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Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

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Agricultural Water Management

Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges --Manuscript Draft--

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|-------------------------------|--|
| Manuscript Number: | AGWAT-D-22-01024R1 |
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| Corresponding Author: | Monica Pinaridi, PhD ITALY |
| First Author: | Monica Pinaridi, PhD |
| Order of Authors: | Monica Pinaridi, PhD Elisa Soana Edoardo Severini Erica Racchetti Fulvio Celico Marco Bartoli |
| Abstract: | <p>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.</p> |
| Suggested Reviewers: | Romero Estela estela.romero@creaf.uab.cat Anna Malagò anna.malago@ec.europa.eu Luis Lassaletta luis.lassaletta@upm.es |
| Opposed Reviewers: | |
| 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

Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

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 ($\pm 15\%$) 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 ($\pm 2\%$) pigs and 28,000 ($\pm 4\%$) 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

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4 **Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges**
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6 Monica Pinardi^{1*}, Elisa Soana², Edoardo Severini³, Erica Racchetti³, Fulvio Celico³, Marco Bartoli^{3,4}
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10 ¹ Institute for Electromagnetic Sensing of the Environment, National Research Council, Via Corti 12, 20133 Milan,
11 Italy
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13
14 ² Department of Life Sciences and Biotechnology, University of Ferrara, Via Borsari 46, 44121 Ferrara, Italy
15

16 ³ Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle
17 Scienze 11/A, 43124 Parma, Italy
18

19
20 ⁴ Marine Science and Technology Center, Klaipeda University, Klaipeda, 92294, Lithuania
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24 * Corresponding author
25

26 Address: Via A. Corti 12, 20133 Milan, Italy
27

28 Tel: +39 0223699298
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30 Email: pinardi.m@irea.cnr.it
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1 **Abstract**

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

21
22 **Keywords:** nitrogen; retention; transport; loads; watershed; irrigation; river-groundwater interaction

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4 **24 1. Introduction**

5
6 **25** The dramatic increase of anthropogenic reactive nitrogen (N) inputs in watersheds with intensive agriculture and
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8 **26** animal farming has demonstrated negative effects for inland water and groundwater chemical and biological quality,
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10 **27** drinking water supplies, ecosystem integrity and functioning and human health (Van Grinsven et al., 2006; Galloway
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12 **28** et al., 2008; Rivett et al., 2008; Schlesinger, 2009; Sobota et al., 2015; Huang et al., 2017). Such negative effects are
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14 **29** amplified by the human-derived alteration of the hydrological cycle at the watershed scale and by climate change
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16 **30** (Galloway et al., 2008; Overeem et al., 2013; Woolway and Merchant, 2019; Woolway et al., 2020). Among the
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18 **31** underlying mechanisms are water abstraction for irrigation or industrial purposes or climate change-related drought
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20 **32** reducing river discharge and its capacity to dilute and process N loads (Palmer et al., 2008). Low discharge promotes
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22 **33** also hyporheic anoxia and ammonium recycling from sediments (Hlaváčová et al., 2005). Hydrological extremes
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24 **34** include also short-term, heavy precipitations resulting in high discharge events transferring large N loads from
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26 **35** cultivated areas saturating riverine denitrification capacity (Viaroli et al., 2018; Magri et al., 2019).
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28 **36** Nitrogen budgets calculated for agricultural soils within a river basin allow to evaluate the potential risk of diffuse N
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30 **37** pollution (Oenema et al., 2003; Soana et al., 2011). In agricultural soils, N inputs associated with organic or synthetic
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32 **38** fertilizers, atmospheric deposition or biological fixation can be either temporarily retained in crops or released to the
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34 **39** atmosphere as gaseous losses. Nitrogen inputs in excess to temporary retention or permanent loss can be transferred
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36 **40** via runoff to adjacent aquatic ecosystems (Howarth et al., 1996; Seitzinger et al., 2006; Pinardi et al., 2018, 2020;
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38 **41** Kwon et al., 2022). If soil system budgets in arable land produce reliable snapshots of N pools and fluxes in
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40 **42** cultivated areas, the detailed reconstruction and partitioning of N pools and fluxes within watersheds is a challenging
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42 **43** objective. For example, seasonally variable water inputs to agricultural soils via precipitation and irrigation affect
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44 **44** soil N leaching, horizontal and vertical transport and transformation, N use efficiency as well as river-groundwater
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46 **45** interactions and associated N exchange (Schaefer and Alber, 2007; Chae et al., 2009; Howarth et al., 2012; Sinha and
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48 **46** Michalak, 2016). Moreover, in intensively cultivated floodplains the hydrological cycle has been regulated by the
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50 **47** realization of infrastructures as dams and networks of canals that help buffering climatic anomalies and ensure water
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52 **48** availability for crops. In Italy for example, the Alpine sector of the Po River basin hosts large dams that regulate the
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54 **49** release of water from deep subalpine lakes (Maggiore, Como, Iseo, Idro and Garda Lakes) to their emissaries
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56 **50** (Ticino, Adda, Oglio, Chiese and Mincio Rivers). Winter water retention in subalpine lakes occurs at the cost and
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58 **51** drawbacks of reduced water discharge and contributes to the downward vertical migration of groundwater, often
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4 52 resulting in downwelling river-groundwater interactions (i.e. the river feeds the groundwater) (Rotiroti et al., 2019;
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6 53 Severini et al., 2021). On the contrary summer irrigation, besides representing a vehicle for N transport, produces
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8 54 opposite effects, often reversing the direction of river-groundwater interactions (i.e. upwelling, the groundwater
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10 55 feeds the river). These practices, that characterize anthropogenic, intensively cultivated, and hydraulically regulated
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12 56 watersheds with permeable soil, introduce marked seasonality in N budgets (Lin et al., 2019; Racchetti et al., 2019).
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14 57 Many authors reported a significant correlation between annual N input to croplands and river N export (Neff et al.,
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16 58 2003; Yan et al., 2010; Xu et al., 2013; Stokal et al., 2014; Tong et al., 2017), but they did rarely account for the
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18 59 seasonality of N input and export (McCrackin et al., 2014; Chen et al., 2019). Studies targeting N budgets in
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20 60 agricultural watersheds are generally conceived at the annual scale for mainly practical reasons, as agricultural
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22 61 census data are collected and published by national statistical institutions with annual frequency. Such an approach
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24 62 from one side allows to calculate N use efficiency in cropland and potential N loss, but from the other side, it misses
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26 63 temporal resolution and precludes the understanding of seasonal variations of the array of N-related processes,
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28 64 potentially regulated also by climate change. For example, human activities (e.g., crop production) and altered
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30 65 hydrology may influence the seasonality of N river export (Basu et al., 2010; Compton et al., 2020), together with
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32 66 the seasonal evolution of temperature that influences N losses, retention and removal processes (e.g., denitrification)
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34 67 (McCrackin et al., 2014). Understanding how seasonal variations in human activities and hydrology influence N
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36 68 budgets in agricultural soils and N transport by rivers is important to better understand the mechanisms underlying N
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38 69 transformations along the terrestrial-aquatic path, improve agricultural practices to increase N use efficiency and
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40 70 decrease N pollution, and eventually forecast how climate change will affect N dynamics (Mas-Pla and Menció,
41
42 71 2019). This important set of objectives is a difficult target at the scale of whole watersheds due to scarce resolution
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44 72 of available data and spatial heterogeneity (e.g. pedology, land use, etc). Smaller scales of analysis, targeting specific
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46 73 and homogeneous river and watershed sectors, seem much more promising (McCrackin et al., 2014; Chen et al.,
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48 74 2019; Compton et al., 2020).
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50 75 Different studies carried out at large temporal and spatial scales (Soana et al., 2011; Pinaridi et al., 2018; Viaroli et
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52 76 al., 2018; Lassaletta et al., 2021) have highlighted the presence of hot-spots within watersheds that represent outliers
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54 77 in N budgets (e.g., with very large N excess or very low N use efficiency). They also emphasized the presence of
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56 78 hot-moments within watersheds, that are specific periods during which N mass transfer peaks as a combination of
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58 79 decreased uptake, increased runoff or variation of the water table level, resulting in the reactivation of river-

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

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

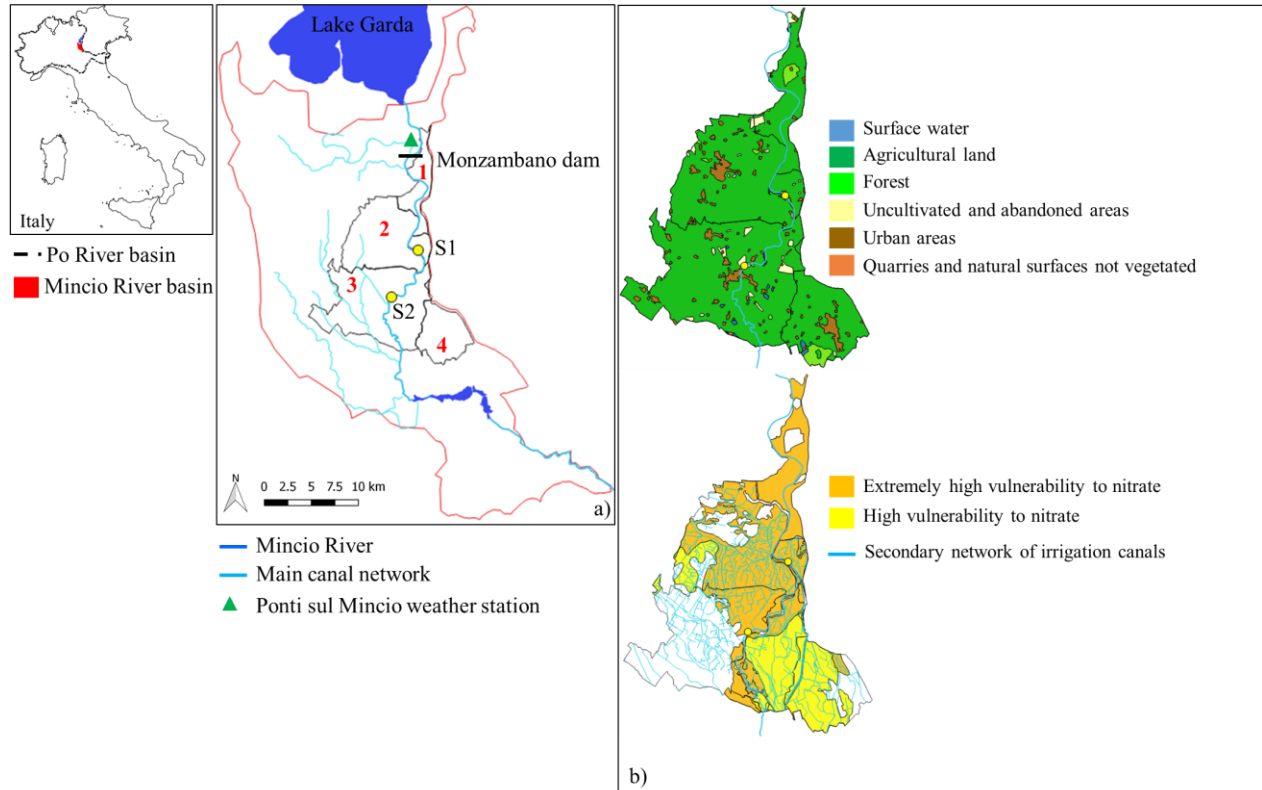
94 In this sector of the Po River, groundwater in the phreatic and shallow aquifer has a short residence time as compared
95 to semiconfined or confined deeper aquifers. This is supported by fast (few days) surface-groundwater dynamics of
96 micro-pollutants (Balderacchi et al., 2016) and low concentrations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

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

2. Study area

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4 108 The Mincio River (~75 km) originates from the Lake Garda, the largest Italian Lake, and is a tributary of the Po River
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6 109 (Fig. 1). The hydrological regime of the Mincio River is regulated upstream by a dam, which controls the water
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8 110 discharge from the Lake Garda. Along the river course, a series of dams and weirs feeds a network of canals for
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10 111 irrigation and industrial purposes and controls discharge variations to avoid the flooding of cities and villages. Water
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12 112 management for Lake Garda recreational activities and for agricultural purposes results in marked flow variations.
13
14 113 Indeed, since the establishment of the river regulation in the 60's of the last century, the Mincio River discharge
15
16 114 averaged ~80 and ~30 m³ s⁻¹ during the irrigation (May to September) and outside the irrigation periods, respectively
17
18 115 (Lombardy Region, 2006). More recently, projections of decreasing water availability resulted in a further reduction of
19
20 116 the Mincio River discharge to ~14 m³ s⁻¹ (www.laghi.net) in autumn and winter to keep water in the Lake Garda and
21
22 117 guarantee water availability for irrigation and tourism in the summer season. During winter, the flow reduction and
23
24 118 absence of irrigation result in a decreased aquifer recharge, a phenomenon described by different authors in this
25
26 119 geographical area at regional (Rotiroti et al., 2019) and local scales (Severini et al., 2021) and, consequently, in a
27
28 120 lowering of the phreatic surface. This water transfer dynamic results in a decrease of groundwater upwelling in winter
29
30 121 and early spring (Balderacchi et al., 2016).
31
32
33 122 A wide segment of the Mincio River, including the portion investigated in this study, flows in a flood plain
34
35 123 characterized by a multilayered aquifer system with a cyclic facies architecture mainly made of fluvial-channel
36
37 124 (gravel and sand) and floodplain (clay) deposits (Amorosi et al., 2008). As a result, the northern part of the plain
38
39 125 (high plain) is locally characterized by shallow phreatic aquifers, while in the southern part (low plain) the floodplain
40
41 126 facies act as aquitards or aquicludes, resulting in confined and semi-confined aquifers (Chelli et al., 2018). The river
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43 127 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-
44
45 128 medium plain of the Mincio watershed and includes four municipalities (1- Valeggio sul Mincio, 2- Volta Mantovana,
46
47 129 3- Goito, and 4- Marmirolo) for a total surface of 184 km² (Fig. 1). Since 2006, these municipalities are classified as
48
49 130 Nitrate Vulnerable Zones (NVZs) according to the European Nitrate Directive (91/676/CEE). The study area is
50
51 131 characterized by fertile soils due to calcareous gravel deposits and is intensively exploited by agriculture (Utilized
52
53 132 Agricultural Area - UAA covers 76% of the study area; Fig. 1) and animal farming (1.2 and 0.6 t of live weight per
54
55 133 hectare for cattle and pigs, respectively). The S1-S2 river segment flows into natural banks, has a mainly gravel bottom,
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57 134 and has transparent waters. The main primary producers are submerged vegetation (e.g., *Vallisneria spiralis*) with
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59 135 associated epiphytes, benthic biofilms and different emergent macrophytes growing along the river banks or forming

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4 136 islands (Pinardi et al., 2009, 2014). The linear development of irrigation canals in S1-S2 river reach sub basin sums
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6 137 ~560 km (Fig. 1). The surface covered by the other aquatic environments, such as quarry lakes is ~0.62 km².



36 139
37 140 Figure 1. Maps of the study area: the Mincio River segment from Pozzolo dam (S1) to Goito village (S2)
38
39 141 (yellow points = water sampling stations). a) Municipalities where the nitrogen mass budget was performed are
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41 142 reported (1 - Valeggio sul Mincio, 2 - Volta Mantovana, 3 – Goito, 4 - Marmirolo). b) Land use and maps of
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43 143 soil vulnerability to nitrate are reported for the four municipalities under study.
44

45 144 46 47 145 **3. Material and methods**

48 49 146 **3.1. Nitrogen budgets and water inputs**

50
51 147 A comprehensive input–output N budget across the Utilized Agricultural Area (UAA) was compiled by using
52
53 148 locally-derived data on farming activity, agronomic coefficients and atmospheric deposition. Nitrogen budget was
54
55 149 first calculated at the municipal scale, i.e., the administrative level at which official agricultural statistics are
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57 150 available, then weighted for the percentage of each municipality surface included within the study area, and finally
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59 151 summed up. Census data were integrated in a nutrient budgeting approach proposed by Oenema et al. (2003),
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4 152 recently reviewed by Zhang et al. (2020), and formerly applied to the whole Mincio River basin (Pinardi et al.,
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6 153 2018). Four inputs of N to the UAA were considered (land application of livestock manure, land application of
7
8 154 synthetic fertilizers, atmospheric deposition, and biological fixation by crops), together with four outputs of N from
9
10 155 UAA (crop harvest, crop stock, ammonia volatilization and denitrification in soils). The difference between N inputs
11
12 156 and outputs results in a net, which represents a condition of equilibrium, surplus or deficit of N across the UAA.
13

14 157

15
16 158 The Soil System Budget (SSB) was calculated as follow:
17

$$18 \text{ 159 } SSB \text{ N} = N_{\text{Man}} + N_{\text{Fert}} + N_{\text{Fix}} + N_{\text{Dep}} - N_{\text{Harv}} - N_{\text{stock}} - N_{\text{Vol}} - N_{\text{Den}}$$

19
20 160 where:
21

22 161 N_{Man} = N in livestock manure applied to agricultural soils
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24 162 N_{Fert} = N in synthetic fertilizer applied to agricultural soils
25

26 163 N_{Fix} = agricultural N_2 fixation associated with N fixing crops
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28 164 N_{Dep} = atmospheric N deposition on agricultural land
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30 165 N_{Harv} = N exported from agricultural soils with crop harvest
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32 166 N_{stock} = organic N in crop's standing stock
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34 167 N_{Vol} = NH_3 volatilization in agricultural soils
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36 168 N_{Den} = denitrification in agricultural soils
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38 169
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40 170 All budget terms were expressed in unit of mass per time ($t \text{ N } y^{-1}$), and on a per-area basis, after normalization for the
41
42 171 UAA ($kg \text{ N } ha^{-1} y^{-1}$). The calculation was based on agriculture and farming data for the year 2015 reported by the
43
44 172 Agricultural Information System of Lombardy Region (SIARL, www.siarl.regione.lombardia.it) and by the Annals
45
46 173 of Agrarian Statistics, published yearly by the National Institute of Statistics (ISTAT, <http://agri.istat.it/>). SIARL
47
48 174 databases, retrieved from the Open Data portal of the Lombardy Region (<https://dati.lombardia.it/>), provided data for
49
50 175 livestock density and agricultural areas at the municipality level, whereas the database of the Annals of Agrarian
51
52 176 Statistics provided data for crop yield and fertilizer application (<http://dati.istat.it/>) at the provincial level. Inputs and
53
54 177 outputs were initially calculated for each municipality and then aggregated at the study area level.
55

56
57 178 Uncertainty in N budget calculations was assessed by a Monte Carlo analysis using Excel and R software (R Core
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59 179 Team 2019). All coefficients used to convert census data into N amounts were assumed to vary stochastically and
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4 180 independently around the average value with a normal probability distribution. For each simulation, a set of
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6 181 coefficients was randomly generated from probability distribution functions and a total of 1000 simulations were run.
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8 182 Budget calculation was conducted both at the annual and at the seasonal scales and compared with seasonal in-
9
10 183 stream N loads. Details about annual budget equations, seasonal calculations and sources of census data and
11
12 184 agronomic coefficients are presented in Supplementary Material A.

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14 185 The N loads produced by the urban areas were not included in the calculation because more than 95% of the sewers
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16 186 in the study area are connected to wastewater treatment plants (WWTP). Nearly 75% of the N inputs to WWTP is
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18 187 removed via denitrification in tertiary treatment (Lombardy Region, 2017). Indeed, the calculation of the urban load
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20 188 produced by the resident population, obtained by the conversion of equivalent inhabitant in kg of N per day, resulted
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22 189 in less than 2% of the total N input by diffuse sources (Pinardi et al., 2018).

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24 190 The daily precipitation data were downloaded from the ARPA Lombardy website
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26 191 (<https://www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx>) at Ponti sul Mincio station (Fig. 1)
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28 192 for the period from 2010 to 2017. The mean annual, seasonal (irrigation and non-irrigation period) and monthly
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30 193 precipitation data were calculated. Irrigation data at the municipality level was obtained from the 6th Agricultural
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32 194 Census (National Institute of Statistics, 2010, <http://dati-censimentoagricoltura.istat.it>) and then aggregated at the
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34 195 study area level.
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37 197 **3.2. Water sampling and analyses**

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39 198 Two stations located at the extremes of the identified river reach (S1 and S2; Fig. 1) were sampled for water
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41 199 analyses. The two stations were selected as they were located upstream and downstream the area where the Mincio
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43 200 River can be considered as a gaining river in groundwater-surface water interaction, that is the river is fed by
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45 201 groundwater (Racchetti et al., 2019). Given the constant discharge between S1 and S2, the identified river reach was
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47 202 more recently characterized as a flow-through reach (Severini et. al., 2022), with groundwater feeding the river in the
48
49 203 western bank and being fed by the river in the eastern bank. Field campaigns were carried out seasonally with a
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51 204 series of daily cycles of repeated samplings carried out on 12-13 August and 15-16 November 2016, 14-15 February,
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53 205 12-13 April and 13-14 June 2017. Water samples were taken in three replicates every 4 hours for a 24-hour period.
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55 206 An aliquot was transferred into a 12 mL exetainer (Labco, UK), added with 100 μ L of HgCl₂, and analyzed for
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57 207 dissolved inorganic carbon (DIC) with Gran titration (0.1 N HCl) within 24 hours from sampling. DIC was measured
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4 208 as it may trace differences between surface and groundwater chemistry. Water aliquots were filtered (GF/F glass
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6 209 fiber filters) and transferred to plastic vials for nitrate (NO₃-N), nitrite (NO₂-N), and ammonium (NH₄-N)
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8 210 determination by spectrophotometric methods (Rodier, 1978; APHA, AWWA, WPCF, 1999). Hourly or daily water
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10 211 flow data were obtained by the Interregional Agency for the Po River (AIPO), and by the Mincio Consortium for
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12 212 Pozzolo and Goito sites.

