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CICLO XXXIV

The effects of aquifer recharge and river-groundwater interaction on contaminants migration in alluvial aquifers: the test sites of Goito and Parma aquifer (northern Italy)

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SERIES XXXIV

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Water does not resist. Water flows. When you plunge your hand into it, all you feel is a caress. Water is not a solid wall, it will not stop you. But water always goes where it wants to go, and nothing in the end can stand against it. Water is patient. Dripping water wears away a stone. Remember that, my child. Remember you are half water. If you can't go through an obstacle, go around it. Water does.

Margaret Atwood, THE PENELOPIAD

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CHAPTER 1: INTRODUCTION

1.1. THE NITROGEN CONTAMINATION

1.1.1. A European perspective

Nature and its biodiversity could only exist because of the availability, even if limited, of nitrogen (N) in the system, which is defined as all nitrogen compounds except for N_2. Because of its limited availability, ecosystems became very efficient in conserving and re-using reactive nitrogen compounds. N can be considered as a limiting factor to produce food, together with other nutrients and water. To support the large increase in global population, the crop production increased over the 20th century. The increase in crop production has been possible thanks to the intensification and extension of agricultural land, but also thanks to the availability of N fertilizers, both organic and synthetic, based on the Haber–Bosch process (Erisman et al., 2008). For instance, Erisman et al. (2008) evaluated that 48% of the global population is indirectly upheld by synthetic fertilizers. European agriculture is one of the largest and most productive worldwide. Here, in 2004, farmers generated 21% of global meat production and 20% of global cereal production (Sutton et al., 2011). This intensive agriculture and animal farming results in higher productivity in Europe compared to other world areas: for example, the average cereal yield in the EU is 60% higher than the worldwide average value (EFMA, 2010).

This intensive agriculture requires a large use of fertilizers, both synthetic and natural. Regarding the synthetic N fertilizers, they are usually created combining atmospheric N (N_2) with hydrogen from methane or gasified coal to produce ammonium nitrate (NH_4NO_3), ammonium sulphate ($(NH_4)_2SO_4$), or urea ($CO(NH_2)_2$). Their use increased worldwide during the 20th century (Davidson, 2009) and Europe is not an exception. Regarding organic fertilizers, their use is strictly connected to livestock production. Animal farming does not cause the input of new N into the environment, as it happens for the synthetic fertilizers, but a redistribution at different spatial and temporal scales. This is particularly true when animal farming is not equally distributed into an area. If N distribution is homogeneous in the area, the N input to the environment is lighter, i.e. more diluted. If intensive animal farming is performed, the same N input is concentrated in a smaller area.

A large portion of the global livestock production is located in Europe (Sutton et al., 2011), e.g. 31.1% and 31.7% of global meat and cow milk production, respectively (FAO, 2020). According to the European Nitrogen Assessment (2011), the regions with the higher livestock production in Europe are Denmark, the Netherlands, Belgium, Brittany, Spain, Poland, the UK, and the Po Valley (Italy). The pigs per unit area density is among the highest in the world together with some regions of China and the USA (Sutton et al., 2011). The intense animal farming performed in Europe results in the accumulation of 8.39 Tg N produced in 2019, of which 4.45 Tg N were applied to the soil (FAO, 2020). It is well known that agriculture provides strong pressures on surface and groundwater due to N pollution. In the first case, all the N applied to the soil can be transported to superficial water bodies due to runoff. In the second case, the leaching of N through the vadose zone can affect groundwater, especially when leaching is fast and N leaches quickly below the root zone (30-100 cm).

These threats are not new to scientists and stakeholders in Europe. Sure enough, the high use of N fertilizers during the 20th century (Sutton et al., 2011) probably resulted in high concentrations of nitrate (NO_3^-) in groundwater. In Europe the background concentrations of NO_3^- in groundwater are low and the aquifers with higher concentrations are the result of anthropogenic pressures, most of which are related to agricultural activities (Sutton et al., 2011). As a consequence, to defend the water for human consumption from the N contamination, the first directives were released in the 1970s ("Council Directive 75/440/EEC of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States" 1975) and 1980s ("Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption" 1980). Despite that, between the 20th and 21st centuries groundwater (and surface waters) in Europe were still threatened by N pollution.

Using various data from the 1990-2000 period, Van Drecht et al. (2003) estimated a remarkable leaching to groundwater of 8 Tg y^{-1} in Europe, of which 40% will reach surface waters. Although very dependent on the typology of agriculture performed and the hydrogeological settings of the aquifers, groundwater in some regions of Europe showed in the past (and shows also nowadays) high nitrate concentrations. To protect surface and groundwater from this threat, the European Union issued the Nitrate Directive (ND, "Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources" 1991), with the aim of reducing and preventing nitrate pollution from agricultural activities. To achieve this, the directive promoted the designation of trigger criteria for surface and groundwater: the Nitrate Vulnerable Zones (NVZ) and a threshold of 50 mg/L NO_3^- for groundwater, and the use of eutrophication indexes for surface waters. As conservative measure, the use of organic N fertilizers above the NVZ was limited to 170 Kg ha^{-1} y^{-1}.

Nevertheless, the European Commission (2007) presented discouraging data during the Third Assessment Report on the Implementation of the Nitrates Directive. The results showed that during the period 2000-2003, almost 17% of the wells in the EU15 exhibited a concentration of NO_3^- exceeding the limit of 50 mg/l. Moreover, almost 40% of the groundwater monitoring stations exceed 25 mg/L NO_3^- and almost 50% of the surface water monitoring stations had concentrations higher than 10 mg/L NO_3^-. As a consequence, Behrendt et al. (2003) estimated that during the period 1998-2000, groundwater contributed to 48% of the total N load for the Danube River. Similarly, Palmeri et al. (2005) estimated a contribution of groundwater of around 36% to the N load of the Po River. After the 91/676/EEC, two more directives were established: the Water Framework Directive (WFD, "EU Water Framework Directive" 2000) and the Groundwater Directive ("Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration," 2006). Although the ND still represents a reference point for the protection of water against N contamination from agricultural activities, the Groundwater Directive and the WFD updated the methods and the socioeconomic and environmental transitions necessary to reach the optimum quality and quantity of surface and groundwater (Tab. 1).

Table 1. Main EU Directives related to N inputs and concentrations in water bodies due to agricultural activities. From Sutton et al. (2011), modified.

Directive	Description	Requirements and Objectives
91/676/EEC	**Nitrates Directive** (**ND**): concerning the protection of waters against pollution caused by nitrates from agricultural sources	• Establishment of a code of good agricultural practice, including balanced N fertilization, to be implemented by farmers voluntarily • Designation of Nitrate Vulnerable Zones (NVZ) • Establishment of action programmes with mandatory measures in vulnerable zones, including N application limits • Water quality trigger criteria: (1) 50 mg/L NO_3^- in groundwater and surface waters, and (2) eutrophic status of surface waters • Application limit for nitrogen from animal manure in NVZ: 170 Kg ha^{-1} y^{-1}
2000/60/EC	**Water Framework Directive** (**WFD**): to establish a framework for the protection of inland surface waters, transitional waters, coastal waters, and groundwater from pollution and depletion	• Maintaining/establishing good ecological status in surface water bodies and good chemical and quantitative status in groundwater bodies • Establishment of river basement management plans • Designation of "protected areas" • For "limit values" and "measures required" reference is made to other Directives
2006/118/EC	**Groundwater Directive**: establishes a regime that sets underground water quality standards and introduces measures to prevent or limit inputs of pollutants into groundwater	• Groundwater quality standards for nitrate and active substances in pesticides, including their relevant metabolites, degradation, and reaction products • Threshold values for all pollutants and indicators of pollution which characterize groundwater as being at risk of failing to achieve good groundwater chemical status • Establishes the 50 mg/l NO_3^- as a binding maximum quality threshold

After almost 30 years, in 2015 38% of European water bodies remained under significant pressure from agricultural pollution (United Nations World Water Assessment Programme, 2015). However, the directives had some impact. The overall NO_3^- values decreased during the period 1992-2015: in groundwater the concentrations returned similar to those in 1992, remedying the concentrations rise measured during the mid-1990s and 2000s, while in surface waters, NO_3^- concentrations decreased by 20% (European Environment Agency, 2018). Nevertheless, 26% of groundwater bodies in Europe had poor chemical status, usually caused by NO_3^- and reported in 24 Member States. These results are quite alarming, since groundwater is still a fundamental resource, providing water for domestic use for about two-thirds of the population (Sutton et al., 2011).

Eventually, more efforts are required to decrease the NO_3^- concentrations in both surface and groundwater across Europe. The key must be the perpetuation of good agricultural and management practices to prevent and contrast the present and future NO_3^- contaminations. An additional improvement can be reached by increasing the monitoring network, which is still not homogeneously distributed and not sufficient to represent the plentiful groundwater and surface water bodies in

Europe, as reported from the environmental agencies at regional and local level, with the European Environment Agency above all.

1.1.2. The Po Plain system

Hydrogeological Setting

The Po plain is a large alluvial valley in northern Italy bordered at North by the Alps, at South by the Apennines, and at East by the Adriatic Sea. At its centre, the Po River flows for 675 km from the western Alps to the Adriatic Sea, collecting water from 141 tributaries. The Po plain is one of the largest aquifers in Europe (Perego et al., 2012). At regional scale, it is a multilayer aquifer composed of gravel and sand separated by silt and clay (Fig. 1). Closer to the mountain chains, where the alluvial fans can be found, the shallow aquifer is generally unconfined and some pieces of evidence of the connection with the deep aquifers (semiconfined and confined) can be found (Martinelli et al., 2018). As reported in Fig. 1, groundwater in the shallow aquifer flows usually towards the Po River. The hydraulic gradient is higher near the mountain chains (8‰ to 10‰), the so-called “higher plain”, while closer to the Po River, the “lower plain” has a lower hydraulic gradient (1‰ to 4‰). Similarly, the higher hydraulic conductivities (1-10×10^{-3} m/s) are measured in the high plain due to the alluvial fan areas, while in the lower plain lower values are reported in the literature (1-10×10^{-5} m/s) (Martinelli et al., 2018). The shift of the hydraulic gradient marks the presence of the typical lowland springs called “fontanili” (Balderacchi et al., 2016; Fumagalli et al., 2017). These are small, semi-

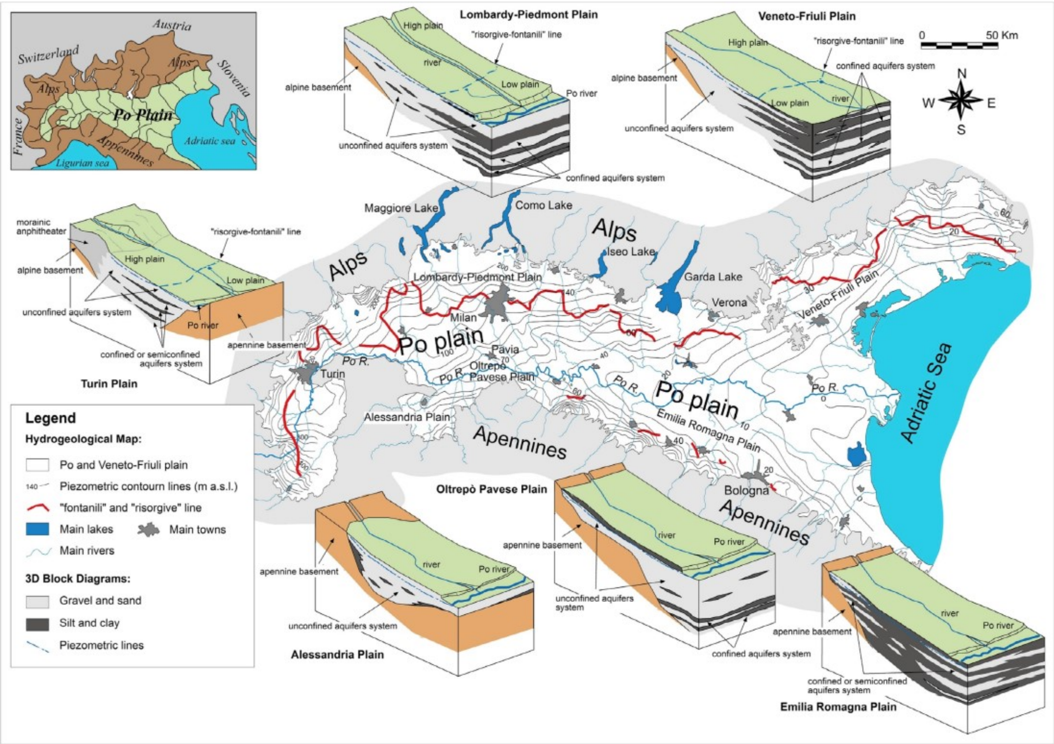

Figure 1. Simplified geological and hydrogeological settings of the Po Plain (from Martinelli et al., 2018).

artificial springs resulting in peculiar aquatic ecosystems (Kløve et al., 2011). They are able, anyway, to promote a fast transport of contaminants from groundwater to surface waters (Balestrini et al., 2021). The Alpine and Apennine foothills represent the main recharge areas of the Po valley aquifer system. Due to the aquifer granulometry (gravel, sand, and silt) and the thickness of the surface alteration layers and/or loess deposits, near the Alpine and Apennine foothills the infiltrability map reported the higher values, pointing out the threat of diffuse contamination of substances from the surface (Giuliano et al., 1998).

Nitrate Contamination

The Po valley represents an important portion of northern Italy, which hosts more than 20 million inhabitants and where almost 50% of the Italian Gross National Product is produced. Among the activities performed in this valley, a significant share is given by intensive agriculture and animal farming, which have been central activities for centuries in this area (Marchetti, 2002). Agriculture was and is still supported by the large water availability in the Po Plain, especially in the central area where, since the Middle Ages, a capillary network of irrigation channels was realised to deliver irrigation water far from springs and rivers (Fantoni, 2008). Nowadays, almost half of the total surface is still devoted to (intensive) agriculture and animal breeding, including mostly cattle, pigs, and chickens (Martinelli et al., 2018). For these reasons, the Po Plain has been always characterized by high N concentrations in groundwater and was considered to be one of the most NO_3^- polluted sites in Europe (Figure 2; Sutton et al., 2011).

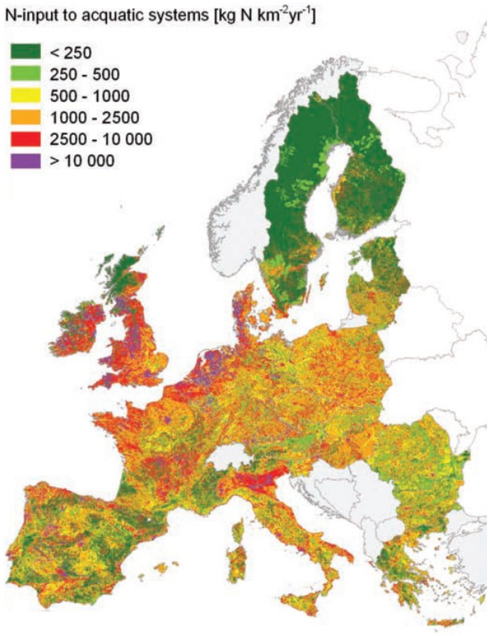

Figure 2. Total reactive N input to the hydrosphere (rivers and groundwater) in EU-27 for the year 2002 (from Sutton et al., 2011, modified).

The Po Plain hosts several NVZs due to the high infiltrability values and the high NO_3^- concentrations in groundwater till the late 1990s. As a matter of fact, the NVZs in the Po Plain represent 70% of the NVZs present in Italy. Nonetheless, Italy asked for a derogation to the ND. The derogation allowed Italian farmers to use inside the NVZs up to 250 kg N ha^{-1} y^{-1} instead of the 170 kg N ha^{-1} y^{-1} specified in the ND, to support the agricultural activity and livestock production in the Po Plain. Namely, two derogations were released. One concerned all the regions of the Po Plain (Emilia Romagna, Lombardia, Piemonte, and Veneto) from 01/01/2012 to 31/12/2015 ("Commission Implementing Decision of 3 November 2011 on granting a derogation requested by Italy with regard to the Regions of Emilia Romagna, Lombardia, Piemonte and Veneto pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (notified under document C(2011) 7770)" 2011). The derogation was successively prolonged, but only for the Lombardia and Veneto regions from 20/01/2016 to 31/12/2019 ("Commission Implementing Decision (EU) 2016/1040 of 24 June 2016 on granting a derogation requested by Italian Republic with regard to the Regions of Lombardia and Piemonte pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (notified under document C(2016) 3820)," 2016). The derogation was successively not extended and from 2020, in the NVZs the amount of manure applied as fertilizer returned to 170 kg N ha^{-1} y^{-1}.

