pm 3.9\ m^3\ s^{-1}$ at S2; $p > 0.05$). No significant differences were also found between water flow during the irrigation and not-irrigation periods ($p > 0.05$, n=34). The water discharge measured during the experimental activities fell within the annual range of flow variation.

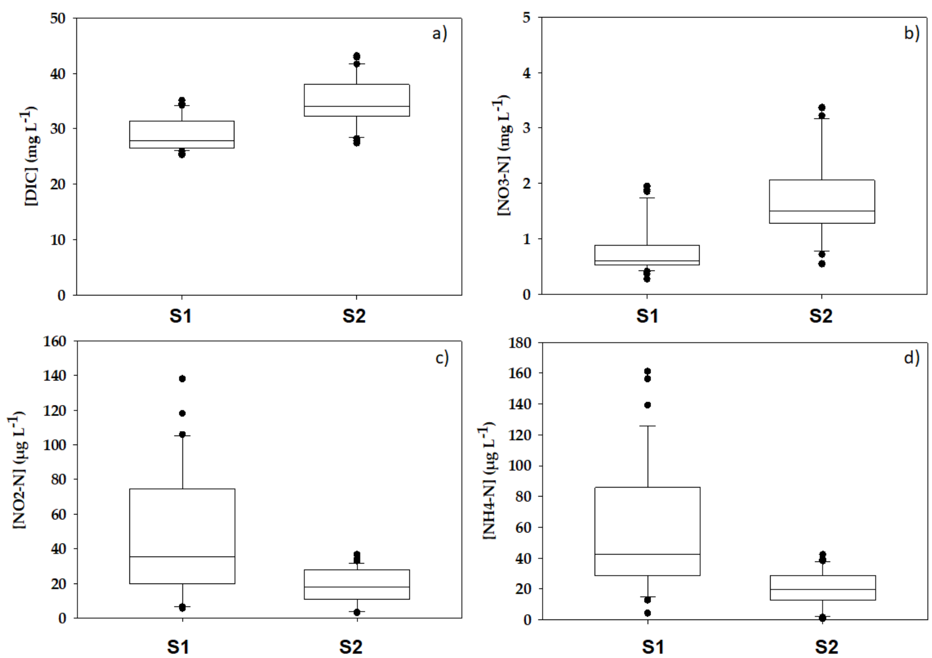

Figure 4. Box plot showing the concentrations of dissolved inorganic carbon (DIC; a), and of nitric (NO3-N; b), nitrous (NO2-N; c) and ammonium (NH4-N; d) nitrogen measured seasonally from August 2016 to June 2017 at S1 and S2. Note different concentration units.

4.3. Dissolved inorganic nitrogen and carbon daily loads

In all seasons, NO3-N and DIC transported loads were higher at S2, whereas NO2-N and NH4-N loads were higher at S1, but by much lower extent (Fig. 5). A positive correlation was found between NO3-N and DIC concentrations (Pearson's correlation coefficient $r = 0.899$, $p < 0.001$, n=68 for each parameter) supporting the possibility of the same origin for the two solutes. A similar seasonal trend was detected for DIC and NO3-N accumulation along the analyzed river reach (Fig. 5). The maximum increase of transported loads (nearly 11,000 and 1500 kg d^{-1} for DIC and NO3-N, respectively) was measured in August 2016, whereas the minimum increase (nearly 2,000 and 200 kg d^{-1} for DIC and NO3-N, respectively) was measured in April 2017 (Fig. 5). The highest NO2-N and NH4-N loads reduction along the stretch were recorded during summer months (Fig. 5).

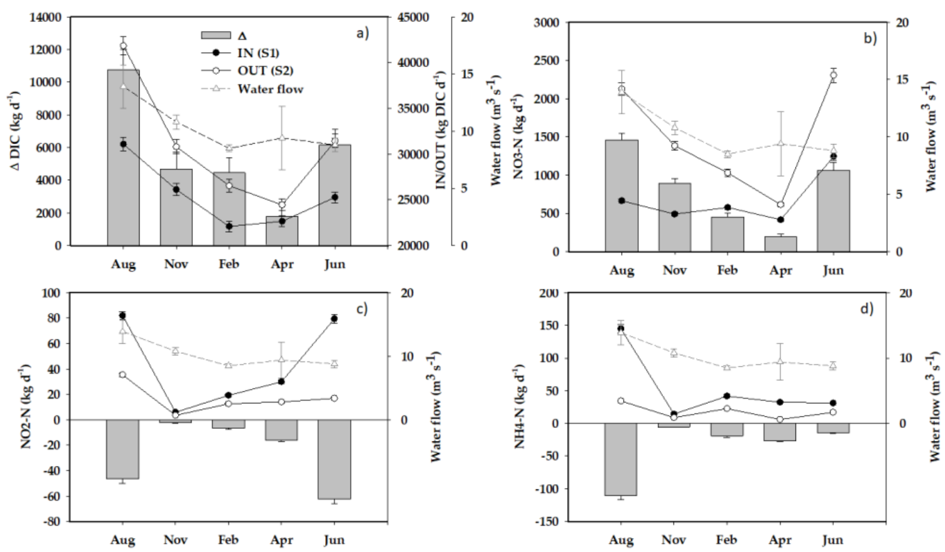

Figure 5. Daily loads of inorganic carbon (DIC; a), nitric nitrogen (NO3-N; b), nitrous nitrogen (NO2-N; c), and ammonium nitrogen (NH4-N; d) transported at the extremes of the studied river reach and their differences (Δ=S2-S1) in the period August 2016 to June 2017. Water flow is also reported. Mean values are given, with error bars corresponding to ±1 standard deviation.

5. Discussion

5.1. The seasonality of soil N budgets

Previous studies carried out in the Po River basin and in other geographical areas characterized by intensive agriculture and animal farming suggest a generalized N surplus and inefficient N use, leading to large N losses to surface and groundwater (Soana et al., 2011; Hou et al., 2015; Özbek et al., 2015; Viaroli et al., 2018; Häußermann et al., 2020). In the Po River plain, to our knowledge, only a few sub-basins (e.g., Ticino and Po di Volano) represent exceptions to this rule due to very limited animal farming and synthetic fertilizers inputs balanced with crop needs (Racchetti et al., 2019; Soana et al., 2021). All those studies were carried out on an annual temporal scale, that potentially masked marked seasonal differences. Our calculations suggest a clear seasonal variation in N soil budgets, changing along seasons with periods with deficit (summer), equilibrium (autumn), moderate (winter) or large (spring) excess. These differences arise from variable seasonal balance among agricultural practices, such as large spring manure and synthetic fertilizer spreading uncoupled to crop uptake, moderate spread of fertilizers during winter with little to no uptake or large summer crop uptake in excess to N inputs (Chen et al., 2019). The N assimilation term is calculated as the product of the standing stock by the biomass-specific uptake rates and peaks in summer. Indeed, the biomass-specific uptake tends to decrease along with the crop's growth, but the crop’s standing stock is much smaller in spring than in summer. Large spring N inputs are therefore coupled to relatively low uptake, resulting in a maximum surplus, exceeding that calculated for winter, when uptake is minimum. Large summer uptake on the contrary exceeds inputs and results in a seasonal deficit of N in the soil system budget. High fertilization is probably driven by the N need of the main cultivated crop, i.e., maize, which is a water and N-demanding species (FAO, 2006). These results on intra-annual variations in N mass budgets support the relevance of seasonal studies in highlighting critical moments in terms of potential water pollution (Lin et al., 2019; Compton et al., 2020). As nitrate water pollution is correlated with N excess in soils, our outcomes indicate a maximum nitrate pollution risk in the spring, a minimum risk in the summer and something intermediate in winter and autumn. Results from seasonal river N transport suggest something different as the highest N accumulation along the stretch was measured in summer and the lowest in spring. Taken together, these apparently contrasting results indicate that more factors are involved in the horizontal transfer of soil N excess to surface water and that such factors determine a temporal lag. In the perspective of efficient N use at the soil level, our results stress that the spring is a critical season that requires a thorough re-thinking of practices by better balancing crops needs with fertilizers inputs. During spring, organic and synthetic N inputs need to be better balanced with N uptake, as the crops have high potential growth but low biomass, which results in an insufficient uptake of the large N inputs (Robertson and Vitousek, 2009).

5.2. The seasonality of inorganic N loads in the Mincio River

Results from this work add to a few seasonal studies coupling land mass budgets of N and net river N export-retention (e.g., Chen et al., 2019; Lin et al., 2019; Compton et al., 2020). For the portion of the Mincio River considered in this study, Fig 6 reports the monthly water inputs, either from precipitation or irrigation, the seasonal soil system N budget and the nitrate accumulation between S1 and S2, which is the difference of the nitrate loads transported past the two river sections.

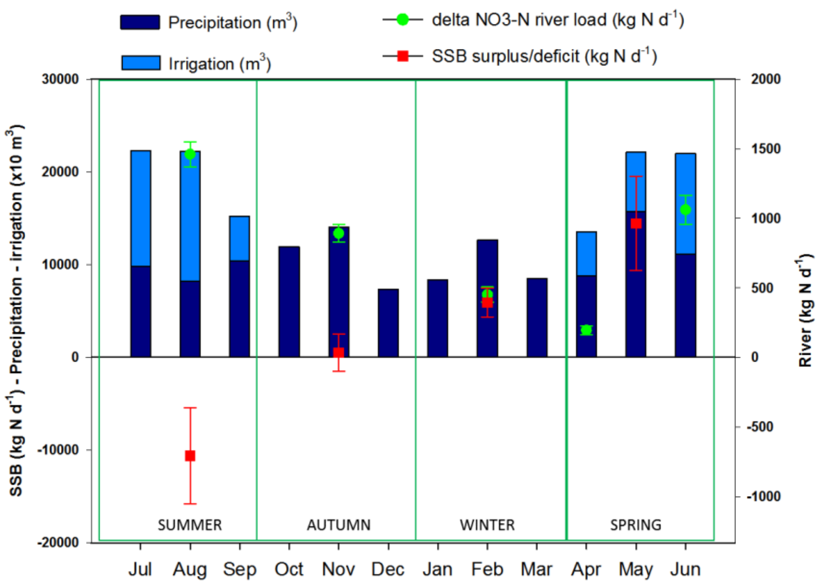

Figure 6. Histograms report monthly water input due to precipitation and irrigation in the municipalities of the Mincio basin under study. Green dots show the delta nitrate loads of the river reach (delta NO3-N river load) in the five sampling dates and red squares show seasonal N budget of agricultural soils (SSB – Soil system budget – surplus/deficit).