13
14 213 The Mann-Whitney Rank Sum Test was used to test the difference between upstream and downstream values of
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16 214 water flow, NO₃-N, NO₂-N, NH₄-N and DIC concentrations. The R software package (R Development Core Team,
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18 215 2019) was used to perform all statistical tests.

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20 216

21 217 ***3.3. Dissolved inorganic nitrogen and carbon daily loads***

22 218 For each sampling date, daily NO₃-N, NO₂-N, NH₄-N and DIC riverine loads transported at S1 and S2 (kg d⁻¹) were
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24 219 calculated multiplying concentrations by river discharge. The difference between loads at S2 and S1 was calculated
25
26 219 according to the following equation:
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28 220

$$29 \Delta\text{NO}_3\text{-N (or NO}_2\text{-N, NH}_4\text{-N, DIC)} = \sum [\text{C}_t \times \Delta t \times \text{Q}]_{\text{S2}} - \sum [\text{C}_t \times \Delta t \times \text{Q}]_{\text{S1}} \quad (1)$$

30 221
31 222 where: C_t = concentration of NO₃-N (or NO₂-N, NH₄-N, DIC) at time t downstream (S2) or upstream (S1) (mg m⁻³);
32 223 Δt = time interval between samplings (h); Q = water flow (m³ h⁻¹). Such difference can be null, suggesting
33 224 equilibrium between inputs and outputs, negative, suggesting net retention or dissipation (e.g., uptake or
34 225 denitrification), or positive, suggesting the occurrence of production or additional inputs along the stretch (e.g.,
35 226 nitrification or point and diffuse inputs). A standard deviation was associated to NO₃-N, NO₂-N, NH₄-N and DIC
36 227 measurements made in replicates. The errors conveyed through the mathematical description were calculated with
37 228 classical error propagation equations.

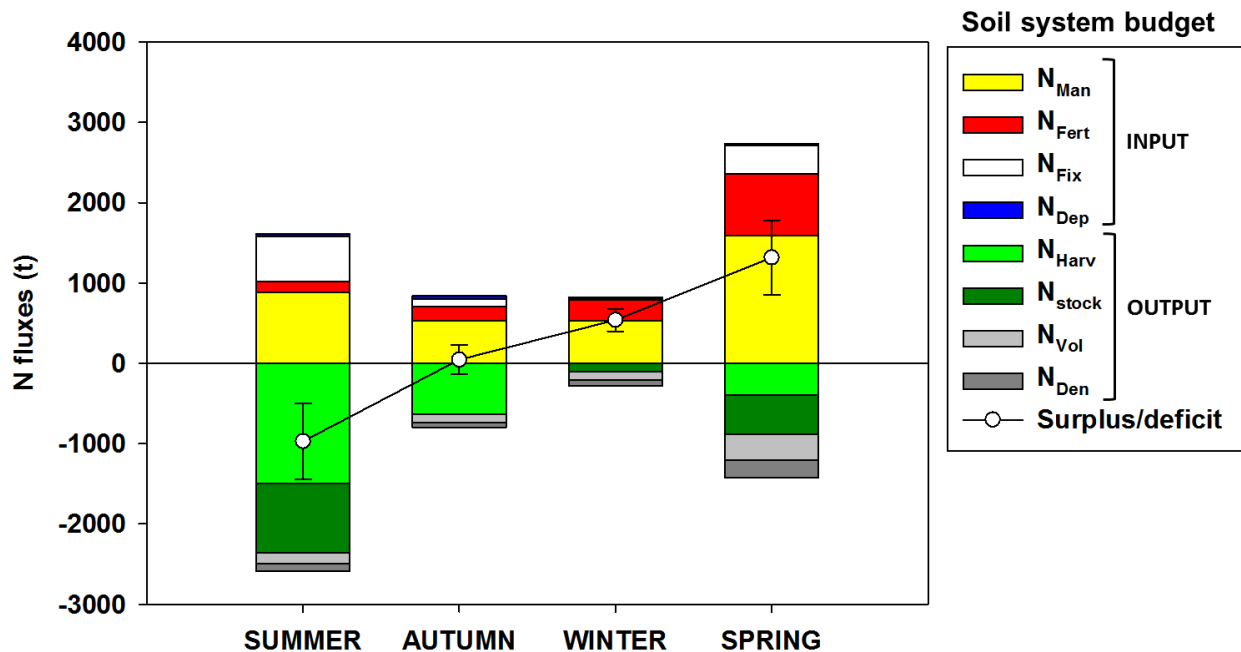
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39 229

40 230 **4. Results**

41 231 ***4.1. Nitrogen budgets and water inputs***

42 232 In the four municipalities under study, the total N inputs to arable land (6010±498 t y⁻¹) were mainly due to livestock
43 233 manure (59%). Nitrogen outputs (5078±494 t y⁻¹) accounted for 84% of the N inputs and were mainly due to crop
44 234 harvest (50% of the total N outputs). The main cultivated crop is maize (63% of arable land) followed by permanent
45 235 grassland. The difference between N inputs and outputs denoted a N soil surplus (932±702 t y⁻¹). The mean areal N

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 4 236 surplus was $67 \pm 50 \text{ kg ha}^{-1} \text{ y}^{-1}$ with values calculated for the different municipalities ranging between -172 and 204 kg
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 6 237 $\text{ha}^{-1} \text{ y}^{-1}$. All these results are reported in Supplementary material B, Table B.1 and Figure B.1.
 7
 8 238 The seasonal SSB is reported in Figure 2. Manure N fertilization was higher in spring ($1590 \pm 268 \text{ t}$) and summer
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 10 239 ($883 \pm 149 \text{ t}$), and similar in autumn and winter ($530 \pm 89 \text{ t}$). Synthetic N fertilization was higher in spring ($769 \pm 190 \text{ t}$)
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 12 240 and winter ($257 \pm 63 \text{ t}$). Biological N fixation was higher in summer ($559 \pm 259 \text{ t}$) and spring ($350 \pm 162 \text{ t}$) whereas
 13
 14 241 atmospheric N depositions were concentrated mainly in summer and autumn (32 ± 4 and $38 \pm 4 \text{ t}$, respectively). In
 15
 16 242 spring, N associated to the crop's standing stock ($487 \pm 98 \text{ t}$) was higher than N in the crop harvest ($398 \pm 80 \text{ t}$). With
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 18 243 respect to N outputs, crop harvest was highest in summer ($1492 \pm 301 \text{ t}$) and autumn ($637 \pm 128 \text{ t}$). Ammonia
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 20 244 volatilization and soil denitrification were quantitatively important in spring ($324 \pm 233 \text{ t}$ and $210 \pm 102 \text{ t}$,
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 22 245 respectively). Coupling the seasonal input and output data, a transition from N deficit to N surplus is evident moving
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 24 246 from summer ($-971 \pm 472 \text{ t}$) to spring ($1317 \pm 464 \text{ t}$).
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52 248 Figure 2. Components of the Soil System Budget (SSB) of nitrogen (N) for the study area within the Mincio River
 53 249 watershed for all seasons. N_{Man} = N in livestock manure applied to agricultural soils; N_{Fert} = N in synthetic fertilizer
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 55 250 applied to agricultural soils; N_{Fix} = agricultural N_2 fixation associated with N fixing crops; N_{Dep} = atmospheric N
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 57 251 deposition on agricultural land; N_{Harv} = N exported from agricultural soils with crop harvest; N_{stock} = organic N in
 58
 59 252

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 $43 \pm 6\%$ occurred during the irrigation period (Fig. 3).

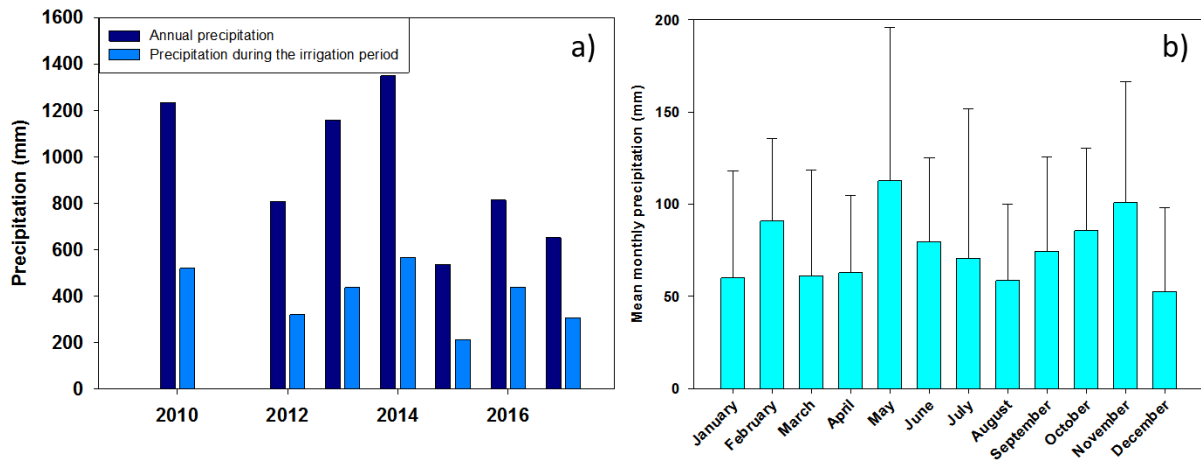


Figure 3. Annual precipitation and the fraction of precipitation during the irrigation period (from May to September) (a) and mean monthly precipitation (\pm standard deviation) (b) in the period from 2010 to 2017 at Ponti sul Mincio meteorological station (see Fig. 1 for the localization) (2011 was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

During the May-September period a water volume of 53.3×10^6 m³ 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 (NO_2-N and NH_4-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 m³ 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 m³ s⁻¹ at S1 and 13.0 ± 3.9 m³ 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.

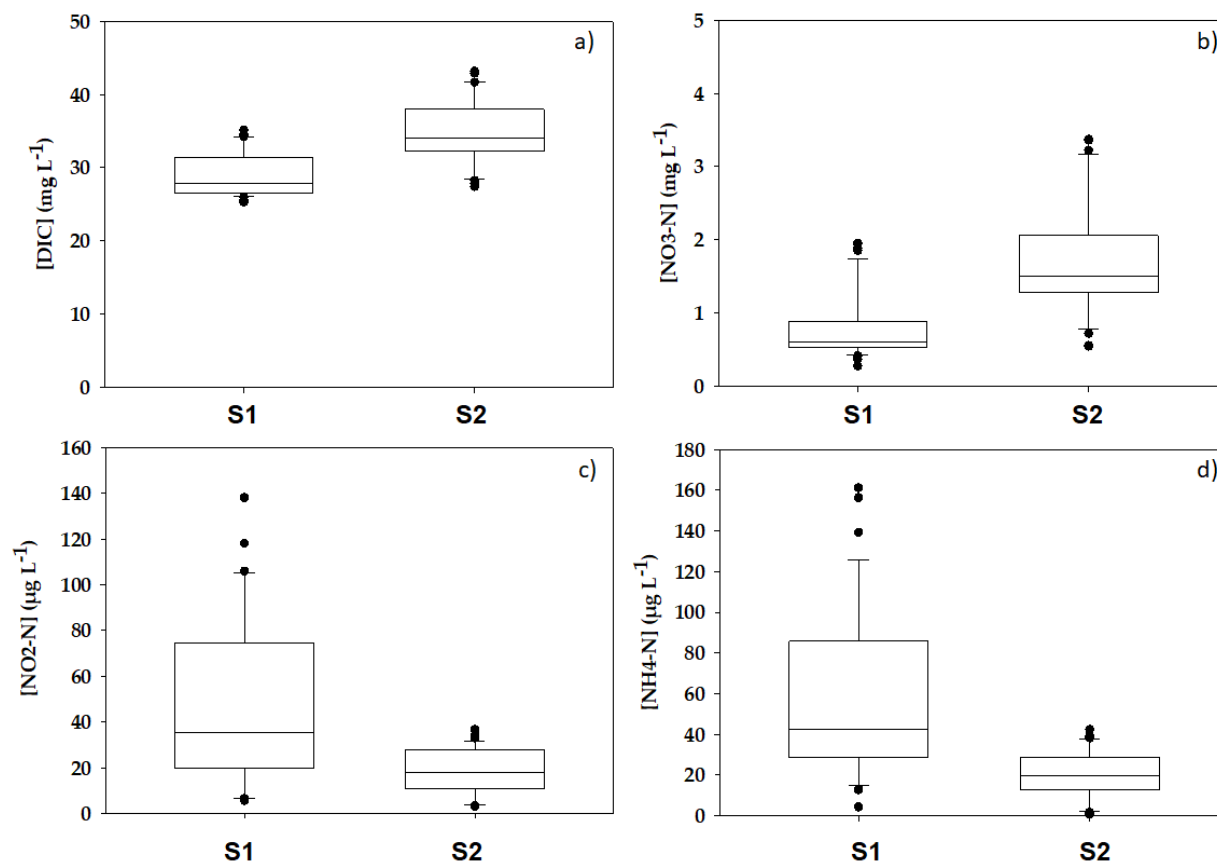


Figure 4. Box plot showing the concentrations of dissolved inorganic carbon (DIC; a), and of nitric (NO₃-N; b),

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).

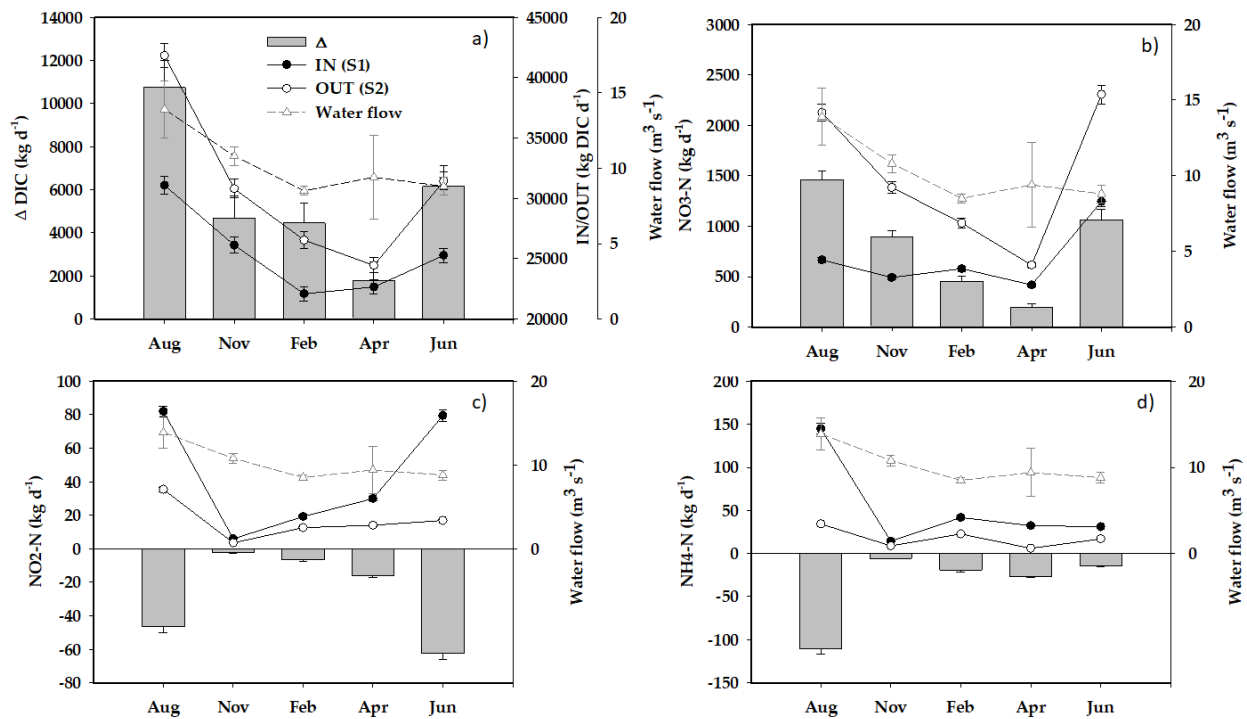


Figure 5. Daily loads of inorganic carbon (DIC; a), nitric nitrogen (NO₃-N; b), nitrous nitrogen (NO₂-N; c), and ammonium nitrogen (NH₄-N; d) transported at the extremes of the studied river reach and their differences ($\Delta=S2-S1$).

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4 302 S1) in the period August 2016 to June 2017. Water flow is also reported. Mean values are given, with error bars
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6 303 corresponding to ± 1 standard deviation.
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8 304 9 10 305 **5. Discussion**

11 12 306 **5.1. The seasonality of soil N budgets**

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14 307 Previous studies carried out in the Po River basin and in other geographical areas characterized by intensive
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16 308 agriculture and animal farming suggest a generalized N surplus and inefficient N use, leading to large N losses to
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18 309 surface and groundwater (Soana et al., 2011; Hou et al., 2015; Özbek et al., 2015; Viaroli et al., 2018; Häußermann
19
20 310 et al., 2020). In the Po River plain, to our knowledge, only a few sub-basins (e.g., Ticino and Po di Volano) represent
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22 311 exceptions to this rule due to very limited animal farming and synthetic fertilizers inputs balanced with crop needs
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24 312 (Racchetti et al., 2019; Soana et al., 2021). All those studies were carried out on an annual temporal scale, that
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26 313 potentially masked marked seasonal differences. Our calculations suggest a clear seasonal variation in N soil
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28 314 budgets, changing along seasons with periods with deficit (summer), equilibrium (autumn), moderate (winter) or
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30 315 large (spring) excess. These differences arise from variable seasonal balance among agricultural practices, such as
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32 316 large spring manure and synthetic fertilizer spreading uncoupled to crop uptake, moderate spread of fertilizers during
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34 317 winter with little to no uptake or large summer crop uptake in excess to N inputs (Chen et al., 2019). The N
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36 318 assimilation term is calculated as the product of the standing stock by the biomass-specific uptake rates and peaks in
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38 319 summer. Indeed, the biomass-specific uptake tends to decrease along with the crop's growth, but the crop's standing
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40 320 stock is much smaller in spring than in summer. Large spring N inputs are therefore coupled to relatively low uptake,
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42 321 resulting in a maximum surplus, exceeding that calculated for winter, when uptake is minimum. Large summer
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44 322 uptake on the contrary exceeds inputs and results in a seasonal deficit of N in the soil system budget. High
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46 323 fertilization is probably driven by the N need of the main cultivated crop, i.e., maize, which is a water and N-
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48 324 demanding species (FAO, 2006). These results on intra-annual variations in N mass budgets support the relevance of
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50 325 seasonal studies in highlighting critical moments in terms of potential water pollution (Lin et al., 2019; Compton et
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52 326 al., 2020). As nitrate water pollution is correlated with N excess in soils, our outcomes indicate a maximum nitrate
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54 327 pollution risk in the spring, a minimum risk in the summer and something intermediate in winter and autumn. Results
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56 328 from seasonal river N transport suggest something different as the highest N accumulation along the stretch was
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58 329 measured in summer and the lowest in spring. Taken together, these apparently contrasting results indicate that more
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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.