Nowadays, high NO_3^- concentrations (> 50 mg/L NO_3^-) are still determined in the Po Plain, especially in the Alpine and Apennine foothills, whereas in the central portion of the Plain NO_3^-concentrations remain low (Martinelli et al., 2018). Anyway, given the fast circulation of groundwater (0.1–5 m d^{-1}), its contamination can be rapidly remediated if the (agricultural) N input is reduced (Martinelli et al., 2018). According to the same authors, samples with high NO_3^- concentrations are enriched in $\delta^{15}N$, suggesting a large use of organic fertilizers, although some studies point out also the presence of synthetic fertilizers (Sacchi et al., 2013). At the basin scale, they found a positive and significant correlation between $\delta^{15}N$ and pigs per Utilized Agricultural Area (UAA), suggesting that the intensive use of pigs slurry in restricted areas is associated with intensive animal farming. The high NO_3^- concentrations can be lowered by denitrification processes, especially in the Piedmont and Lombardy lower plains (Lasagna et al., 2016b; Sacchi et al., 2013).

1.1.3. The Mincio River

In the Po Plain, the scientific and stakeholders community has given particular attention to the rivers (Adda, Chiese, Mincio, Oglio, and Ticino River) originating from the Great Italian Alpine Lakes. These are major contributors to the Po River discharge and provide water for multiple socio-ecological services, from irrigation to recreational activities. They also constitute areas of high biodiversity in the anthropic landscape dominated by industrial sites and intensive agriculture. Different studies investigated the surface and groundwater quality and quantity in their watersheds, using different approaches. These studies are not common for all the rivers and sub-areas of the Po Plain. Most of them are focused on the Piedmont plain with the first stretch of the Po River watershed (e. g. Lasagna et al., 2016a), the Veneto (e.g. Morari et al., 2012), and Lombardy plain with the Ticino, Adda, and Oglio rivers (e.g. Balestrini et al., 2021; Delconte et al., 2014; Racchetti et al., 2019; Rotiroti et al., 2019; Sacchi et al., 2013).

Among them, the Mincio River remained an understudied area. Few authors investigated its dynamics (Pinardi et al., 2018b; Racchetti et al., 2019). Along the Mincio River course, between the 18th and 33rd km (Fig. 3), the NO_3^- concentrations in the 2010s increased in summer by a factor of 10, from 0.84 to 9.39 mg L^{-1} NO_3^- (Racchetti et al., 2019). These values, besides being close to the threshold NO_3^- concentration of the Third Assessment Report on the Implementation of the Nitrates Directive European Commission (2007), constitute also a threat due to the high NO_3^- load increase in the river and subsequent eutrophication problems for the downstream water bodies. In fact, eutrophic and hypereutrophic status are reported for the Mantua Fluvial Lakes fed by the Mincio River (Bolpagni et al., 2014; Bresciani et al., 2013; Pinardi et al., 2018a). Moreover the increase of NO_3^- concentrations in the Mincio River constitute a serious threat to the environmental quality of the downstream water bodies in the sense of WFD such as the Po River (Tavernini et al., 2011; Viaroli et al., 2018) and the Po River Delta (Naldi & Viaroli, 2002; Viaroli et al., 1993). The explanation for these results came from the analyses of the fertilizers application and nutrients export from the watershed. Using the Soil System Budget (SSB, Oenema et al., 2003) a N surplus in the watershed of 132 kg N ha^{-1} y^{-1} (Pinardi et al., 2018b) and 136 kg N ha^{-1} y^{-1} (Racchetti et al., 2019) was calculated and associated with the excess manure availability and use. The cultivation of such high water-demanding and profitable cultivars (e.g. *Zea mays*, the main cultivar in the watershed) over highly permeable soils (pebble, gravel, and sand) requires high water volumes, which fortunately are available thanks to the Lake Garda and the Mincio River. In the Mincio River basin, the irrigation is performed from April to September through flooding irrigation. In

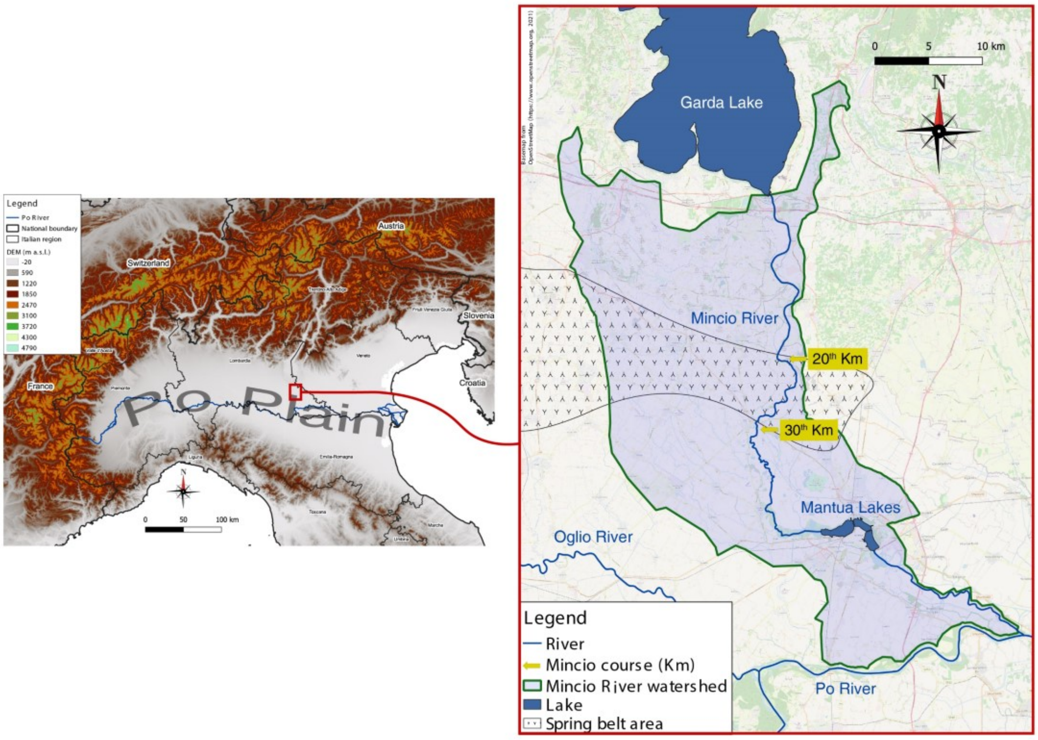

Figure 3. Location of the Mincio River basin and section where the NO3- concentrations increase was found in the Mincio River (20th-30th km). The Spring belt area is derived from Bischetti et al. (2012).

other words, a capillary network of artificial and natural channels divert water from the Mincio River, which is used to flood the agricultural fields usually two or three times per week (as reported by farmers' interviews). The NO_3^- enrichment in the Mincio River was then explained connecting those dots. In Pinardi et al. (2018b) and Racchetti et al. (2019), the underlying mechanism is the so-called *irrigation loop*. The high recharge provided during the irrigation period promotes the percolation of nitrate-rich water to groundwater, fostering the vertical and horizontal transport of NO_3^- from fertilizers. In a short time, groundwater flows to the Mincio River, which is contaminated from both diffuse (groundwater) and point (groundwater from springs) N sources. This proposed mechanism was consistent with the SSB and the NO_3^- concentrations in the river. Anyway, some open questions remained. How does groundwater respond to the high recharge given by irrigation from a quantitative and qualitative perspective? If fed by groundwater, why the Mincio River did not show a flow increase? Is this phenomenon related only to summer? To answer these questions, a hydrogeological investigation was started.

1.2. THE MICROPLASTICS CONTAMINATION

During the pandemic, the access to the study area and laboratories of the university was not allowed. This time was used to conceptualize a side line of research. The idea was to investigate other contaminants which migration is strictly related to the aquifer recharge and river-groundwater interaction. Thus, the attention was posed to the microplastics contamination in groundwater.

Since this was a fully new argument, it started with detailed research in the available literature published. From this search, it was found that microplastics are drawing the attention of the scientific community, which anyway is not in agreement on standardized investigation, analyses, and report methods (Hartmann et al., 2019). Most of the investigations are performed in marine environment, where those kinds of studies started in the 1970s (Carpenter et al., 1972). Paradoxically, other important aquatic compartments such as groundwater, which is the single most important supply for drinking water in many areas of the world, have received almost no attention. The first case of microplastics contamination in groundwater was reported in a fractured medium, with karst crevices and conduits reportedly allowing the transfer of microplastics (Panno et al., 2019). In other aquifers with lower porosity, soil can be assumed to work as a barrier for microplastics, probably dissuading researchers from further investigations. This hypothesis has recently been challenged, with two recent articles reporting microplastic contamination in alluvial aquifers, raising new questions on microplastics contamination and migration processes, especially from the hydrogeological perspective. Goeppert and Goldscheider (2021) simulated microplastics spheres contamination in an alluvial aquifer, demonstrating the possible microplastics transport, thus invalidating the role of the aquifer (and soil) as a priori barrier for microplastics. Samandra et al. (2022) were the first and, so far the only to report on microplastics contamination in an alluvial unconfined aquifer. Most of the studies on microplastics and groundwater have a mainly chemical approach and little attention is given to the hydrogeological features possibly influencing the contamination (e.g. microplastics shape and recharge of the aquifer). Moreover, most of the methods of investigation proposed in the literature are suitable only for research groups with a dedicated analytical chemistry laboratory or with fluorescence microscopes.

CHAPTER 2: PhD RESEARCH RATIONALE AND GOALS

2.1. THESIS STRUCTURE

This thesis is organized into three main sections. The first one (Chapter 1) is committed to the introduction to the thesis subjects. The first is the NO_3^- contamination in a stretch of the Mincio River and surrounding aquifer; the second is the microplastics contamination, with regards to new methods and contamination characterization. These themes are connected by the same processes affecting their contamination: the aquifer recharge and the river-groundwater interaction. Regarding the NO_3^- problematic, it is introduced from European to local scale, focusing on the N source from agricultural and livestock activities and the relative European directives. Regarding microplastics, there are briefly introduced and contextualized in the framework of this doctoral thesis and will be better debated in the relative article. The second section (Chapter 2 and 3) presents the rationale and goals of the PhD course and thesis. It presents the PhD aims and the publications submitted regarding the main research. A Gantt diagram (Fig. 4) explaining the overall activities, publications, and participation to conferences is also presented. Successively, the main publications are reported in full. Since the material and methods are presented in detail in the manuscripts, a dedicated chapter has not been included. In the third section (Chapter 4), the overall conclusions of the thesis are reported and contextualised. In addition, other research activities carried out during the PhD course but not related to the main research are reported.

2.2. RESEARCH DEVELOPMENT

2.2.1. NO_3^- contamination in the Goito aquifer and Mincio River

The overall aim of this doctoral thesis is to explain the hydrogeological behaviour of the Mincio River and the surrounding groundwater around the spring bel area. To achieve this, different sequential objectives were proposed, which can be summarised as follows:

- Carry out an extensive hydrogeological monitoring to create the hydrogeological conceptual model of the investigated area.
- Perform a detailed monitoring of NO_3^- concentrations in both surface and groundwater.
- Identify a tracer of the manure spreading and NO_3^-.
- Characterize the surface-groundwater interaction in the study area.
- Understand the dynamics of the Mincio River NO_3^- loads in the study area.
- Evaluate how changes in the ND fertilization limits can affect the quality of surface and groundwater in a short period.
- Conceptualize and complete a side project, focused on other possible groundwater contaminations from agricultural activities.

During the three years of PhD course, the following manuscripts on this thematic were submitted:

- **Severini, E**., Bartoli, M.; Pinardi, M.; Celico, F. Short-term effects of the Nitrate Directive reintroduction: a case of study in a contaminated alluvial aquifer in northern Italy. 2022. Submitted.
- **Severini**, **E**., Bartoli, M., Celico, F. Can't tell a book by its cover: disjointed groundwater contamination and land use in an alluvial aquifer of northern Italy. Italian Journal of Engineering Geology and Environment 2021, 1, 229-238, doi:10.4408/IJEGE.2021-01.S-21.
- **Severini, E**., Bartoli, M.; Pinardi, M.; Celico, F. Reactive Silica Traces Manure Spreading in Alluvial Aquifers Affected by Nitrate Contamination: A Case Study in a High Plain of northern Italy. 2020, 12, 2511, doi:10.3390/w12092511.
- Pinardi, M., Soana, E., **Severini**, **E**., Racchetti, E., Celico, F., Bartoli, M. Agricultural practices regulate the seasonality of groundwater-river interactions and nitrogen exchange. 2022. Submitted.
- Racchetti, E., Salmaso, F., Pinardi, M., Quadroni, S., Soana, E., Sacchi, E., **Severini**, **E**., Celico, F., Viaroli, P., Bartoli, M. Is flood irrigation a potential driver of river-groundwater interactions and diffuse nitrate pollution in agricultural watersheds? Water 2019, 11, 2304. doi:10.3390/w11112304**.**

2.2.2. Microplastics: method and application

Given the absence of a standardized method for microplastics typification and the few studies related, this research was structured with different aims:

- Methodological aim: to define a protocol for the separation, quantitative analysis, and geometrical characterization of microplastics in water samples, with particular attention to groundwater.
- Practical aim: to apply the protocol to a study area to:
  - verify the operational pros and cons of the protocol
  - provide preliminary results on the microplastics contamination in the investigated area
  - verify if the hydrogeological features of the aquifer (i.e. the recharge and recession period) affect the microplastics contamination.

The initial proposal was to perform this investigation in the same study area characterized by NO_3^- contamination, but due to pandemic restrictions (travel between different municipalities), the study was performed in the alluvial aquifer of the Parma province. This investigation was then carried out in the Parma alluvial aquifer (Po Plain, Fig. 1).

The interesting results led to the following publication:

- **Severini**, **E**., Ducci, L., Sutti, A., Robottom, S., Sutti. S., Celico, F. Microplastics contamination in a confined alluvial aquifer: insights through a hydrogeological approach. 2022. Under submission.

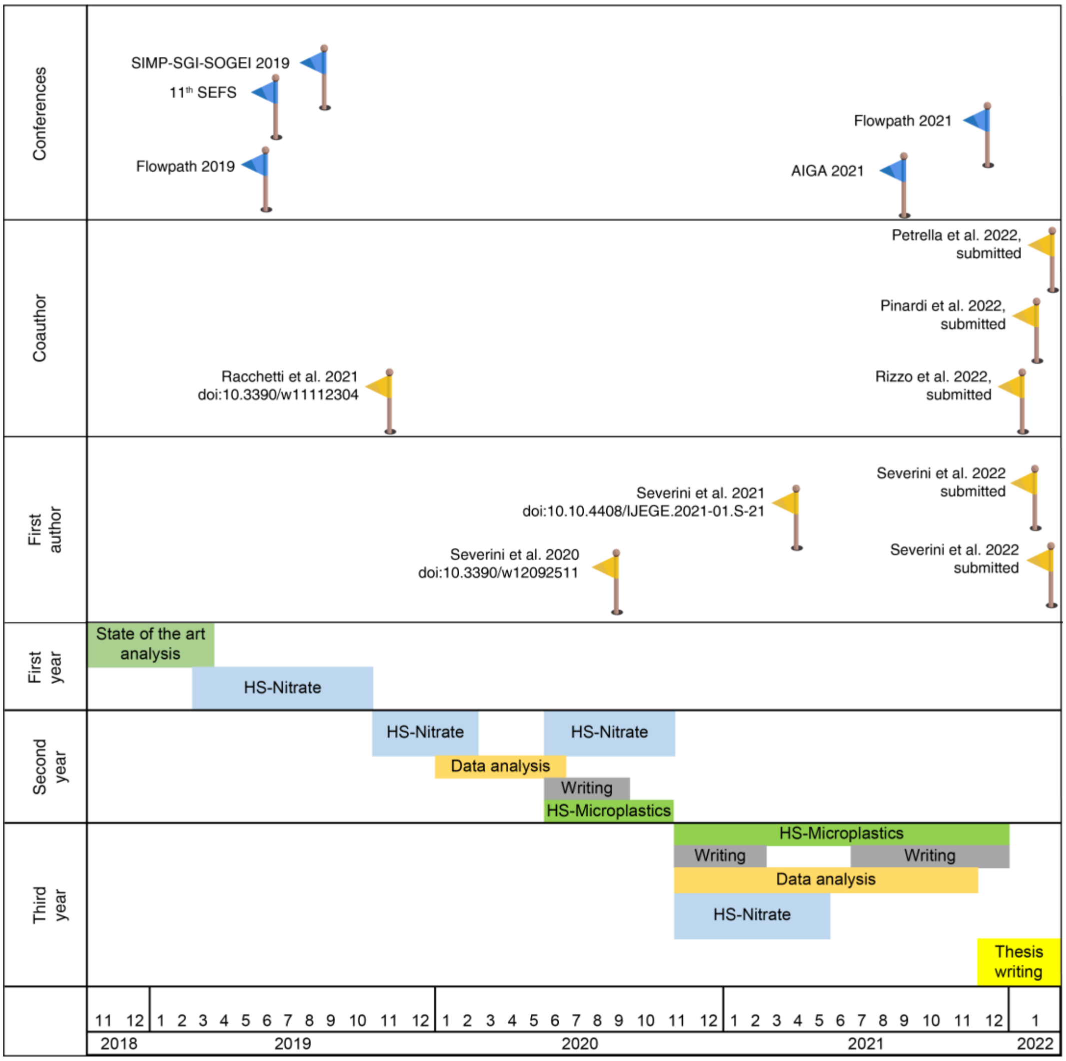

Figure 4. Gantt diagram presenting the main activities and publications of the PhD triennium. The hydrogeological survey for both the contaminants (nitrate and microplastics) is reported as “HS”.