In the regulated river reach under study, which is characterized by gravel bottom and colonized by submerged macrophytes, ammonium and nitrite loads were higher upstream and suggested net retention in all seasons, peaking in summer when the primary producers' activity is maximum. Differently, dissolved inorganic carbon and nitrate loads evidenced a significant increase from upstream to downstream in all the investigated seasons. Nitrate and inorganic carbon net export decreased from August to April, with August as the central month for irrigation and April the last month before the start of the irrigation period. From these results, we calculated the annual net export of inorganic carbon and nitrate multiplying the daily values by the number of days between consecutive samplings, and then integrating the results over one year. Despite 6-8 repeated water samplings during the 24 hours, these calculations are based on measurements carried out in single days along different seasons. However, the nitrate concentrations measured in this study are consistent with the seasonal NO3-N concentrations measured by ARPA Lombardy (the authority that manages water monitoring for the WFD) in the period 2009-2017 and by the Laboratory of Aquatic Ecology of the University of Parma in the period 2011-2017 (Fig. C.1 in Supplementary material C).

It was estimated that 2153±174 t DIC y^{-1} and 317±12 t NO3-N y^{-1} were net exported from the 8 km long reach between S1 and S2. These amounts can be explained by element transformation (e.g., respiration or nitrification), and lateral or vertical inputs. As the loads of the other two inorganic N forms were negligible as compared to nitrate, this calculation was done only for the latter. Using dissolved oxygen budgets (not reported in this work) we converted dark river respiration rates (night oxygen uptake along this stretch; from -1.3 to -5.7 mm O_2 m^{-2} h^{-1} in winter and summer, respectively) into potential nitrification rates (~75 t N y^{-1}) according to nitrification stoichiometry. To this purpose, we assumed that 100% of the oxygen consumed was used to oxidize ammonium to nitrate. Results suggest that microbes-mediated processes as nitrification can explain at most 23% of the nitrate accumulation in the river reach in all seasons. Such percentage is a large overestimation of the real value as it was obtained neglecting all other oxygen-consuming processes, including macrophytes, fish, macroinvertebrates, and the whole heterotrophic microbial community respiration. A comparable nitrate production (~80 t N y^{-1}) was obtained using the nitrification rate set by Taherisoudejani et al. (2018) in the QUAL2Kw model applied to the Oglio River, a nearby Po River tributary with similar hydrological characteristics. Pinardi et al. (2014) found that the processes in the hyporheic zone or the microbial metabolism of carbonate dissolution could explain up to 15% of the DIC increase, including the role of macrophytes in modulating dissolved CO_2 saturation values and fixation of C.

If biological processes cannot explain inorganic carbon and nitrate increase, also point pollution sources can be excluded, due to low discharge of small tributaries along this stretch with water chemistry comparable to that of the Mincio River. Another potential source of N and DIC is groundwater, via seasonally variable river-groundwater interactions. Indeed, the level of the phreatic surface increases and interacts with the river due to precipitation, flooding and sprinkler irrigation during the spring-summer period (Racchetti et al., 2019; Severini et al., 2020) (Fig. 7). In a period before and after fertilization (from March to May 2021), Severini et al. (2022) measured in the same area of the Mincio River significantly higher HCO_3^- concentrations in groundwater than in surface water (Appelo and Postma, 2005). Hence, the DIC increase in the investigated river stretch can be associated to the groundwater feeding the Mincio River. Considering the abundant use of organic fertilizers in the area, the higher DIC in groundwater can be related to the presence of calcite ($CaCO_3$) in the mineralogical composition of the aquifer and to the oxidation of the organic matter, which promotes a higher DIC concentration in groundwater. Future investigations should include the mineralogical composition of the aquifer.

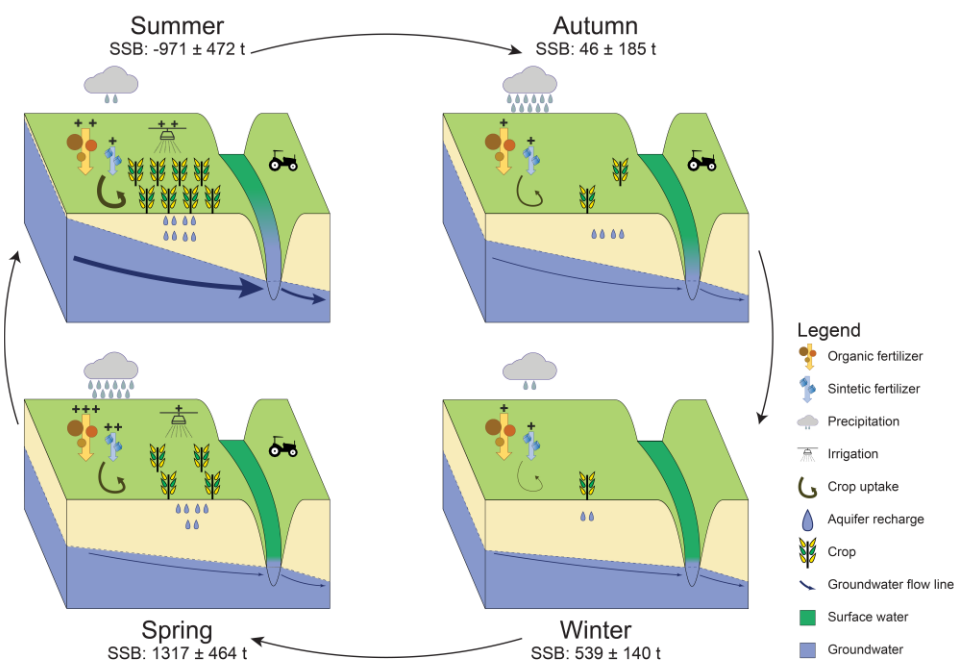

Figure 7. Synthesis of the seasonal nitrogen budgets at agricultural land and at river section. The relation with the groundwater is also reported. SSB = Soil System Budget.

5.3. Linking soil N budgets and N riverine export: the key role of the aquifer

The main period of N fertilization is in spring when there is the highest N soil surplus and surface and groundwater pollution risk due to limited crop uptake. The crop harvest occurs mainly in summer and autumn when the N soil budget is in deficit or close to equilibrium, respectively. These data are reflected by an increasing N export by the river reach from spring to summer, favored by large volumes of nitrate-enriched water displaced through the irrigation across the aquifer-river continuum (Isidoro et al., 2006). Using SiO_2 as tracer, Severini et al. (2022) typified the investigated river stretch as a flow-through system, where groundwater feeds the Mincio River in its west bank and it is fed from the river's east bank (Fig. 7). As a result, N-rich groundwater can displace N-poor water from the Mincio River without a significant modification of the river flow (Fig. 7). Our data are consistent with this hydrogeological conceptual model, since the higher NO3-N delta loads were found in summer, when the groundwater level are the highest and there is the maximum groundwater seeping to the Mincio River, highlighting the deep effects of the recharge given by irrigation. On the contrary, some differences were found during the rest of the year, characterized by a less anthropic recharge of the aquifer. These differences are more related to the dissimilar distribution of precipitation and percolation of water and N to groundwater, which fosters the migration of N to the Mincio River. In fact, as we move away from the end of the irrigation period (September), the lower groundwater heads reported in Severini et al. (2022) could result in a lower groundwater seepage to the river (Fig. 7). Having less nutrient enriched water available guarantees a minor nitrate surplus in the river reach, even if N fertilization starts again in winter, resulting in another period with N soil surplus (Figs. 6, 7).

Considering that the mean annual N surplus on the agricultural land in the study area averaged 67 kg ha^{-1} y^{-1}, it is possible to speculate that the agricultural land surface that can potentially generate the NO3-N river export (317±12 t NO3-N y^{-1}) is equivalent to ~4700 ha^{-1}. In addition, dividing this surface by the length of the river reach investigated, it is possible to estimate the width from the river, which is about 2.9 km for each side, that might be involved in the N surplus production. These data allow to speculate that the N surplus was generated in the 25% of the surface of municipalities under study, giving useful information to better address arable land management.

Our results on N input and output trends in agricultural soils and into the river reach at annual and seasonal basis allow to better understand N patterns from land to river and the potential nitrate pollution to surface and groundwater. This information at seasonal resolution can help policy-makers in developing effective plans to improve N management at the macroscale. In fact, this combination of information can guide the identification of proper spatial-temporal management strategies to reduce N pollution and river export to avoid eutrophication processes of water bodies. For example, our results suggest that more nitrate was delivered downstream in summer because of spring soil N excess coupled to flood irrigation over permeable soils. Hence, it is important to focus on agricultural sources (manure and synthetic fertilizers in particular) to better balance N inputs and output by crop harvest (or stock). Our approach was applied in a pilot study at the sub basin level, but it is exportable to the whole basin and to other rivers. It becomes very important to have local information and basin-specific data to perform seasonal analysis on N patterns (Lassaletta et al., 2021).

5.4. Possible remediation strategies in the context of climate change and the regulation of river discharge

Temporal disconnections between N fertilization, transport and uptake in agricultural land can result in low N use efficiency (Robertson and Vitousek, 2009). Specific monitoring of crop growth, nutrient demand and soil availability is useful to obtain a more synchronous nutrient supply in response to crop needs (Quemada et al., 2013). Alternative practices arranged to implement nutrient management directly in the field include actions such as variable rate or split of fertilizer applications matched to crop growth demand, improvements in efficiency of irrigation practices and use of nitrification inhibitors (Lacey and Armstrong, 2015; Fernández et al., 2016). The nutrient best management practices, for N, should be designed in view of seasonal N leaching losses and hydrologic export to properly depict crop growth dynamics and N demand, soil conditions and hydrology (Lin et al., 2019).

It is during summer that the investigated reach experienced the highest water nitrate accumulation. An action useful to buffer the N export is the implementation of riparian buffer strips that can promote N retention during the spring-summer irrigation period or the use of cover crops in winter (Dabney et al., 2010; Cole et al., 2020). During winter, our calculations suggest N soil excess in a period where uptake is minimum, and denitrification is likely limited by low temperatures and by the thick unsaturated soil. The latter follows the downward winter migration of the water table, previously discussed. For this reason, the adoption of practices that can favor water retention to increase soil humidity in the cold season and the presence of water in canals, commonly dry in autumn and winter, might be a solution that can favor denitrification process. As an example, the construction of artificial ponds or wetlands can be useful to intercept N runoff from agricultural lands (Nõges et al., 2003; Carstensen et al., 2020).