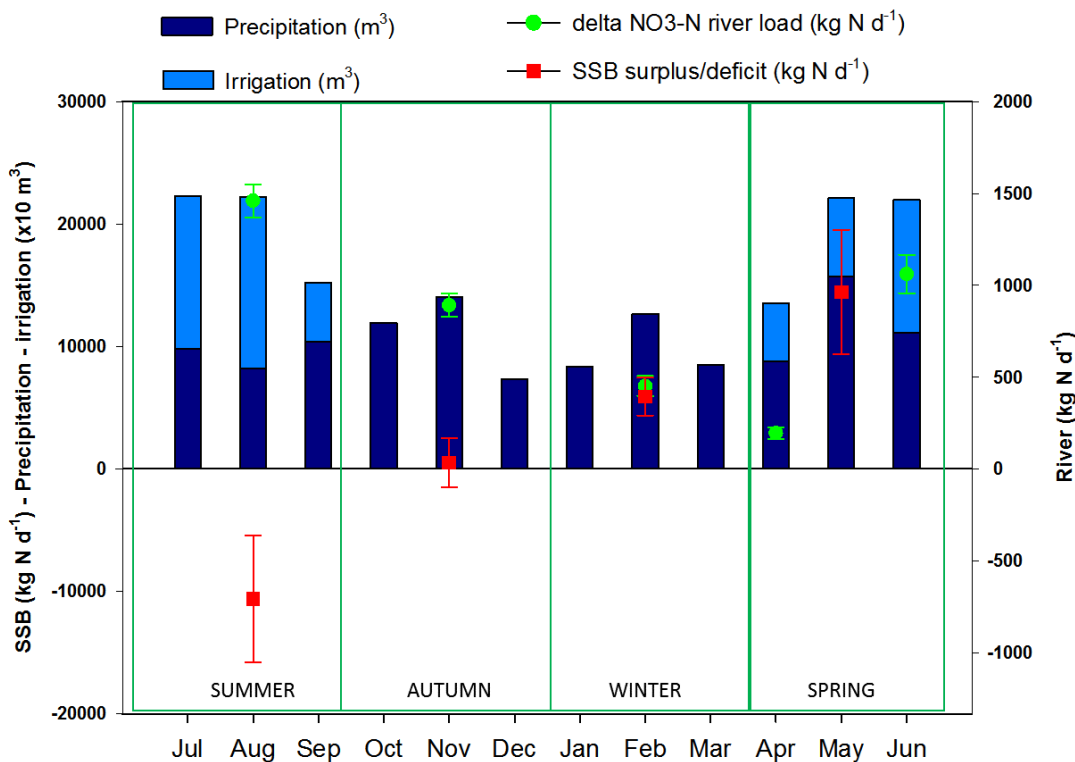


Figure 6. Histograms report monthly water input due to precipitation and irrigation in the municipalities of the

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4 346 Mincio basin under study. Green dots show the delta nitrate loads of the river reach (delta NO₃-N river load) in the
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6 347 five sampling dates and red squares show seasonal N budget of agricultural soils (SSB – Soil system budget –
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8 348 surplus/deficit).
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12 350 In the regulated river reach under study, which is characterized by gravel bottom and colonized by submerged
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14 351 macrophytes, ammonium and nitrite loads were higher upstream and suggested net retention in all seasons, peaking
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16 352 in summer when the primary producers' activity is maximum. Differently, dissolved inorganic carbon and nitrate
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18 353 loads evidenced a significant increase from upstream to downstream in all the investigated seasons. Nitrate and
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20 354 inorganic carbon net export decreased from August to April, with August as the central month for irrigation and
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22 355 April the last month before the start of the irrigation period. From these results, we calculated the annual net export
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24 356 of inorganic carbon and nitrate multiplying the daily values by the number of days between consecutive samplings,
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26 357 and then integrating the results over one year. Despite 6-8 repeated water samplings during the 24 hours, these
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28 358 calculations are based on measurements carried out in single days along different seasons. However, the nitrate
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30 359 concentrations measured in this study are consistent with the seasonal NO₃-N concentrations measured by ARPA
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32 360 Lombardy (the authority that manages water monitoring for the WFD) in the period 2009-2017 and by the
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34 361 Laboratory of Aquatic Ecology of the University of Parma in the period 2011-2017 (Fig. C.1 in Supplementary
35
36 362 material C).
37
38 363 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
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40 364 between S1 and S2. These amounts can be explained by element transformation (e.g., respiration or nitrification),
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42 365 and lateral or vertical inputs. As the loads of the other two inorganic N forms were negligible as compared to nitrate,
43
44 366 this calculation was done only for the latter. Using dissolved oxygen budgets (not reported in this work) we
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46 367 converted dark river respiration rates (night oxygen uptake along this stretch; from -1.3 to -5.7 mm O₂ m⁻² h⁻¹ in
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48 368 winter and summer, respectively) into potential nitrification rates (~ 75 t N y^{-1}) according to nitrification
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50 369 stoichiometry. To this purpose, we assumed that 100% of the oxygen consumed was used to oxidize ammonium to
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52 370 nitrate. Results suggest that microbes-mediated processes as nitrification can explain at most 23% of the nitrate
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54 371 accumulation in the river reach in all seasons. Such percentage is a large overestimation of the real value as it was
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56 372 obtained neglecting all other oxygen-consuming processes, including macrophytes, fish, macroinvertebrates, and the
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58 373 whole heterotrophic microbial community respiration. A comparable nitrate production (~ 80 t N y^{-1}) was obtained
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4 374 using the nitrification rate set by Taherisoudejani et al. (2018) in the QUAL2Kw model applied to the Oglio River, a
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6 375 nearby Po River tributary with similar hydrological characteristics. Pinardi et al. (2014) found that the processes in
7
8 376 the hyporheic zone or the microbial metabolism of carbonate dissolution could explain up to 15% of the DIC
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10 377 increase, including the role of macrophytes in modulating dissolved CO₂ saturation values and fixation of C.
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12 378 If biological processes cannot explain inorganic carbon and nitrate increase, also point pollution sources can be
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14 379 excluded, due to low discharge of small tributaries along this stretch with water chemistry comparable to that of the
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16 380 Mincio River. Another potential source of N and DIC is groundwater, via seasonally variable river-groundwater
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18 381 interactions. Indeed, the level of the phreatic surface increases and interacts with the river due to precipitation,
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20 382 flooding and sprinkler irrigation during the spring-summer period (Racchetti et al., 2019; Severini et al., 2020) (Fig.
21
22 383 7). In a period before and after fertilization (from March to May 2021), Severini et al. (2022) measured in the same
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24 384 area of the Mincio River significantly higher HCO₃⁻ concentrations in groundwater than in surface water (Appelo
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26 385 and Postma, 2005). Hence, the DIC increase in the investigated river stretch can be associated to the groundwater
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28 386 feeding the Mincio River. Considering the abundant use of organic fertilizers in the area, the higher DIC in
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30 387 groundwater can be related to the presence of calcite (CaCO₃) in the mineralogical composition of the aquifer and to
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32 388 the oxidation of the organic matter, which promotes a higher DIC concentration in groundwater. Future
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34 389 investigations should include the mineralogical composition of the aquifer.

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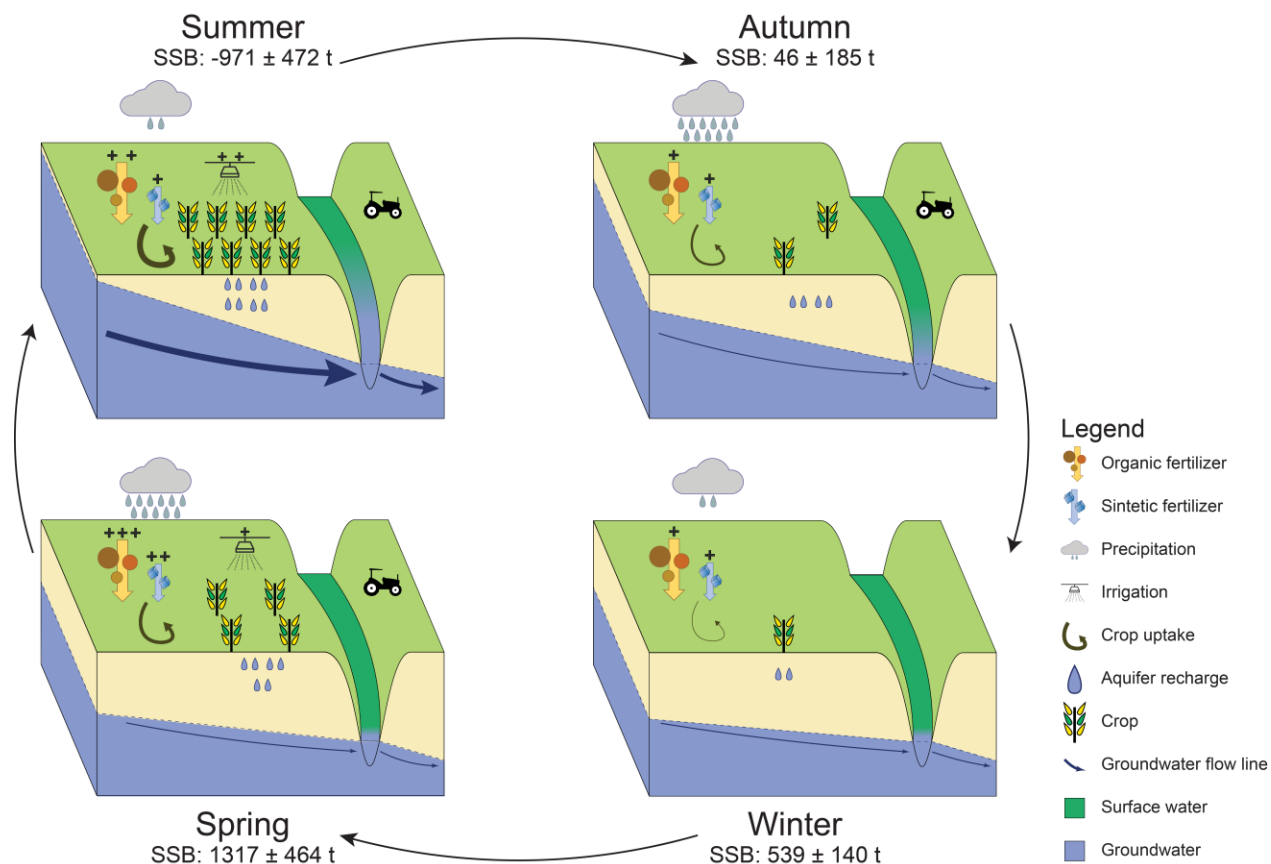


Figure 7. Synthesis of the seasonal nitrogen budgets at agricultural land and at river section. The relation with the groundwater is also reported. SSB = Soil System Budget.

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 $\text{NO}_3\text{-N}$ delta loads were found in summer, when the

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4 406 groundwater level are the highest and there is the maximum groundwater seeping to the Mincio River, highlighting
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6 407 the deep effects of the recharge given by irrigation. On the contrary, some differences were found during the rest of
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8 408 the year, characterized by a less anthropic recharge of the aquifer. These differences are more related to the
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10 409 dissimilar distribution of precipitation and percolation of water and N to groundwater, which fosters the migration of
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12 410 N to the Mincio River. In fact, as we move away from the end of the irrigation period (September), the lower
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14 411 groundwater heads reported in Severini et al. (2022) could result in a lower groundwater seepage to the river (Fig. 7).
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16 412 Having less nutrient enriched water available guarantees a minor nitrate surplus in the river reach, even if N
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18 413 fertilization starts again in winter, resulting in another period with N soil surplus (Figs. 6, 7).
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20 414 Considering that the mean annual N surplus on the agricultural land in the study area averaged $67 \text{ kg ha}^{-1} \text{ y}^{-1}$, it is
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22 415 possible to speculate that the agricultural land surface that can potentially generate the $\text{NO}_3\text{-N}$ river export ($317 \pm 12 \text{ t}$
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24 416 $\text{NO}_3\text{-N y}^{-1}$) is equivalent to $\sim 4700 \text{ ha}^{-1}$. In addition, dividing this surface by the length of the river reach investigated,
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26 417 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
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28 418 N surplus production. These data allow to speculate that the N surplus was generated in the 25% of the surface of
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30 419 municipalities under study, giving useful information to better address arable land management.
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32 420 Our results on N input and output trends in agricultural soils and into the river reach at annual and seasonal basis
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34 421 allow to better understand N patterns from land to river and the potential nitrate pollution to surface and
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36 422 groundwater. This information at seasonal resolution can help policy-makers in developing effective plans to
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38 423 improve N management at the macroscale. In fact, this combination of information can guide the identification of
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40 424 proper spatial-temporal management strategies to reduce N pollution and river export to avoid eutrophication
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42 425 processes of water bodies. For example, our results suggest that more nitrate was delivered downstream in summer
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44 426 because of spring soil N excess coupled to flood irrigation over permeable soils. Hence, it is important to focus on
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46 427 agricultural sources (manure and synthetic fertilizers in particular) to better balance N inputs and output by crop
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48 428 harvest (or stock). Our approach was applied in a pilot study at the sub basin level, but it is exportable to the whole
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50 429 basin and to other rivers. It becomes very important to have local information and basin-specific data to perform
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52 430 seasonal analysis on N patterns (Lassaletta et al., 2021).
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57 432 **5.4. Possible remediation strategies in the context of climate change and the regulation of river discharge**

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59 433 Temporal disconnections between N fertilization, transport and uptake in agricultural land can result in low N use
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4 434 efficiency (Robertson and Vitousek, 2009). Specific monitoring of crop growth, nutrient demand and soil availability
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6 435 is useful to obtain a more synchronous nutrient supply in response to crop needs (Quemada et al., 2013). Alternative
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8 436 practices arranged to implement nutrient management directly in the field include actions such as variable rate or
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10 437 split of fertilizer applications matched to crop growth demand, improvements in efficiency of irrigation practices and
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12 438 use of nitrification inhibitors (Lacey and Armstrong, 2015; Fernández et al., 2016). The nutrient best management
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14 439 practices, for N, should be designed in view of seasonal N leaching losses and hydrologic export to properly depict
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16 440 crop growth dynamics and N demand, soil conditions and hydrology (Lin et al., 2019).
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18 441 It is during summer that the investigated reach experienced the highest water nitrate accumulation. An action useful
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20 442 to buffer the N export is the implementation of riparian buffer strips that can promote N retention during the spring-
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22 443 summer irrigation period or the use of cover crops in winter (Dabney et al., 2010; Cole et al., 2020). During winter,
23
24 444 our calculations suggest N soil excess in a period where uptake is minimum, and denitrification is likely limited by
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26 445 low temperatures and by the thick unsaturated soil. The latter follows the downward winter migration of the water
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28 446 table, previously discussed. For this reason, the adoption of practices that can favor water retention to increase soil
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30 447 humidity in the cold season and the presence of water in canals, commonly dry in autumn and winter, might be a
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32 448 solution that can favor denitrification process. As an example, the construction of artificial ponds or wetlands can be
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34 449 useful to intercept N runoff from agricultural lands (Nõges et al., 2003; Carstensen et al., 2020).
35
36 450 In the geographical area of our case study, the Alps host a series of large lakes regulated by dams that feed rivers
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38 451 among which the Mincio River. The dams regulate the lake water level and the river discharge with rules that aims to
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40 452 accumulate water in winter and release it during the irrigation period, adapting also to local meteorology and water
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42 453 inputs to lakes. This regulation practice guarantees sufficient summer level in lakes for tourism and navigation
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44 454 purposes and large water availability for irrigation and electricity production in the downstream river section.
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46 455 Irrigation is supported by several water abstraction infrastructures that facilitated agriculture and animal farming
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48 456 activities. Irrigation practices, supported mainly by flooding irrigation in the sector of the Po River plain including
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50 457 our study area are carried out over permeable soils, and favor the recharge of the aquifer mainly in summer (Rotiroti
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52 458 et al., 2019). Therefore, water retention upstream during non-irrigation periods and flood irrigation with large water
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54 459 volumes during summer are probably the main drivers of the groundwater head variation, which is subject to strong
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56 460 seasonal differences (Taherisoudejani et al., 2018). Under the current climate change scenario, also in this
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58 461 geographical area, the rapid changes of global warming are manifested with lower precipitation, dry winters,
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4 462 heatwaves and storms events, and an increasing number of consecutive days with high temperatures (Cifrodelli et al.,
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6 463 2015; Pedro-Monzonís et al., 2016; Lassaletta et al., 2021; Ranasinghe et al., 2021). The response to these global
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8 464 trends could increase or decrease river nitrate concentration depending on regional or site-specific linkage between N
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10 465 concentration and discharge (Stelzer et al., 2020). For this reason, the geographical sector under study seems
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12 466 extremely vulnerable to climate change as the system is depicted and managed for large water availability (i.e., large
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14 467 lakes regulation, high water demanding crops, and flooding as main irrigation practice) and therefore a discussion on
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16 468 water management at political level is urgent. Predicting scenarios on the fate of the N excess with different water
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18 469 availability is difficult, we can hypothesize a reduction of water discharge from rivers and consequently from
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20 470 irrigation that will not recharge sufficiently the groundwater due to its deep level (Taherisoudejani et al., 2018). This
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22 471 condition will lead to more thick vadose zones, fostering a higher nitrification rate and nitrate accumulation in soil
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24 472 during winter, whereas denitrification, the main process that removes N permanently from the system, is favored in
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26 473 water saturated soils with high organic matter (Ascott et al., 2017). In soil and rivers close to N saturation, it is
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28 474 expected a lower nitrate retention efficiency and therefore an increase in N availability and vertical and horizontal
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30 475 transfer (Stelzer et al., 2020). Moreover, for the future it can be expected a delay in the river feeding by groundwater
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32 476 with hot-moments of N mass transfer. In fact, we can expect that with the increment of unsaturated zone, the rate of
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34 477 soil denitrification will be reduced and conversely the nitrification process will be favored supplying a short N mass-
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36 478 transfer as soon as the first rainfall or flooding irrigation will occur, carrying water with high N concentration to
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38 479 surface or groundwater. A possible solution to limit this hot-moment can be the improvement of irrigation practices
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40 480 with less water consumption and a more widespread use of precision farming supported for example with remote
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42 481 sensing technique (Nutini et al., 2021). Such a new vision on the irrigation practices can allow a lower winter water
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44 482 retention in the Lake Garda, that can be partially used in the non-irrigation period to guarantee a minimal vital flow
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46 483 in a certain number of drainage canals as well as in the Mincio River favoring denitrification process also in autumn
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48 484 and winter, although with lower rates driven by lower temperatures.
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51 485 52 53 486 **6. Conclusions**

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55 487 Published soil system budgets in agricultural areas generally reveal net N excess on an annual basis, whereas the
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57 488 present study reveals seasonally variable inventories of inputs and outputs, resulting in periods of large N excess and
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59 489 periods of pronounced deficit. The export of N excess via the river draining the investigated area has a temporal lag
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4 490 that depends on irrigation, vertical migration of the water table and subsurface water flow. Flood irrigation first fills
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6 491 the unsaturated zone and then favors river-groundwater interactions. Subsurface water flow replaces N-poor river
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8 492 water with N-rich groundwater. Seasonal soil N budget and the mechanisms of N transfer described in this study
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10 493 should foster more efficient agricultural practices, minimizing N losses and improving N use. Results from this work
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12 494 should also be carefully considered in future planning of agricultural and irrigation activities, in a scenario of climate
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14 495 change and variable availability of water. Winter retention of water in lakes, upstream the agricultural areas, has
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16 496 serious drawbacks as it will increase the volume of the unsaturated soil and the production of nitrate via organic N
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18 497 ammonification and nitrification. Adaptive strategies based on precision farming, new material to retain soil
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20 498 humidity, irrigation techniques alternative to flooding and a management of the canal network targeting the
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22 499 restoration of biogeochemical services (e.g., N-uptake and denitrification) seem effective and sustainable options.
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38 507 water flow data of the Mincio River, respectively.
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42 509 **Declarations**

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46 511 **Consent to Participate:** Not applicable.
47
48 512 **Consent to Publish:** Not applicable.
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57
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Declarations

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Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

Monica Pinardi^{1*}, Elisa Soana², Edoardo Severini³, Erica Racchetti³, Fulvio Celico³, Marco Bartoli^{3,4}

¹ Institute for Electromagnetic Sensing of the Environment, National Research Council, Via Corti 12, 20133 Milan, Italy

² Department of Life Sciences and Biotechnology, University of Ferrara, Via Borsari 46, 44121 Ferrara, Italy

³ Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy

⁴ Marine Science and Technology Center, Klaipeda University, Klaipeda, 92294, Lithuania

* Corresponding author

Address: Via A. Corti 12, 20133 Milan, Italy

Tel: +39 0223699298

Email: pinardi.m@irea.cnr.it

1 **Abstract**

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

20

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

22

23 **1. Introduction**

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

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

51 river-groundwater interactions (i.e. the river feeds the groundwater) (Rotiroti et al., 2019; Severini et al., 2021). On
52 the contrary summer irrigation, besides representing a vehicle for N transport, produces opposite effects, often
53 reversing the direction of river-groundwater interactions (i.e. upwelling, the groundwater feeds the river). These
54 practices, that characterize anthropogenic, intensively cultivated, and hydraulically regulated watersheds with
55 permeable soil, introduce marked seasonality in N budgets (Lin et al., 2019; Racchetti et al., 2019).

56 Many authors reported a significant correlation between annual N input to croplands and river N export (Neff et al.,
57 2003; Yan et al., 2010; Xu et al., 2013; Stokal et al., 2014; Tong et al., 2017), but they did rarely account for the
58 seasonality of N input and export (McCrackin et al., 2014; Chen et al., 2019). Studies targeting N budgets in
59 agricultural watersheds are generally conceived at the annual scale for mainly practical reasons, as agricultural census
60 data are collected and published by national statistical institutions with annual frequency. Such an approach from one
61 side allows to calculate N use efficiency in cropland and potential N loss, but from the other side, it misses temporal
62 resolution and precludes the understanding of seasonal variations of the array of N-related processes, potentially
63 regulated also by climate change. For example, human activities (e.g., crop production) and altered hydrology may
64 influence the seasonality of N river export (Basu et al., 2010; Compton et al., 2020), together with the seasonal
65 evolution of temperature that influences N losses, retention and removal processes (e.g., denitrification) (McCrackin
66 et al., 2014). Understanding how seasonal variations in human activities and hydrology influence N budgets in
67 agricultural soils and N transport by rivers is important to better understand the mechanisms underlying N
68 transformations along the terrestrial-aquatic path, improve agricultural practices to increase N use efficiency and
69 decrease N pollution, and eventually forecast how climate change will affect N dynamics (Mas-Pla and Menció, 2019).