CHAPTER 3: RESEARCH MAIN OUTPUTS

Note: the following articles are the pre-print version of those submitted to the scientific journals. They have not been through the copyediting, typesetting, pagination and proofreading process of the journals, which may lead to differences between these and the published versions.

The presented pre-prints refer to the following articles:

- **Severini, E.**, Bartoli, M.; Pinardi, M.; Celico, F. Reactive Silica Traces Manure Spreading in Alluvial Aquifers Affected by Nitrate Contamination: A Case Study in a High Plain of northern Italy. 2020, 12, 2511, doi:10.3390/w12092511.
- **Severini, E.**, Bartoli, M.; Pinardi, M.; Celico, F. Short-term effects of the Nitrate Directive reintroduction: a case of study in a contaminated alluvial aquifer in northern Italy. 2022. Submitted.
- **Severini**, **E.**, Ducci, L., Sutti, A., Robottom, S., Sutti. S., Celico, F. Microplastics contamination in a confined alluvial aquifer: insights through a hydrogeological approach. 2022. Under submission.

Article

Using silica as tracer of manure spreading in alluvial aquifers affected by nitrate contamination: a case of study in a high plain of northern Italy

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Abstract: The Alpine Lakes, their emissaries, and the irrigation network they feed support in the northern sector of the Po River Plain (Italy) intensive agriculture and animal farming. Flood irrigation and large availability of manure are thought to be responsible for diffuse nitrogen pollution of surface and groundwater. However, rigorous hydrogeological approaches analyzing the mechanisms linking agricultural practices with vertical and horizontal nitrogen paths are scarce. In this study groundwater quality and quantity were studied in an unconfined, coarse-grained alluvial aquifer close to the Mincio River (a tributary of the Po River), where steep summer gradients of nitrate (NO_3^-) concentrations are reported. The effects of manure on solutes vertical transport during precipitation events in fertilized and in control soils was simulated in laboratory conditions, which results showed high SiO_2 and NO_3^- leaching in fertilized samples. Similarly, field data are characterized by high SiO2 and NO_3^- concentrations, with a comparable spatial distribution but a different temporal evolution, suggesting their common origin but different processes affecting their concentrations in the study area. Our results show that SiO_2 can be used as a conservative tracer of manure spreading, as it does not undergo biogeochemical processes that alter significantly its concentrations. On the contrary, nitrate displays large short-term variations related to the aquifer recharge (i.e. flood irrigation and precipitations). It may promote immediate solubilization and stimulate nitrification, resulting in high NO_3^- concentrations, up to 95.9 mg/L and exceeding admitted thresholds. When recharge ends, anoxic conditions likely establish in the saturated zone, favoring denitrification and resulting in a steep decrease of NO_3^- concentrations.

Keywords: groundwater; nitrogen; silica; flood irrigation; agricultural practices

1 Introduction

For decades, water pollution has been claiming the attention of scientists and stakeholders all around the world as a growing threat to both environment and human health (Ward et al., 2018). In developing countries, the rising demand for food leads to the expansion and intensifications of cropping and animal husbandry (Bruinsma, 2003; Tilman et al., 2011). As a result, 70% of agricultural land worldwide is committed to livestock production, making it one of the major factors contributing to the environmental challenges from local to global scale (Steinfeld et al., 2006). Such anthropogenic pressures determine the alteration of nitrogen (N), phosphorous (P), and silica (Si) biogeochemical cycles, leading to eutrophication and degradation of the chemical and biological quality of the water bodies (Bernot & Dodds, 2005; Han & Allan, 2012; Mulholland et al., 2008; Paerl, 2009). In the European Union, two directives have been enacted to protect both superficial and groundwater ("EU Water Framework Directive," 2000; "Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration," 2006), nonetheless, according to the United Nations World Water Assessment Programme (2015) 38% of European water bodies remain under significant pressure from agricultural pollution.

Hydrogeology and freshwater ecology have often worked as detached fields, ignoring that they are naturally interconnected, both from a quantitative and qualitative point of view (Winter, 1999). Only in recent years, the contribution of hydrogeology for the interpretation of the surface water quality issues and the development of successful remediation strategies has been explored (e.g. Holman et al., 2008; Smolders et al., 2010), and the opportunity for a more collaborative and holistic approach has been understood. Regarding surface waters, it is well known that the excessive use of fertilizers in agriculture leads to a nitrogen over-enrichment in water via runoff and leaching (Cassman et al., 2002; Hart et al., 2004), promoting eutrophication in lakes (Hutchins, 2012) and coastal waters (Ruehl et al., 2007).

In the same way, several agricultural pressures can affect groundwater chemical quality, especially in areas where the aquifer is shallow (Bouwer, 1987; Bouwer et al., 1990). The copious use of fertilizers, including both synthetic fertilizers and organic manure, has been indicated as the main non-point source of contamination of groundwater (Baker, 1992; Chowdary et al., 2005; Liao et al., 2012; Postma et al., 1991). This is particularly true for nitrates, which are considered to be stored globally in great quantities in aquifers (Ascott et al., 2017). At the same time, hydrodynamic alterations are given by the groundwater abstraction from the aquifer for irrigation purposes (Leng et al., 2014) and the use of surface water for irrigation causing the recharge of the aquifer (Rotiroti et al., 2019).

The Po Plain is an alluvial plain in Northern Italy which represents a good example of an area with agricultural pressures, for both surface and groundwater, characterized by deep hydrological and land use alterations, with areas identified as vulnerable to nitrates pollution ("Commission Implementing Decision (EU) 2016/1040 of 24 June 2016 on granting a derogation requested by Italian Republic with regard to the Regions of Lombardia and Piemonte pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (notified under document C(2016) 3820)," 2016; "Commission Implementing Decision of 3 November 2011 on granting a derogation requested by Italy with regard to the Regions of Emilia Romagna, Lombardia, Piemonte and Veneto pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (notified under document C(2011) 7770)," 2011; "Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources," 1991). Several authors analyzed the groundwater and surface water pressures in various regions of the Po Plain and at various (temporal and spatial) scales, using different approaches (Lasagna et al., 2016; Martinelli et al., 2018; Sacchi et al., 2013; Soana et al., 2011; Zanini et al., 2018). The Lombardy region (north-center part of the plain), represents an interesting study case, due to the intensive agricultural production and irrigation practices. Here, since the middle ages, agricultural activities have been supported by a capillary network of irrigation channels (Fantoni, 2008), leading to a deep modification of the natural hydrological cycle, due to the enormous amount of water deflected from the Northern Alpine lakes and their emissaries. Moreover, several articles where soil system budgets have been calculated at the watershed level, report widespread and large nutrients surplus (Pinardi et al., 2018; Soana et al., 2011; Viaroli et al., 2018). These articles postulate a mechanism linking fertilizer surplus with river quality in specific river sectors, mediated by large water demanding irrigation techniques and precipitation, favored by the permeability of soils (Bartoli et al., 2012; Laini et al., 2011; Racchetti et al., 2019). However, such mechanism is hypothesized but not verified through a rigorous approach including the hydrogeological characterization of specific portions of the watershed and specific river sectors.

This work aims to fill this gap, providing scientific support to the nitrates contamination of groundwater postulated in these previous works. To this purpose, we coupled seasonal groundwater analyses with laboratory tests to investigate solutes leaching from fertilized soils under controlled conditions. Besides inorganic nitrogen, we focused also on reactive dissolved silica due to discrepancies between soil system mass budgets of this element and its loads in surface waters [37]. In particular, the latter resulted in much higher concentrations than those expected from mass budgets, suggesting overlooked release from fertilizers.

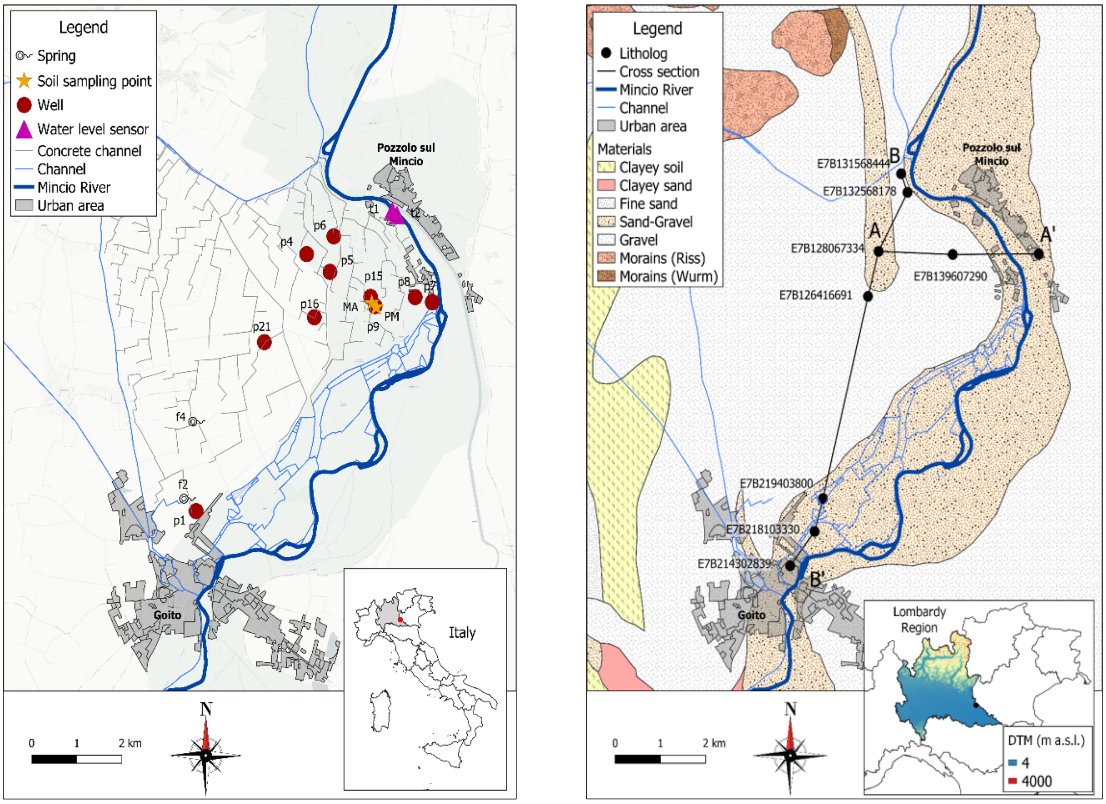
We hypothesized that both high SiO_2 and NO_3^- concentrations in groundwater can be related to manure spreading. We also hypothesized that groundwater chemistry depends upon the aquifer recharge rate. In particular, during flood irrigation or abundant precipitations, high recharge rates occur with oxic water, favoring large conversion of organic nitrogen into soluble nitrate via coupled ammonification and nitrification and large solutes vertical transport, including reactive silica. After the recharge phase, water saturation and redox changes may promote the loss of nitrates, where the concentrations of other solutes may remain unaltered.

2 Materials and Methods

2.1 Study area

The experimental site (Figure 1a) lays on the right bank of the Mincio River, between the villages of Pozzolo sul Mincio and Goito (Mantua province, Lombardy).

The Mincio River is a 75 km long river originating from Lake Garda and flowing to the Po River. From the geological point of view (Figure 1b), the Po plain is characterized by an extended multilayer aquifer made up of middle-upper Quaternary succession sediments of the Po Basin. Generally, the shallow aquifer is unconfined, unlike the deeper aquifers which are semiconfined or confined (Martinelli et al., 2018). Remaining on a regional scale, the aquifer is made of alluvial plain deposits characterized by a cyclic architecture of fluvial-channel facies (made of gravel and sand) and overbank facies (made of silts and clays) (Amorosi et al., 2008), which act as aquitards or aquicludes from local to regional scale (Chelli et al., 2018). From a geomorphological perspective, the area is characterized by the Po plain main level (Guzzetti et al., 1997) dissected by the Mincio River, whose erosive process formed four orders of fluvial terraces (Chelli et al., 2018) and which now flows in Holocene deposits (Marchetti, 2002). The Po plain is also characterized by several springs (the so-called *fontanili*), that are small, semi-artificial, aquatic ecosystems sensu Kløve et al. (2011). In the study area, several *fontanili* have been found, generally exploited by farmers for irrigation purposes.



(**a**) (**b**)

Figure 1. Schematic map of the study area (a) Location of monitoring and sampling points; (b) Geological map with digital terrain model of the Lombardy Region. AA' and BB' indicate the cross-sections shown in Figure 2.

The hydrological regime of the Mincio River is controlled by a dam immediately downstream the Lake Garda and another one just upstream Pozzolo sul Mincio village. These dams regulate the river flow according to the irrigation needs of the surrounding agricultural area during the irrigation period, which starts before the end of April and ends within September. As consequence, the Mincio River has a higher discharge during the irrigation period (Pinardi et al., 2018). The dams, together with several weirs, provide water for the bountiful network of irrigation channels, summing 173 linear km in the study area and including both natural (e.g. with vegetated banks and bottom sediments) and artificial ones (e.g. made of concrete). The Mincio River basin is characterized by intensive agriculture and livestock farming. The Utilized Agricultural Area covers ~ 70% of the watershed (850 km^2), where the main cultivated crops are maize (30%), feed crops (27%), wheat (11%), and permanent grassland (9%) (Italian National Institute of Statistics, 2010). The livestock farming accounts for 136×10^3 cattle and 483×10^3 swine, whose manure together with synthetic fertilizers, is used in the Mincio River basin to improve crop production (Pinardi et al., 2018). In the Mantua area, manure is applied usually in autumn, when the volumetric capacity of the slurry tanks is usually reached, forcing farmers to spread (Perego et al., 2012).

The climate of the study area is humid subtropical (Cfa), according to the Köppen classification. The climatic data from the Goito meteorological station of the Regional Environmental Protection Agency of Lombardy (ARPA Lombardy) mean annual temperature is 14.2° C, while the mean annual precipitation is 765.2 mm/y. The precipitations are higher in spring and autumn than in summer and winter.

2.2 Hydrogeological characterization

The geological model of the study area has been reconstructed through 20 lithological logs of boreholes from the Lombardy region dataset [49]. The hydraulic heads have been measured monthly, from May to December 2019, in ten wells screened within the shallow unconfined aquifer. The heads were measured through a water level meter to reconstruct the groundwater flow net and analyze the effect of irrigation on groundwater dynamics. Hourly based precipitations data in the study area were downloaded from the Goito meteorological station (ARPA Lombardy). Wells altitude (m a.s.l.) were obtained from the national Lidar dataset (± 15 cm altimetric accuracy and ± 30 cm planimetric accuracy) and compared to the Regional Technical Map (CTR; 1:10.000 scale). The Mincio River stages were measured with two radar water level sensors from the Interregional Agency for the Po River (AIPo), located upstream and downstream the Pozzolo dam. Data from these two sensors have been integrated in the phreatic surface reconstruction.