In the geographical area of our case study, the Alps host a series of large lakes regulated by dams that feed rivers among which the Mincio River. The dams regulate the lake water level and the river discharge with rules that aims to accumulate water in winter and release it during the irrigation period, adapting also to local meteorology and water inputs to lakes. This regulation practice guarantees sufficient summer level in lakes for tourism and navigation purposes and large water availability for irrigation and electricity production in the downstream river section. Irrigation is supported by several water abstraction infrastructures that facilitated agriculture and animal farming activities. Irrigation practices, supported mainly by flooding irrigation in the sector of the Po River plain including our study area are carried out over permeable soils, and favor the recharge of the aquifer mainly in summer (Rotiroti et al., 2019). Therefore, water retention upstream during non-irrigation periods and flood irrigation with large water volumes during summer are probably the main drivers of the groundwater head variation, which is subject to strong seasonal differences (Taherisoudejani et al., 2018). Under the current climate change scenario, also in this geographical area, the rapid changes of global warming are manifested with lower precipitation, dry winters, heatwaves and storms events, and an increasing number of consecutive days with high temperatures (Cifrodelli et al., 2015; Pedro-Monzonís et al., 2016; Lassaletta et al., 2021; Ranasinghe et al., 2021). The response to these global trends could increase or decrease river nitrate concentration depending on regional or site-specific linkage between N concentration and discharge (Stelzer et al., 2020). For this reason, the geographical sector under study seems extremely vulnerable to climate change as the system is depicted and managed for large water availability (i.e., large lakes regulation, high water demanding crops, and flooding as main irrigation practice) and therefore a discussion on water management at political level is urgent. Predicting scenarios on the fate of the N excess with different water availability is difficult, we can hypothesize a reduction of water discharge from rivers and consequently from irrigation that will not recharge sufficiently the groundwater due to its deep level (Taherisoudejani et al., 2018). This condition will lead to more thick vadose zones, fostering a higher nitrification rate and nitrate accumulation in soil during winter, whereas denitrification, the main process that removes N permanently from the system, is favored in water saturated soils with high organic matter (Ascott et al., 2017). In soil and rivers close to N saturation, it is expected a lower nitrate retention efficiency and therefore an increase in N availability and vertical and horizontal transfer (Stelzer et al., 2020). Moreover, for the future it can be expected a delay in the river feeding by groundwater with hot-moments of N mass transfer. In fact, we can expect that with the increment of unsaturated zone, the rate of soil denitrification will be reduced and conversely the nitrification process will be favored supplying a short N mass-transfer as soon as the first rainfall or flooding irrigation will occur, carrying water with high N concentration to surface or groundwater. A possible solution to limit this hot-moment can be the improvement of irrigation practices with less water consumption and a more widespread use of precision farming supported for example with remote sensing technique (Nutini et al., 2021). Such a new vision on the irrigation practices can allow a lower winter water retention in the Lake Garda, that can be partially used in the non-irrigation period to guarantee a minimal vital flow in a certain number of drainage canals as well as in the Mincio River favoring denitrification process also in autumn and winter, although with lower rates driven by lower temperatures.

6. Conclusions

Published soil system budgets in agricultural areas generally reveal net N excess on an annual basis, whereas the present study reveals seasonally variable inventories of inputs and outputs, resulting in periods of large N excess and periods of pronounced deficit. The export of N excess via the river draining the investigated area has a temporal lag that depends on irrigation, vertical migration of the water table and subsurface water flow. Flood irrigation first fills the unsaturated zone and then favors river-groundwater interactions. Subsurface water flow replaces N-poor river water with N-rich groundwater. Seasonal soil N budget and the mechanisms of N transfer described in this study should foster more efficient agricultural practices, minimizing N losses and improving N use. Results from this work should also be carefully considered in future planning of agricultural and irrigation activities, in a scenario of climate change and variable availability of water. Winter retention of water in lakes, upstream the agricultural areas, has serious drawbacks as it will increase the volume of the unsaturated soil and the production of nitrate via organic N ammonification and nitrification. Adaptive strategies based on precision farming, new material to retain soil humidity, irrigation techniques alternative to flooding and a management of the canal network targeting the restoration of biogeochemical services (e.g., N-uptake and denitrification) seem effective and sustainable options.

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Declarations

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

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Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

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Abstract

Soil System Budgets (SSB) of nutrients are generally performed annually over arable land to infer their use efficiency and water pollution risk in highly exploited agricultural watersheds. They are seldom partitioned into seasonal budgets and matched with seasonal nutrient transport in adjacent river reaches. We calculated seasonal soil nitrogen (N) budgets in a Mincio River sub-basin (Italy), and we analyzed the dissolved inorganic N net export in the river reach draining such sub-basin. Our results show seasonal differences of SSB with N excess in winter and even more in spring, equilibrium among sources and sinks during autumn and N deficit during summer. Seasonal inorganic N loads transported by the river were not correlated with SSB as they peaked in late summer and were at their minimum in early spring. Fertilization uncoupled to significant uptake supports N excess in winter and spring, whereas crop uptake uncoupled to N inputs supports summer N deficit. Nitrification cannot explain nitrate accumulation in the river reach, suggesting alternative dynamics driving the local hydrology. Flood irrigation results in large soil nitrate solubilization, transport and in upward migration of the groundwater piezometric head during spring and summer periods. River water is likely replaced by nitrate-rich groundwater when the groundwater recharge exceeds a certain threshold coinciding with late summer. Irrigation is then interrupted and the piezometric head, together with nitrate exchange, decreases. This work suggests that a deep understanding of N dynamics in agricultural watersheds with flooding irrigation on permeable soils needs the reconstruction of the vertical pathways of nitrate and of river-groundwater interactions. Moreover, the partitioning of annual into seasonal N budgets and their combination with irrigation practices allows the identification of hot moments in N cycling. Agricultural practices minimizing nitrate excess, its mobility and the risk of surface and groundwater pollution are suggested for this area.

Keywords: nitrogen; retention; transport; loads; irrigation; river-groundwater interaction

1. Introduction

The dramatic increase of anthropogenic reactive nitrogen (N) inputs in watersheds with intensive agriculture and animal farming has demonstrated negative effects for inland water and groundwater chemical and biological quality, drinking water supplies, ecosystem integrity and functioning and human health (Van Grinsven et al., 2006; Galloway et al., 2008; Rivett et al., 2008; Schlesinger, 2009; Sobota et al., 2015; Huang et al., 2017). Such negative effects are amplified by the human-derived alteration of the hydrological cycle at the watershed scale and by climate change (Galloway et al., 2008; Overeem et al., 2013; Woolway and Merchant, 2019; Woolway et al., 2020). Among the underlying mechanisms are water abstraction for irrigation or industrial purposes or climate change-related drought reducing river discharge and its capacity to dilute and process N loads (Palmer et al., 2008). Low discharge promotes also hyporheic anoxia and ammonium recycling from sediments (Hlaváčová et al., 2005). Hydrological extremes include also short-term, heavy precipitations resulting in high discharge events transferring large N loads from cultivated areas saturating riverine denitrification capacity (Viaroli et al., 2018; Magri et al., 2019).

Nitrogen budgets calculated for agricultural soils within a river basin allow to evaluate the potential risk of diffuse N pollution (Oenema et al., 2003; Soana et al., 2011). In agricultural soils, N inputs associated with organic or synthetic fertilizers, atmospheric deposition or biological fixation can be either temporarily retained in crops or released to the atmosphere as gaseous losses. Nitrogen inputs in excess to temporary retention or permanent loss can be transferred via runoff to adjacent aquatic ecosystems (Howarth et al., 1996; Seitzinger et al., 2006; Pinardi et al., 2018, 2020; Kwon et al., 2022). If soil system budgets in arable land produce reliable snapshots of N pools and fluxes in cultivated areas, the detailed reconstruction and partitioning of N pools and fluxes within watersheds is a challenging objective. For example, seasonally variable water inputs to agricultural soils via precipitation and irrigation affect soil N leaching, horizontal and vertical transport and transformation, N use efficiency as well as river-groundwater interactions and associated N exchange (Schaefer and Alber, 2007; Chae et al., 2009; Howarth et al., 2012; Sinha and Michalak, 2016). Moreover, in intensively cultivated floodplains the hydrological cycle has been regulated by the realization of infrastructures as dams and networks of canals that help buffering climatic anomalies and ensure water availability for crops. In Italy for example, the Alpine sector of the Po River basin hosts large dams that regulate the release of water from deep subalpine lakes (Maggiore, Como, Iseo, Idro and Garda Lakes) to their emissaries (Ticino, Adda, Oglio, Chiese and Mincio Rivers). Winter water retention in subalpine lakes occurs at the cost and drawbacks of reduced water discharge and contributes to the downward vertical migration of groundwater, often resulting in downwelling river-groundwater interactions (i.e. the river feeds the groundwater) (Rotiroti et al., 2019; Severini et al., 2021). On the contrary summer irrigation, besides representing a vehicle for N transport, produces opposite effects, often reversing the direction of river-groundwater interactions (i.e. upwelling, the groundwater feeds the river). These practices, that characterize anthropogenic, intensively cultivated, and hydraulically regulated watersheds with permeable soil, introduce marked seasonality in N budgets (Lin et al., 2019; Racchetti et al., 2019).

Many authors reported a significant correlation between annual N input to croplands and river N export (Neff et al., 2003; Yan et al., 2010; Xu et al., 2013; Strokal et al., 2014; Tong et al., 2017), but they did rarely account for the seasonality of N input and export (McCrackin et al., 2014; Chen et al., 2019). Studies targeting N budgets in agricultural watersheds are generally conceived at the annual scale for mainly practical reasons, as agricultural census data are collected and published by national statistical institutions with annual frequency. Such an approach from one side allows to calculate N use efficiency in cropland and potential N loss, but from the other side, it misses temporal resolution and precludes the understanding of seasonal variations of the array of N-related processes, potentially regulated also by climate change. For example, human activities (e.g., crop production) and altered hydrology may influence the seasonality of N river export (Basu et al., 2010; Compton et al., 2020), together with the seasonal evolution of temperature that influences N losses, retention and removal processes (e.g., denitrification) (McCrackin et al., 2014). Understanding how seasonal variations in human activities and hydrology influence N budgets in agricultural soils and N transport by rivers is important to better understand the mechanisms underlying N transformations along the terrestrial-aquatic path, improve agricultural practices to increase N use efficiency and decrease N pollution, and eventually forecast how climate change will affect N dynamics (Mas-Pla and Menció, 2019). This important set of objectives is a difficult target at the scale of whole watersheds due to scarce resolution of available data and spatial heterogeneity (e.g. pedology, land use, etc). Smaller scales of analysis, targeting specific and homogeneous river and watershed sectors, seem much more promising (McCrackin et al., 2014; Chen et al., 2019; Compton et al., 2020).

Different studies carried out at large temporal and spatial scales (Soana et al., 2011; Pinardi et al., 2018; Viaroli et al., 2018; Lassaletta et al., 2021) have highlighted the presence of hot-spots within watersheds that represent outliers in N budgets (e.g., with very large N excess or very low N use efficiency). They also emphasized the presence of hot-moments within watersheds, that are specific periods during which N mass transfer peaks as a combination of decreased uptake, increased runoff or variation of the water table level, resulting in the reactivation of river-

groundwater interaction (Rosenzweig et al., 2008; Preisendanz et al., 2020; Taherisoudejani et al., 2018). The analysis of N hot-spots and hot-moments in watersheds require specific studies, focusing on small spatial and temporal scales. In Northern Italy, the Po River valley is an alluvial plain heavily exploited by human activities such as agriculture, animal farming, industry, and tourism. Land use change and hydrological alterations determined high pressure on both surface and groundwater (May, 2013; Pérez-Martín et al., 2014; Lasagna and De Luca, 2019) and a wide portion of the plain is classified as vulnerable to nitrate pollution (Martinelli et al., 2018). The main aim of this study is to analyze the seasonal evolution of dissolved inorganic N loads in a fluvial segment of the Mincio River, a tributary of the Po River, characterized by natural banks, gravel bottom with submerged vegetation, and regulated discharge. This segment crosses a transitional area between permeable and non-permeable soils, characterized by springs and classified as an area of river-groundwater interactions (Balestrini et al., 2021). Due to its hydrogeological features and the large water availability, the considered sub basin is a hotspot of intensive agriculture and animal farming and represents a key study area to analyze if and how the seasonality of agricultural practices affects N dynamics.