70 This important set of objectives is a difficult target at the scale of whole watersheds due to scarce resolution of available
71 data and spatial heterogeneity (e.g. pedology, land use, etc). Smaller scales of analysis, targeting specific and
72 homogeneous river and watershed sectors, seem much more promising (McCrackin et al., 2014; Chen et al., 2019;
73 Compton et al., 2020).

74 Different studies carried out at large temporal and spatial scales (Soana et al., 2011; Pinardi et al., 2018; Viaroli et al.,
75 2018; Lassaletta et al., 2021) have highlighted the presence of hot-spots within watersheds that represent outliers in N
76 budgets (e.g., with very large N excess or very low N use efficiency). They also emphasized the presence of hot-
77 moments within watersheds, that are specific periods during which N mass transfer peaks as a combination of
78 decreased uptake, increased runoff or variation of the water table level, resulting in the reactivation of river-

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

91 In this sector of the Po River, groundwater in the phreatic and shallow aquifer has a short residence time as compared
92 to semiconfined or confined deeper aquifers. This is supported by fast (few days) surface-groundwater dynamics of
93 micro-pollutants (Balderacchi et al., 2016) and low concentrations of total dissolved solids (Martinelli et al., 2018).
94 Results of Balderacchi et al. (2016) suggest also fast response of shallow aquifers to changing conditions; as such they
95 allow to trace agricultural practices (e.g., use of herbicides or fertilization) and they respond quickly to hydrologic
96 variations (e.g., drought, precipitations, irrigation). It can be assumed that macrocontaminants as nitrates undergo the
97 same fast transfer mechanisms, also due to their elevated solubility and absence of interaction with soil and sediment.
98 The main hypotheses of this work are that river-groundwater interactions affect N transport in specific river sectors
99 and vary seasonally due to combination of irrigation practices and inorganic nitrogen excess in soil. We also
100 hypothesized that the seasonal dynamics of such variable interactions can be captured analyzing comparatively
101 seasonal N budget in agricultural soils and seasonal riverine N transport. In this context, the main aim of the present
102 study was to contrast seasonal N soil budget in an agricultural area drained by a river stretch with seasonal N loads
103 transported by the same draining river stretch, to assess riverine N dynamics in relation to agricultural practices.

104

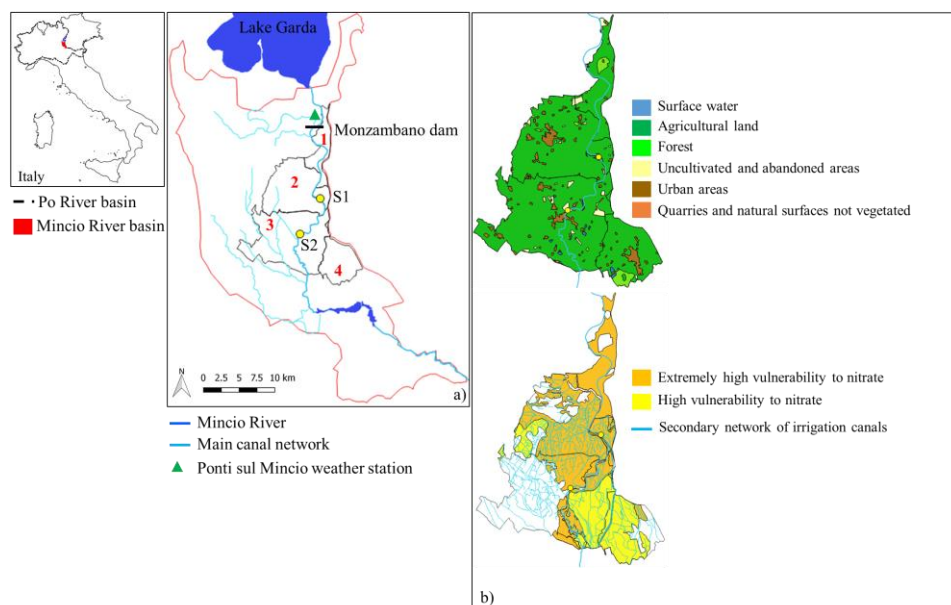
105 2. Study area

106 The Mincio River (~75 km) originates from the Lake Garda, the largest Italian Lake, and is a tributary of the Po River
107 (Fig. 1). The hydrological regime of the Mincio River is regulated upstream by a dam, which controls the water discharge
108 from the Lake Garda. Along the river course, a series of dams and weirs feeds a network of canals for irrigation and
109 industrial purposes and controls discharge variations to avoid the flooding of cities and villages. Water management for
110 Lake Garda recreational activities and for agricultural purposes results in marked flow variations. Indeed, since the
111 establishment of the river regulation in the 60's of the last century, the Mincio River discharge averaged ~80 and ~30 m³
112 s⁻¹ during the irrigation (May to September) and outside the irrigation periods, respectively (Lombardy Region, 2006).
113 More recently, projections of decreasing water availability resulted in a further reduction of the Mincio River discharge
114 to ~14 m³ s⁻¹ (www.laghi.net) in autumn and winter to keep water in the Lake Garda and guarantee water availability for
115 irrigation and tourism in the summer season. During winter, the flow reduction and absence of irrigation result in a
116 decreased aquifer recharge, a phenomenon described by different authors in this geographical area at regional (Rotiroti et
117 al., 2019) and local scales (Severini et al., 2021) and, consequently, in a lowering of the phreatic surface. This water
118 transfer dynamic results in a decrease of groundwater upwelling in winter and early spring (Balderacchi et al., 2016).
119 A wide segment of the Mincio River, including the portion investigated in this study, flows in a flood plain
120 characterized by a multilayered aquifer system with a cyclic facies architecture mainly made of fluvial-channel (gravel
121 and sand) and floodplain (clay) deposits (Amorosi et al., 2008). As a result, the northern part of the plain (high plain)
122 is locally characterized by shallow phreatic aquifers, while in the southern part (low plain) the floodplain facies act as
123 aquitards or aquicludes, resulting in confined and semi-confined aquifers (Chelli et al., 2018). The river reach
124 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
125 plain of the Mincio watershed. ~~This river segment drains the surface and includes of~~ four municipalities (1- Valeggio sul
126 Mincio, 2- Volta Mantovana, 3- Goito, and 4- Marmirolo; ~~total area 184 km²~~) ~~for a total surface of 184 km²~~ (Fig. 1). Since
127 2006, these municipalities are classified as Nitrate Vulnerable Zones (NVZs) according to the European Nitrate Directive
128 (91/676/CEE). ~~The study-area of the four municipalities investigated in mainly classified as agricultural land withis~~
129 ~~characterized by~~ fertile soils due to calcareous gravel deposits ~~with favor and is~~ intensively exploit~~ationed~~ed by agriculture
130 (Utilized Agricultural Area - UAA covers 76% of the study area; Fig. 1) and animal farming (1.2 and 0.6 t of live weight
131 per hectare for cattle and pigs, respectively). ~~The urban, infrastructural and industrial areas cover only 5% of the total~~
132 ~~surface of the four municipalities~~ (Fig. 1). The S1-S2 river segment flows into natural banks, has a mainly gravel bottom,
133 and has transparent waters. The main primary producers are submerged vegetation (e.g., *Vallisneria spiralis*) with

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134 associated epiphytes, benthic biofilms and different emergent macrophytes growing along the river banks or forming
135 islands (Pinardi et al., 2009, 2014). The linear development of irrigation canals in S1-S2 river reach sub basin sums ~560
136 km (Fig. 1). The surface covered by the other aquatic environments, such as quarry lakes is ~0.62 km².

137



138

139 Figure 1. Maps of the study area: the Mincio River segment from Pozzolo dam (S1) to Goito village (S2) (yellow
140 points = water sampling stations). a) Municipalities where the nitrogen mass budget was performed are reported
141 (1 - Valeggio sul Mincio, 2 - Volta Mantovana, 3 – Goito, 4 - Marmirolo). b) Land use and maps of soil
142 vulnerability to nitrate are reported for the four municipalities under study.

143

144 3. Material and methods

145 3.1. Nitrogen budgets and water inputs

146 A comprehensive input–output N budget across the Utilized Agricultural Area (UAA) was compiled by using locally-
147 derived data on farming activity, agronomic ~~coefficients~~coefficients, and atmospheric deposition. [One of the elements](#)
148 [of strength of the present work is the reliability of the soil N budget, that is built on a detailed dataset of statistical data](#)

149 [and on an accurate set of species-specific coefficients taken from the literature in the field.](#) Nitrogen budget was first
150 calculated at the municipal scale, i.e., the administrative level at which official agricultural statistics are available, then
151 weighted for the percentage of each municipality surface included within the study area, and finally summed up.
152 Census data were integrated in a nutrient budgeting approach proposed by Oenema et al. (2003), recently reviewed by
153 Zhang et al. (2020), and formerly applied to the whole Mincio River basin (Pinardi et al., 2018). Four inputs of N to
154 the UAA were considered (land application of livestock manure, land application of synthetic fertilizers, atmospheric
155 deposition, and biological fixation by crops), together with four outputs of N from UAA (crop harvest, crop stock,
156 ammonia volatilization and denitrification in soils). The difference between N inputs and outputs results in a net, which
157 represents a condition of equilibrium, surplus or deficit of N across the UAA.

158
159 The Soil System Budget (SSB) was calculated as follow:

$$160 \text{SSB N} = N_{\text{Man}} + N_{\text{Fert}} + N_{\text{Fix}} + N_{\text{Dep}} - N_{\text{Harv}} - N_{\text{stock}} - N_{\text{Vol}} - N_{\text{Den}}$$

161 where:

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

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

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

165 N_{Dep} = atmospheric N deposition on agricultural land

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

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

168 N_{Vol} = NH_3 volatilization in agricultural soils

169 N_{Den} = denitrification in agricultural soils

170
171 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

172 UAA ($kg N ha^{-1} y^{-1}$). [The soil N budget was estimated by employing agricultural census data referring to the](#)
173 [agricultural year 2015-2016. The agricultural year overlaps the vegetative cropping cycle, covering two consecutive](#)
174 [years, i.e., from November of the first year to October of the following one. The N budget calculated for the agricultural](#)
175 [year 2015-2016 is relevant in the present day because, in the last decade, only minor variations occurred in crop](#)
176 [surfaces and livestock densities of the study area and fertilization rates did not change appreciably.](#)

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

184
185 Uncertainty in N budget calculations was assessed by a Monte Carlo analysis using Excel and R software (R Core
186 Team 2019). All coefficients used to convert census data into N amounts were assumed to vary stochastically and
187 independently around the average value with a normal probability distribution. For each simulation, a set of coefficients
188 was randomly generated from probability distribution functions and a total of 1000 simulations were run. Budget
189 calculation was conducted both at the annual and at the seasonal scales and compared with seasonal in-stream N loads.
190 Details about annual budget [data](#), equations, [seasonal breakdown](#), [seasonal calculations](#) and sources of census data and
191 agronomic coefficients are presented in Supplementary Material A.

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

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

203

204 **3.2. Water sampling and analyses**

205 Two stations located at the extremes of the identified river reach (S1 and S2; Fig. 1) were sampled for water analyses.
206 The two stations were selected as they were located upstream and downstream the area where the Mincio River can be
207 considered as a gaining river in groundwater-surface water interaction, that is the river is fed by groundwater (Racchetti
208 et al., 2019). Given the constant discharge between S1 and S2, the identified river reach was more recently
209 characterized as a flow-through reach (Severini et. al., 2022), with groundwater feeding the river in the western bank
210 and being fed by the river in the eastern bank. Field campaigns were carried out seasonally with a series of daily cycles
211 of repeated samplings carried out on 12-13 August and 15-16 November 2016, 14-15 February, 12-13 April and 13-
212 14 June 2017. Water samples were taken in three replicates every 4 hours for a 24-hour period. An aliquot was
213 transferred into a 12 mL exetainer (Labco, UK), added with 100 μ L of HgCl₂, and analyzed for dissolved inorganic
214 carbon (DIC) with Gran titration (0.1 N HCl) within 24 hours from sampling. DIC was measured as it may trace
215 differences between surface and groundwater chemistry. Water aliquots were filtered (GF/F glass fiber filters) and
216 transferred to plastic vials for nitrate (NO₃-N), nitrite (NO₂-N), and ammonium (NH₄-N) determination by
217 spectrophotometric methods (Rodier, 1978; APHA, AWWA, WPCF, 1999). There are two main reasons for focusing
218 on inorganic nitrogen. The first is that from our database and from those of national monitoring agencies in the Mincio
219 river nearly 80% of the total N load is made of inorganic N (DIN, and within the DIN pool >90% is nitrate). The
220 second is that from the same dataset nitrate represents more than 95% of the total dissolved N in groundwater.

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

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

226

227 *3.3. Dissolved inorganic nitrogen and carbon daily loads*

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

$$231 \Delta \text{NO}_3\text{-N (or NO}_2\text{-N, NH}_4\text{-N, DIC)} = \sum [\text{C}_t \times \Delta t \times Q]_{S2} - \sum [\text{C}_t \times \Delta t \times Q]_{S1} \quad (1)$$

232 where: C_t = concentration of NO₃-N (or NO₂-N, NH₄-N, DIC) at time t downstream (S2) or upstream (S1) (mg m⁻³);

233 Δt = time interval between samplings (h); Q = water flow ($\text{m}^3 \text{h}^{-1}$). Such difference can be null, suggesting equilibrium
234 between inputs and outputs, negative, suggesting net retention or dissipation (e.g., uptake or denitrification), or
235 positive, suggesting the occurrence of production or additional inputs along the stretch (e.g., nitrification or point and
236 diffuse inputs). A standard deviation was associated to $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$ and DIC measurements made in
237 replicates. The errors conveyed through the mathematical description were calculated with classical error propagation
238 equations.

239

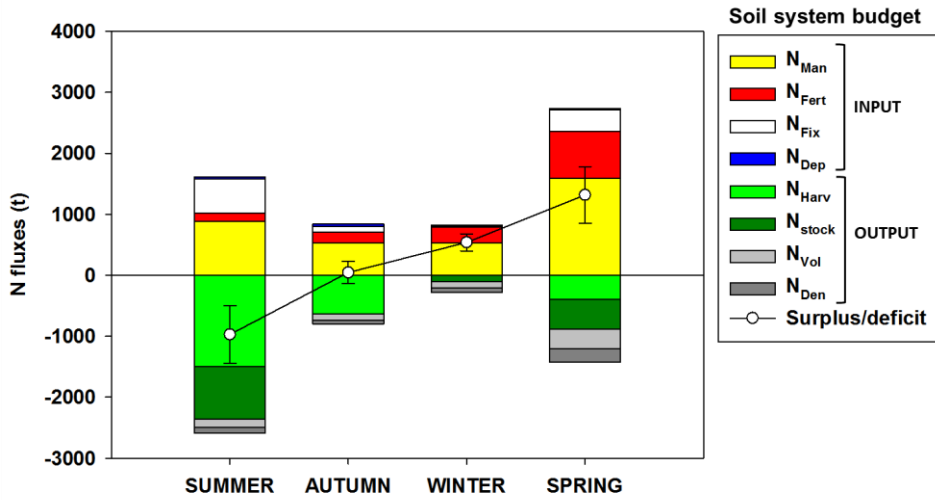
240 **4. Results**

241 *4.1. Nitrogen budgets and water inputs*

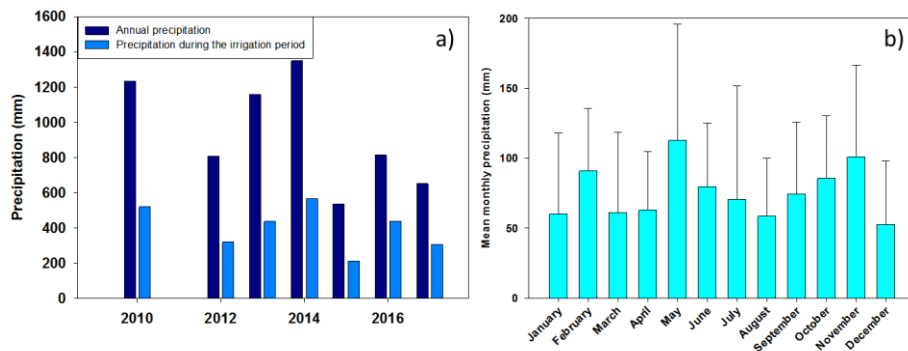
242 In the four municipalities under study, the total N inputs to arable land ($6010 \pm 498 \text{ t y}^{-1}$) were mainly due to livestock
243 manure (59%). Nitrogen outputs ($5078 \pm 494 \text{ t y}^{-1}$) accounted for 84% of the N inputs and were mainly due to crop
244 harvest (50% of the total N outputs). The main cultivated crop is maize (63% of arable land) followed by permanent
245 grassland. The difference between N inputs and outputs denoted a N soil surplus ($932 \pm 702 \text{ t y}^{-1}$). The mean areal N
246 surplus was $67 \pm 50 \text{ kg ha}^{-1} \text{ y}^{-1}$ with values calculated for the different municipalities ranging between -172 and 204 kg
247 $\text{ha}^{-1} \text{ y}^{-1}$. All these results are reported in Supplementary material B, Table B.1 and Figure B.1.

248 The seasonal SSB is reported in Figure 2. Manure N fertilization was higher in spring ($1590 \pm 268 \text{ t}$) and summer
249 ($883 \pm 149 \text{ t}$), and similar in autumn and winter ($530 \pm 89 \text{ t}$). Synthetic N fertilization was higher in spring ($769 \pm 190 \text{ t}$)
250 and winter ($257 \pm 63 \text{ t}$). Biological N fixation was higher in summer ($559 \pm 259 \text{ t}$) and spring ($350 \pm 162 \text{ t}$) whereas
251 atmospheric N depositions were concentrated mainly in summer and autumn (32 ± 4 and $38 \pm 4 \text{ t}$, respectively). In spring,
252 N associated to the crop's standing stock ($487 \pm 98 \text{ t}$) was higher than N in the crop harvest ($398 \pm 80 \text{ t}$). With respect to
253 N outputs, crop harvest was highest in summer ($1492 \pm 301 \text{ t}$) and autumn ($637 \pm 128 \text{ t}$). Ammonia volatilization and
254 soil denitrification were quantitatively important in spring ($324 \pm 233 \text{ t}$ and $210 \pm 102 \text{ t}$, respectively). Coupling the
255 seasonal input and output data, a transition from N deficit to N surplus is evident moving from summer ($-971 \pm 472 \text{ t}$)
256 to spring ($1317 \pm 464 \text{ t}$).

257



258
 259 Figure 2. Components of the Soil System Budget (SSB) of nitrogen (N) for the study area within the Mincio River
 260 watershed for all seasons. N_{Man} = N in livestock manure applied to agricultural soils; N_{Fert} = N in synthetic fertilizer
 261 applied to agricultural soils; N_{Fix} = agricultural N_2 fixation associated with N fixing crops; N_{Dep} = atmospheric N
 262 deposition on agricultural land; N_{Harv} = N exported from agricultural soils with crop harvest; N_{stock} = organic N in
 263 crop's standing stock; N_{Vol} = NH_3 volatilization in agricultural soils; N_{Den} = denitrification in agricultural soils. White
 264 dots represent seasonal N budgets (Σ INPUTS - Σ OUTPUTS); positive values suggest surplus whereas negative values
 265 suggest deficit.
 266
 267 During the 2010-2017 period the mean annual precipitation in the study area was $910 \pm 197 \text{ mm y}^{-1}$, of which about
 268 $43 \pm 6\%$ occurred during the irrigation period (Fig. 3).

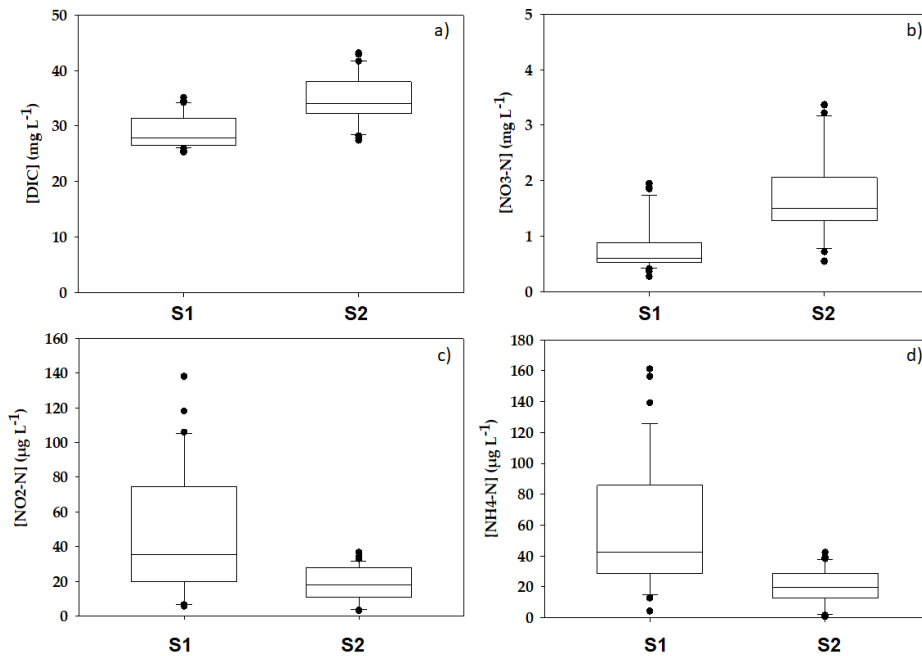


269
 270 Figure 3. Annual precipitation and the fraction of precipitation during the irrigation period (from May to September)
 271 (a) and mean monthly precipitation (\pm standard deviation) (b) in the period from 2010 to 2017 at Ponti sul Mincio
 272 meteorological station (see Fig. 1 for the localization) (2011 was not considered due to missing data for August and
 273 September).