2.3 Sampling and physico-chemical analyses

During the hydraulic head measurement campaigns, groundwater samples in wells were taken using a bailer sampler, while the two springs were directly sampled using a 1 L polyethylene bottle. For every sample, water was collected using 1 L polyethylene bottles, where EC, pH, and temperature were measured using a multi-parameter probe (HI9829 HANNA Instruments). Water samples were then filtered with a 0.7 µm pore size glass fiber paper filter and stored in a refrigerated case till analyses, carried out within 24 hours from collection. Water samples for ions analyses were further filtered with a 0.2 µm pore size glass fiber paper filter. For silica, filtered water samples were stored in 50 ml polyethylene bottles. For the analyses of ions, 20 ml plastic vials have been used and then the samples for cations were acidified with 2M HNO_3 to avoid metals precipitation (A.P.H.A. et al., 1981). In the laboratory, anions and cations were analyzed by ion chromatography (883 Basic IC plus Metrohm) and silica was determined by spectrophotometry with the same method explained in laboratory tests (see the next paragraph).

2.4 Soil leaching tests

A physical analogue model was carried out using column experiments to analyze the changes in water chemistry after percolation through bare soil and soil amended with manure. To this purpose, two columns were packed with different types of bare soil (control), while two columns were packed with the same soils amended with manure to simulate fertilization. Simultaneously, the effects of longer interaction time between water, soil, and manure were investigated using soil suspensions in water.

In the column experiments, soil samples were taken from adjacent maize agricultural areas (indicated as MA) and permanent grasslands (indicated as PM) reported in Figure 1a. The soil samples were immediately transferred to the laboratory where they were mixed into a single homogenate for each typology (MA vs PM), from which subsamples were placed in a standard permeameter (81.07 cm^2 by 11 cm deep; MaTest, Treviolo, Italy). Water was applied from the top of the permeameter, simulating precipitation in the agricultural field. To this purpose, a peristaltic pump (Watson-Marlow, Wilmington, Mass) provided a constant flow through the soil sample. We simulated a single precipitation event (3 hours and 20 minutes long) of 70 mm, which was registered by the Goito meteorological station in November.

Two additional columns were realized by mixing manure with the soil from maize agricultural areas (indicated as MAM) and permanent grasslands (indicated as PMM) before placing the soil in the permeameter, to simulate plowing, which is usually carried out after the manure distribution on soil. Before mixing, manure was dried in oven until constant weight and milled to a fine powder. The percolating water was collected every 20 minutes and stored in 50 ml polyethylene bottles. After the measurement of EC and pH, it was filtered and analyzed for dissolved inorganic carbon, reactive silica, nitrate, and ammonium. Dissolved inorganic carbon was analyzed by titration with 0.1 N HCl (TIM 90 Radiometer; Anderson et al., 1986). SiO_2 was analyzed by spectrophotometry (Novaspec II Pharmacia; Lienig, 1980). NO_3^- and NH_4^+ were determined by spectrophotometry according to Rodier et al. (1975) and A.P.H.A. et al. (1981), respectively. Considering a mean percentage of N in manure equal to 1.5% (Eghball et al., 2002), in these two simulations 16.21 g of manure were added, equivalent to 250 kg N ha^{-1} y^{-1} set by the derogation to the Nitrates Directive in the Lombardy region ("Commission Implementing Decision (EU) 2016/1040 of 24 June 2016 on granting a derogation requested by Italian Republic with regard to the Regions of Lombardia and Piemonte pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (notified under document C(2016) 3820)," 2016).

For the flask tests, nearly 1.7 g of soil was suspended in 100 ml of water in 250 ml flasks and left on an orbital shaker for 2 h at 600 rpm. This test was carried out in four different treatments (MA, PM, MAM, PMM, as detailed above) with three replicates each. In the MAM and PMM flasks, 0.2 g of manure were added besides soil, equivalent to 250 kg N ha^{-1}. After the 2 hours incubation, samples were centrifuged until the separation of liquid from solid phase (5 min at 3000 rpm), then the EC and pH were measured and samples were filtered and analyzed for reactive silica, dissolved inorganic carbon, nitrates and ammonium as described for the column tests methods.

For all the soil leaching tests, the leachate was characterized by a brown color given by the dissolved organic matter and was heavily diluted to minimize the interference given in spectrophotometric analyses (from 1:10 to 1:50, according to color intensity and expected sample concentrations). Nonetheless, the sample's background color was registered and subtracted for every wavelength in all the samples. Each chemical analysis was performed by the same operator on the same instrument using the same standards. For both column and flask tests, soil and manure were sampled in the study area.

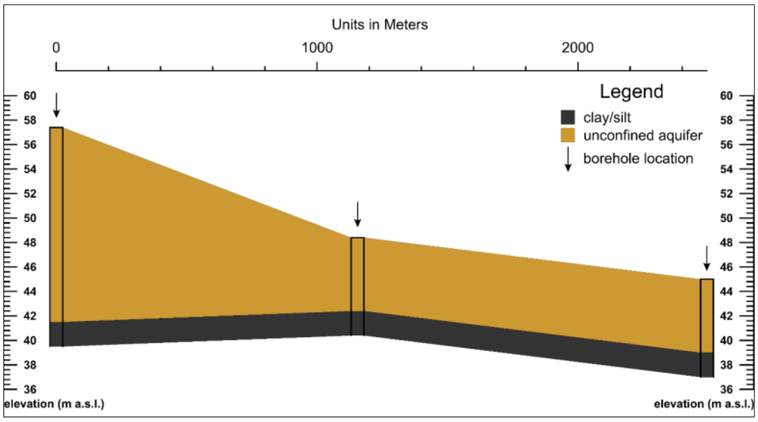
3 Results

3.1 Geological and hydrogeological settings

study site from which two geological cross-sections are presented (Figure 2). Based on logs data, the unconfined aquifer is made of gravels and subordinately sands. Moving from regional to local scale, the cyclic architecture proposed by Amorosi et al. (2008) is confirmed: below the ground surface the stratigraphic sequence begins with gravels and sands belonging to the fluvial-channel facies. The shallow unconfined aquifer has a thickness varying from 22 to 2 m moving from N-W to S-E. Beneath the shallow aquifer, a continuous bed of fine-grained-sized deposits has been found, which belongs to the overbank facies and is constituted by clay and silt, with a thickness varying from 2 to 10 m.

The hydraulic properties of the unconfined aquifer have not been investigated yet at the study site. However, a transmissivity of 1.0×10^{-2} m^2s^{-1} and a storativity of 1.49×10^{-2} were calculated through pumping tests in a nearby area (Chelli et al., 2018).

Overall, the shallow groundwater flows from NW to SE, towards the Mincio River (Figure 3). The groundwater heads show seasonal fluctuations of different proportions (Figure 4). Irrigation water is provided in negligible volumes when rainfalls are abundant. Thus, in May 2019 groundwater heads were affected more by precipitation (that was unusually abundant) than by irrigation. From June to August 2019, considering the low amount of precipitation and the relatively high evapotranspiration, groundwater was mainly recharged by irrigation (Figure 4), rising groundwater heads to their maximum during the observation period.



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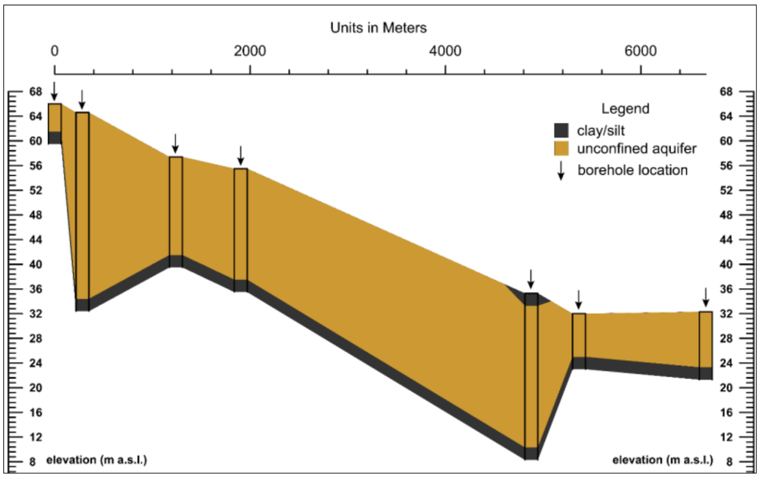


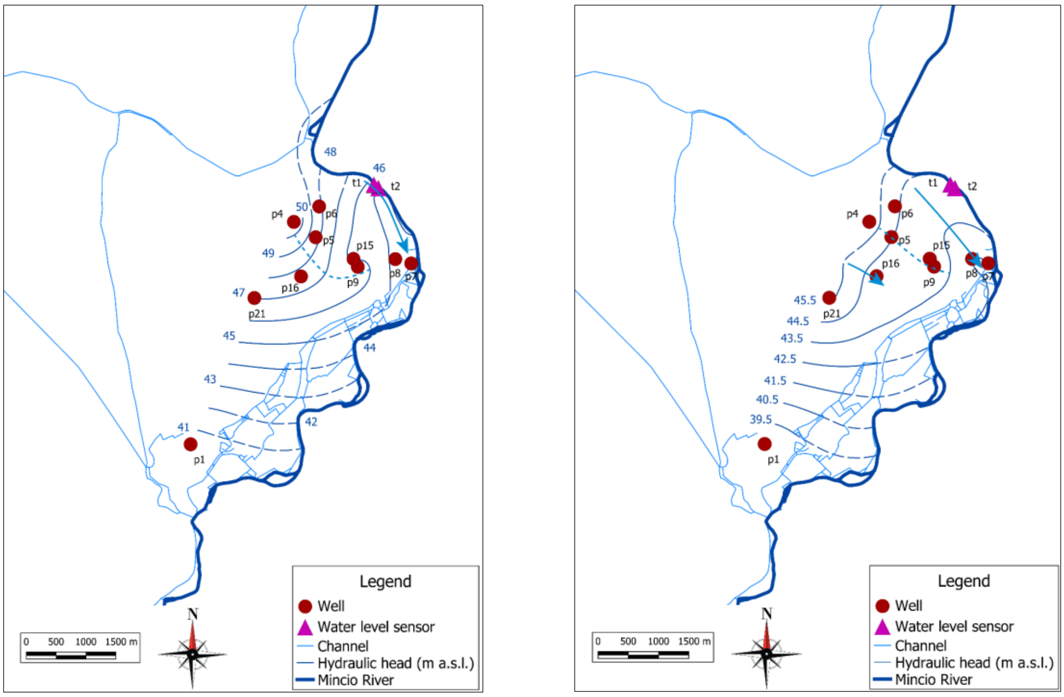
(**b**)

Figure 2. (a) Geological cross-section AA'; (b) Geological cross-section BB'. The location of both sections is shown in Figure 1.

According to similar timing reported around the Lombardy plain (Perego et al., 2014), from late August within the end of September maize, which is the main cultivated crop, is harvested. Thus, water from the Mincio River is no longer used for irrigation purposes and groundwater heads in the study area begin to lower. In November, the abundant precipitations slow the lowering of the groundwater surface, stabilizing groundwater heads, which remain nearly constant until December. The rapid response of the phreatic surface agrees with the high permeability of the aquifer and the low thickness of the unsaturated zone.

The groundwater flow net showed some minor differences between the irrigation and non-irrigation period (Figure 3a, b). In the northern part of the study area, a main drainage axis and a groundwater divide have been identified. Their shapes are accentuated in summer by the higher hydraulic gradient, due to higher groundwater heads and nearly constant (fixed and regulated) water level in the Mincio River.

During the irrigation period, near the Pozzolo dam the hydraulic gradient has an average of 0.02, while at W of the drainage axis is slightly higher (0.03). In the rest of the investigated area, a nearly constant hydraulic gradient is inferred (0.002), lowering S-SE towards the Mincio River. During the non-irrigation period, the hydraulic gradient slightly decreases to 0.001 in this zone.



(**a**) (**b**)

Figure 3. Groundwater flow net during irrigation [July 2019] (a) and non-irrigation [December 2019] (b) periods.

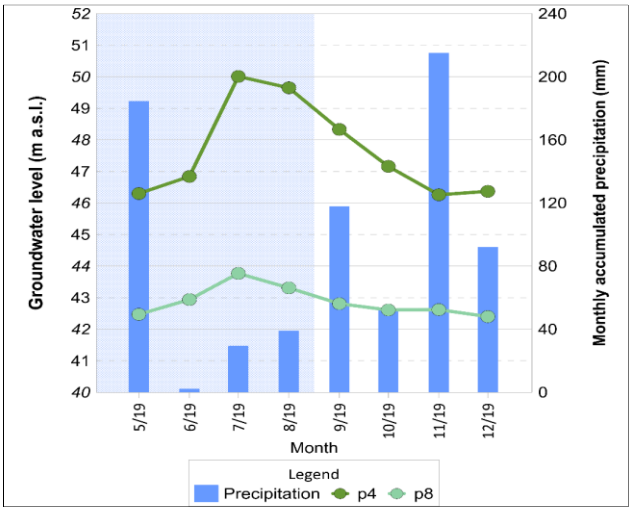


Figure 4. Groundwater level fluctuations in wells p1, p4, and p8 (markers with straight lines) and monthly precipitations from the Goito meteorological station (bars). The shaded area includes the irrigation period.

3.2 Flasks tests

The soil suspension in flasks has a slightly different meaning as it provides the potential release to or retention from the water of solutes. It is a potential measurement as the soil is suspended in water and continuously mixed, something that does not happen under in situ conditions. However, due to oxic conditions during the suspension, the amounts of released solutes (e.g. nitrates) should be comparable to those of the percolation test.

Despite the different interaction between water and soil, flask tests produced results similar to those of the column tests, with some differences in NO_3^- concentrations (Figure 5, Table S1).

SiO_2 concentrations were similar to those found in the column tests, with higher values in fertilized samples. NO_3^- displayed a higher variability from the collected soil, emphasized by the small amount used. Here, the differentiation between fertilized and non-fertilized flasks was less marked. EC data showed a net difference between fertilized and non-fertilized soils, like in column tests, with a small variability inside the three replicates. pH results were characterized by a higher variability within replicates, especially PM, with a standard deviation – SD = 0.44. Nevertheless, fertilized soils produced higher pH values. In HCO_3^- results, the differentiation between tests is clear, with higher dissolved inorganic carbon in PMM and MAM, like in the column tests. NH_4^+ results are similar to those from column tests, with higher concentrations in samples amended with manure.

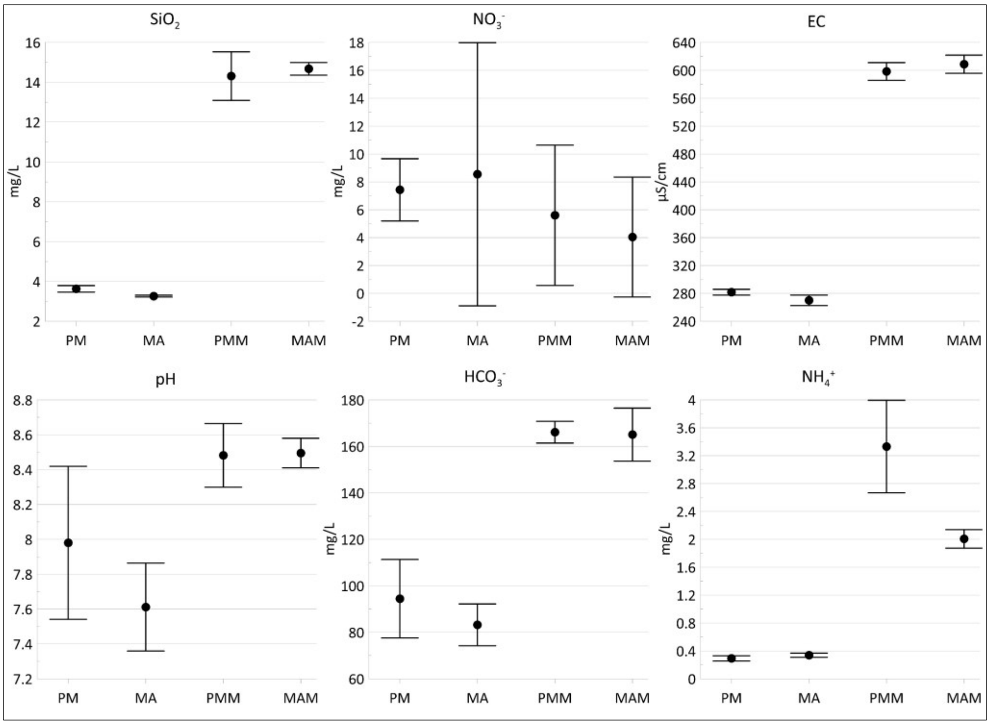


Figure 5. Physico-chemical parameters analysed during the flask tests. Means and standard deviations (n=3) are presented.