In this sector of the Po River, groundwater in the phreatic and shallow aquifer has a short residence time as compared to semiconfined or confined deeper aquifers. This is supported by fast (few days) surface-groundwater dynamics of micro-pollutants (Balderacchi et al., 2016) and low concentrations of total dissolved solids (Martinelli et al., 2018). Results of Balderacchi et al. (2016) suggest also fast response of shallow aquifers to changing conditions; as such they allow to trace agricultural practices (e.g., use of herbicides or fertilization) and they respond quickly to hydrologic variations (e.g., drought, precipitations, irrigation). It can be assumed that macrocontaminants as nitrates undergo the same fast transfer mechanisms, also due to their elevated solubility and absence of interaction with soil and sediment.

The main hypotheses of this work are that river-groundwater interactions affect N transport in specific river sectors and vary seasonally due to combination of irrigation practices and inorganic nitrogen excess in soil. We also hypothesized that the seasonal dynamics of such variable interactions can be captured analyzing comparatively seasonal N budget in agricultural soils and seasonal riverine N transport. In this context, the main aim of the present study was to contrast seasonal N soil budget in an agricultural area drained by a river stretch with seasonal N loads transported by the same draining river stretch, to assess riverine N dynamics in relation to agricultural practices.

2. Study area

The Mincio River (~75 km) originates from the Lake Garda, the largest Italian Lake, and is a tributary of the Po River (Fig. 1). The hydrological regime of the Mincio River is regulated upstream by a dam, which controls the water discharge from the Lake Garda. Along the river course, a series of dams and weirs feeds a network of canals for irrigation and industrial purposes and controls discharge variations to avoid the flooding of cities and villages. Water management for Lake Garda recreational activities and for agricultural purposes results in marked flow variations. Indeed, since the establishment of the river regulation in the 60's of the last century, the Mincio River discharge averaged ~80 and ~30 $m^3 s^{-1}$ during the irrigation (May to September) and outside the irrigation periods, respectively (Lombardy Region, 2006). More recently, projections of decreasing water availability resulted in a further reduction of the Mincio River discharge to ~14 $m^3 s^{-1}$ (www.laghi.net) in autumn and winter to keep water in the Lake Garda and guarantee water availability for irrigation and tourism in the summer season. During winter, the flow reduction and absence of irrigation result in a decreased aquifer recharge, a phenomenon described by different authors in this geographical area at regional (Rotiroti et al., 2019) and local scales (Severini et al., 2021) and, consequently, in a lowering of the phreatic surface. This water transfer dynamic results in a decrease of groundwater upwelling in winter and early spring (Balderacchi et al., 2016).

A wide segment of the Mincio River, including the portion investigated in this study, flows in a flood plain characterized by a multilayered aquifer system with a cyclic facies architecture mainly made of fluvial-channel (gravel and sand) and floodplain (clay) deposits (Amorosi et al., 2008). As a result, the northern part of the plain (high plain) is locally characterized by shallow phreatic aquifers, while in the southern part (low plain) the floodplain facies act as aquitards or aquicludes, resulting in confined and semi-confined aquifers (Chelli et al., 2018). The river reach investigated, from S1 to S2 (length 8.1 km, mean depth ~1 m, mean water velocity ~1.0 m s^{-1}) is in the high-medium plain of the Mincio watershed. This river segment drains the surface of four municipalities (1- Valeggio sul Mincio, 2- Volta Mantovana, 3- Goito, and 4- Marmirolo; total area 184 km^2) (Fig. 1). Since 2006, these municipalities are classified as Nitrate Vulnerable Zones (NVZs) according to the European Nitrate Directive (91/676/CEE). The area of the four municipalities investigated in mainly classified as agricultural land with fertile soils due to calcareous gravel deposits with favor intensively exploitation by agriculture (Utilized Agricultural Area - UAA covers 76% of the study area; Fig. 1) and animal farming (1.2 and 0.6 t of live weight per hectare for cattle and pigs, respectively). The urban, infrastructural and industrial areas cover only 5% of the total surface (Fig. 1). The S1-S2 river segment flows into natural banks, has a mainly gravel bottom, and has transparent waters. The main primary producers are submerged vegetation (e.g., *Vallisneria spiralis*) with associated epiphytes, benthic biofilms and different emergent macrophytes growing along the river banks or forming islands (Pinardi et al., 2009, 2014). The linear development of irrigation canals in S1-S2 river reach sub basin sums ~560 km (Fig. 1). The surface covered by the other aquatic environments, such as quarry lakes is ~0.62 km^2.

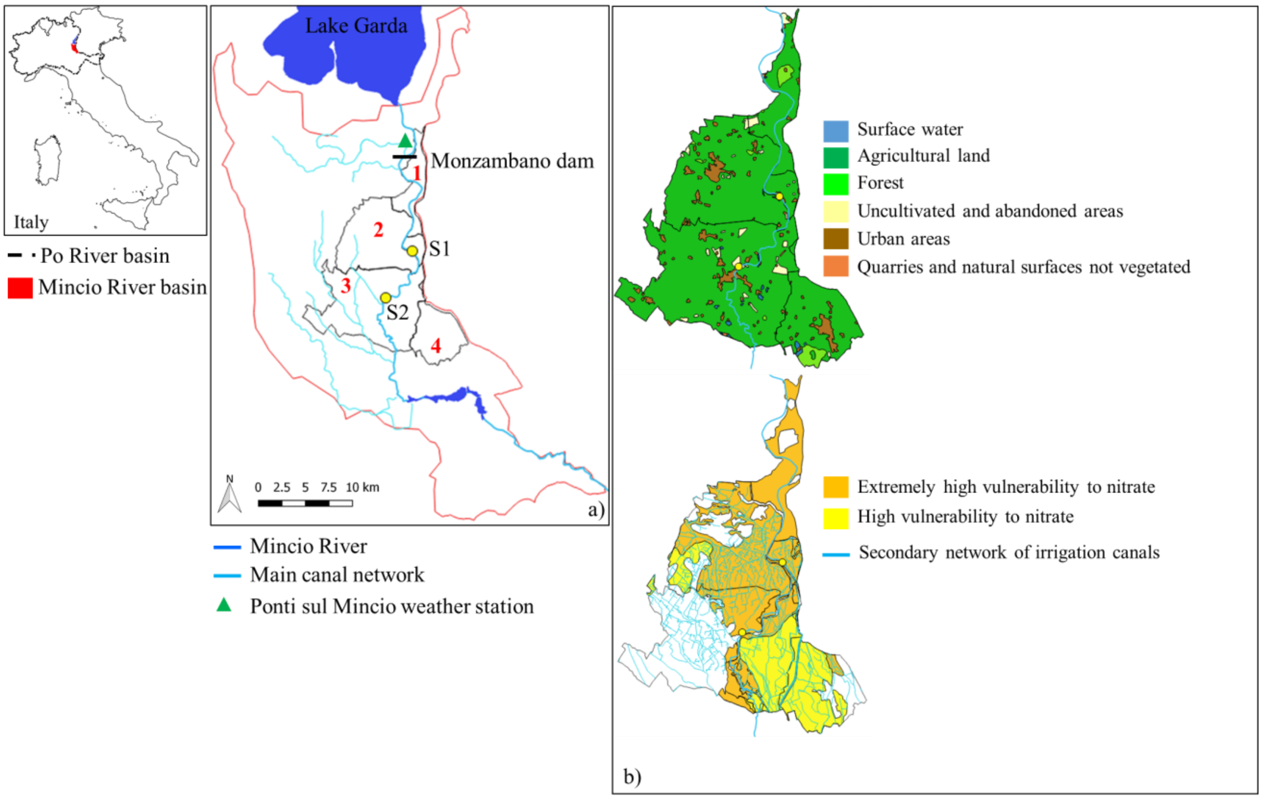

Figure 1. Maps of the study area: the Mincio River segment from Pozzolo dam (S1) to Goito village (S2) (yellow points = water sampling stations). a) Municipalities where the nitrogen mass budget was performed are reported (1 - Valeggio sul Mincio, 2 - Volta Mantovana, 3 – Goito, 4 - Marmirolo). b) Land use and maps of soil vulnerability to nitrate are reported for the four municipalities under study.

3. Material and methods

3.1. Nitrogen budgets and water inputs

A comprehensive input–output N budget across the Utilized Agricultural Area (UAA) was compiled by using locally-derived data on farming activity, agronomic coefficients, and atmospheric deposition. One of the elements of strength of the present work is the reliability of the soil N budget, that is built on a detailed dataset of statistical data and on an accurate set of species-specific coefficients taken from the literature in the field. Nitrogen budget was first calculated at the municipal scale, i.e., the administrative level at which official agricultural statistics are available, then weighted for the percentage of each municipality surface included within the study area, and finally summed up. Census data were integrated in a nutrient budgeting approach proposed by Oenema et al. (2003), recently reviewed by Zhang et al. (2020), and formerly applied to the whole Mincio River basin (Pinardi et al., 2018). Four inputs of N to the UAA were considered (land application of livestock manure, land application of synthetic fertilizers, atmospheric deposition, and biological fixation by crops), together with four outputs of N from UAA (crop harvest, crop stock, ammonia volatilization and denitrification in soils). The difference between N inputs and outputs results in a net, which represents a condition of equilibrium, surplus or deficit of N across the UAA.

The Soil System Budget (SSB) was calculated as follow:

$$SSB\ N = N_{Man} + N_{Fert} + N_{Fix} + N_{Dep} - N_{Harv} - N_{stock} - N_{Vol} - N_{Den}$$

where:

N_{Man} = N in livestock manure applied to agricultural soils

N_{Fert} = N in synthetic fertilizer applied to agricultural soils

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

N_{Dep} = atmospheric N deposition on agricultural land

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

N_{stock} = organic N in crop’s standing stock

N_{Vol} = NH_3 volatilization in agricultural soils

N_{Den} = denitrification in agricultural soils

All budget terms were expressed in unit of mass per time (t N y^{-1}), and on a per-area basis, after normalization for the UAA (kg N ha^{-1} y^{-1}). The soil N budget was estimated by employing agricultural census data referring to the agricultural year 2015-2016. The agricultural year overlaps the vegetative cropping cycle, covering two consecutive years, i.e., from November of the first year to October of the following one. The N budget calculated for the agricultural year 2015-2016 is relevant in the present day because, in the last decade, only minor variations occurred in crop surfaces and livestock densities of the study area and fertilization rates did not change appreciably.