274
 275 During the May-September period a water volume of $53.3 \times 10^6 \text{ m}^3$ was used to irrigate 13,513 ha of UAA, which
 276 represent 75% of the total arable land. Flooding and sprinkler were the main irrigation typologies (72% and 27% of
 277 the irrigated surface, respectively) (data from the National Institute of Statistics).

278
 279 **4.2. Water physico-chemical features of sampling sites**
 280 Nitrate and DIC concentrations were significantly higher at the downstream site for all sampling dates (Mann-Whitney
 281 Rank Sum Test, $p < 0.001$, $n=68$ for each parameter; Fig. 4). On the contrary, the concentrations of the other dissolved
 282 inorganic forms of nitrogen ($\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$) were significantly higher at the upstream site ($p < 0.001$, $n=68$ for
 283 each parameter; Fig. 4). The highest $\text{NO}_3\text{-N}$ and DIC concentrations were measured in June 2017 at both sampling
 284 sites (1.6 and $3.0 \text{ mg NO}_3\text{-N L}^{-1}$, and 33 and 41 mg DIC L^{-1} at S1 and S2, respectively). The highest values of $\text{NO}_2\text{-N}$
 285 were recorded in summer at S1 and S2 (up to 101 and $31 \mu\text{g L}^{-1}$, respectively), whereas $\text{NH}_4\text{-N}$ concentrations
 286 peaked in August 2016 at S1 (up to $122 \mu\text{g L}^{-1}$) and were high at both sites in February 2017. Nitrate was always the
 287 main form of inorganic nitrogen, accounting on average for 88% and 98% of the total N at S1 and S2, respectively.
 288 The mean annual water flow was $10.3 \pm 2.5 \text{ m}^3 \text{ s}^{-1}$ in the S1-S2 river reach (whole dataset 2016-2017, $n=140$). During

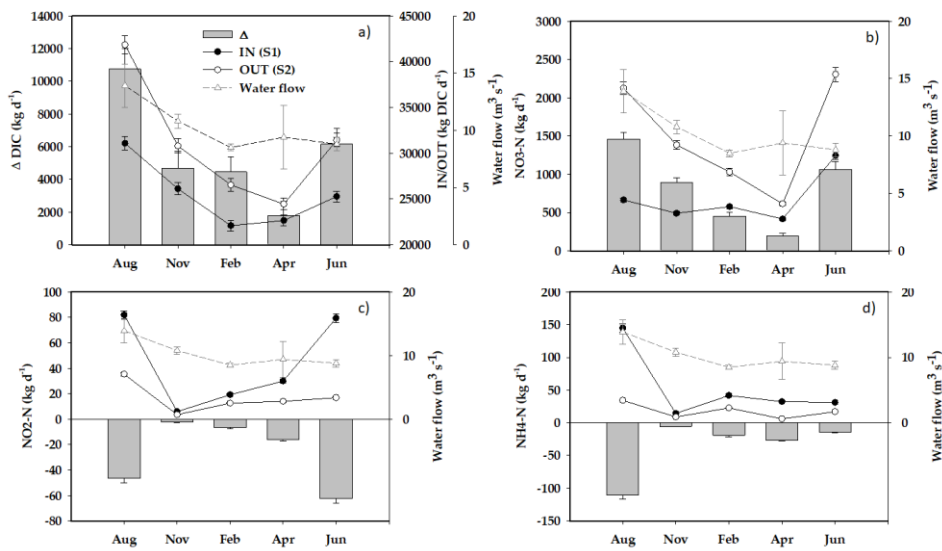
289 the irrigation period the water flow was not significantly different upstream and downstream ($11.7 \pm 2.0 \text{ m}^3 \text{ s}^{-1}$ at S1
 290 and $13.0 \pm 3.9 \text{ m}^3 \text{ s}^{-1}$ at S2; $p > 0.05$). No significant differences were also found between water flow during the
 291 irrigation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



293
 294 Figure 4. Box plot showing the concentrations of dissolved inorganic carbon (DIC; a), and of nitric (NO₃-N; b), nitrous
 295 (NO₂-N; c) and ammonium (NH₄-N; d) nitrogen measured seasonally from August 2016 to June 2017 at S1 and S2.
 296 Note different concentration units.

297
 298 **4.3. Dissolved inorganic nitrogen and carbon daily loads**
 299 In all seasons, NO₃-N and DIC transported loads were higher at S2, whereas NO₂-N and NH₄-N loads were higher at
 300 S1, but by much lower extent (Fig. 5). A positive correlation was found between NO₃-N and DIC concentrations
 301 (Pearson's correlation coefficient $r = 0.899$, $p < 0.001$, $n=68$ for each parameter) supporting the possibility of the same

302 origin for the two solutes. A similar seasonal trend was detected for DIC and NO₃-N accumulation along the analyzed
 303 river reach (Fig. 5). The maximum increase of transported loads (nearly 11,000 and 1500 kg d⁻¹ for DIC and NO₃-N,
 304 respectively) was measured in August 2016, whereas the minimum increase (nearly 2,000 and 200 kg d⁻¹ for DIC and
 305 NO₃-N, respectively) was measured in April 2017 (Fig. 5). The highest NO₂-N and NH₄-N loads reduction along the
 306 stretch were recorded during summer months (Fig. 5).
 307



308
 309 Figure 5. Daily loads of inorganic carbon (DIC; a), nitric nitrogen (NO₃-N; b), nitrous nitrogen (NO₂-N; c), and
 310 ammonium nitrogen (NH₄-N; d) transported at the extremes of the studied river reach and their differences (Δ=S₂-S₁)
 311 in the period August 2016 to June 2017. Water flow is also reported. Mean values are given, with error bars
 312 corresponding to ±1 standard deviation.

313

314 5. Discussion

315 5.1. The seasonality of soil N budgets

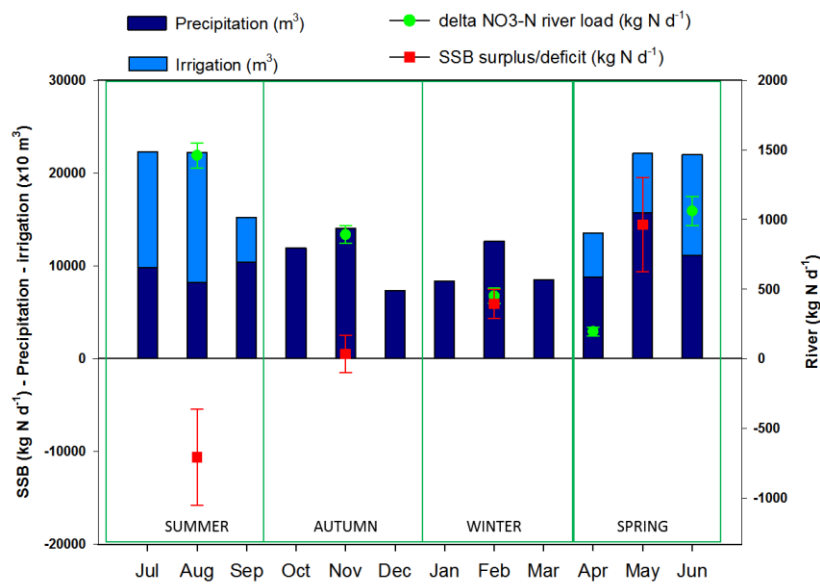
316 Previous studies carried out in the Po River basin and in other geographical areas characterized by intensive agriculture
 317 and animal farming suggest a generalized N surplus and inefficient N use, leading to large N losses to surface and

318 groundwater (Soana et al., 2011; Hou et al., 2015; Özbek et al., 2015; Viaroli et al., 2018; Häußermann et al., 2020).
319 In the Po River plain, to our knowledge, only a few sub-basins (e.g., Ticino and Po di Volano) represent exceptions to
320 this rule due to very limited animal farming and synthetic fertilizers inputs balanced with crop needs (Racchetti et al.,
321 2019; Soana et al., 2021). All those studies were carried out on an annual temporal scale, that potentially masked
322 marked seasonal differences. Our calculations suggest a clear seasonal variation in N soil budgets, changing along
323 seasons with periods with deficit (summer), equilibrium (autumn), moderate (winter) or large (spring) excess. These
324 differences arise from variable seasonal balance among agricultural practices, such as large spring manure and
325 synthetic fertilizer spreading uncoupled to crop uptake, moderate spread of fertilizers during winter with little to no
326 uptake or large summer crop uptake in excess to N inputs (Chen et al., 2019). The N assimilation term is calculated as
327 the product of the standing stock by the biomass-specific uptake rates and peaks in summer. Indeed, the biomass-
328 specific uptake tends to decrease along with the crop's growth, but the crop's standing stock is much smaller in spring
329 than in summer. Large spring N inputs are therefore coupled to relatively low uptake, resulting in a maximum surplus,
330 exceeding that calculated for winter, when uptake is minimum. Large summer uptake on the contrary exceeds inputs
331 and results in a seasonal deficit of N in the soil system budget. High fertilization is probably driven by the N need of
332 the main cultivated crop, i.e., maize, which is a water and N-demanding species (FAO, 2006). These results on intra-
333 annual variations in N mass budgets support the relevance of seasonal studies in highlighting critical moments in terms
334 of potential water pollution (Lin et al., 2019; Compton et al., 2020). As nitrate water pollution is correlated with N
335 excess in soils, our outcomes indicate a maximum nitrate pollution risk in the spring, a minimum risk in the summer
336 and something intermediate in winter and autumn. Results from seasonal river N transport suggest something different
337 as the highest N accumulation along the stretch was measured in summer and the lowest in spring. Taken together,
338 these apparently contrasting results indicate that more factors are involved in the horizontal transfer of soil N excess
339 to surface water and that such factors determine a temporal lag. In the perspective of efficient N use at the soil level,
340 our results stress that the spring is a critical season that requires a thorough re-thinking of practices by better balancing
341 crops needs with fertilizers inputs. During spring, organic and synthetic N inputs need to be better balanced with N
342 uptake, as the crops have high potential growth but low biomass, which results in an insufficient uptake of the large N
343 inputs (Robertson and Vitousek, 2009).

344

345 **5.2. The seasonality of inorganic N loads in the Mincio River**

346 Results from this work add to a few seasonal studies coupling land mass budgets of N and net river N export-retention
 347 (e.g., Chen et al., 2019; Lin et al., 2019; Compton et al., 2020). For the portion of the Mincio River considered in this
 348 study, Fig 6 reports the monthly water inputs, either from precipitation or irrigation, the seasonal soil system N budget
 349 and the nitrate accumulation between S1 and S2, which is the difference of the nitrate loads transported past the two
 350 river sections.
 351



352
 353 Figure 6. Histograms report monthly water input due to precipitation and irrigation in the municipalities of the Mincio
 354 basin under study. Green dots show the delta nitrate loads of the river reach (delta NO₃-N river load) in the five
 355 sampling dates and red squares show seasonal N budget of agricultural soils (SSB – Soil system budget –
 356 surplus/deficit).
 357
 358 In the regulated river reach under study, which is characterized by gravel bottom and colonized by submerged
 359 macrophytes, ammonium and nitrite loads were higher upstream and suggested net retention in all seasons, peaking in
 360 summer when the primary producers' activity is maximum. Differently, dissolved inorganic carbon and nitrate loads
 361 evidenced a significant increase from upstream to downstream in all the investigated seasons. Nitrate and inorganic

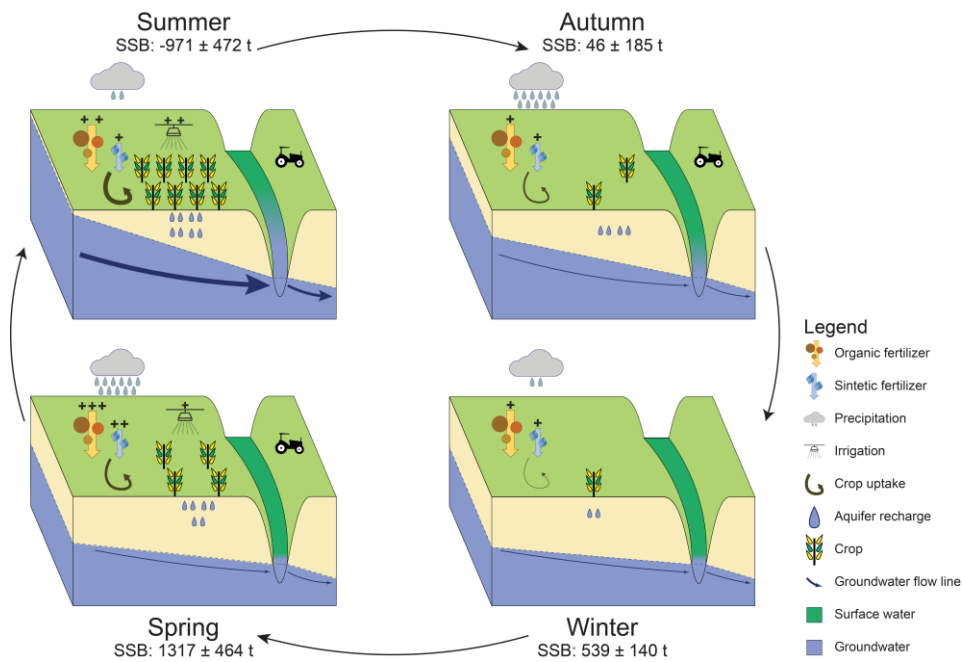
362 carbon net export decreased from August to April, with August as the central month for irrigation and April the last
363 month before the start of the irrigation period. From these results, we calculated the annual net export of inorganic
364 carbon and nitrate multiplying the daily values by the number of days between consecutive samplings, and then
365 integrating the results over one year. Despite 6-8 repeated water samplings during the 24 hours, these calculations are
366 based on measurements carried out in single days along different seasons. However, the nitrate concentrations
367 measured in this study are consistent with the seasonal NO₃-N concentrations measured by ARPA Lombardy (the
368 authority that manages water monitoring for the WFD) in the period 2009-2017 and by the Laboratory of Aquatic
369 Ecology of the University of Parma in the period 2011-2017 (Fig. C.1 in Supplementary material C).

370 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
371 S1 and S2. These amounts can be explained by element transformation (e.g., respiration or nitrification), and lateral or
372 vertical inputs. As the loads of the other two inorganic N forms were negligible as compared to nitrate, this calculation
373 was done only for the latter. Using dissolved oxygen budgets (not reported in this work) we converted dark river
374 respiration rates (night oxygen uptake along this stretch; from -1.3 to -5.7 mm O₂ m⁻² h⁻¹ in winter and summer,
375 respectively) into potential nitrification rates (~75 t N y⁻¹) according to nitrification stoichiometry. To this purpose, we
376 assumed that 100% of the oxygen consumed was used to oxidize ammonium to nitrate. Results suggest that microbes-
377 mediated processes as nitrification can explain at most 23% of the nitrate accumulation in the river reach in all seasons.
378 Such percentage is a large overestimation of the real value as it was obtained neglecting all other oxygen-consuming
379 processes, including macrophytes, fish, macroinvertebrates, and the whole heterotrophic microbial community
380 respiration. A comparable nitrate production (~80 t N y⁻¹) was obtained using the nitrification rate set by
381 Taherisoudejani et al. (2018) in the QUAL2Kw model applied to the Oglio River, a nearby Po River tributary with
382 similar hydrological characteristics. Pinaridi et al. (2014) found that the processes in the hyporheic zone or the microbial
383 metabolism of carbonate dissolution could explain up to 15% of the DIC increase, including the role of macrophytes
384 in modulating dissolved CO₂ saturation values and fixation of C.

385 If biological processes cannot explain inorganic carbon and nitrate increase, also point pollution sources can be
386 excluded, due to low discharge of small tributaries along this stretch with water chemistry comparable to that of the
387 Mincio River. Another potential source of N and DIC is groundwater, via seasonally variable river-groundwater
388 interactions. Indeed, the level of the phreatic surface increases and interacts with the river due to precipitation, flooding
389 and sprinkler irrigation during the spring-summer period (Racchetti et al., 2019; Severini et al., 2020) (Fig. 7). In a

390 period before and after fertilization (from March to May 2021), Severini et al. (2022) measured in the same area of the
 391 Mincio River significantly higher HCO_3^- concentrations in groundwater than in surface water (Appelo and Postma,
 392 2005). Hence, the DIC increase in the investigated river stretch can be associated to the groundwater feeding the Mincio
 393 River. Considering the abundant use of organic fertilizers in the area, the higher DIC in groundwater can be related to
 394 the presence of calcite (CaCO_3) in the mineralogical composition of the aquifer and to the oxidation of the organic
 395 matter, which promotes a higher DIC concentration in groundwater. Future investigations should include the
 396 mineralogical composition of the aquifer.

397



398

399

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

402

403 5.3. Linking soil N budgets and N riverine export: the key role of the aquifer

404 The main period of N fertilization is in spring when there is the highest N soil surplus and surface and groundwater

405 pollution risk due to limited crop uptake. The crop harvest occurs mainly in summer and autumn when the N soil
406 budget is in deficit or close to equilibrium, respectively. These data are reflected by an increasing N export by the river
407 reach from spring to summer, favored by large volumes of nitrate-enriched water displaced through the irrigation
408 across the aquifer-river continuum (Isidoro et al., 2006). Using SiO₂ as tracer, Severini et al. (2022) typified the
409 investigated river stretch as a flow-through system, where groundwater feeds the Mincio River in its west bank and it
410 is fed from the river's east bank (Fig. 7). As a result, N-rich groundwater can displace N-poor water from the Mincio
411 River without a significant modification of the river flow (Fig. 7). Our data are consistent with this hydrogeological
412 conceptual model, since the higher NO₃-N delta loads were found in summer, when the groundwater level are the
413 highest and there is the maximum groundwater seeping to the Mincio River, highlighting the deep effects of the
414 recharge given by irrigation. On the contrary, some differences were found during the rest of the year, characterized
415 by a less anthropic recharge of the aquifer. These differences are more related to the dissimilar distribution of
416 precipitation and percolation of water and N to groundwater, which fosters the migration of N to the Mincio River. In
417 fact, as we move away from the end of the irrigation period (September), the lower groundwater heads reported in
418 Severini et al. (2022) could result in a lower groundwater seepage to the river (Fig. 7). Having less nutrient enriched
419 water available guarantees a minor nitrate surplus in the river reach, even if N fertilization starts again in winter,
420 resulting in another period with N soil surplus (Figs. 6, 7).

421 Considering that the mean annual N surplus on the agricultural land in the study area averaged 67 kg ha⁻¹ y⁻¹, it is
422 possible to speculate that the agricultural land surface that can potentially generate the NO₃-N river export (317±12 t
423 NO₃-N y⁻¹) is equivalent to ~4700 ha⁻¹. In addition, dividing this surface by the length of the river reach investigated,
424 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
425 surplus production. These data allow to speculate that the N surplus was generated in the 25% of the surface of
426 municipalities under study, giving useful information to better address arable land management.

427 Our results on N input and output trends in agricultural soils and into the river reach at annual and seasonal basis allow
428 to better understand N patterns from land to river and the potential nitrate pollution to surface and groundwater. This
429 information at seasonal resolution can help policy-makers in developing effective plans to improve N management at
430 the macroscale. In fact, this combination of information can guide the identification of proper spatial-temporal
431 management strategies to reduce N pollution and river export to avoid eutrophication processes of water bodies. For
432 example, our results suggest that more nitrate was delivered downstream in summer because of spring soil N excess

433 coupled to flood irrigation over permeable soils. Hence, it is important to focus on agricultural sources (manure and
434 synthetic fertilizers in particular) to better balance N inputs and output by crop harvest (or stock). Our approach was
435 applied in a pilot study at the sub basin level, but it is exportable to the whole basin and to other rivers. It becomes
436 very important to have local information and basin-specific data to perform seasonal analysis on N patterns (Lassaletta
437 et al., 2021).