3.3 Column tests

When analyzing the variation of physico-chemical parameters in the column tests, large variations of solute concentrations are observed (Figure 6), with values greater than those measured in the study area during the sampling period. These differences are due to the amount of fertilizer used in each test, equivalent to the bulk that should be used in 1 year, according to the derogation to the Nitrates Directive in Lombardy region ("Commission Implementing Decision (EU) 2016/1040 of 24 June 2016 on granting a derogation requested by Italian Republic with regard to the Regions of Lombardia and Piemonte pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (notified under document C(2016) 3820)," 2016). Remarkable differences between fertilized and non-fertilized soil were apparent in all the column tests (Figure 6 and Table S1).

Starting from SiO_2, results showed a net discrepancy between fertilized and non-fertilized soils, with decreasing concentrations along with the PMM, MAM, PM, and MA treatments, respectively. In the column tests, MAM and PMM presented higher NO_3^- concentrations in the first samples, reaching 1014.55 mg/L NO_3^-, and comparable to those found in similar studies such as Mantovi et al. (2006). After that, concentrations decreased rapidly along time, reaching values even smaller than those obtained from non-fertilized tests. In the last MAM sample, NO_3^- concentrations were lower than the detection limit of the method and approximated to 0. In MA and PM tests, NO_3^- concentrations were initially lower than fertilized tests samples, but with a less pronounced decrease. As a consequence, NO_3^- concentrations in the last samples were higher, suggesting a slower and more constant leaching. The analyses of NH_4^+ concentrations, that are generally low in the study area, revealed marked differences between fertilized and non-fertilized samples. MA and PM conditions have a nearly constant concentration in all the collected samples, with mean values of 1.07 mg/L and 2.06 mg/L, respectively. MAM samples were almost constant too, with a mean value of 5.69 mg/L. On the other hand, PMM samples showed the highest concentrations but with a decreasing trend, varying from 14.5 mg/L to 9.99 mg/L.

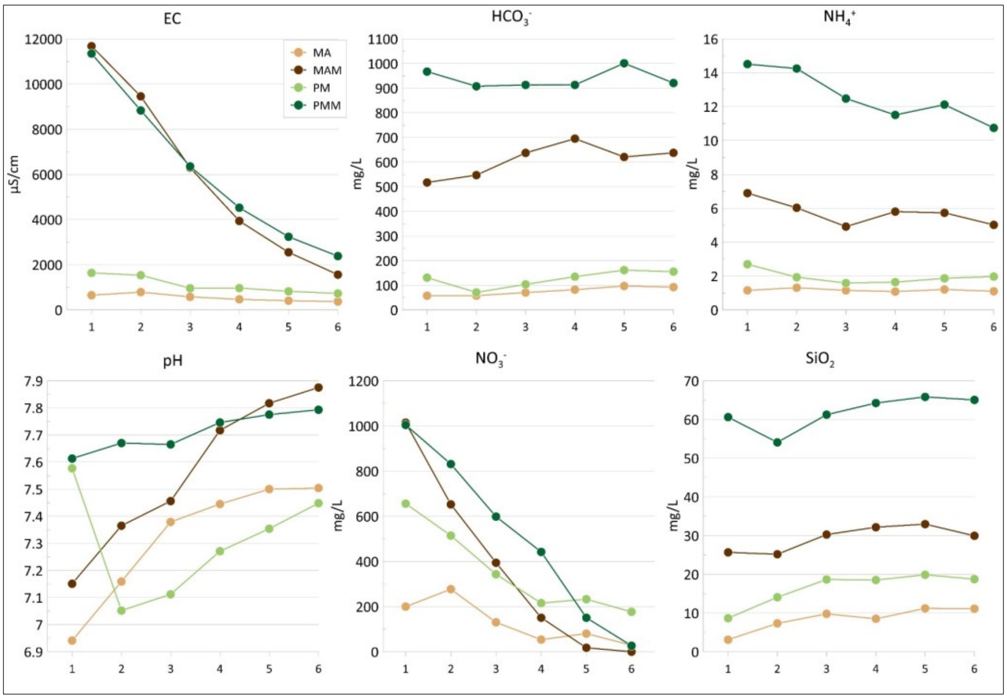


Figure 6. Physico-chemical parameters analysed during the four column tests and their variation in the samples in succession.

NO_3^- concentrations in the percolated water showed a trend similar to EC. Regarding EC, the first leached water collected from the permeameter is marked by high conductivity for all the tests, reaching 11347 µS/cm. PM and MA tests produced leached water with similar EC trends, even if PM values are higher, especially in the first collected samples. Manure addition led to similar results within fertilized and non-fertilized tests, minimizing the soil differences from different agricultural soils.

In the PM test, HCO_3^- concentrations initially decreased and then slowly increased, returning to the initial values, while MA test is marked by a slight increase during time. The HCO_3^- values measured during the fertilized tests were unexpectedly high, underlying the effect of manure spreading on dissolved inorganic carbon concentrations in leachate water. In all the tests, an increase in pH was observed during time, even if PM test showed an initial drop. MAM samples displayed the highest increase, varying by 0.7 pH units.

3.4 Groundwater contamination

During the monitoring period, the concentrations of some analytes remained nearly constant, e.g. Na^+, K^+, F^- and NO_2^-. NH_4^+ was detected only sporadically. On the contrary, EC, NO_3^-, SO_4^{2-}, Ca_2^+ and SiO_2 were characterized by substantial variations (Table S2, Figure 7).

EC is characterized by high fluctuations (min = 328 µS/cm, mean = 515 µS/cm, max = 687 µS/cm), with higher values in autumn. Among the analyzed cations, only Ca^{2+} varied during time (min = 18.96 mg/L, mean = 64.9 mg/L, max = 120.51 mg/L), with lower values during summer and higher concentrations in November and December. Within the analyzed anions, only sulphates (min = 7.63 mg/L, mean = 16.25 mg/L, max = 22.18 mg/L) and nitrates (min = 0.79 mg/L, mean = 26.59 mg/L, max = 95.94 mg/L) varied during sampling time. SO_4^{2-} concentrations are marked by lower values during the irrigation period and higher concentrations from September to November.

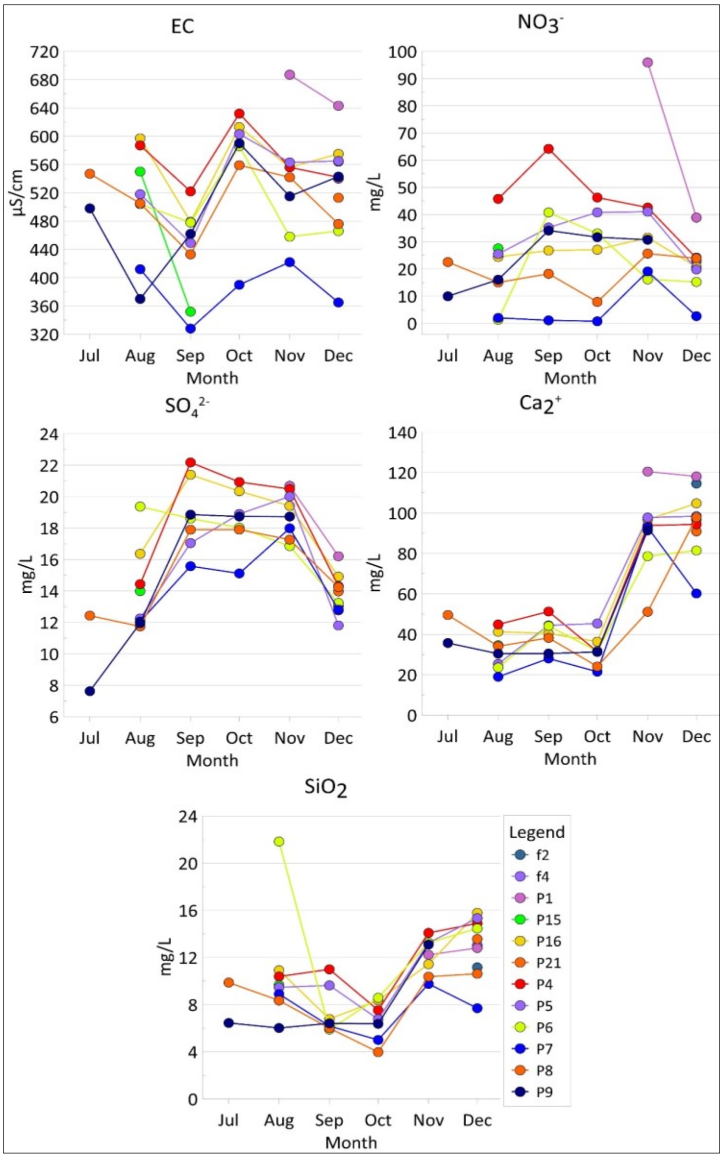


Figure 7. Main physico-chemical parameters of groundwater in the study site.

NO_3^- concentrations showed an increase in September and November, exceeding in some cases the limits of 50 mg/L set by the WFD ("Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources," 1991). In the area where the wells network allows a reliable analysis, the spatial distribution of NO_3^- concentrations is in agreement with the groundwater flow net (Figure 8a), suggesting that the main origin of the contamination is located upstream the study area. The concentrations progressively decrease along the groundwater flow path, according to the expected dispersion within the saturated medium. In the study area, SiO_2 showed marked variations during sampling time (min = 3.97 mg/L, mean = 10.17 mg/L, max = 21.84 mg/L), with higher values measured in November and December. The spatial distribution of silica (Figure 8b) overlaps that of nitrates, indicating a possible common origin.

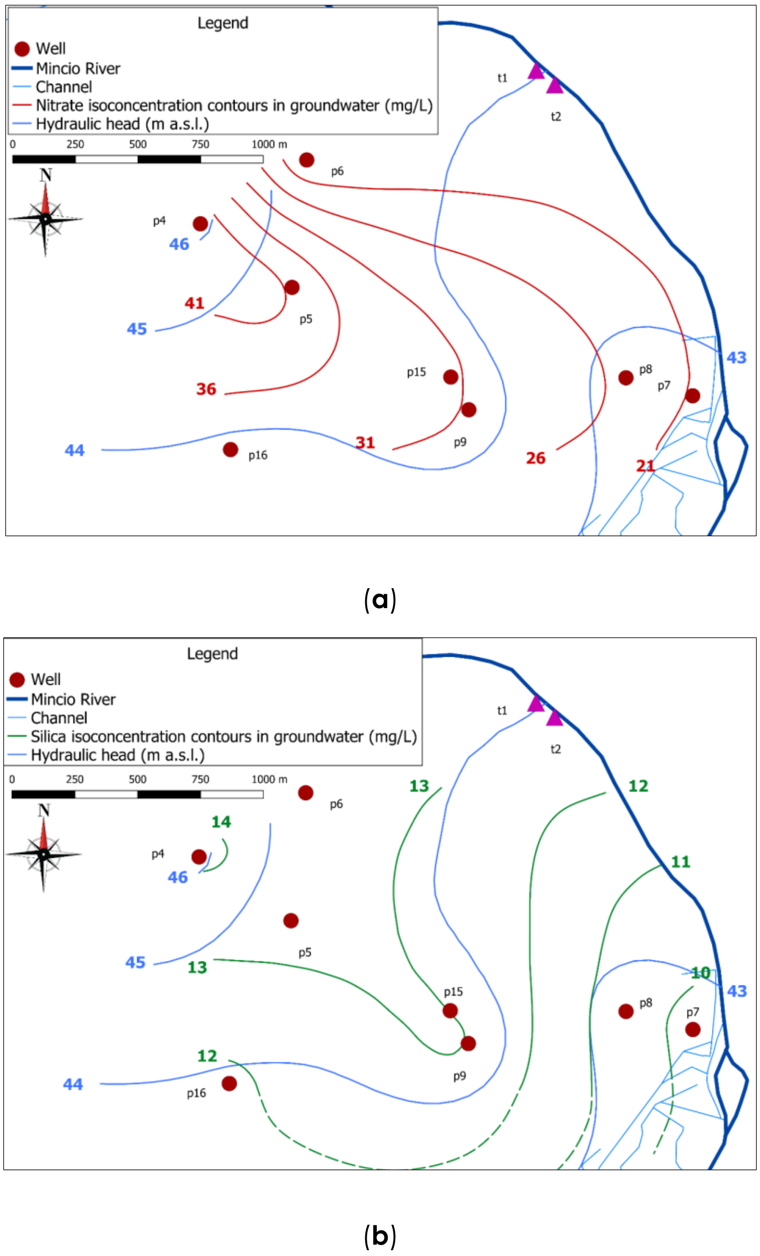


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Figure 8. (a) Distribution of nitrate concentrations in the northern part of the study area (November 2019); (b) Distribution of silica concentrations in the northern part of the study area (November 2019).

4 Discussion

4.1 Manure, soil, and water interactions

Column tests were already reported as a useful tool to analyze the chemical evolution of water percolating through the soil for hydrogeological purposes

(Boschetti et al., 2014). Thus, we performed column tests to investigate how the manure addition can change leachate physico-chemical characteristics. Repeated water sampling and analyses showed how water chemistry varies over time along its vertical path in the upper unsaturated aquifer. It is expected that soluble compounds are firstly leached showing a peak and then concentrations decrease, whilst other solutes might be lost from soil due to water saturation and redox-dependent processes, and therefore display a delayed release. Soil might also retain some chemical forms, decreasing their concentrations in the percolating water.

Moreover, the comparison between these results and those acquired in the field allows us to understand further processes affecting groundwater hydrochemical parameters in the study site. In column tests, permanent grasslands samples (PM and PMM) showed higher concentrations compared to maize agricultural fields (MA and MAM), likely due to the greater organic matter content.

SiO_2 results from both laboratory tests reported a clear differentiation between bare soils and soils amended with manure. Thus, the origin of silica in the column tests can be related to the manure spread. Moreover, the rapid increase in SiO_2 concentrations could be due to more soluble Si forms in livestock diet, such as biogenic silica (BSi) (Cornelis et al., 2011). These findings are in agreement with other studies, which reported SiO_2-rich manure spread as a useful tool to restore the Si content in depleted agricultural soils (Song et al., 2014).

In the column experiments, high NO_3^- concentrations were expected, as this consequence of manure spreading in agricultural areas is well known (Wick et al., 2012). Interesting insights on NO_3^- concentrations can be inferred from the NH_4^+. Given the larger amount of NH_4^+ in manure, compared to NO_3^- (Kirchmann et al., 1989), PMM and MAM column tests showed higher nitrification potential, resulting in a rapid NO_3^- production from the applied NH_4^+. In the PM and MA column tests, the low NH_4^+ concentrations suggest the non-addition of manure, but the high NO_3^- concentrations are evidence of previous spreads to the fields, and of complete ammonium oxidation via nitrification. Additionally, their low NH_4^+ levels can be caused by irrigation events and the consequent leaching in the days preceding the sampling, resulting in a depletion of cation exchange sites and higher mobility of ammonium (Finck, 1983). Moreover, the higher NO_3^- concentrations in PM can be explained by higher numbers of nitrifying bacteria in this soil, which might be explained by higher ammonium and oxygen availability (Estavillo et al., 2002). These results suggest that fertilizer spread could be performed not only after the crop harvesting, due to the uncoupled number of farmed animals and arable land surface for manure spreading. The former, in this area of northern Italy, is in large excess (Viaroli et al., 2018). In addition, the high concentrations and the nitrate fast decrease in fertilized column tests suggest the quick leaching of recently applied manure when water applied to the top of the soil is abundant, as in Summer (due to irrigation, e.g. Racchetti et al. (2019)) or Spring and Autumn (due to precipitation, e.g. (Hess et al., 2020; Jabloun et al., 2015)). On the other hand, previous manure spreads with consequent NH_4^+ loss could bring to a lower leaching of nitrates. Conversely, flask tests resulted in no obvious differences in NO_3^- concentrations. In the fertilized flask tests, manure addition stimulated ammonia oxidation. However, the bacterial growth and associated nitrogen uptake might explain similar concentrations in the 4 treatments; alternatively, the higher organic content may have stimulated microbial respiration and the use of nitrate as electron acceptor. Thus, at the end of the incubation period nitrates concentrations were similar between fertilized and non-fertilized tests. Analogous nitrogen courses have been found by other authors in clayey soils of the Lombardy Region, where high residence time in soil leads to denitrification of manure (Zilio et al., 2020). Processes occurring in the flasks during the soil suspension did not significantly altered the ammonium concentrations. NH_4^+ has different values between fertilized and non-fertilized flasks, with the highest values in PMM test, as shown in the column test. Also, due to the high pH values in the PMM and MAM flasks tests, ammonium concentrations could have been underestimated. Differently from the permeameter where the NH_4^+ has been lost via nitrification and included in NO_3^- concentrations, here at high pH values part of the ammonium is converted from NH_4^+ to NH_3 (Emerson et al., 1975) (e.g. 18.2% at pH 8.5 at 28° C) which is lost via volatilization, underestimating the overall nitrogen concentrations.