The calculation was based on agriculture and farming data reported by the Agricultural Information System of

Lombardy Region (SIARL, www.siarl.regione.lombardia.it) and by the Annals of Agrarian Statistics, published yearly by the National Institute of Statistics (ISTAT, http://agri.istat.it/). SIARL databases, retrieved from the Open Data portal of the Lombardy Region (https://dati.lombardia.it/), provided data for livestock density and agricultural areas at the municipality level, whereas the database of the Annals of Agrarian Statistics provided data for crop yield and fertilizer application (http://dati.istat.it/) at the provincial level. Inputs and outputs were initially calculated for each municipality and then aggregated at the study area level.

Uncertainty in N budget calculations was assessed by a Monte Carlo analysis using Excel and R software (R Core Team 2019). All coefficients used to convert census data into N amounts were assumed to vary stochastically and independently around the average value with a normal probability distribution. For each simulation, a set of coefficients was randomly generated from probability distribution functions and a total of 1000 simulations were run. Budget calculation was conducted both at the annual and at the seasonal scales and compared with seasonal in-stream N loads. Details about annual budget data, equations, seasonal breakdown, and sources of census data and agronomic coefficients are presented in Supplementary Material A.

The N loads produced by the urban areas were not included in the calculation because more than 95% of the sewers in the study area are connected to wastewater treatment plants (WWTP). Nearly 75% of the N inputs to WWTP is removed via denitrification in tertiary treatment (Lombardy Region, 2017). Indeed, the calculation of the urban load produced by the resident population, obtained by the conversion of equivalent inhabitant in kg of N per day, resulted in less than 2% of the total N input by diffuse sources (Pinardi et al., 2018).

The daily precipitation data were downloaded from the ARPA Lombardy website (https://www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx) at Ponti sul Mincio station (Fig. 1) for the period from 2010 to 2017. The mean annual, seasonal (irrigation and non-irrigation period) and monthly precipitation data were calculated. Irrigation data at the municipality level was obtained from the 6th Agricultural Census (National Institute of Statistics, 2010, http://dati-censimentoagricoltura.istat.it) and then aggregated at the study area level.

3.2. Water sampling and analyses

Two stations located at the extremes of the identified river reach (S1 and S2; Fig. 1) were sampled for water analyses. The two stations were selected as they were located upstream and downstream the area where the Mincio River can be considered as a gaining river in groundwater-surface water interaction, that is the river is fed by groundwater (Racchetti et al., 2019). Given the constant discharge between S1 and S2, the identified river reach was more recently characterized as a flow-through reach (Severini et. al., 2022), with groundwater feeding the river in the western bank and being fed by the river in the eastern bank. Field campaigns were carried out seasonally with a series of daily cycles of repeated samplings carried out on 12-13 August and 15-16 November 2016, 14-15 February, 12-13 April and 13-14 June 2017. Water samples were taken in three replicates every 4 hours for a 24-hour period. An aliquot was transferred into a 12 mL exetainer (Labco, UK), added with 100 µL of $HgCl_2$, and analyzed for dissolved inorganic carbon (DIC) with Gran titration (0.1 N HCl) within 24 hours from sampling. DIC was measured as it may trace differences between surface and groundwater chemistry. Water aliquots were filtered (GF/F glass fiber filters) and transferred to plastic vials for nitrate (NO3-N), nitrite (NO2-N), and ammonium (NH4-N) determination by spectrophotometric methods (Rodier, 1978; APHA, AWWA, WPCF, 1999). There are two main reasons for focusing on inorganic nitrogen. The first is that from our database and from those of national monitoring agencies in the Mincio river nearly 80% of the total N load is made of inorganic N (DIN, and within the DIN pool >90% is nitrate). The second is that from the same dataset nitrate represents more than 95% of the total dissolved N in groundwater.

Hourly or daily water flow data were obtained by the Interregional Agency for the Po River (AIPO), and by the Mincio Consortium for Pozzolo and Goito sites.

The Mann-Whitney Rank Sum Test was used to test the difference between upstream and downstream values of water flow, NO3-N, NO2-N, NH4-N and DIC concentrations. The R software package (R Development Core Team, 2019) was used to perform all statistical tests.

3.3. Dissolved inorganic nitrogen and carbon daily loads

For each sampling date, daily NO3-N, NO2-N, NH4-N and DIC riverine loads transported at S1 and S2 (kg d^{-1}) were calculated multiplying concentrations by river discharge. The difference between loads at S2 and S1 was calculated according to the following equation:

$$\Delta\text{NO3-N (or NO2-N, NH4-N, DIC)} = \sum [\text{Ct x } \Delta\text{t x Q}]_{S2} - \sum [\text{Ct x } \Delta\text{t x Q}]_{S1} \quad (1)$$

where: Ct = concentration of NO3-N (or NO2-N, NH4-N, DIC) at time t downstream (S2) or upstream (S1) (mg m^{-3}); Δt = time interval between samplings (h); Q = water flow (m^3 h^{-1}). Such difference can be null, suggesting equilibrium between inputs and outputs, negative, suggesting net retention or dissipation (e.g., uptake or denitrification), or positive, suggesting the occurrence of production or additional inputs along the stretch (e.g., nitrification or point and diffuse inputs). A standard deviation was associated to NO3-N, NO2-N, NH4-N and DIC measurements made in replicates. The errors conveyed through the mathematical description were calculated with classical error propagation equations.

4. Results

4.1. Nitrogen budgets and water inputs

In the four municipalities under study, the total N inputs to arable land (6010±498 t y^{-1}) were mainly due to livestock manure (59%). Nitrogen outputs (5078±494 t y^{-1}) accounted for 84% of the N inputs and were mainly due to crop harvest (50% of the total N outputs). The main cultivated crop is maize (63% of arable land) followed by permanent grassland. The difference between N inputs and outputs denoted a N soil surplus (932±702 t y^{-1}). The mean areal N surplus was 67±50 kg ha^{-1} y^{-1} with values calculated for the different municipalities ranging between -172 and 204 kg ha^{-1} y^{-1}. All these results are reported in Supplementary material B, Table B.1 and Figure B.1.

The seasonal SSB is reported in Figure 2. Manure N fertilization was higher in spring (1590±268 t) and summer (883±149 t), and similar in autumn and winter (530±89 t). Synthetic N fertilization was higher in spring (769±190 t) and winter (257±63 t). Biological N fixation was higher in summer (559±259 t) and spring (350±162 t) whereas atmospheric N depositions were concentrated mainly in summer and autumn (32±4 and 38±4 t, respectively). In spring, N associated to the crop's standing stock (487±98 t) was higher than N in the crop harvest (398±80 t). With respect to N outputs, crop harvest was highest in summer (1492±301 t) and autumn (637±128 t). Ammonia volatilization and soil denitrification were quantitatively important in spring (324±233 t and 210±102 t, respectively). Coupling the seasonal input and output data, a transition from N deficit to N surplus is evident moving from summer (-971±472 t) to spring (1317±464 t).

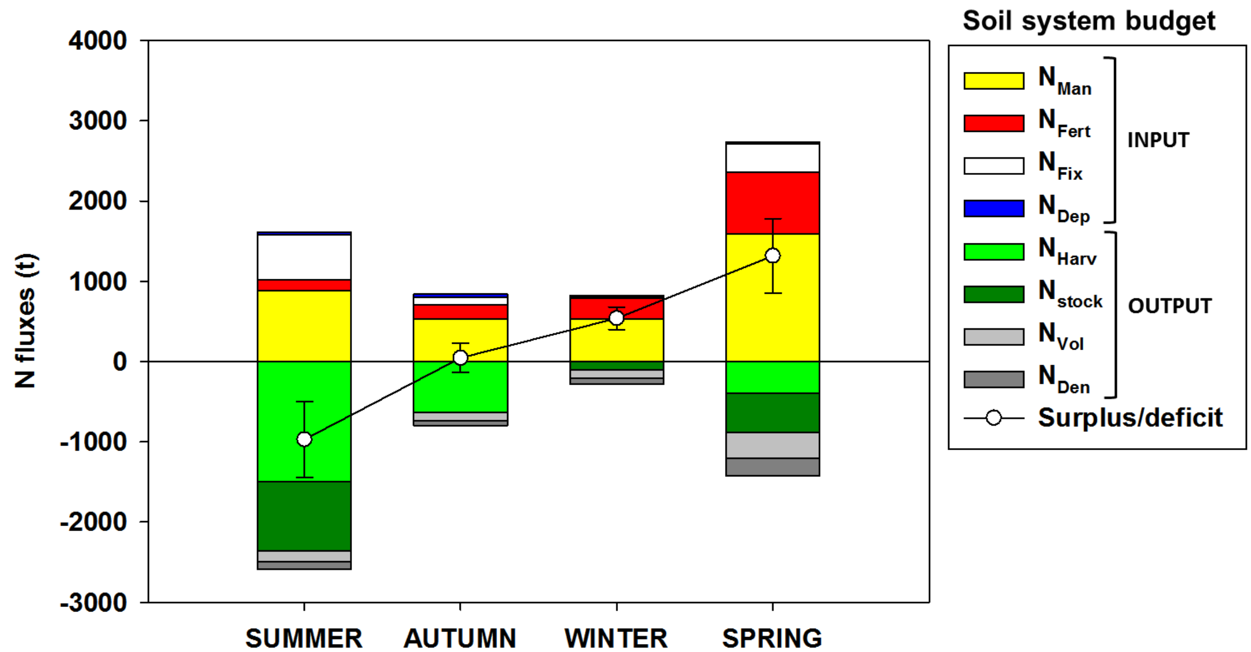

Figure 2. Components of the Soil System Budget (SSB) of nitrogen (N) for the study area within the Mincio River watershed for all seasons. N_{Man} = N in livestock manure applied to agricultural soils; N_{Fert} = N in synthetic fertilizer applied to agricultural soils; N_{Fix} = agricultural N_2 fixation associated with N fixing crops; N_{Dep} = atmospheric N deposition on agricultural land; N_{Harv} = N exported from agricultural soils with crop harvest; N_{stock} = organic N in crop's standing stock; N_{Vol} = NH_3 volatilization in agricultural soils; N_{Den} = denitrification in agricultural soils. White dots represent seasonal N budgets (Σ INPUTS - Σ OUTPUTS); positive values suggest surplus whereas negative values suggest deficit.

During the 2010-2017 period the mean annual precipitation in the study area was 910 ± 197 mm y^{-1}, of which about 43 ± 6% occurred during the irrigation period (Fig. 3).