438

439 **5.4. Possible remediation strategies in the context of climate change and the regulation of river discharge**

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

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

457 In the geographical area of our case study, the Alps host a series of large lakes regulated by dams that feed rivers
458 among which the Mincio River. The dams regulate the lake water level and the river discharge with rules that aims to
459 accumulate water in winter and release it during the irrigation period, adapting also to local meteorology and water
460 inputs to lakes. This regulation practice guarantees sufficient summer level in lakes for tourism and navigation purposes

461 and large water availability for irrigation and electricity production in the downstream river section. Irrigation is
462 supported by several water abstraction infrastructures that facilitated agriculture and animal farming activities.
463 Irrigation practices, supported mainly by flooding irrigation in the sector of the Po River plain including our study area
464 are carried out over permeable soils, and favor the recharge of the aquifer mainly in summer (Rotiroti et al., 2019).
465 Therefore, water retention upstream during non-irrigation periods and flood irrigation with large water volumes during
466 summer are probably the main drivers of the groundwater head variation, which is subject to strong seasonal
467 differences (Taherisoudejani et al., 2018). Under the current climate change scenario, also in this geographical area,
468 the rapid changes of global warming are manifested with lower precipitation, dry winters, heatwaves and storms events,
469 and an increasing number of consecutive days with high temperatures (Cifrodelli et al., 2015; Pedro-Monzonís et al.,
470 2016; Lassaletta et al., 2021; Ranasinghe et al., 2021). The response to these global trends could increase or decrease
471 river nitrate concentration depending on regional or site-specific linkage between N concentration and discharge
472 (Stelzer et al., 2020). For this reason, the geographical sector under study seems extremely vulnerable to climate change
473 as the system is depicted and managed for large water availability (i.e., large lakes regulation, high water demanding
474 crops, and flooding as main irrigation practice) and therefore a discussion on water management at political level is
475 urgent. Predicting scenarios on the fate of the N excess with different water availability is difficult, we can hypothesize
476 a reduction of water discharge from rivers and consequently from irrigation that will not recharge sufficiently the
477 groundwater due to its deep level (Taherisoudejani et al., 2018). This condition will lead to more thick vadose zones,
478 fostering a higher nitrification rate and nitrate accumulation in soil during winter, whereas denitrification, the main
479 process that removes N permanently from the system, is favored in water saturated soils with high organic matter
480 (Ascott et al., 2017). In soil and rivers close to N saturation, it is expected a lower nitrate retention efficiency and
481 therefore an increase in N availability and vertical and horizontal transfer (Stelzer et al., 2020). Moreover, for the future
482 it can be expected a delay in the river feeding by groundwater with hot-moments of N mass transfer. In fact, we can
483 expect that with the increment of unsaturated zone, the rate of soil denitrification will be reduced and conversely the
484 nitrification process will be favored supplying a short N mass-transfer as soon as the first rainfall or flooding irrigation
485 will occur, carrying water with high N concentration to surface or groundwater. A possible solution to limit this hot-
486 moment can be the improvement of irrigation practices with less water consumption and a more widespread use of
487 precision farming supported for example with remote sensing technique (Nutini et al., 2021). Such a new vision on the
488 irrigation practices can allow a lower winter water retention in the Lake Garda, that can be partially used in the non-

489 irrigation period to guarantee a minimal vital flow in a certain number of drainage canals as well as in the Mincio River
490 favoring denitrification process also in autumn and winter, although with lower rates driven by lower temperatures.

491

492 **6. Conclusions**

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

506

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514

515 **Declarations**

516 **Ethical Approval:** Not applicable.

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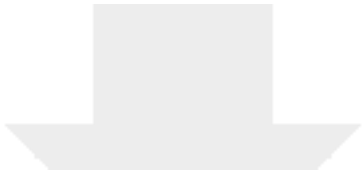
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Agricultural practices regulate the seasonality of groundwater-river nitrogen exchanges

Monica Pinardi^{1*}, Elisa Soana², Edoardo Severini³, Erica Racchetti³, Fulvio Celico³, Marco Bartoli^{3,4}

¹ Institute for Electromagnetic Sensing of the Environment, National Research Council, Via Corti 12, 20133 Milan, Italy

² Department of Life Sciences and Biotechnology, University of Ferrara, Via Borsari 46, 44121 Ferrara, Italy

³ Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy

⁴ Marine Science and Technology Center, Klaipeda University, Klaipeda, 92294, Lithuania

* Corresponding author

Address: Via A. Corti 12, 20133 Milan, Italy

Tel: +39 0223699298

Email: pinardi.m@irea.cnr.it

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1 **Abstract**

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

20
21 **Keywords:** nitrogen; retention; transport; loads; irrigation; river-groundwater interaction

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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

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4 51 river-groundwater interactions (i.e. the river feeds the groundwater) (Rotiroti et al., 2019; Severini et al., 2021). On
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6 52 the contrary summer irrigation, besides representing a vehicle for N transport, produces opposite effects, often
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8 53 reversing the direction of river-groundwater interactions (i.e. upwelling, the groundwater feeds the river). These
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10 54 practices, that characterize anthropogenic, intensively cultivated, and hydraulically regulated watersheds with
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12 55 permeable soil, introduce marked seasonality in N budgets (Lin et al., 2019; Racchetti et al., 2019).
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14 56 Many authors reported a significant correlation between annual N input to croplands and river N export (Neff et al.,
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16 57 2003; Yan et al., 2010; Xu et al., 2013; Stokal et al., 2014; Tong et al., 2017), but they did rarely account for the
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18 58 seasonality of N input and export (McCrackin et al., 2014; Chen et al., 2019). Studies targeting N budgets in
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20 59 agricultural watersheds are generally conceived at the annual scale for mainly practical reasons, as agricultural census
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22 60 data are collected and published by national statistical institutions with annual frequency. Such an approach from one
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24 61 side allows to calculate N use efficiency in cropland and potential N loss, but from the other side, it misses temporal
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26 62 resolution and precludes the understanding of seasonal variations of the array of N-related processes, potentially
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28 63 regulated also by climate change. For example, human activities (e.g., crop production) and altered hydrology may
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30 64 influence the seasonality of N river export (Basu et al., 2010; Compton et al., 2020), together with the seasonal
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32 65 evolution of temperature that influences N losses, retention and removal processes (e.g., denitrification) (McCrackin
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34 66 et al., 2014). Understanding how seasonal variations in human activities and hydrology influence N budgets in
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36 67 agricultural soils and N transport by rivers is important to better understand the mechanisms underlying N
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38 68 transformations along the terrestrial-aquatic path, improve agricultural practices to increase N use efficiency and
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40 69 decrease N pollution, and eventually forecast how climate change will affect N dynamics (Mas-Pla and Menció, 2019).
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42 70 This important set of objectives is a difficult target at the scale of whole watersheds due to scarce resolution of available
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44 71 data and spatial heterogeneity (e.g. pedology, land use, etc). Smaller scales of analysis, targeting specific and
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46 72 homogeneous river and watershed sectors, seem much more promising (McCrackin et al., 2014; Chen et al., 2019;
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48 73 Compton et al., 2020).
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50 74 Different studies carried out at large temporal and spatial scales (Soana et al., 2011; Pinardi et al., 2018; Viaroli et al.,
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52 75 2018; Lassaletta et al., 2021) have highlighted the presence of hot-spots within watersheds that represent outliers in N
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54 76 budgets (e.g., with very large N excess or very low N use efficiency). They also emphasized the presence of hot-
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56 77 moments within watersheds, that are specific periods during which N mass transfer peaks as a combination of
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58 78 decreased uptake, increased runoff or variation of the water table level, resulting in the reactivation of river-

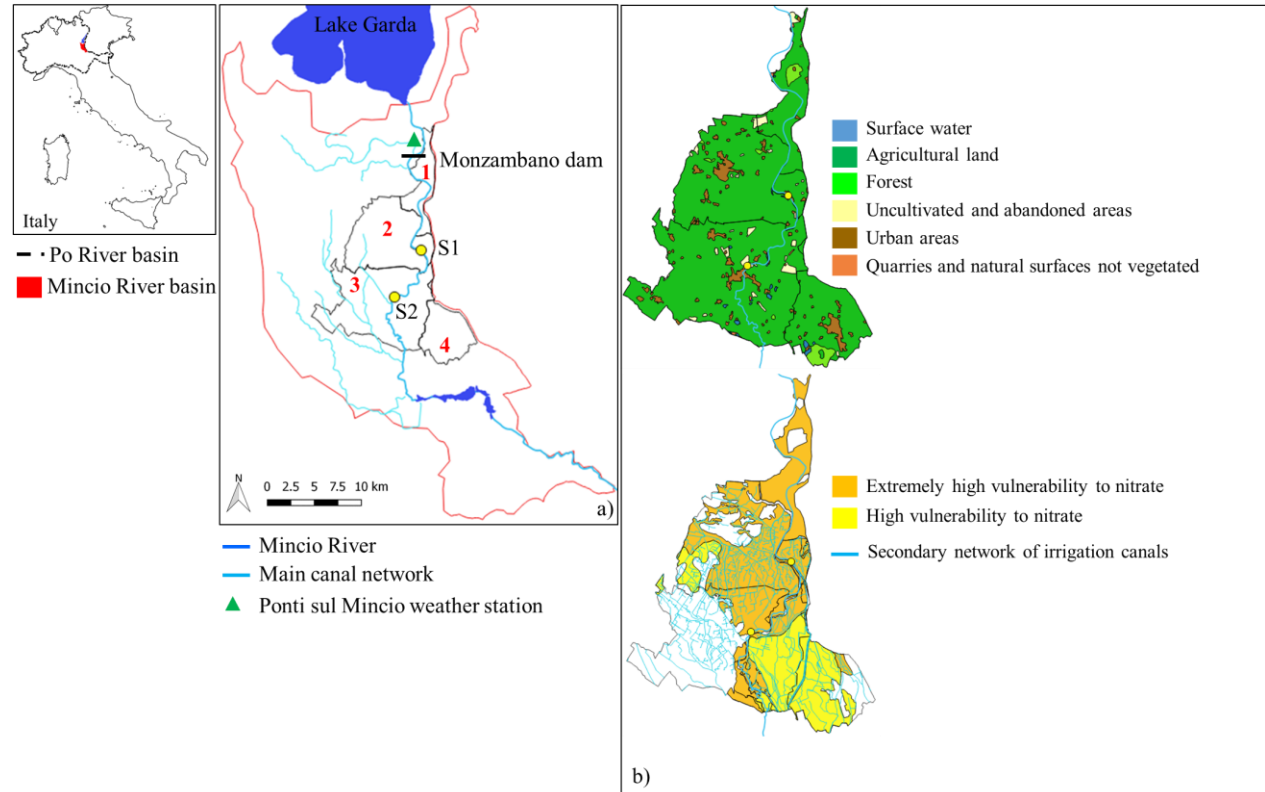
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4 79 groundwater interaction (Rosenzweig et al., 2008; Preisendanz et al., 2020; Taherisoudejani et al., 2018). The analysis
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6 80 of N hot-spots and hot-moments in watersheds require specific studies, focusing on small spatial and temporal scales.
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8 81 In Northern Italy, the Po River valley is an alluvial plain heavily exploited by human activities such as agriculture,
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10 82 animal farming, industry, and tourism. Land use change and hydrological alterations determined high pressure on both
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12 83 surface and groundwater (May, 2013; Pérez-Martín et al., 2014; Lasagna and De Luca, 2019) and a wide portion of
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14 84 the plain is classified as vulnerable to nitrate pollution (Martinelli et al., 2018). The main aim of this study is to analyze
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16 85 the seasonal evolution of dissolved inorganic N loads in a fluvial segment of the Mincio River, a tributary of the Po
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18 86 River, characterized by natural banks, gravel bottom with submerged vegetation, and regulated discharge. This
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20 87 segment crosses a transitional area between permeable and non-permeable soils, characterized by springs and classified
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22 88 as an area of river-groundwater interactions (Balestrini et al., 2021). Due to its hydrogeological features and the large
23
24 89 water availability, the considered sub basin is a hotspot of intensive agriculture and animal farming and represents a
25
26 90 key study area to analyze if and how the seasonality of agricultural practices affects N dynamics.
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28 91 In this sector of the Po River, groundwater in the phreatic and shallow aquifer has a short residence time as compared
29
30 92 to semiconfined or confined deeper aquifers. This is supported by fast (few days) surface-groundwater dynamics of
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32 93 micro-pollutants (Balderacchi et al., 2016) and low concentrations of total dissolved solids (Martinelli et al., 2018).
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34 94 Results of Balderacchi et al. (2016) suggest also fast response of shallow aquifers to changing conditions; as such they
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36 95 allow to trace agricultural practices (e.g., use of herbicides or fertilization) and they respond quickly to hydrologic
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38 96 variations (e.g., drought, precipitations, irrigation). It can be assumed that macrocontaminants as nitrates undergo the
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40 97 same fast transfer mechanisms, also due to their elevated solubility and absence of interaction with soil and sediment.
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42 98 The main hypotheses of this work are that river-groundwater interactions affect N transport in specific river sectors
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44 99 and vary seasonally due to combination of irrigation practices and inorganic nitrogen excess in soil. We also
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46 100 hypothesized that the seasonal dynamics of such variable interactions can be captured analyzing comparatively
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48 101 seasonal N budget in agricultural soils and seasonal riverine N transport. In this context, the main aim of the present
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50 102 study was to contrast seasonal N soil budget in an agricultural area drained by a river stretch with seasonal N loads
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52 103 transported by the same draining river stretch, to assess riverine N dynamics in relation to agricultural practices.
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57 105 **2. Study area**

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4 106 The Mincio River (~75 km) originates from the Lake Garda, the largest Italian Lake, and is a tributary of the Po River
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6 107 (Fig. 1). The hydrological regime of the Mincio River is regulated upstream by a dam, which controls the water discharge
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8 108 from the Lake Garda. Along the river course, a series of dams and weirs feeds a network of canals for irrigation and
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10 109 industrial purposes and controls discharge variations to avoid the flooding of cities and villages. Water management for
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12 110 Lake Garda recreational activities and for agricultural purposes results in marked flow variations. Indeed, since the
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14 111 establishment of the river regulation in the 60's of the last century, the Mincio River discharge averaged ~80 and ~30 m³
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16 112 s⁻¹ during the irrigation (May to September) and outside the irrigation periods, respectively (Lombardy Region, 2006).
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18 113 More recently, projections of decreasing water availability resulted in a further reduction of the Mincio River discharge
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20 114 to ~14 m³ s⁻¹ (www.laghi.net) in autumn and winter to keep water in the Lake Garda and guarantee water availability for
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22 115 irrigation and tourism in the summer season. During winter, the flow reduction and absence of irrigation result in a
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24 116 decreased aquifer recharge, a phenomenon described by different authors in this geographical area at regional (Rotiroti et
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26 117 al., 2019) and local scales (Severini et al., 2021) and, consequently, in a lowering of the phreatic surface. This water
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28 118 transfer dynamic results in a decrease of groundwater upwelling in winter and early spring (Balderacchi et al., 2016).
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31 119 A wide segment of the Mincio River, including the portion investigated in this study, flows in a flood plain
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33 120 characterized by a multilayered aquifer system with a cyclic facies architecture mainly made of fluvial-channel (gravel
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35 121 and sand) and floodplain (clay) deposits (Amorosi et al., 2008). As a result, the northern part of the plain (high plain)
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37 122 is locally characterized by shallow phreatic aquifers, while in the southern part (low plain) the floodplain facies act as
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39 123 aquitards or aquicludes, resulting in confined and semi-confined aquifers (Chelli et al., 2018). The river reach
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41 124 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
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43 125 plain of the Mincio watershed. This river segment drains the surface of four municipalities (1- Valeggio sul Mincio, 2-
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45 126 Volta Mantovana, 3- Goito, and 4- Marmirolo; total area 184 km²) (Fig. 1). Since 2006, these municipalities are classified
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47 127 as Nitrate Vulnerable Zones (NVZs) according to the European Nitrate Directive (91/676/CEE). The area of the four
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49 128 municipalities investigated in mainly classified as agricultural land with fertile soils due to calcareous gravel deposits
50
51 129 with favor intensively exploitation by agriculture (Utilized Agricultural Area - UAA covers 76% of the study area; Fig.
52
53 130 1) and animal farming (1.2 and 0.6 t of live weight per hectare for cattle and pigs, respectively). The urban, infrastructural
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55 131 and industrial areas cover only 5% of the total surface (Fig. 1). The S1-S2 river segment flows into natural banks, has a
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57 132 mainly gravel bottom, and has transparent waters. The main primary producers are submerged vegetation (e.g., *Vallisneria*
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59 133 *spiralis*) with associated epiphytes, benthic biofilms and different emergent macrophytes growing along the river banks
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4 134 or forming islands (Pinardi et al., 2009, 2014). The linear development of irrigation canals in S1-S2 river reach sub basin
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6 135 sums ~560 km (Fig. 1). The surface covered by the other aquatic environments, such as quarry lakes is ~0.62 km².



38 138 Figure 1. Maps of the study area: the Mincio River segment from Pozzolo dam (S1) to Goito village (S2) (yellow
39 points = water sampling stations). a) Municipalities where the nitrogen mass budget was performed are reported
40 139 (1 - Valeggio sul Mincio, 2 - Volta Mantovana, 3 – Goito, 4 - Marmirolo). b) Land use and maps of soil
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42 140 vulnerability to nitrate are reported for the four municipalities under study.
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48 143 3. Material and methods

49 144 3.1. Nitrogen budgets and water inputs

50 145 A comprehensive input–output N budget across the Utilized Agricultural Area (UAA) was compiled by using locally-
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52 146 derived data on farming activity, agronomic coefficients, and atmospheric deposition. One of the elements of strength
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54 147 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
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56 148 accurate set of species-specific coefficients taken from the literature in the field. Nitrogen budget was first calculated
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4 149 at the municipal scale, i.e., the administrative level at which official agricultural statistics are available, then weighted
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6 150 for the percentage of each municipality surface included within the study area, and finally summed up. Census data
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8 151 were integrated in a nutrient budgeting approach proposed by Oenema et al. (2003), recently reviewed by Zhang et al.
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10 152 (2020), and formerly applied to the whole Mincio River basin (Pinardi et al., 2018). Four inputs of N to the UAA were
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12 153 considered (land application of livestock manure, land application of synthetic fertilizers, atmospheric deposition, and
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14 154 biological fixation by crops), together with four outputs of N from UAA (crop harvest, crop stock, ammonia
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16 155 volatilization and denitrification in soils). The difference between N inputs and outputs results in a net, which
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18 156 represents a condition of equilibrium, surplus or deficit of N across the UAA.
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22 158 The Soil System Budget (SSB) was calculated as follow:

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

26 160 where:

28 161 N_{Man} = N in livestock manure applied to agricultural soils

30 162 N_{Fert} = N in synthetic fertilizer applied to agricultural soils

32 163 N_{Fix} = agricultural N_2 fixation associated with N fixing crops

34 164 N_{Dep} = atmospheric N deposition on agricultural land

36 165 N_{Harv} = N exported from agricultural soils with crop harvest

38 166 N_{stock} = organic N in crop's standing stock

40 167 N_{Vol} = NH_3 volatilization in agricultural soils

42 168 N_{Den} = denitrification in agricultural soils

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46 170 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
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49 171 UAA ($kg\ N\ ha^{-1}\ y^{-1}$). The soil N budget was estimated by employing agricultural census data referring to the
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51 172 agricultural year 2015-2016. The agricultural year overlaps the vegetative cropping cycle, covering two consecutive
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53 173 years, i.e., from November of the first year to October of the following one. The N budget calculated for the agricultural
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55 174 year 2015-2016 is relevant in the present day because, in the last decade, only minor variations occurred in crop
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57 175 surfaces and livestock densities of the study area and fertilization rates did not change appreciably.

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59 176 The calculation was based on agriculture and farming data reported by the Agricultural Information System of

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4 177 Lombardy Region (SIARL, www.siarl.regione.lombardia.it) and by the Annals of Agrarian Statistics, published yearly
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6 178 by the National Institute of Statistics (ISTAT, <http://agri.istat.it/>). SIARL databases, retrieved from the Open Data
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8 179 portal of the Lombardy Region (<https://dati.lombardia.it/>), provided data for livestock density and agricultural areas at
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10 180 the municipality level, whereas the database of the Annals of Agrarian Statistics provided data for crop yield and
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12 181 fertilizer application (<http://dati.istat.it/>) at the provincial level. Inputs and outputs were initially calculated for each
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14 182 municipality and then aggregated at the study area level.

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16 183 Uncertainty in N budget calculations was assessed by a Monte Carlo analysis using Excel and R software (R Core
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18 184 Team 2019). All coefficients used to convert census data into N amounts were assumed to vary stochastically and
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20 185 independently around the average value with a normal probability distribution. For each simulation, a set of coefficients
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22 186 was randomly generated from probability distribution functions and a total of 1000 simulations were run. Budget
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24 187 calculation was conducted both at the annual and at the seasonal scales and compared with seasonal in-stream N loads.
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26 188 Details about annual budget data, equations, seasonal breakdown, and sources of census data and agronomic
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28 189 coefficients are presented in Supplementary Material A.

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30 190 The N loads produced by the urban areas were not included in the calculation because more than 95% of the sewers in
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32 191 the study area are connected to wastewater treatment plants (WWTP). Nearly 75% of the N inputs to WWTP is
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34 192 removed via denitrification in tertiary treatment (Lombardy Region, 2017). Indeed, the calculation of the urban load
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36 193 produced by the resident population, obtained by the conversion of equivalent inhabitant in kg of N per day, resulted
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38 194 in less than 2% of the total N input by diffuse sources (Pinaridi et al., 2018).

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40 195 The daily precipitation data were downloaded from the ARPA Lombardy website
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42 196 (<https://www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx>) at Ponti sul Mincio station (Fig. 1)
43
44 197 for the period from 2010 to 2017. The mean annual, seasonal (irrigation and non-irrigation period) and monthly
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46 198 precipitation data were calculated. Irrigation data at the municipality level was obtained from the 6th Agricultural
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48 199 Census (National Institute of Statistics, 2010, <http://dati-censimentoagricoltura.istat.it>) and then aggregated at the study
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50 200 area level.