Laboratory tests clearly underline how both SiO_2 and NO_3^- in leachate water originate from manure application on soil and that their temporal evolution undergoes a different regulation. If regarding NO_3^- the matter is well documented (Spalding & Exner, 1993), SiO_2 concentrations in groundwater were usually ascribed to mineral weathering, never taking into account the contribution of manure, especially where it is spread in high quantities. More importantly, our results show that SiO_2 concentrations in leachate water are nearly constant during time, both in column and flask tests. Such result, in the column test, is particularly interesting if compared to the temporal trends of most solutes. Nitrate is immediately solubilized and exported from the column with the percolating water; its concentrations decrease rapidly along with the temporal series. Reactive silica concentrations display a similar initial peak but they do not show a similar decrease suggesting much constant and slower release either from manure or from the soil. Moreover, reactive silica concentrations are not affected by biogeochemical processes in groundwater, such as nitrification or denitrification for nitrates. These results make SiO_2 a useful tracer (at least in the study area) when studying the impact of manure spreading on groundwater quality, with emphasis on nitrate contamination. They also may allow to explain discrepancies observed in recent comparison between Si mass budgets performed at the watershed level and Si loads measured in the Mincio River, with the latter much higher than those expected from mass budgets [Pinardi]

Our tests reported EC values in the flask tests closer to those registered in the field, while in column tests they were sensibly higher. Column experiments samples are characterized by a low correlation between EC and NO_3^- ($R^2 = 0.49$), while field samples are characterized by low values in the first three months (mean $R^2 = 0.455$, SD = 0.101) and higher values in the last three (mean $R^2 = 0.670$, SD = 0.082), supporting the idea of a major contribution of nitrates to the EC in groundwater compared to other ions.

Even if not analyzed for field samples, HCO_3^- analyses were performed due to the high amount of organic matter in the collected laboratory samples. It is well known that organic matter can modify pH in soil, which can consequently affect processes considered to be highly pH-sensitive such as nitrification (Curtin et al., 1998; Paul, 2014; Strayer et al., 1981). The HCO_3^- concentrations in column and flasks tests are in agreement among them and suggest also other effects of manure application. The high HCO_3^- concentrations could be function also of organic acid anions and CO_2 production. Regarding the first factor, this difference is given by the high amount of organic matter in leached water, suggested by the dark color of samples. The method for HCO_3^- analysis reported above is based on the total alkalinity, which can be altered by the organic acid anions present in water samples rich in organic matter (Lozovik, 2005), resulting in an overestimation of HCO_3^- concentration. Regarding the second factor, in other similar tests the manure addition caused an increase of HCO_3^-, due to higher production of CO_2 in soil (Guenzi et al., 1978), which is favored by irrigations (Chang et al., 1991). Flasks tests show pH values similar to the last samples of the column tests, suggesting the persistence of these conditions in more extended interaction times.

4.2 Groundwater contamination in the study area: insights from laboratory tests

The study area is characterized by an unconfined aquifer located below intensively cultivated agricultural fields. Here, from late spring to autumn the main source of aquifer recharge is the Mincio River, which largely exceeds the natural recharge given by precipitation during the irrigation period. The high volumes of water used for flood irrigation entail an unnatural rise of groundwater heads, incremented up to ~4 m during July. During the non-irrigation period, the phreatic surface is sensibly lower, with some shallow wells remaining dry (e.g. p15).

Regarding SiO_2 concentrations, the continuous and thick bed of fine sediments below the unconfined aquifer excludes the contribution given by deeper and slower groundwater circuits enriched in SiO_2. Their values in the study area started to rise in autumn, after manure spread. The highest concentrations are reached in the rainier months, i.e. November and December, as precipitations promote manure-associated solutes percolation in groundwater. SiO_2 concentrations were unexpectedly high and distributed in the study area similarly to NO_3^- (Figure 8b). Thus, according to its spatial distribution and the laboratory tests, its origin is attributed to manure spreading in agricultural fields, in which silica content may vary accordingly to the livestock diet. Additionally, high SiO_2 concentrations have been reported to increase nitrate mobility (Reithmaier et al., 2017) and water residence time in soil, promoting also denitrification (Schaller et al., 2020). This information, coupled with the data presented in this study, highlights the connection between SiO_2 and NO_3^- in the study area.

The NO_3^- concentrations have a spatial distribution similar to SiO_2 (Figure 8a). NO_3^- concentrations are characterized by higher values upstream the study area, decreasing progressively along the groundwater flow path. This result suggests the absence of groundwater contaminations from agricultural areas above the study sites, or at least not as significant as upstream. The progressive decrease of nitrate concentrations is due to hydrodynamic dispersion, probably coupled with denitrification and/or nitrate reduction. In fact, nitrate concentrations decrease faster than silica concentration along the groundwater flow path, therefore suggesting that the spatial distribution of NO_3^- must be influenced by biological factors together with the hydrodynamic ones.

On the contrary, NO_3^- and SiO_2 concentrations are characterized by dissimilar trends during sampling time, even if they both rise in September and November. As demonstrated in column and flask tests, N undergoes biogeochemical processes that do not affect Si, such as nitrification and denitrification according to redox conditions of groundwater. Simultaneously, nitrate is highly soluble and its production is limited by ammonium and oxygen availability whereas reactive silica production and accumulation undergoes a completely different regulation that is independent from redox conditions and may derive from both organic and mineral sources. The high NO_3^- concentrations in September and November can be the expression of manure spread and recharge given by irrigation and abundant precipitation, respectively. The recharge can lead to the vertical zoning of physico-chemical features within the shallower saturated zone, as reported by other authors (Petrella & Celico, 2013; Petrella et al., 2009). Here, the upper groundwater layer (where groundwater samples were collected for chemical analyses through a bailer) is mainly made of rainwater or irrigation water and characterized by oxic conditions. This implies that microbial communities cannot perform denitrification and/or nitrate reduction, and NO_3^- concentrations remain high. This process is similar to that observed in the PMM and MAM column tests, where applied NH_4^+ in manure were immediately converted in NO_3^- due to nitrification, resulting in high concentrations of the latter. However, when the aquifer recharge is minor or absent, the thickness of the upper and oxic groundwater layer becomes negligible, and microbial communities can perform denitrification and/or nitrate reduction. This can happen in October and December when there is no irrigation and precipitations are less abundant. This process is like that observed in the PMM and MAM flask tests, which can be associated with groundwater without recharge. Here, possible combinations of different factors, including low oxygen availability, limit the oxidation of NH_4^+ to NO_3^-, or favor the assimilation of nitrate or its reduction by microbial communities, resulting in no significant differences between fertilized and non-fertilized samples.

Summing up, manure spreading and the complex hydrogeological features of the study area, with flood irrigation as the dominant recharge path during summer, are drivers of groundwater chemistry in the study area. Groundwater monitoring alone is not sufficient to catch underlying processes whereas combined in situ characterization and laboratory experiments suggest the presence of solute-specific differential paths. Results from this work confirm the large manure excess in this geographical area and risk of water contamination, as indicated by nitrate exceeding concentration thresholds. More interestingly, it provides for the first time the evidence of manure-dependent SiO_2 accumulation in groundwater. In particular, our data support a similar origin of silica and nitrate (from manure spreading) and similar mechanisms of silica and nitrate transfer to groundwater (via irrigation and precipitation) but different temporal dynamics of the two solutes.

Nitrate dynamics strictly depend upon recharge and the recharge-dependent redox status and include rapid accumulation periods (positive redox, solubilization, and nitrification as dominant processes) followed by consumption phases (negative redox, denitrification as dominant process). Comparatively, reactive silica dynamics seem slower, as suggested by a much-delayed peak in groundwater concentrations.

5 Statement and open questions

This study points out that SiO_2 could be used as an effective tracer when studying the impact of manure spreading on groundwater quality. At the same time, it opens new questions regarding the processes affecting nitrate contamination of groundwater in the study area, as well as in other agricultural areas worldwide. The main open questions can be synthesized as follows. How much recharge can influence the vertical zoning of redox conditions in the saturated zone and how fast do these conditions evolve? How much the vertical heterogeneity of redox conditions in groundwater can allow the coexistence of nitrification or denitrification processes within the same saturated zone? How much the possible variations of the hydraulic head with depth (typical of heterogeneous alluvial aquifers) can minimize the vertical zoning of redox conditions, due to vertical flow and mixing within the saturated zone? How to characterize the microbial communities and activities in soil and groundwater to refine knowledge about biogeochemical processes discussed in this work? To start refining knowledge about these open questions, further investigations have been planned at the study site, through the adjustment of established interdisciplinary approaches (Bucci et al., 2011) merging hydrogeological (with emphasis on the utilization of multilevel piezometers; (Rizzo et al., 2020)), isotopic (mainly stable isotopes of oxygen and deuterium, and tritium), hydrochemical and biomolecular investigations usually applied in microbiological studies (e.g., DDGE (Crescenzo et al., 2017) and NGS (Mancabelli et al., 2020)).

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Laboratory tests samples physico-chemical parameters; Table S2: Groundwater physico-chemical parameters.

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Article

Short-term effects of the Nitrate Directive reintroduction: a case of study in a contaminated alluvial aquifer in northern Italy

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Abstract: The Po Plain (northern Italy) is one of the largest aquifers in Europe and 67% of the utilized agricultural land in this area is classified as Nitrate Vulnerable Zone (NVZ). However, it hosts intensive agriculture and livestock farming. In a stretch of the Mincio River (a tributary of the Po River), hydraulic heads, physico-chemical parameters of river and groundwater were monitored for a hydrologic year (2020-2021), to evaluate the effects of manure fertilization and flooding irrigation on surface- and groundwater chemistry. From 2020 the Nitrate Directive's fertilization limit was reintroduced and a comparison has been performed comparing surface- and groundwater data from the 2019 fertilization period (before limit reintroduction) and 2020 (after). Results suggest that in 2021 the phreatic aquifer displayed elevated nitrate (NO_3^-) concentrations, exceeding 50 mg L-1, although average values were lower than those of 2019. Nitrate loads in the Mincio River reached 6670 kg NO_3^- d^{-1} and resulted from the overfertilization in the surrounding area and the quick transfer of nitrogen from groundwater to the river. Compared to 2019, the river loads decreased by 59%, suggesting that the introduction of fertilization limits can produce measurable, short-term responses in alluvial aquifers.

Keywords: nitrate; European directive; river-groundwater interaction; Po Plain

1 Introduction

Although vital for society, agriculture and livestock are considered stressors for the hydrological and nutrient cycles in lowland ecosystems, especially when performed using intensive practices. Due to their fast response capability to increasing demand, intensive livestock production systems now account for prevailing shares of global swine, poultry meat, and egg production (56%, 72%, and 61%, respectively) and a significant share of milk production (Steinfeld et al., 2006). To maintain this production, livestock requests water, energy (in the form of carbohydrates), proteins, and 18 mineral nutrients, including phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), selenium (Se), copper (Cu), and zinc (Zn) (Steinfeld et al., 2013). A large percentage of the ingested substances, typically between 70% and 90% of the nitrogen (N) and the mineral nutrients present in the feed, are excreted via manure and urine. A large portion of the harmful environmental effects of intensive livestock production is related to the inadequate management of this livestock excreta, which contain large amounts of undigested organic matter and mineral nutrients (Schröder, 2005; Sims et al., 2005). The Po plain (northern Italy) is one of the most productive areas of Italy from the animal farming perspective, accounting for approximately 3.1×10^{6}cattle (about 50% of the national stock) and 6.0×10^{6} swine (about 65% of the national stock) (Bartoli et al., 2012). The livestock by-products are a precious resource for the intensive agriculture performed in the area. Together, livestock and agriculture contribute to almost 80% of the total N load in the Po plain, which is appraised at 550,000 t y^{-1}, leading to a diffuse NO_3^- contamination of both surface and groundwater (Cinnirella et al., 2005; Sacchi et al., 2013). In addition, the Po plain is characterized by one of the largest aquifers in Europe (Perego et al., 2012) and 67% of the Utilized Agricultural Area (UAA) was classified as Nitrate Vulnerable Zone (NVZ) (Italian National Institute of Statistics, 2010).

The NVZs were characterized during the reception of the Council Directive 91/676/EEC (Nitrate Directive) concerning the protection of water against pollution caused by nitrates from agricultural sources ("Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources," 1991). They are described as areas draining into "*waters affected and at risk of being affected by nitrates pollution as well […] where agriculture contributes significantly to this pollution*". One of the most important issues of the Directive is that in the NVZ, farmers cannot spread more than 170 kg N ha^{-1} y^{-1} derived from manure. This limit is applied all over the EU, notwithstanding whether waters are polluted or at risk of pollution, regardless of the climatic and soil conditions and the crops grown. Anyway, EU Member States can exceed the proposed limit requesting a derogation to the EU and proving "*with scientific evidence that for defined crops, in the local conditions the use of higher amounts will not hamper in any way water quality*". Italy requested this derogation and was successively granted to the regions of Lombardy and Piedmont. Here, farmers were allowed to exceed the 91/676/EEC limits and spread till 250 kg N ha^{-1} y^{-1}. Although the derogation fostered intensive agriculture in these regions, it also resulted in a (possible) worsening of the environmental conditions regarding the contamination by N and other nutrients *lato sensu*. Indeed, several authors described different anthropic pressures to the hydrosphere at different (temporal and spatial) scales and areas, using hydrological, biogeochemical, and isotopic approaches (Lasagna et al., 2016; Martinelli et al., 2018; Sacchi et al., 2013; Soana et al., 2011; Viaroli et al., 2018; Zanini et al., 2018). The overall picture describes a quite diffuse contamination (<50 mg NO_3^- L^{-1}) and non-homogeneous pollution (>50 mg NO_3^- L^{-1}) from nitrate in all the Po Plain in both surface- and groundwater. The

Italian derogation to the Nitrate Directive was active up to 2019. From 2020, farmers from the above-mentioned regions are obligated to the previous fertilization limit of 170 kg N ha^{-1} y^{-1}, which is expected to better protect surface- and groundwater from NO_3^- pollution.

Among the emissaries from the Italian northern alpine lakes, one of the relatively less investigated areas by the hydrogeological perspective is the Mincio River basin (Po plain). Here, livestock farming accounts for 136×10^3 cattle and 483×10^3 swine, whose manure is used in the Mincio River basin to improve crop production, consisting of maize, feed crops, wheat, and permanent grassland (Italian National Institute of Statistics, 2010). Previous work reported an anomalous rise of NO_3^- concentrations at the 33rd km of the Mincio River course (Pinardi et al., 2018b). The authors, after performing soil system budgets of the area, calculated a high surplus of NO_3^- in the system and subsequently postulated that the NO_3^- anomalies could be related to the groundwater feeding the river. This mechanism, likely linked to the high amount of manure used as fertilizer, was still unverified. In addition, the area is poorly monitored by the Regional Environmental Protection Agency of Lombardy (ARPA Lombardia), which groundwater quality monitoring network is limited in the northern part of the Mincio basin (Monti et al., 2021), and it is not possible to verify the contamination trend along time.

In this context, the main aims of this study were: i) the characterization of water quality between the 18th and 33rd km of the Mincio River through the entire hydrological year in both surface- and groundwater, to assess the possible NO_3^- contamination; ii) to explore the possible dynamics of the river-groundwater interaction, comparing the variations of the physico-chemical parameters along time in both surface- and groundwater; iii) the evaluation of the short-term effects of the limits' reintroduction on the use of fertilizers imposed by the Nitrate Directive in both surface and groundwater. The comparison regards a fertilizing period (autumn) in 2019 (250 kg N ha^{-1} y^{-1}) and 2020 (170 kg N ha^{-1} y^{-1}) and the effects on the surface- and groundwater NO_3^- concentrations. In surface waters, the comparison was performed between upstream and downstream the investigated reach of the Mincio River, comparing the NO_3^- loads between autumn 2019 and 2020.