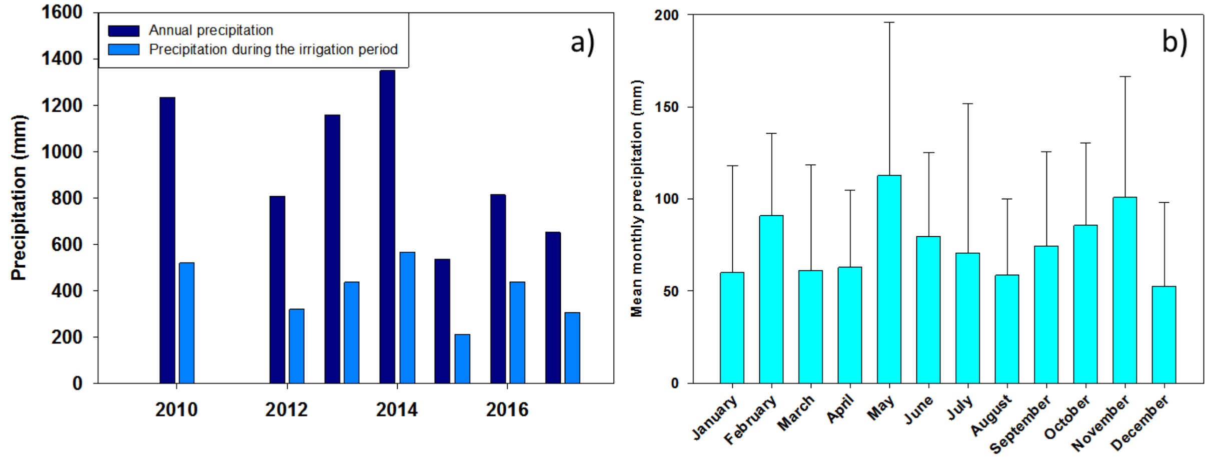

Figure 3. Annual precipitation and the fraction of precipitation during the irrigation period (from May to September) (a) and mean monthly precipitation (± standard deviation) (b) in the period from 2010 to 2017 at Ponti sul Mincio meteorological station (see Fig. 1 for the localization) (2011 was not considered due to missing data for August and September).

During the May-September period a water volume of 53.3 x10^6 m^3 was used to irrigate 13,513 ha of UAA, which represent 75% of the total arable land. Flooding and sprinkler were the main irrigation typologies (72% and 27% of the irrigated surface, respectively) (data from the National Institute of Statistics).

4.2. Water physico-chemical features of sampling sites

Nitrate and DIC concentrations were significantly higher at the downstream site for all sampling dates (Mann-Whitney Rank Sum Test, $p < 0.001$, n=68 for each parameter; Fig. 4). On the contrary, the concentrations of the other dissolved inorganic forms of nitrogen (NO2-N and NH4-N) were significantly higher at the upstream site ($p < 0.001$, n=68 for each parameter; Fig. 4). The highest NO3-N and DIC concentrations were measured in June 2017 at both sampling sites (1.6 and 3.0 mg NO3-N L^{-1}, and 33 and 41 mg DIC L^{-1} at S1 and S2, respectively). The highest values of NO2-N were recorded in summer at S1 and S2 (up to 101 and 31 µg L^{-1}, respectively), whereas NH4-N concentrations peaked in August 2016 at S1 (up to 122 µg L^{-1}) and were high at both sites in February 2017. Nitrate was always the main form of inorganic nitrogen, accounting on average for 88% and 98% of the total N at S1 and S2, respectively.
The mean annual water flow was 10.3±2.5 m^3 s^{-1} in the S1-S2 river reach (whole dataset 2016-2017, n=140). During

the irrigation period the water flow was not significantly different upstream and downstream (11.7 ± 2.0 m^3 s^{-1} at S1 and 13.0 ± 3.9 m^3 s^{-1} at S2; $p > 0.05$). No significant differences were also found between water flow during the irrigation and not-irrigation periods ($p > 0.05$, n=34). The water discharge measured during the experimental activities fell within the annual range of flow variation.

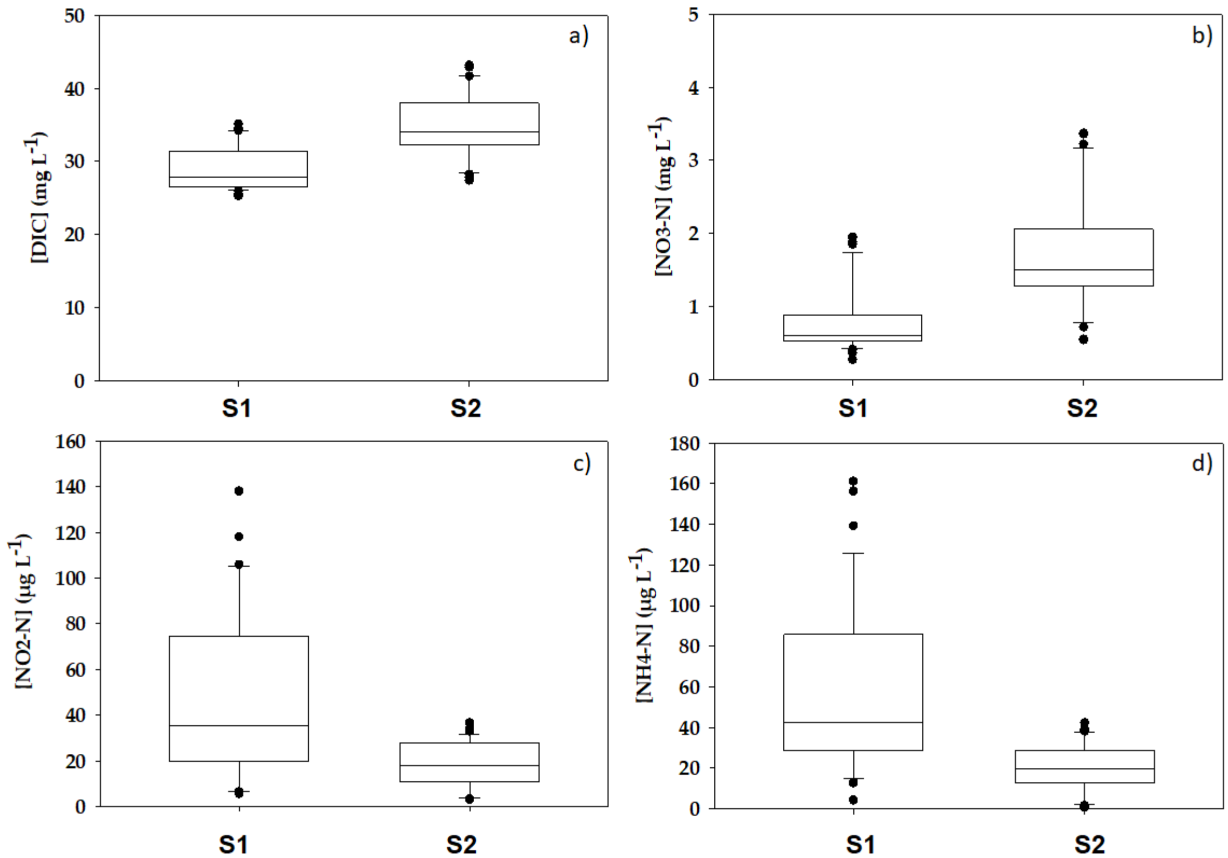

Figure 4. Box plot showing the concentrations of dissolved inorganic carbon (DIC; a), and of nitric (NO3-N; b), nitrous (NO2-N; c) and ammonium (NH4-N; d) nitrogen measured seasonally from August 2016 to June 2017 at S1 and S2. Note different concentration units.

4.3. Dissolved inorganic nitrogen and carbon daily loads

In all seasons, NO3-N and DIC transported loads were higher at S2, whereas NO2-N and NH4-N loads were higher at S1, but by much lower extent (Fig. 5). A positive correlation was found between NO3-N and DIC concentrations (Pearson's correlation coefficient $r = 0.899$, $p < 0.001$, n=68 for each parameter) supporting the possibility of the same origin for the two solutes. A similar seasonal trend was detected for DIC and NO3-N accumulation along the analyzed river reach (Fig. 5). The maximum increase of transported loads (nearly 11,000 and 1500 kg d^{-1} for DIC and NO3-N, respectively) was measured in August 2016, whereas the minimum increase (nearly 2,000 and 200 kg d^{-1} for DIC and NO3-N, respectively) was measured in April 2017 (Fig. 5). The highest NO2-N and NH4-N loads reduction along the stretch were recorded during summer months (Fig. 5).

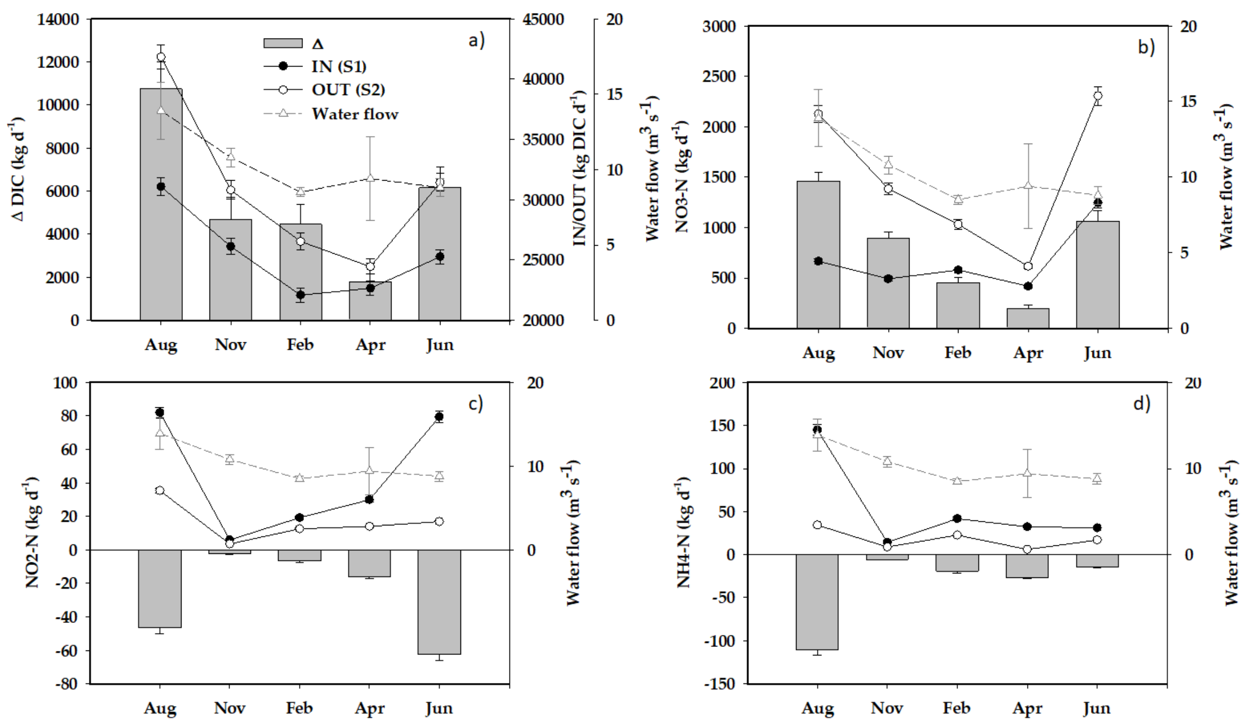

Figure 5. Daily loads of inorganic carbon (DIC; a), nitric nitrogen (NO3-N; b), nitrous nitrogen (NO2-N; c), and ammonium nitrogen (NH4-N; d) transported at the extremes of the studied river reach and their differences (Δ=S2-S1) in the period August 2016 to June 2017. Water flow is also reported. Mean values are given, with error bars corresponding to ±1 standard deviation.