51 201 52 53 202 **3.2. Water sampling and analyses**

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55 203 Two stations located at the extremes of the identified river reach (S1 and S2; Fig. 1) were sampled for water analyses.
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57 204 The two stations were selected as they were located upstream and downstream the area where the Mincio River can be
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4 205 considered as a gaining river in groundwater-surface water interaction, that is the river is fed by groundwater (Racchetti
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6 206 et al., 2019). Given the constant discharge between S1 and S2, the identified river reach was more recently
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8 207 characterized as a flow-through reach (Severini et. al., 2022), with groundwater feeding the river in the western bank
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10 208 and being fed by the river in the eastern bank. Field campaigns were carried out seasonally with a series of daily cycles
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12 209 of repeated samplings carried out on 12-13 August and 15-16 November 2016, 14-15 February, 12-13 April and 13-
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14 210 14 June 2017. Water samples were taken in three replicates every 4 hours for a 24-hour period. An aliquot was
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16 211 transferred into a 12 mL exetainer (Labco, UK), added with 100 μL of HgCl_2 , and analyzed for dissolved inorganic
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18 212 carbon (DIC) with Gran titration (0.1 N HCl) within 24 hours from sampling. DIC was measured as it may trace
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20 213 differences between surface and groundwater chemistry. Water aliquots were filtered (GF/F glass fiber filters) and
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22 214 transferred to plastic vials for nitrate ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), and ammonium ($\text{NH}_4\text{-N}$) determination by
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24 215 spectrophotometric methods (Rodier, 1978; APHA, AWWA, WPCF, 1999). There are two main reasons for focusing
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26 216 on inorganic nitrogen. The first is that from our database and from those of national monitoring agencies in the Mincio
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28 217 river nearly 80% of the total N load is made of inorganic N (DIN, and within the DIN pool >90% is nitrate). The
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30 218 second is that from the same dataset nitrate represents more than 95% of the total dissolved N in groundwater.
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32 219 Hourly or daily water flow data were obtained by the Interregional Agency for the Po River (AIPO), and by the Mincio
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34 220 Consortium for Pozzolo and Goito sites.
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36 221 The Mann-Whitney Rank Sum Test was used to test the difference between upstream and downstream values of water
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38 222 flow, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$ and DIC concentrations. The R software package (R Development Core Team, 2019)
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40 223 was used to perform all statistical tests.
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44 225 *3.3. Dissolved inorganic nitrogen and carbon daily loads*

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46 226 For each sampling date, daily $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$ and DIC riverine loads transported at S1 and S2 (kg d^{-1}) were
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48 227 calculated multiplying concentrations by river discharge. The difference between loads at S2 and S1 was calculated
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51 228 according to the following equation:

$$52 \quad \Delta\text{NO}_3\text{-N (or NO}_2\text{-N, NH}_4\text{-N, DIC)} = \sum [\text{Ct} \times \Delta t \times \text{Q}]_{\text{S2}} - \sum [\text{Ct} \times \Delta t \times \text{Q}]_{\text{S1}} \quad (1)$$

53 229 where: Ct = concentration of $\text{NO}_3\text{-N}$ (or $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, DIC) at time t downstream (S2) or upstream (S1) (mg m^{-3});
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55 230 Δt = time interval between samplings (h); Q = water flow ($\text{m}^3 \text{h}^{-1}$). Such difference can be null, suggesting equilibrium
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57 231 between inputs and outputs, negative, suggesting net retention or dissipation (e.g., uptake or denitrification), or
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4 233 positive, suggesting the occurrence of production or additional inputs along the stretch (e.g., nitrification or point and
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6 234 diffuse inputs). A standard deviation was associated to NO₃-N, NO₂-N, NH₄-N and DIC measurements made in
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8 235 replicates. The errors conveyed through the mathematical description were calculated with classical error propagation
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10 236 equations.

14 238 **4. Results**

16 239 **4.1. Nitrogen budgets and water inputs**

18 240 In the four municipalities under study, the total N inputs to arable land (6010±498 t y⁻¹) were mainly due to livestock
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20 241 manure (59%). Nitrogen outputs (5078±494 t y⁻¹) accounted for 84% of the N inputs and were mainly due to crop
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22 242 harvest (50% of the total N outputs). The main cultivated crop is maize (63% of arable land) followed by permanent
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24 243 grassland. The difference between N inputs and outputs denoted a N soil surplus (932±702 t y⁻¹). The mean areal N
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26 244 surplus was 67±50 kg ha⁻¹ y⁻¹ with values calculated for the different municipalities ranging between -172 and 204 kg
27
28 245 ha⁻¹ y⁻¹. All these results are reported in Supplementary material B, Table B.1 and Figure B.1.

30 246 The seasonal SSB is reported in Figure 2. Manure N fertilization was higher in spring (1590±268 t) and summer
31
32 247 (883±149 t), and similar in autumn and winter (530±89 t). Synthetic N fertilization was higher in spring (769±190 t)
33
34 248 and winter (257±63 t). Biological N fixation was higher in summer (559±259 t) and spring (350±162 t) whereas
35
36 249 atmospheric N depositions were concentrated mainly in summer and autumn (32±4 and 38±4 t, respectively). In spring,
37
38 250 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
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40 251 N outputs, crop harvest was highest in summer (1492±301 t) and autumn (637±128 t). Ammonia volatilization and
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42 252 soil denitrification were quantitatively important in spring (324±233 t and 210±102 t, respectively). Coupling the
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44 253 seasonal input and output data, a transition from N deficit to N surplus is evident moving from summer (-971±472 t)
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46 254 to spring (1317±464 t).

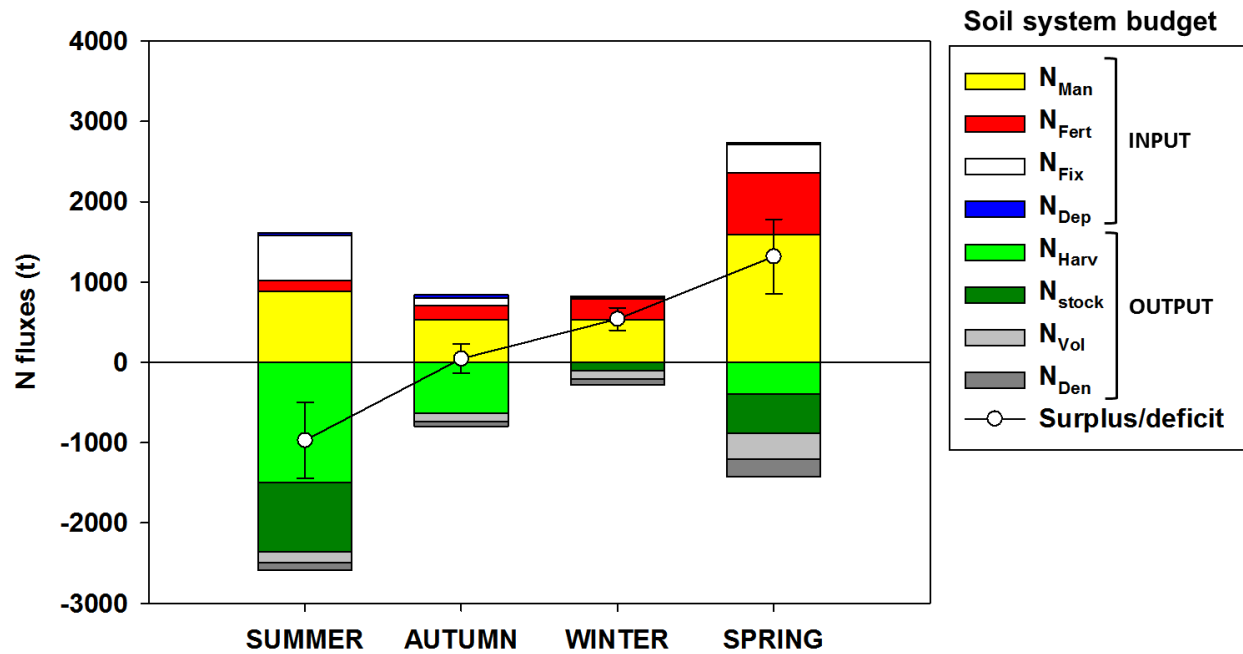
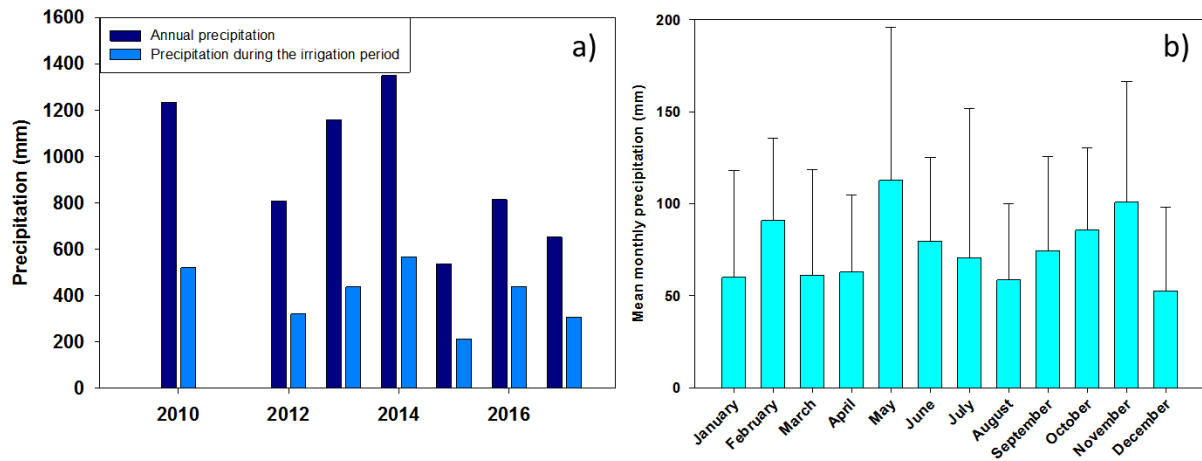


Figure 2. Components of the Soil System Budget (SSB) of nitrogen (N) for the study area within the Mincio River watershed for all seasons. N_{Man} = N in livestock manure applied to agricultural soils; N_{Fert} = N in synthetic fertilizer applied to agricultural soils; N_{Fix} = agricultural N_2 fixation 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 $43 \pm 6\%$ occurred during the irrigation period (Fig. 3).



267
268 Figure 3. Annual precipitation and the fraction of precipitation during the irrigation period (from May to September)
269 (a) and mean monthly precipitation (\pm standard deviation) (b) in the period from 2010 to 2017 at Ponti sul Mincio
270 meteorological station (see Fig. 1 for the localization) (2011 was not considered due to missing data for August and
271 September).

272
273 During the May-September period a water volume of $53.3 \times 10^6 \text{ m}^3$ was used to irrigate 13,513 ha of UAA, which
274 represent 75% of the total arable land. Flooding and sprinkler were the main irrigation typologies (72% and 27% of
275 the irrigated surface, respectively) (data from the National Institute of Statistics).

277 4.2. Water physico-chemical features of sampling sites

278 Nitrate and DIC concentrations were significantly higher at the downstream site for all sampling dates (Mann-Whitney
279 Rank Sum Test, $p < 0.001$, $n=68$ for each parameter; Fig. 4). On the contrary, the concentrations of the other dissolved
280 inorganic forms of nitrogen ($\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$) were significantly higher at the upstream site ($p < 0.001$, $n=68$ for
281 each parameter; Fig. 4). The highest $\text{NO}_3\text{-N}$ and DIC concentrations were measured in June 2017 at both sampling
282 sites (1.6 and $3.0 \text{ mg NO}_3\text{-N L}^{-1}$, and 33 and 41 mg DIC L^{-1} at S1 and S2, respectively). The highest values of $\text{NO}_2\text{-N}$
283 N were recorded in summer at S1 and S2 (up to 101 and $31 \text{ } \mu\text{g L}^{-1}$, respectively), whereas $\text{NH}_4\text{-N}$ concentrations
284 peaked in August 2016 at S1 (up to $122 \text{ } \mu\text{g L}^{-1}$) and were high at both sites in February 2017. Nitrate was always the
285 main form of inorganic nitrogen, accounting on average for 88% and 98% of the total N at S1 and S2, respectively.

286 The mean annual water flow was $10.3 \pm 2.5 \text{ m}^3 \text{ s}^{-1}$ 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 ($11.7 \pm 2.0 \text{ m}^3 \text{ s}^{-1}$ at S1 and $13.0 \pm 3.9 \text{ m}^3 \text{ s}^{-1}$ 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.

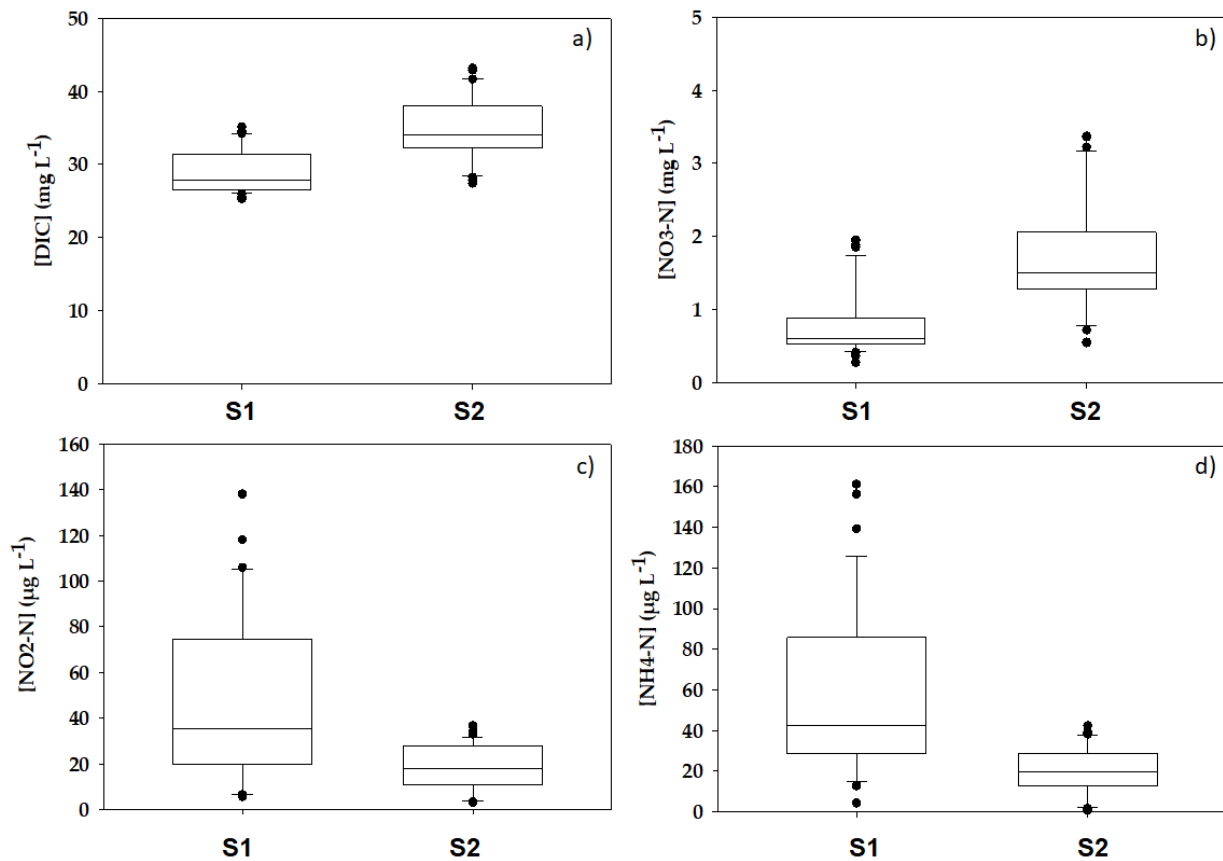
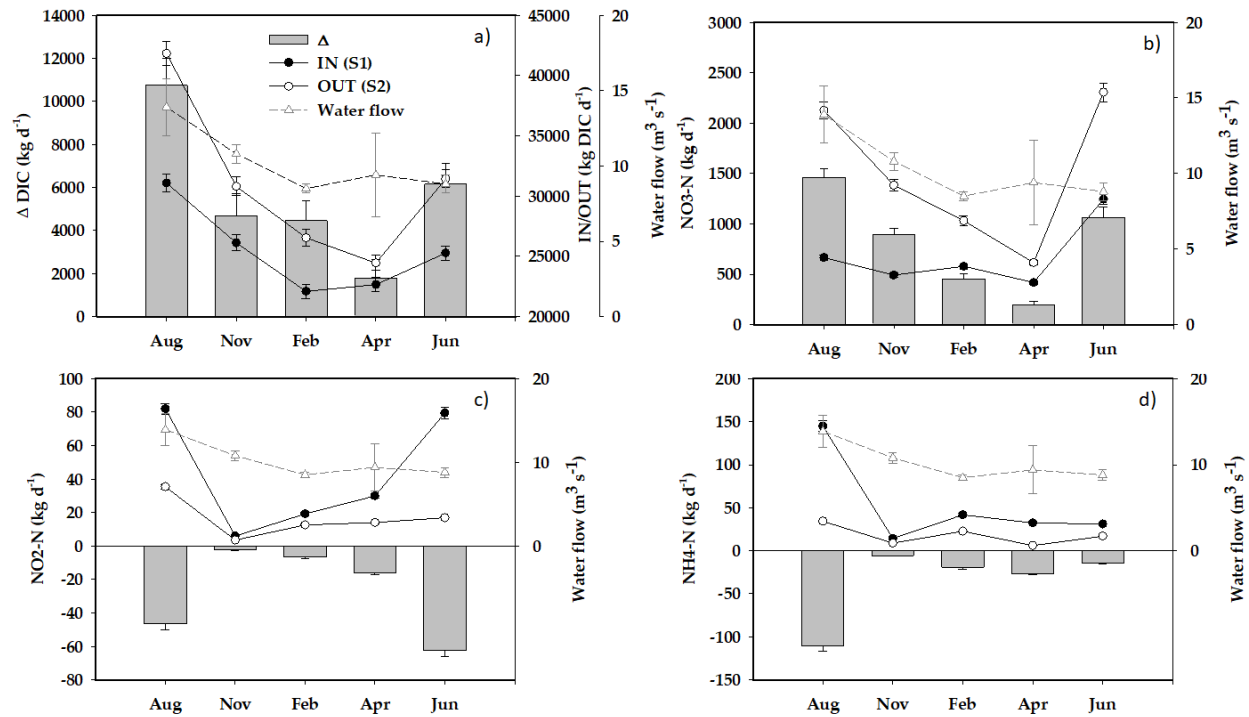


Figure 4. Box plot showing the concentrations of dissolved inorganic carbon (DIC; a), and of nitric (NO₃-N; b), nitrous (NO₂-N; c) and ammonium (NH₄-N; d) nitrogen measured seasonally from August 2016 to June 2017 at S1 and S2. Note different concentration units.

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

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4 300 origin for the two solutes. A similar seasonal trend was detected for DIC and NO₃-N accumulation along the analyzed
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6 301 river reach (Fig. 5). The maximum increase of transported loads (nearly 11,000 and 1500 kg d⁻¹ for DIC and NO₃-N,
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8 302 respectively) was measured in August 2016, whereas the minimum increase (nearly 2,000 and 200 kg d⁻¹ for DIC and
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10 303 NO₃-N, respectively) was measured in April 2017 (Fig. 5). The highest NO₂-N and NH₄-N loads reduction along the
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12 304 stretch were recorded during summer months (Fig. 5).
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43 307 Figure 5. Daily loads of inorganic carbon (DIC; a), nitric nitrogen (NO₃-N; b), nitrous nitrogen (NO₂-N; c), and
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45 308 ammonium nitrogen (NH₄-N; d) transported at the extremes of the studied river reach and their differences (Δ=S2-S1)
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47 309 in the period August 2016 to June 2017. Water flow is also reported. Mean values are given, with error bars
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49 310 corresponding to ±1 standard deviation.
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53 312 **5. Discussion**

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55 313 **5.1. The seasonality of soil N budgets**

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57 314 Previous studies carried out in the Po River basin and in other geographical areas characterized by intensive agriculture
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59 315 and animal farming suggest a generalized N surplus and inefficient N use, leading to large N losses to surface and
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4 316 groundwater (Soana et al., 2011; Hou et al., 2015; Özbek et al., 2015; Viaroli et al., 2018; Häußermann et al., 2020).
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6 317 In the Po River plain, to our knowledge, only a few sub-basins (e.g., Ticino and Po di Volano) represent exceptions to
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8 318 this rule due to very limited animal farming and synthetic fertilizers inputs balanced with crop needs (Racchetti et al.,
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10 319 2019; Soana et al., 2021). All those studies were carried out on an annual temporal scale, that potentially masked
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12 320 marked seasonal differences. Our calculations suggest a clear seasonal variation in N soil budgets, changing along
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14 321 seasons with periods with deficit (summer), equilibrium (autumn), moderate (winter) or large (spring) excess. These
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16 322 differences arise from variable seasonal balance among agricultural practices, such as large spring manure and
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18 323 synthetic fertilizer spreading uncoupled to crop uptake, moderate spread of fertilizers during winter with little to no
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20 324 uptake or large summer crop uptake in excess to N inputs (Chen et al., 2019). The N assimilation term is calculated as
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22 325 the product of the standing stock by the biomass-specific uptake rates and peaks in summer. Indeed, the biomass-
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24 326 specific uptake tends to decrease along with the crop's growth, but the crop's standing stock is much smaller in spring
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26 327 than in summer. Large spring N inputs are therefore coupled to relatively low uptake, resulting in a maximum surplus,
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28 328 exceeding that calculated for winter, when uptake is minimum. Large summer uptake on the contrary exceeds inputs
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30 329 and results in a seasonal deficit of N in the soil system budget. High fertilization is probably driven by the N need of
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32 330 the main cultivated crop, i.e., maize, which is a water and N-demanding species (FAO, 2006). These results on intra-
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34 331 annual variations in N mass budgets support the relevance of seasonal studies in highlighting critical moments in terms
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36 332 of potential water pollution (Lin et al., 2019; Compton et al., 2020). As nitrate water pollution is correlated with N
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38 333 excess in soils, our outcomes indicate a maximum nitrate pollution risk in the spring, a minimum risk in the summer
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40 334 and something intermediate in winter and autumn. Results from seasonal river N transport suggest something different
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42 335 as the highest N accumulation along the stretch was measured in summer and the lowest in spring. Taken together,
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44 336 these apparently contrasting results indicate that more factors are involved in the horizontal transfer of soil N excess
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46 337 to surface water and that such factors determine a temporal lag. In the perspective of efficient N use at the soil level,
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48 338 our results stress that the spring is a critical season that requires a thorough re-thinking of practices by better balancing
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50 339 crops needs with fertilizers inputs. During spring, organic and synthetic N inputs need to be better balanced with N
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52 340 uptake, as the crops have high potential growth but low biomass, which results in an insufficient uptake of the large N
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54 341 inputs (Robertson and Vitousek, 2009).
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59 343 **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.