We hypothesized a fast response of this system to decreased rates of fertilization. In this area, the flood irrigation and abundant precipitation over permeable soil determine simultaneously large vertical and horizontal transport of contaminants and relatively fast turnover of groundwater. We speculate that decreased nitrogen excess in cultivated soil would result in a lowering of groundwater nitrate concentrations thanks to less nitrate-rich percolating water. This mechanism is different from that described in other, less permeable cultivated floodplains where the response is much slower. We also hypothesize that groundwater feeds the Mincio River, especially in the central and southern part of the investigated area, where a joined effects of springs and diffuse feeding could be more appreciable.

2 Material and Methods

The investigated area (Figure 1) is located between the 18th and 33rd km of the Mincio River, between the Pozzolo sul Mincio and Goito villages (northern Italy). The area was already investigated in some portions by the hydrogeological perspective (Severini et al., 2021; Severini et al., 2020). The overall hydrogeological conceptual model is characterized by a phreatic aquifer with a thickness varying between 2 and 30 m, which thinner part is located near the Mincio River. The unconfined aquifer is made of gravels and, subordinately sands and corresponds to the fluvial-channel facies reported in Amorosi et al. (2008). Beneath the shallow aquifer, a continuous bed of fine grain-sized deposits is present, belonging to the overbank facies and constituted by clay and silt, with a thickness always larger than 2 m. In the area, the greater recharge is provided by the irrigation waters. During the irrigation period (April-September) the hydraulic heads raised up to 4 m in 2019 (Severini et al., 2020).

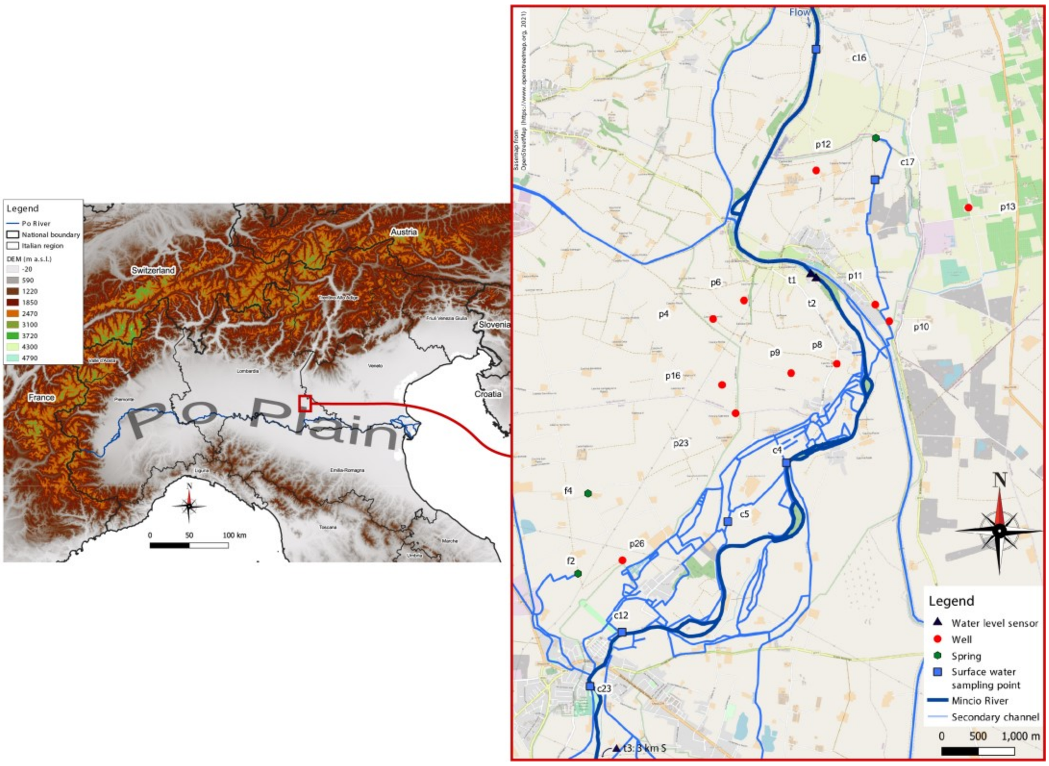
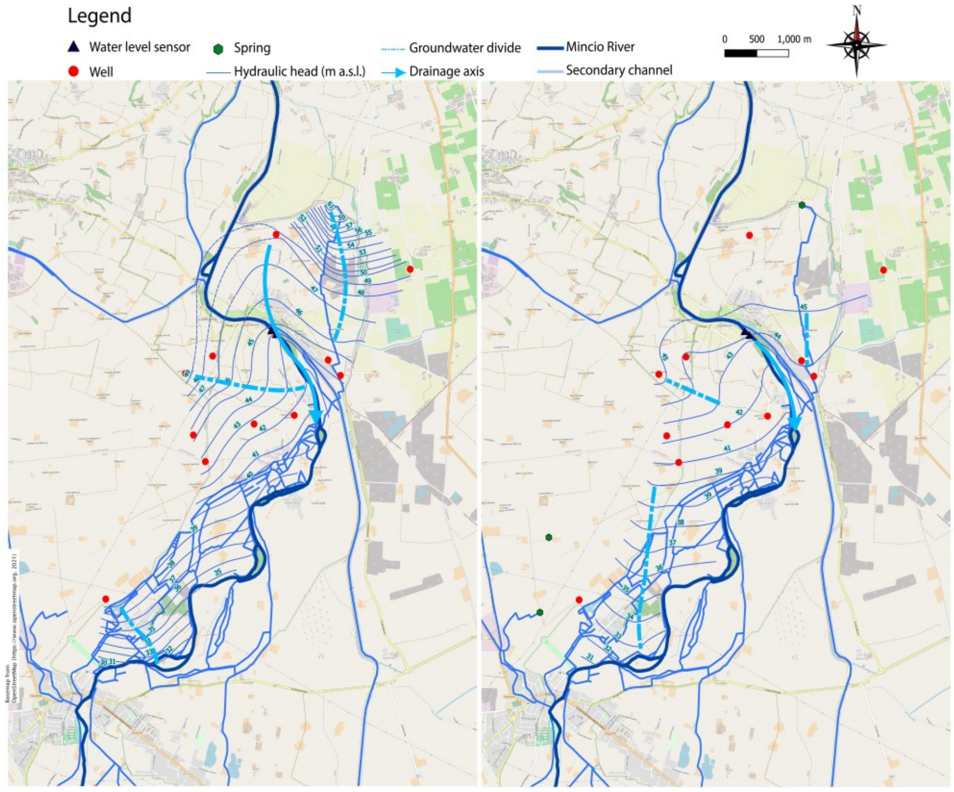


Figure 1. Digital Elevation Model of northern Italy. The investigated area is highlighted in red and reported in detail with the sampling points. Basemap from OpenStreetMap (https://www.openstreetmap.org, 2021).

The hydrogeological characterization was performed measuring the groundwater head in 11 wells to reconstruct the groundwater potentiometric map variations during the hydrological year, from June 2020 to May 2021. To include the Mincio River stages in the phreatic map, data from three water level sensors were downloaded from the Interregional Agency for the Po River ("Monitoraggio idrografico AIPo," 2021). The first two are located upstream (t1) and downstream

(t2) the dam of Pozzolo, whereas the third (t3) is located ~ 3 km S the investigated area (Figure 2). Given the absence of waterfalls and other similar altitudinal discontinuities along the river course, its water levels were interpolated using a constant gradient between t2 and t3. Together with quantitative monitoring, a qualitative sampling was performed. Wells (n=11) were sampled using a bailer sampler, whereas the other samples (n=8) were directly collected using 1 L polyethylene bottles. In the field, electrical conductivity (EC), pH, and temperature were measured using a multi-parameter probe (HI9829 HANNA Instruments, Woonsocket, RI, USA), calibrated the day before sampling. Successively, samples were prepared according to the required analyses. For the silica (SiO_2) analysis, samples were stored in 50 mL polyethylene bottles without filtration. For the other analytes, samples were filtered with 0.7 µm pore size glass microfiber filters. A sub-sample was stored in glass vials for the alkalinity analyse through 0.1 N HCl titration (Pearson, 1981), performed only during three samplings. For ions, samples were filtered with 0.2 µm pore size nylon filters and stored in plastic vials. In the case of cations, vials were acidified with 2 M HNO_3 till pH 2 to avoid metals precipitation (American Public Health et al., 2005). All the samples were stored in a refrigerated box until analyses, carried out within 24 h from the collection. In the laboratory, SiO_2 was analysed by spectrophotometry (Novaspec II Pharmacia) according to Golterman et al. (1980), waiting 10 min after the addition of reagents to permit the colloidal silica depolymerization and reaction with molybdic acid (Okamoto et al., 1957). Samples were successively centrifuged at 2000 rpm for 3 min to allow sedimentation of suspended material and the upper part of the vial was poured in cuvettes for the spectrophotometric reading. Cations and anions were analysed by ion chromatography (883 Basic IC plus Metrohm, Herisau, Switzerland). Data of the Mincio River concentrations from September to December 2019, used for comparison with the ones collected in 2020, were analysed with the same methodology. Each chemical analysis was performed by the same operator on the same instrument using the same standards. Meteorological data were collected from the meteorological station in Goito and downloaded from ARPA Lombardy (https://www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx).
In the investigated river reach more than 80 branches were identified, making the calculation of river flow difficult during the samplings. Water flow data were obtained by the Interregional Agency for the Po River (AIPO, https://www.agenziapo.it/) and by the Mincio Consortium (https://www.territoridelmincio.it/index.php).



(**a**) (**b**)

Figure 2. Potentiometric map. (a) Potentiometric map of August 2020; (b) Potentiometric map of March 2021. Basemap from OpenStreetMap (https://www.openstreetmap.org, 2021).

3 Results

3.1 Surface- and Groundwater Quantitative Monitoring

During the hydrological year, the groundwater flows from outer the investigated area to the Mincio River (Figure 2). The potentiometric surface map, although the variation of hydraulic heads, showed minor differences between the irrigation and non-irrigation period, i.e. between the recharge and recession period, with most of the characteristics constant along time. A main drainage axis is always located in the northern part of the study area (oriented N-S) along the Mincio River course, whereas a groundwater divide is always present in the eastern portion of the area (oriented N-S), although with different entities between the recharge and recession period. Some differences are reported for the western area. Here, two groundwater divides are identified, one near the main drainage axis (oriented W-E) and another one in the southern area (oriented NW-SE). The last one has a greater extent and a different orientation (N-S) during the recession period.

The recharge provided by precipitation is negligible during the irrigation period, also due to the low amount of rain and the relatively high evapotranspiration but can be significant during the rest of the hydrological year. The maximum recharge is given by the flooding irrigation, which deeply modifies the groundwater level from April to September (Figure 3).

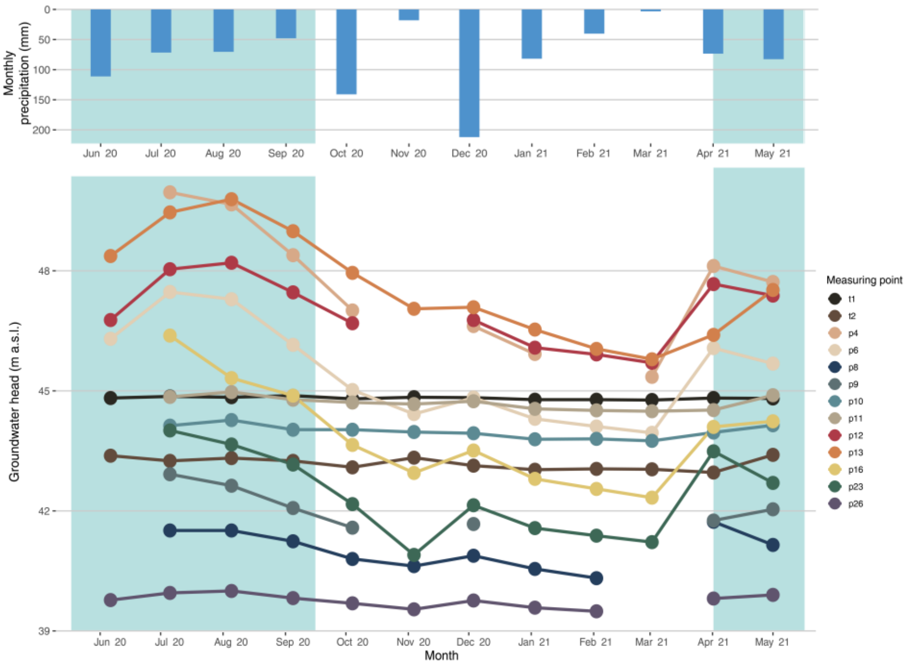


Figure 3. Groundwater level fluctuations in the monitored wells. The bar plot shows the monthly precipitation from the Goito meteorological station. The shaded area includes the irrigation period.

During this period, the highest hydraulic heads are reported (July 2020), whereas the recession period peaks in March, when the lowest groundwater heads were measured. The regression coefficients distribution in the study area highlighted two different zones. One is described by the wells near the Mincio River, with an average regression coefficient between the recharge and recession period of 2.92 × 10^{-3} d^{-1}. The other one is located around the wells more distant by the Mincio River, with an average regression coefficient of 0.02 d^{-1}. The hydraulic gradient shows three main areas of variation during the hydrologic year. The first is the western portion of the area, where it varies from a mean value of 0.002 during the recession period to 0.0046 during the recharge period. Near the groundwater divide in the northern area, two other hydraulic gradient values are identified. At East, a deep change is observed between the recharge period (0.009) and the recession period (0.001). On the contrary, at West, the hydraulic gradient remains constant during the investigated period. The overall variation between the recession and recharge period, is more emphasised far to the Mincio River, as inferable by the groundwater level fluctuations reported in Figure 3.

3.2 Monitoring of Water Chemistry of Surface- and Groundwater

A statistical summary is presented in Table 1, with median values (Median) and standard deviation (Std dev) for each measured parameter for all the 19 sampling points during the monitored period. Among the parameters measured in the field (Figure S1), the pH did not show specific trends. Interestingly, more basic values were measured in surface waters, up to pH 8.8, whereas groundwater samples had more neutral values. No specific trends were detectable along time but a general rise from February to May. The EC showed a clear difference between groundwater and surface water, with the last having lower values as expected. A heterogeneous but visible rise of the EC values was measured in December and March, whereas no peculiar lowering periods were detected. The temperature was homogeneous between surface- and groundwater samples during summer and spring. On the contrary, a net difference in temperature was measured during autumn and winter samples, with surface waters colder (up to 4.82 °C) than groundwater, especially in January. In summer, some surface water samples showed high temperatures (up to 25.89 °C), but within the expected seasonal variation of groundwater (Figure S2). Among the above-mentioned parameters, some sampling points showed peculiar trends. C5, a channel in the centre of the investigated area, showed usually values closer to groundwater than to surface waters, questioning the origin of its waters. F2, a spring in the southern part, showed intermediate values. EC and pH were like those of groundwater, whereas temperature was close to surface waters range. Lastly, P12 showed EC values like surface waters during the irrigation period, whereas the rest of the hydrologic year was similar to groundwater.

Table 1. Statistical summary median value (Median) and standard deviation (Std dev) for each measured parameter for all the 19 sampling points during the hydrological year. HCO_3^- were measured only in March, April, and May.