5. Discussion

5.1. The seasonality of soil N budgets

Previous studies carried out in the Po River basin and in other geographical areas characterized by intensive agriculture and animal farming suggest a generalized N surplus and inefficient N use, leading to large N losses to surface and groundwater (Soana et al., 2011; Hou et al., 2015; Özbek et al., 2015; Viaroli et al., 2018; Häußermann et al., 2020). In the Po River plain, to our knowledge, only a few sub-basins (e.g., Ticino and Po di Volano) represent exceptions to this rule due to very limited animal farming and synthetic fertilizers inputs balanced with crop needs (Racchetti et al., 2019; Soana et al., 2021). All those studies were carried out on an annual temporal scale, that potentially masked marked seasonal differences. Our calculations suggest a clear seasonal variation in N soil budgets, changing along seasons with periods with deficit (summer), equilibrium (autumn), moderate (winter) or large (spring) excess. These differences arise from variable seasonal balance among agricultural practices, such as large spring manure and synthetic fertilizer spreading uncoupled to crop uptake, moderate spread of fertilizers during winter with little to no uptake or large summer crop uptake in excess to N inputs (Chen et al., 2019). The N assimilation term is calculated as the product of the standing stock by the biomass-specific uptake rates and peaks in summer. Indeed, the biomass-specific uptake tends to decrease along with the crop's growth, but the crop’s standing stock is much smaller in spring than in summer. Large spring N inputs are therefore coupled to relatively low uptake, resulting in a maximum surplus, exceeding that calculated for winter, when uptake is minimum. Large summer uptake on the contrary exceeds inputs and results in a seasonal deficit of N in the soil system budget. High fertilization is probably driven by the N need of the main cultivated crop, i.e., maize, which is a water and N-demanding species (FAO, 2006). These results on intra-annual variations in N mass budgets support the relevance of seasonal studies in highlighting critical moments in terms of potential water pollution (Lin et al., 2019; Compton et al., 2020). As nitrate water pollution is correlated with N excess in soils, our outcomes indicate a maximum nitrate pollution risk in the spring, a minimum risk in the summer and something intermediate in winter and autumn. Results from seasonal river N transport suggest something different as the highest N accumulation along the stretch was measured in summer and the lowest in spring. Taken together, these apparently contrasting results indicate that more factors are involved in the horizontal transfer of soil N excess to surface water and that such factors determine a temporal lag. In the perspective of efficient N use at the soil level, our results stress that the spring is a critical season that requires a thorough re-thinking of practices by better balancing crops needs with fertilizers inputs. During spring, organic and synthetic N inputs need to be better balanced with N uptake, as the crops have high potential growth but low biomass, which results in an insufficient uptake of the large N inputs (Robertson and Vitousek, 2009).

5.2. The seasonality of inorganic N loads in the Mincio River

Results from this work add to a few seasonal studies coupling land mass budgets of N and net river N export-retention (e.g., Chen et al., 2019; Lin et al., 2019; Compton et al., 2020). For the portion of the Mincio River considered in this study, Fig 6 reports the monthly water inputs, either from precipitation or irrigation, the seasonal soil system N budget and the nitrate accumulation between S1 and S2, which is the difference of the nitrate loads transported past the two river sections.

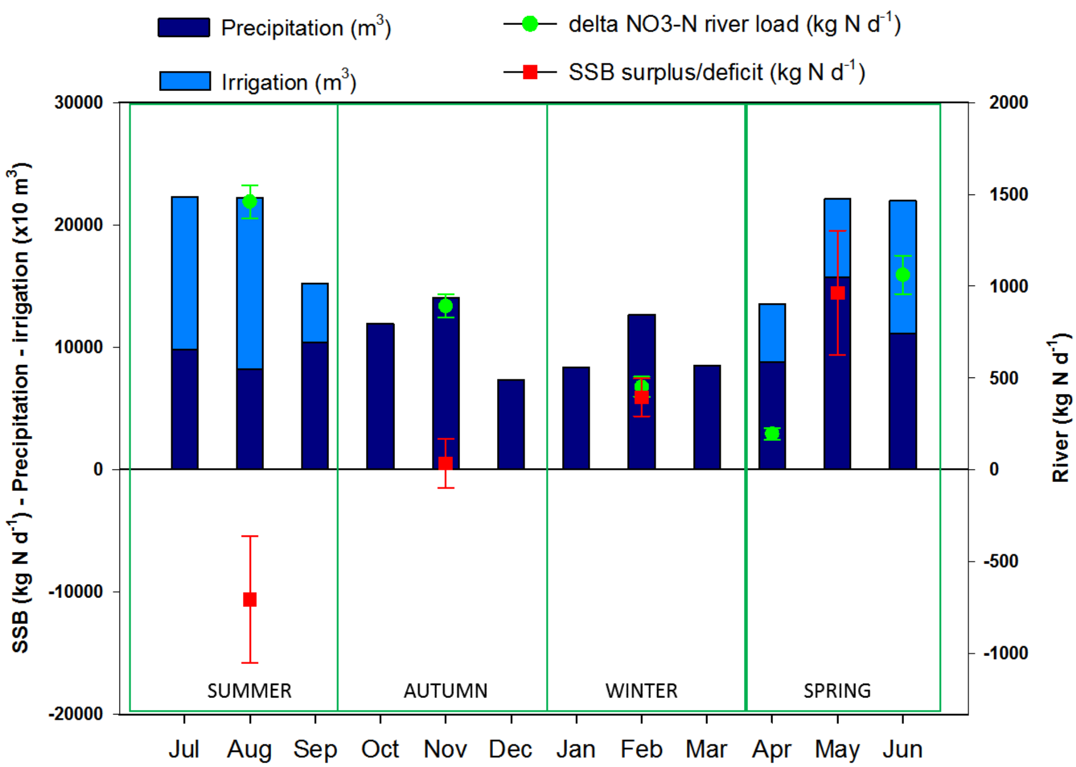

Figure 6. Histograms report monthly water input due to precipitation and irrigation in the municipalities of the Mincio basin under study. Green dots show the delta nitrate loads of the river reach (delta NO3-N river load) in the five sampling dates and red squares show seasonal N budget of agricultural soils (SSB – Soil system budget – surplus/deficit).

In the regulated river reach under study, which is characterized by gravel bottom and colonized by submerged macrophytes, ammonium and nitrite loads were higher upstream and suggested net retention in all seasons, peaking in summer when the primary producers' activity is maximum. Differently, dissolved inorganic carbon and nitrate loads evidenced a significant increase from upstream to downstream in all the investigated seasons. Nitrate and inorganic carbon net export decreased from August to April, with August as the central month for irrigation and April the last month before the start of the irrigation period. From these results, we calculated the annual net export of inorganic carbon and nitrate multiplying the daily values by the number of days between consecutive samplings, and then integrating the results over one year. Despite 6-8 repeated water samplings during the 24 hours, these calculations are based on measurements carried out in single days along different seasons. However, the nitrate concentrations measured in this study are consistent with the seasonal NO3-N concentrations measured by ARPA Lombardy (the authority that manages water monitoring for the WFD) in the period 2009-2017 and by the Laboratory of Aquatic Ecology of the University of Parma in the period 2011-2017 (Fig. C.1 in Supplementary material C).

It was estimated that 2153±174 t DIC y^{-1} and 317±12 t NO3-N y^{-1} were net exported from the 8 km long reach between S1 and S2. These amounts can be explained by element transformation (e.g., respiration or nitrification), and lateral or vertical inputs. As the loads of the other two inorganic N forms were negligible as compared to nitrate, this calculation was done only for the latter. Using dissolved oxygen budgets (not reported in this work) we converted dark river respiration rates (night oxygen uptake along this stretch; from -1.3 to -5.7 mm O_2 m^{-2} h^{-1} in winter and summer, respectively) into potential nitrification rates (~75 t N y^{-1}) according to nitrification stoichiometry. To this purpose, we assumed that 100% of the oxygen consumed was used to oxidize ammonium to nitrate. Results suggest that microbes-mediated processes as nitrification can explain at most 23% of the nitrate accumulation in the river reach in all seasons. Such percentage is a large overestimation of the real value as it was obtained neglecting all other oxygen-consuming processes, including macrophytes, fish, macroinvertebrates, and the whole heterotrophic microbial community respiration. A comparable nitrate production (~80 t N y^{-1}) was obtained using the nitrification rate set by Taherisoudejani et al. (2018) in the QUAL2Kw model applied to the Oglio River, a nearby Po River tributary with similar hydrological characteristics. Pinardi et al. (2014) found that the processes in the hyporheic zone or the microbial metabolism of carbonate dissolution could explain up to 15% of the DIC increase, including the role of macrophytes in modulating dissolved CO_2 saturation values and fixation of C.

If biological processes cannot explain inorganic carbon and nitrate increase, also point pollution sources can be excluded, due to low discharge of small tributaries along this stretch with water chemistry comparable to that of the Mincio River. Another potential source of N and DIC is groundwater, via seasonally variable river-groundwater interactions. Indeed, the level of the phreatic surface increases and interacts with the river due to precipitation, flooding and sprinkler irrigation during the spring-summer period (Racchetti et al., 2019; Severini et al., 2020) (Fig. 7). In a period before and after fertilization (from March to May 2021), Severini et al. (2022) measured in the same area of the Mincio River significantly higher HCO_3^- concentrations in groundwater than in surface water (Appelo and Postma, 2005). Hence, the DIC increase in the investigated river stretch can be associated to the groundwater feeding the Mincio River. Considering the abundant use of organic fertilizers in the area, the higher DIC in groundwater can be related to the presence of calcite ($CaCO_3$) in the mineralogical composition of the aquifer and to the oxidation of the organic matter, which promotes a higher DIC concentration in groundwater. Future investigations should include the mineralogical composition of the aquifer.