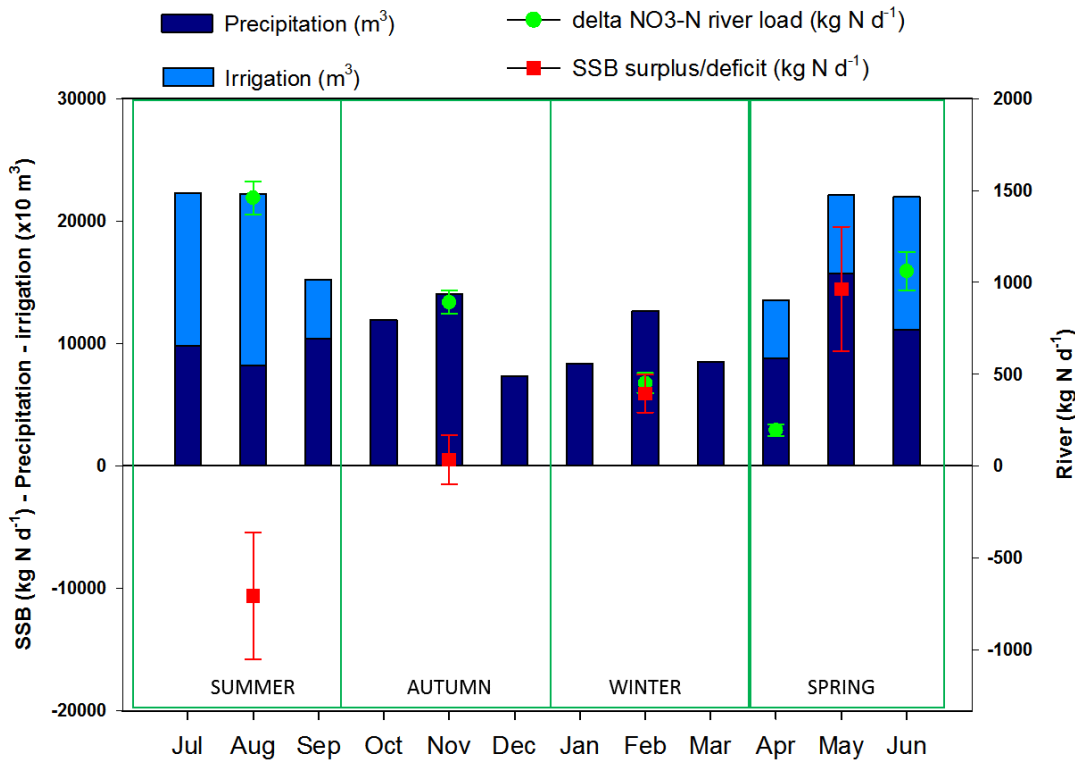


Figure 6. Histograms report monthly water input due to precipitation and irrigation in the municipalities of the Mincio basin under study. Green dots show the delta nitrate loads of the river reach (delta NO₃-N river load) in the five sampling dates and red squares show seasonal N budget of agricultural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

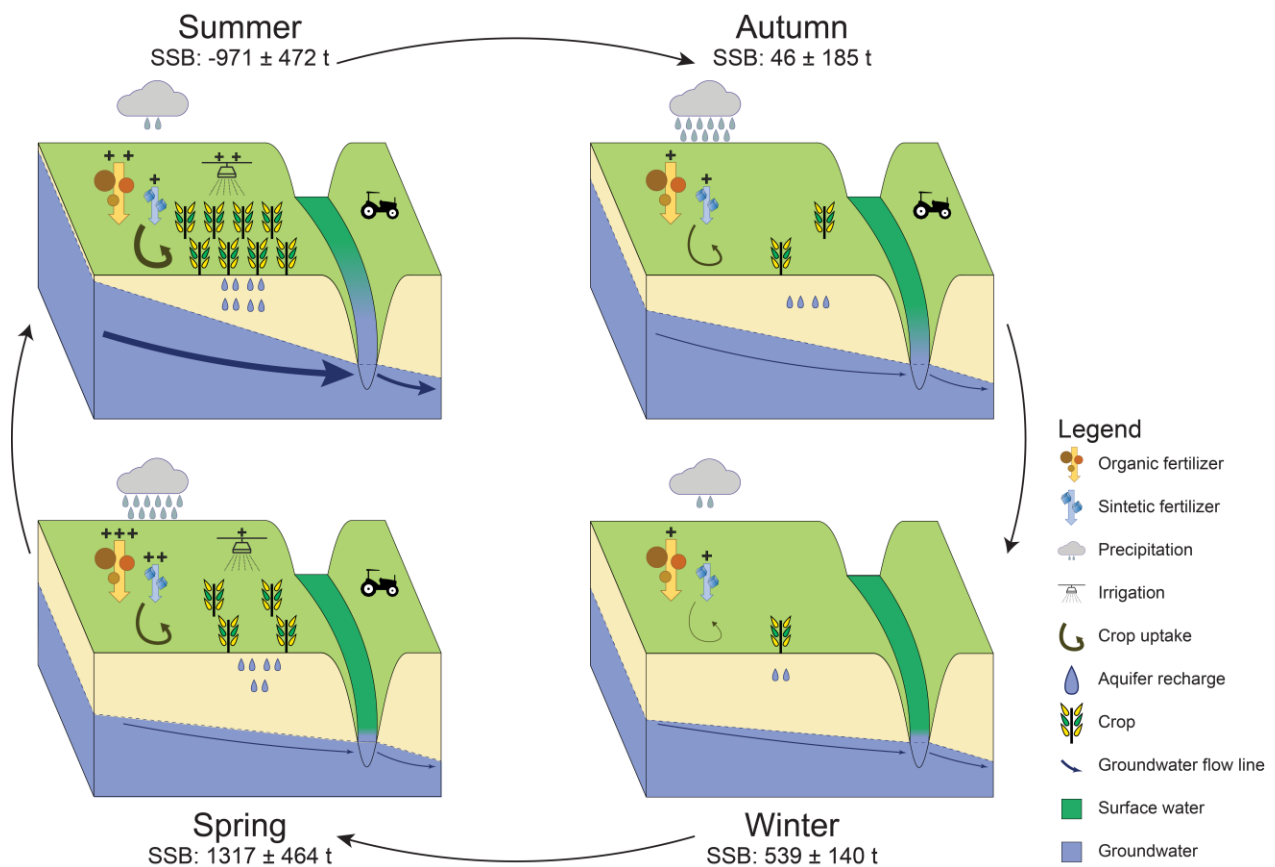
In the regulated river reach under study, which is characterized by gravel bottom and colonized by submerged macrophytes, ammonium and nitrite loads were higher upstream and suggested net retention in all seasons, peaking in summer when the primary producers' activity is maximum. Differently, dissolved inorganic carbon and nitrate loads evidenced a significant increase from upstream to downstream in all the investigated seasons. Nitrate and inorganic

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4 360 carbon net export decreased from August to April, with August as the central month for irrigation and April the last
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6 361 month before the start of the irrigation period. From these results, we calculated the annual net export of inorganic
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8 362 carbon and nitrate multiplying the daily values by the number of days between consecutive samplings, and then
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10 363 integrating the results over one year. Despite 6-8 repeated water samplings during the 24 hours, these calculations are
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12 364 based on measurements carried out in single days along different seasons. However, the nitrate concentrations
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14 365 measured in this study are consistent with the seasonal NO₃-N concentrations measured by ARPA Lombardy (the
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16 366 authority that manages water monitoring for the WFD) in the period 2009-2017 and by the Laboratory of Aquatic
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18 367 Ecology of the University of Parma in the period 2011-2017 (Fig. C.1 in Supplementary material C).

20 368 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
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22 369 S1 and S2. These amounts can be explained by element transformation (e.g., respiration or nitrification), and lateral or
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24 370 vertical inputs. As the loads of the other two inorganic N forms were negligible as compared to nitrate, this calculation
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26 371 was done only for the latter. Using dissolved oxygen budgets (not reported in this work) we converted dark river
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28 372 respiration rates (night oxygen uptake along this stretch; from -1.3 to -5.7 mm O₂ m⁻² h⁻¹ in winter and summer,
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30 373 respectively) into potential nitrification rates (~75 t N y⁻¹) according to nitrification stoichiometry. To this purpose, we
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32 374 assumed that 100% of the oxygen consumed was used to oxidize ammonium to nitrate. Results suggest that microbes-
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34 375 mediated processes as nitrification can explain at most 23% of the nitrate accumulation in the river reach in all seasons.
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36 376 Such percentage is a large overestimation of the real value as it was obtained neglecting all other oxygen-consuming
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38 377 processes, including macrophytes, fish, macroinvertebrates, and the whole heterotrophic microbial community
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40 378 respiration. A comparable nitrate production (~80 t N y⁻¹) was obtained using the nitrification rate set by
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42 379 Taherisoudejani et al. (2018) in the QUAL2Kw model applied to the Oglio River, a nearby Po River tributary with
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44 380 similar hydrological characteristics. Pinaridi et al. (2014) found that the processes in the hyporheic zone or the microbial
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46 381 metabolism of carbonate dissolution could explain up to 15% of the DIC increase, including the role of macrophytes
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48 382 in modulating dissolved CO₂ saturation values and fixation of C.

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51 383 If biological processes cannot explain inorganic carbon and nitrate increase, also point pollution sources can be
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53 384 excluded, due to low discharge of small tributaries along this stretch with water chemistry comparable to that of the
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55 385 Mincio River. Another potential source of N and DIC is groundwater, via seasonally variable river-groundwater
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57 386 interactions. Indeed, the level of the phreatic surface increases and interacts with the river due to precipitation, flooding
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59 387 and sprinkler irrigation during the spring-summer period (Racchetti et al., 2019; Severini et al., 2020) (Fig. 7). In a
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4 388 period before and after fertilization (from March to May 2021), Severini et al. (2022) measured in the same area of the
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6 389 Mincio River significantly higher HCO_3^- concentrations in groundwater than in surface water (Appelo and Postma,
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8 390 2005). Hence, the DIC increase in the investigated river stretch can be associated to the groundwater feeding the Mincio
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10 391 River. Considering the abundant use of organic fertilizers in the area, the higher DIC in groundwater can be related to
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12 392 the presence of calcite (CaCO_3) in the mineralogical composition of the aquifer and to the oxidation of the organic
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14 393 matter, which promotes a higher DIC concentration in groundwater. Future investigations should include the
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16 394 mineralogical composition of the aquifer.
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52 398 Figure 7. Synthesis of the seasonal nitrogen budgets at agricultural land and at river section. The relation with the
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54 399 groundwater is also reported. SSB = Soil System Budget.
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58 401 **5.3. Linking soil N budgets and N riverine export: the key role of the aquifer**

59
60 402 The main period of N fertilization is in spring when there is the highest N soil surplus and surface and groundwater
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4 403 pollution risk due to limited crop uptake. The crop harvest occurs mainly in summer and autumn when the N soil
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6 404 budget is in deficit or close to equilibrium, respectively. These data are reflected by an increasing N export by the river
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8 405 reach from spring to summer, favored by large volumes of nitrate-enriched water displaced through the irrigation
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10 406 across the aquifer-river continuum (Isidoro et al., 2006). Using SiO₂ as tracer, Severini et al. (2022) typified the
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12 407 investigated river stretch as a flow-through system, where groundwater feeds the Mincio River in its west bank and it
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14 408 is fed from the river's east bank (Fig. 7). As a result, N-rich groundwater can displace N-poor water from the Mincio
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16 409 River without a significant modification of the river flow (Fig. 7). Our data are consistent with this hydrogeological
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18 410 conceptual model, since the higher NO₃-N delta loads were found in summer, when the groundwater level are the
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20 411 highest and there is the maximum groundwater seeping to the Mincio River, highlighting the deep effects of the
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22 412 recharge given by irrigation. On the contrary, some differences were found during the rest of the year, characterized
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24 413 by a less anthropic recharge of the aquifer. These differences are more related to the dissimilar distribution of
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26 414 precipitation and percolation of water and N to groundwater, which fosters the migration of N to the Mincio River. In
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28 415 fact, as we move away from the end of the irrigation period (September), the lower groundwater heads reported in
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30 416 Severini et al. (2022) could result in a lower groundwater seepage to the river (Fig. 7). Having less nutrient enriched
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32 417 water available guarantees a minor nitrate surplus in the river reach, even if N fertilization starts again in winter,
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34 418 resulting in another period with N soil surplus (Figs. 6, 7).

36 419 Considering that the mean annual N surplus on the agricultural land in the study area averaged 67 kg ha⁻¹ y⁻¹, it is
37
38 420 possible to speculate that the agricultural land surface that can potentially generate the NO₃-N river export (317±12 t
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40 421 NO₃-N y⁻¹) is equivalent to ~4700 ha⁻¹. In addition, dividing this surface by the length of the river reach investigated,
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43 422 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
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45 423 surplus production. These data allow to speculate that the N surplus was generated in the 25% of the surface of
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47 424 municipalities under study, giving useful information to better address arable land management.

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49 425 Our results on N input and output trends in agricultural soils and into the river reach at annual and seasonal basis allow
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51 426 to better understand N patterns from land to river and the potential nitrate pollution to surface and groundwater. This
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53 427 information at seasonal resolution can help policy-makers in developing effective plans to improve N management at
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55 428 the macroscale. In fact, this combination of information can guide the identification of proper spatial-temporal
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57 429 management strategies to reduce N pollution and river export to avoid eutrophication processes of water bodies. For
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59 430 example, our results suggest that more nitrate was delivered downstream in summer because of spring soil N excess

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4 431 coupled to flood irrigation over permeable soils. Hence, it is important to focus on agricultural sources (manure and
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6 432 synthetic fertilizers in particular) to better balance N inputs and output by crop harvest (or stock). Our approach was
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8 433 applied in a pilot study at the sub basin level, but it is exportable to the whole basin and to other rivers. It becomes
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10 434 very important to have local information and basin-specific data to perform seasonal analysis on N patterns (Lassaletta
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12 435 et al., 2021).

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16 437 **5.4. Possible remediation strategies in the context of climate change and the regulation of river discharge**

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18 438 Temporal disconnections between N fertilization, transport and uptake in agricultural land can result in low N use
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20 439 efficiency (Robertson and Vitousek, 2009). Specific monitoring of crop growth, nutrient demand and soil availability
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22 440 is useful to obtain a more synchronous nutrient supply in response to crop needs (Quemada et al., 2013). Alternative
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24 441 practices arranged to implement nutrient management directly in the field include actions such as variable rate or split
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26 442 of fertilizer applications matched to crop growth demand, improvements in efficiency of irrigation practices and use
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28 443 of nitrification inhibitors (Lacey and Armstrong, 2015; Fernández et al., 2016). The nutrient best management
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30 444 practices, for N, should be designed in view of seasonal N leaching losses and hydrologic export to properly depict
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32 445 crop growth dynamics and N demand, soil conditions and hydrology (Lin et al., 2019).

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34 446 It is during summer that the investigated reach experienced the highest water nitrate accumulation. An action useful to
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36 447 buffer the N export is the implementation of riparian buffer strips that can promote N retention during the spring-
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38 448 summer irrigation period or the use of cover crops in winter (Dabney et al., 2010; Cole et al., 2020). During winter,
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40 449 our calculations suggest N soil excess in a period where uptake is minimum, and denitrification is likely limited by
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42 450 low temperatures and by the thick unsaturated soil. The latter follows the downward winter migration of the water
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44 451 table, previously discussed. For this reason, the adoption of practices that can favor water retention to increase soil
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46 452 humidity in the cold season and the presence of water in canals, commonly dry in autumn and winter, might be a
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48 453 solution that can favor denitrification process. As an example, the construction of artificial ponds or wetlands can be
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50 454 useful to intercept N runoff from agricultural lands (Nøges et al., 2003; Carstensen et al., 2020).

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53 455 In the geographical area of our case study, the Alps host a series of large lakes regulated by dams that feed rivers
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55 456 among which the Mincio River. The dams regulate the lake water level and the river discharge with rules that aims to
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57 457 accumulate water in winter and release it during the irrigation period, adapting also to local meteorology and water
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59 458 inputs to lakes. This regulation practice guarantees sufficient summer level in lakes for tourism and navigation purposes

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4 459 and large water availability for irrigation and electricity production in the downstream river section. Irrigation is
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6 460 supported by several water abstraction infrastructures that facilitated agriculture and animal farming activities.
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8 461 Irrigation practices, supported mainly by flooding irrigation in the sector of the Po River plain including our study area
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10 462 are carried out over permeable soils, and favor the recharge of the aquifer mainly in summer (Rotiroti et al., 2019).
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12 463 Therefore, water retention upstream during non-irrigation periods and flood irrigation with large water volumes during
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14 464 summer are probably the main drivers of the groundwater head variation, which is subject to strong seasonal
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16 465 differences (Taherisoudejani et al., 2018). Under the current climate change scenario, also in this geographical area,
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18 466 the rapid changes of global warming are manifested with lower precipitation, dry winters, heatwaves and storms events,
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20 467 and an increasing number of consecutive days with high temperatures (Cifrodelli et al., 2015; Pedro-Monzonís et al.,
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22 468 2016; Lassaletta et al., 2021; Ranasinghe et al., 2021). The response to these global trends could increase or decrease
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24 469 river nitrate concentration depending on regional or site-specific linkage between N concentration and discharge
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26 470 (Stelzer et al., 2020). For this reason, the geographical sector under study seems extremely vulnerable to climate change
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28 471 as the system is depicted and managed for large water availability (i.e., large lakes regulation, high water demanding
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30 472 crops, and flooding as main irrigation practice) and therefore a discussion on water management at political level is
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32 473 urgent. Predicting scenarios on the fate of the N excess with different water availability is difficult, we can hypothesize
33
34 474 a reduction of water discharge from rivers and consequently from irrigation that will not recharge sufficiently the
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36 475 groundwater due to its deep level (Taherisoudejani et al., 2018). This condition will lead to more thick vadose zones,
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38 476 fostering a higher nitrification rate and nitrate accumulation in soil during winter, whereas denitrification, the main
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40 477 process that removes N permanently from the system, is favored in water saturated soils with high organic matter
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42 478 (Ascott et al., 2017). In soil and rivers close to N saturation, it is expected a lower nitrate retention efficiency and
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44 479 therefore an increase in N availability and vertical and horizontal transfer (Stelzer et al., 2020). Moreover, for the future
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46 480 it can be expected a delay in the river feeding by groundwater with hot-moments of N mass transfer. In fact, we can
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48 481 expect that with the increment of unsaturated zone, the rate of soil denitrification will be reduced and conversely the
49
50 482 nitrification process will be favored supplying a short N mass-transfer as soon as the first rainfall or flooding irrigation
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52 483 will occur, carrying water with high N concentration to surface or groundwater. A possible solution to limit this hot-
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54 484 moment can be the improvement of irrigation practices with less water consumption and a more widespread use of
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56 485 precision farming supported for example with remote sensing technique (Nutini et al., 2021). Such a new vision on the
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58 486 irrigation practices can allow a lower winter water retention in the Lake Garda, that can be partially used in the non-
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487 irrigation period to guarantee a minimal vital flow in a certain number of drainage canals as well as in the Mincio River
488 favoring denitrification process also in autumn and winter, although with lower rates driven by lower temperatures.

489
490 **6. Conclusions**

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

504
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510 Region and the Interregional Agency for the Po River for having provided physico-chemical data and water flow data
511 of the Mincio River, respectively.

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513 **Declarations**

514 **Ethical Approval:** Not applicable.

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20 528 **References**
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