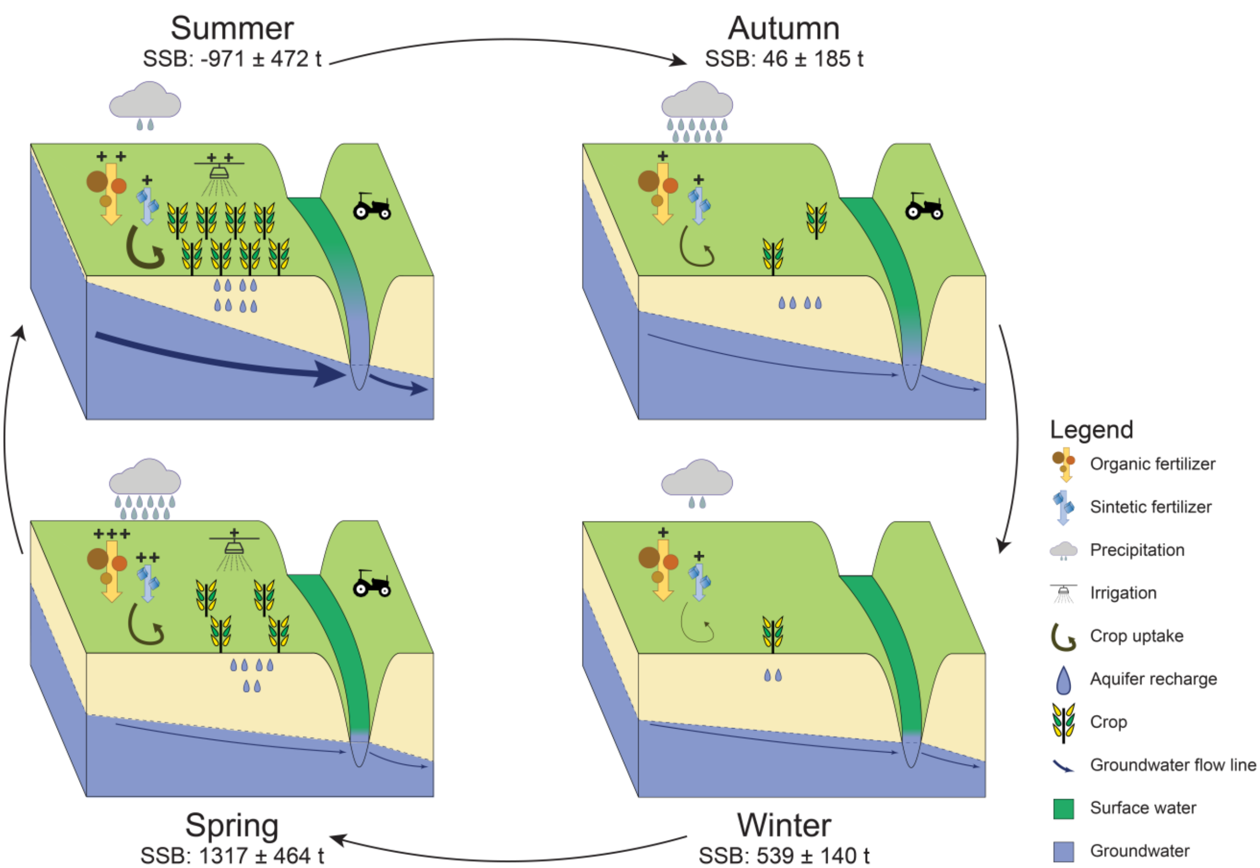

Figure 7. Synthesis of the seasonal nitrogen budgets at agricultural land and at river section. The relation with the groundwater is also reported. SSB = Soil System Budget.

5.3. Linking soil N budgets and N riverine export: the key role of the aquifer

The main period of N fertilization is in spring when there is the highest N soil surplus and surface and groundwater pollution risk due to limited crop uptake. The crop harvest occurs mainly in summer and autumn when the N soil budget is in deficit or close to equilibrium, respectively. These data are reflected by an increasing N export by the river reach from spring to summer, favored by large volumes of nitrate-enriched water displaced through the irrigation across the aquifer-river continuum (Isidoro et al., 2006). Using SiO_2 as tracer, Severini et al. (2022) typified the investigated river stretch as a flow-through system, where groundwater feeds the Mincio River in its west bank and it is fed from the river's east bank (Fig. 7). As a result, N-rich groundwater can displace N-poor water from the Mincio River without a significant modification of the river flow (Fig. 7). Our data are consistent with this hydrogeological conceptual model, since the higher NO3-N delta loads were found in summer, when the groundwater level are the highest and there is the maximum groundwater seeping to the Mincio River, highlighting the deep effects of the recharge given by irrigation. On the contrary, some differences were found during the rest of the year, characterized by a less anthropic recharge of the aquifer. These differences are more related to the dissimilar distribution of precipitation and percolation of water and N to groundwater, which fosters the migration of N to the Mincio River. In fact, as we move away from the end of the irrigation period (September), the lower groundwater heads reported in Severini et al. (2022) could result in a lower groundwater seepage to the river (Fig. 7). Having less nutrient enriched water available guarantees a minor nitrate surplus in the river reach, even if N fertilization starts again in winter, resulting in another period with N soil surplus (Figs. 6, 7).

Considering that the mean annual N surplus on the agricultural land in the study area averaged 67 kg ha^{-1} y^{-1}, it is possible to speculate that the agricultural land surface that can potentially generate the NO3-N river export (317±12 t NO3-N y^{-1}) is equivalent to ~4700 ha^{-1}. In addition, dividing this surface by the length of the river reach investigated, it is possible to estimate the width from the river, which is about 2.9 km for each side, that might be involved in the N surplus production. These data allow to speculate that the N surplus was generated in the 25% of the surface of municipalities under study, giving useful information to better address arable land management.

Our results on N input and output trends in agricultural soils and into the river reach at annual and seasonal basis allow to better understand N patterns from land to river and the potential nitrate pollution to surface and groundwater. This information at seasonal resolution can help policy-makers in developing effective plans to improve N management at the macroscale. In fact, this combination of information can guide the identification of proper spatial-temporal management strategies to reduce N pollution and river export to avoid eutrophication processes of water bodies. For example, our results suggest that more nitrate was delivered downstream in summer because of spring soil N excess coupled to flood irrigation over permeable soils. Hence, it is important to focus on agricultural sources (manure and synthetic fertilizers in particular) to better balance N inputs and output by crop harvest (or stock). Our approach was applied in a pilot study at the sub basin level, but it is exportable to the whole basin and to other rivers. It becomes very important to have local information and basin-specific data to perform seasonal analysis on N patterns (Lassaletta et al., 2021).

5.4. Possible remediation strategies in the context of climate change and the regulation of river discharge

Temporal disconnections between N fertilization, transport and uptake in agricultural land can result in low N use efficiency (Robertson and Vitousek, 2009). Specific monitoring of crop growth, nutrient demand and soil availability is useful to obtain a more synchronous nutrient supply in response to crop needs (Quemada et al., 2013). Alternative practices arranged to implement nutrient management directly in the field include actions such as variable rate or split of fertilizer applications matched to crop growth demand, improvements in efficiency of irrigation practices and use of nitrification inhibitors (Lacey and Armstrong, 2015; Fernández et al., 2016). The nutrient best management practices, for N, should be designed in view of seasonal N leaching losses and hydrologic export to properly depict crop growth dynamics and N demand, soil conditions and hydrology (Lin et al., 2019).

It is during summer that the investigated reach experienced the highest water nitrate accumulation. An action useful to buffer the N export is the implementation of riparian buffer strips that can promote N retention during the spring-summer irrigation period or the use of cover crops in winter (Dabney et al., 2010; Cole et al., 2020). During winter, our calculations suggest N soil excess in a period where uptake is minimum, and denitrification is likely limited by low temperatures and by the thick unsaturated soil. The latter follows the downward winter migration of the water table, previously discussed. For this reason, the adoption of practices that can favor water retention to increase soil humidity in the cold season and the presence of water in canals, commonly dry in autumn and winter, might be a solution that can favor denitrification process. As an example, the construction of artificial ponds or wetlands can be useful to intercept N runoff from agricultural lands (Nõges et al., 2003; Carstensen et al., 2020).

In the geographical area of our case study, the Alps host a series of large lakes regulated by dams that feed rivers among which the Mincio River. The dams regulate the lake water level and the river discharge with rules that aims to accumulate water in winter and release it during the irrigation period, adapting also to local meteorology and water inputs to lakes. This regulation practice guarantees sufficient summer level in lakes for tourism and navigation purposes and large water availability for irrigation and electricity production in the downstream river section. Irrigation is supported by several water abstraction infrastructures that facilitated agriculture and animal farming activities. Irrigation practices, supported mainly by flooding irrigation in the sector of the Po River plain including our study area are carried out over permeable soils, and favor the recharge of the aquifer mainly in summer (Rotiroti et al., 2019). Therefore, water retention upstream during non-irrigation periods and flood irrigation with large water volumes during summer are probably the main drivers of the groundwater head variation, which is subject to strong seasonal differences (Taherisoudejani et al., 2018). Under the current climate change scenario, also in this geographical area, the rapid changes of global warming are manifested with lower precipitation, dry winters, heatwaves and storms events, and an increasing number of consecutive days with high temperatures (Cifrodelli et al., 2015; Pedro-Monzonís et al., 2016; Lassaletta et al., 2021; Ranasinghe et al., 2021). The response to these global trends could increase or decrease river nitrate concentration depending on regional or site-specific linkage between N concentration and discharge (Stelzer et al., 2020). For this reason, the geographical sector under study seems extremely vulnerable to climate change as the system is depicted and managed for large water availability (i.e., large lakes regulation, high water demanding crops, and flooding as main irrigation practice) and therefore a discussion on water management at political level is urgent. Predicting scenarios on the fate of the N excess with different water availability is difficult, we can hypothesize a reduction of water discharge from rivers and consequently from irrigation that will not recharge sufficiently the groundwater due to its deep level (Taherisoudejani et al., 2018). This condition will lead to more thick vadose zones, fostering a higher nitrification rate and nitrate accumulation in soil during winter, whereas denitrification, the main process that removes N permanently from the system, is favored in water saturated soils with high organic matter (Ascott et al., 2017). In soil and rivers close to N saturation, it is expected a lower nitrate retention efficiency and therefore an increase in N availability and vertical and horizontal transfer (Stelzer et al., 2020). Moreover, for the future it can be expected a delay in the river feeding by groundwater with hot-moments of N mass transfer. In fact, we can expect that with the increment of unsaturated zone, the rate of soil denitrification will be reduced and conversely the nitrification process will be favored supplying a short N mass-transfer as soon as the first rainfall or flooding irrigation will occur, carrying water with high N concentration to surface or groundwater. A possible solution to limit this hot-moment can be the improvement of irrigation practices with less water consumption and a more widespread use of precision farming supported for example with remote sensing technique (Nutini et al., 2021). Such a new vision on the irrigation practices can allow a lower winter water retention in the Lake Garda, that can be partially used in the non-irrigation period to guarantee a minimal vital flow in a certain number of drainage canals as well as in the Mincio River favoring denitrification process also in autumn and winter, although with lower rates driven by lower temperatures.

6. Conclusions

Published soil system budgets in agricultural areas generally reveal net N excess on an annual basis, whereas the present study reveals seasonally variable inventories of inputs and outputs, resulting in periods of large N excess and periods of pronounced deficit. The export of N excess via the river draining the investigated area has a temporal lag that depends on irrigation, vertical migration of the water table and subsurface water flow. Flood irrigation first fills the unsaturated zone and then favors river-groundwater interactions. Subsurface water flow replaces N-poor river water with N-rich groundwater. Seasonal soil N budget and the mechanisms of N transfer described in this study should foster more efficient agricultural practices, minimizing N losses and improving N use. Results from this work should also be carefully considered in future planning of agricultural and irrigation activities, in a scenario of climate change and variable availability of water. Winter retention of water in lakes, upstream the agricultural areas, has serious drawbacks as it will increase the volume of the unsaturated soil and the production of nitrate via organic N ammonification and nitrification. Adaptive strategies based on precision farming, new material to retain soil humidity, irrigation techniques alternative to flooding and a management of the canal network targeting the restoration of biogeochemical services (e.g., N-uptake and denitrification) seem effective and sustainable options.

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Declarations

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

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