Parameter	Median	Std dev
t (°C)	17.430	3.466
EC (μS/cm)	526.500	136.375
pH	7.476	0.322
F^-	0.280	0.110
Cl^-	7.792	2.816
NO_3^-	12.072	15.977
SO_4^{2-}	12.918	7.002
HCO^{3-}	257.436	63.613
Na^+	7.111	1.076
K^+	2.108	2.295
Mg^{2+}	15.199	4.151
Ca^{2+}	81.978	22.549
SiO_2	8.470	4.374

Regarding the analysed ions, only a few showed notable trends along time, whereas the others were almost constant or vague (Figure 4). NO_3^- showed the most variable concentrations, peaking in three different moments (August 2020, December 2020, and April 2021) with different values (up to 95 mg L^{-1} NO_3^-), higher in groundwater and lower in surface waters. Similarly, sulphate (SO_4^{2-}) peaked in the same moments, but only in the right bank (eastern area) of the Mincio River and with higher concentrations, while in the left bank, only a peak in March and April 2021 was discernible, with lower concentrations. From a spatial point of view, the distribution of NO_3^- was constant along, time. The contamination source is in general located upgradient with reference to the investigated area, and the highest concentrations were measured in the wells at its boundaries, both in the eastern area (P13, 88.34 mg NO_3^- L^{-1} in December 2020) and western area (P4, 37.18 mg NO_3^- L^{-1} in December 2020). This suggests that the contamination is significantly higher outside the investigated area than inside. Two main source directions were identified upstream the wells P4 and P13. The only sensibly high contamination source inside the investigated area is located between the spring F2 and the well P26, which reached 95.03 mg NO_3^- L^{-1} in December 2020 and showed lower but high values in the other month. Moreover, some local and temporary contamination sources were found, like near the well P16 (75.54 mg NO_3^- L^{-1} in April 2021) or P23 (47.69 mg NO_3^- L^{-1} in June 2020). The complete dataset of NO_3^- concentrations during the investigated period is reported in Table S1. Silica (SiO_2), since it does not undergo biogeochemical processes (in groundwater), showed a different trend, but is still associated with those of NO_3^- and SO_4^{2-}. Starting from August, a small increase of concentrations was measured, but they decreased immediately and remained constant till December. Its concentration then increased from December till March, where the maximum values were detected, after which it started to decrease. During winter and spring, surface- and groundwater showed different trends. In groundwater, SiO_2 concentrations decreased from April but surface waters SiO_2 concentrations increased. A peculiar temporal trend of SiO_2 was distinguished in F2. Here, silica concentration surprisingly fluctuated like in surface waters, but with much lower concentrations (up to 0.16 mg SiO_2 L^{-1}). SiO_2 showed a spatial distribution like NO_3^- in almost all the investigated area. Thus, the contamination source is generally located upgradient with reference to the study area, with similar pathways from upstream wells P13 and P4. On the contrary, P26 was characterized by low SiO_2 concentrations. As a matter of fact, P26 and F2 are close and they both showed low SiO_2 concentrations but high NO_3^- values.

Point C5 (a secondary natural channel) reported anomalous values for the analytes in Figure 4, as previously described also for EC, temperature, and pH, confirming its similarity with groundwater. Fluoride (F^-) and chloride (Cl^-) exhibited unexpected and peculiar trends over time. F^- showed a concentration decrease in August, December, and April, but higher concentrations in September and March. On the contrary, the concentration of Cl^- raised in August, decreased in February, and finally reached the maximum values in March and April, similarly to constant concentration from September to January, to Cl^-, NO_3^- and SO_4^{2-}. Although not particularly noteworthy, calcium (Ca^{2+}) and magnesium (Mg^{2+}) did not show trends over time, but their concentrations were higher in groundwater than surface water, as expected.

Finally, in March, April, and May the HCO_3^- concentrations were calculated from total alkalinity and results were used to evaluate hydrochemical changes through Piper plots (Figure S3). The aim was the evaluation of potential chemical evolution of samples from the recession period till the recharge started in April. Some samples in April showed a consistent negative error in the ionic balance (P13, P16, and P23) probably correlated to titration of other alkalinities. Above all, the possible source of error can be given by ions from organic compounds like humic and fulvic acids, present in manure and percolating in groundwater with the aquifer recharge. Therefore, these samples were not considered in the Piper plot graphs. The recharge didn’t provide any chemical alteration to groundwater (and surface waters) and all the analysed samples can be considered as calcium-bicarbonate waters.

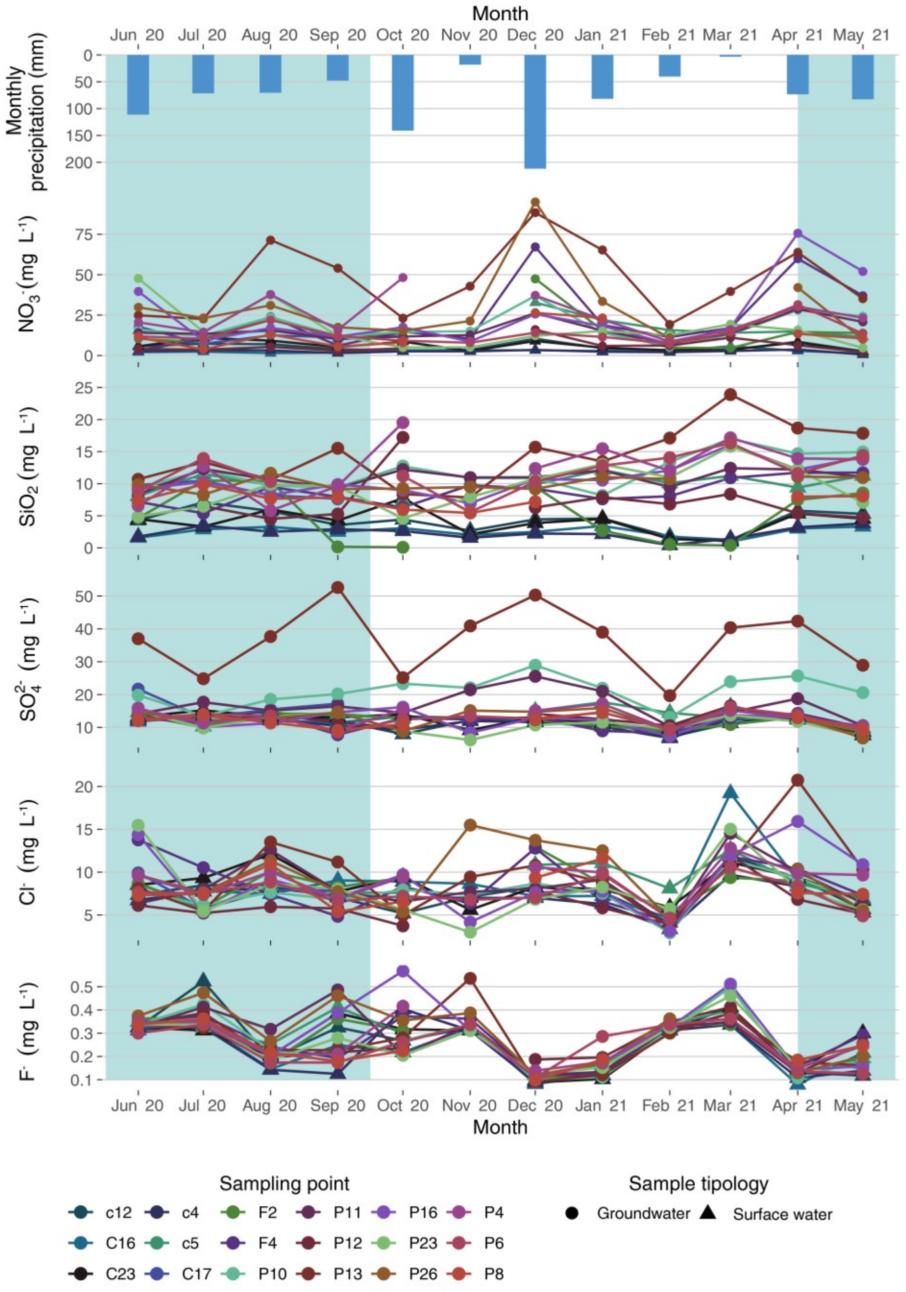


Figure 4. Chemical parameters variation along the hydrological year.

3.3 Mincio River NO_3^- Loads

An interesting result came from the comparison of NO_3^- and SiO_2 concentrations between C16 and C12 (Figure 5, Table 2-3). The first represents the Mincio River waters entering the investigated area and the second characterizes the same waters downstream the study area (Figure 1). In general, C16 showed always lower concentrations of the above-mentioned analytes, supporting an enrichment along the river course. An exception is reported for the NO_3^- concentrations in October, and March, when C12 had a lower NO_3^- concentration than C16. In February, they had the same concentration. On the contrary, SiO_2 concentrations were always higher in C12. For both, a clear temporal trend was observed, like that of NO_3^- concentrations in groundwater. Although this fact is intuitive for NO_3^- (the river load has a trend like groundwater concentration), it is not for SiO_2 (the river load has a trend similar to NO_3^- and not SiO_2 in groundwater). This evidence constitutes an essential proof to understand the river-groundwater interaction in the area. Assuming a constant river flow during the month, during the hydrological year the Mincio River potentially gained a total of 916.06 × 10^3 kg of NO_3^- and 614.18 × 10^3 kg of SiO_2 between C16 and C12. Thus, a clear increase in the concentration has been observed between C16 and C12.

Table 2. NO_3^- and SiO_2 concentrations and loads during the hydrological year 2020-2021. The C12 and C16 data represent the Mincio River concentrations, while their difference is expressed as daily load.

		River flow	C12	C16	Difference
	Month	m^3/s	mg L^{-1}	mg L^{-1}	kg d^{-1}
NO_3^-	Jun-20	8.8	5.60	2.10	2660.10
	Jul-20	13.9	7.32	2.40	5907.92
	Aug-20	13.9	7.01	1.52	6599.39
	Sep-20	13.9	3.84	1.84	2411.15
	Oct-20	10.8	3.13	3.27	-128.61
	Nov-20	10.8	3.48	3.23	237.43
	Dec-20	10.8	10.41	3.26	6670.37
	Jan-21	8.5	4.53	2.81	1269.14
	Feb-21	8.5	2.74	2.74	0.00
	Mar-21	8.5	3.55	5.73	-1603.94
	Apr-21	9.4	8.57	2.91	4593.20
	May-21	9.4	2.61	1.25	1105.51
SiO_2	Jun-20	8.8	4.275	1.579	2049.66
	Jul-20	13.9	7.099	2.892	5052.30
	Aug-20	13.9	5.736	3.270	2961.27
	Sep-20	13.9	3.562	2.512	1260.80
	Oct-20	10.8	4.411	3.066	1254.63
	Nov-20	10.8	2.682	2.024	614.11
	Dec-20	10.8	4.440	2.488	1821.82
	Jan-21	8.5	4.581	3.415	856.06
	Feb-21	8.5	1.805	1.755	36.45
	Mar-21	8.5	1.223	0.961	192.57
	Apr-21	9.4	5.783	3.014	2249.54
	May-21	9.4	5.291	3.329	1593.18

The comparison between two fertilization periods (autumn 2019 and 2020) resulted in interesting and clear differences between the Mincio River loads before and after the reintroduction of the fertilization limits of the Nitrate Directive. This evaluation was performed between a complete season instead of a single month to better handle the variability connected to meteorological conditions and fertilizations timing. Precipitation was similar between 2019 and 2020, with a total of 477.0 and 418.8 mm measured during the autumn (Wilcoxon signed-rank test: Z= 2163.5, *p* = 0.5109, performed using the R software for statistical computing (R Core Team, 2020)).

From September 2019, the difference in NO_3^- load between C16 and C12 was constant till December, with an average value of 5603.63 ± 953.48 kg NO_3^- d^{-1} (from here, average ± standard deviation). This value is more than double of the load measured in the same period in 2020, with 2297.59 ± 953.48 kg NO_3^- d^{-1}. These dissimilarities, taking into account a similar recharge given by precipitation, point out more frequent and abundant manure fertilization in 2019 than 2020.

Table 2. NO_3^- concentrations and loads during the autumnal fertilization period in 2019. River flow data are derived from the Interregional Agency for the Po River and by the Mincio Consortium. Concentration is expressed in mg NO_3^- $L^{-1}$and load is reported as kg NO_3^- d^{-1}.

Sample	Value	Sep	Oct	Nov	Dec
C12	Concentration	11.72	11.18	8.22	8.76
C16	Concentration	6.88	4.15	3.63	2.58
Difference	Load	5806.89	6562.66	4283.96	5761.00

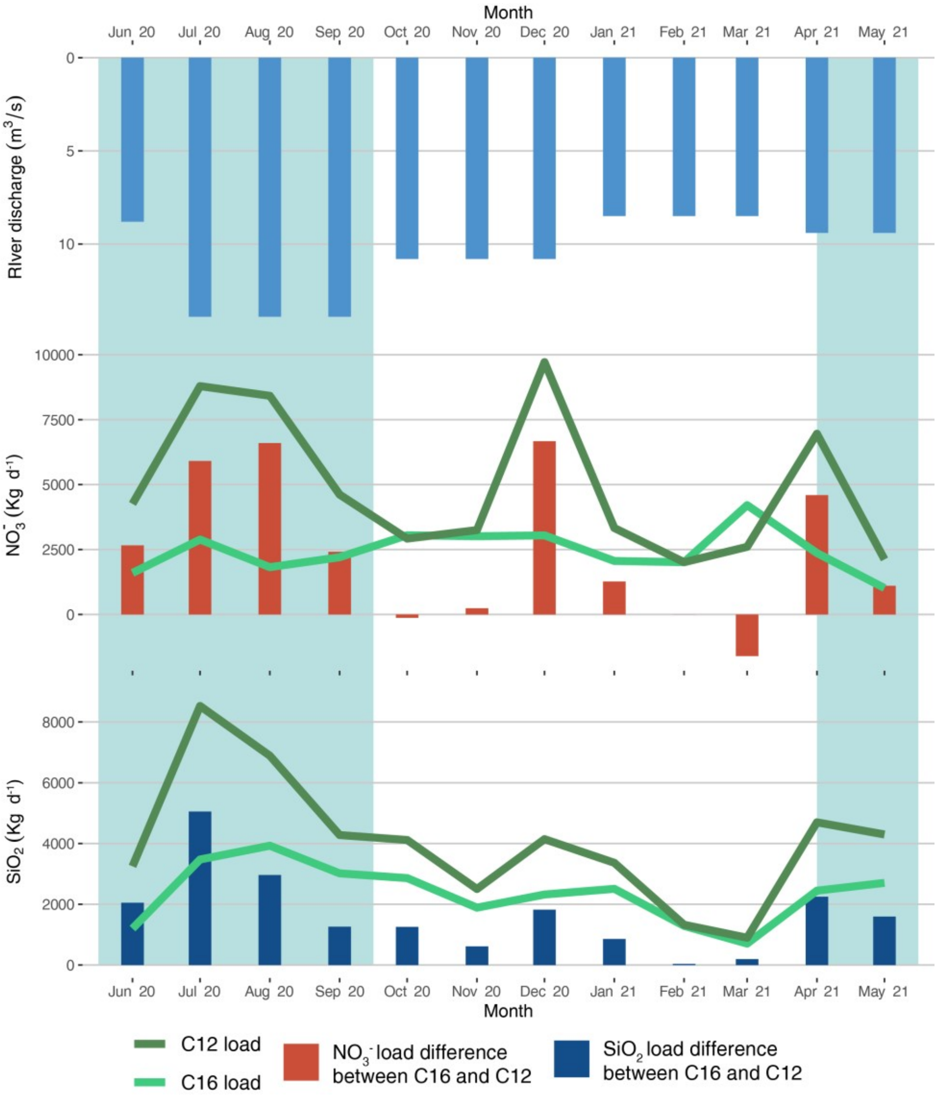


Figure 5. Discharge, NO_3^-, and SiO_2 loads in the Mincio River.

4 Discussion

4.1 Characterization of the NO_3^- Contamination: Source and Trend

The investigated area belongs to a lowland multilayer aquifer where from the middle age agricultural activities have been supported by a capillary network of irrigation channels (Fantoni, 2008), leading to a deep modification of the natural hydrological cycle. For these reasons, the effects of such modification on the aquifer recharge are known since centuries and were studied by different authors (e.g. Balestrini et al., 2021; Rotiroti et al., 2019). In the investigated area, those effects were already explored, but only for limited zones and periods (Severini et al., 2021; Severini et al., 2020). The monitoring of the hydrological year provided a trend of groundwater heads consistent with the above-mentioned authors. The recharge during the irrigation period deeply altered the groundwater level, rising the hydraulic heads up to 4.6 m (well P4) during the maximum recharge period

(August). The recharge is homogeneous along with time and space, as described by the constant raise of hydraulic heads from April and the widespread network of irrigation channels providing homogeneous recharge volumes. Nevertheless, the variation of groundwater heads and regression coefficient is not uniform. The Mincio River is a regulated river, but with variations of the hydrometric levels up to ~ 1m in the investigated area. These variations are anyway considerably smaller than those measured in the wells. The Mincio River has a major influence on the wells near its course. Therefore, the effects of aquifer recharge are detectable only in those wells far from the Mincio River. Different pieces of evidence suggest the high permeability of the investigated aquifer, such as the rapid increase and decrease of hydraulic heads or the limited thickness of the vadose zone. Moreover, the hydraulic characterization of the same aquifer (Chelli et al., 2018) reported a transmissivity of 1.0×10^{-2} m^2 s^{-1} and a storativity of 1.49×10^{-2}, calculated through pumping tests in a nearby area. Nevertheless, a constant hydraulic head during the recession time was never recorded, rather a lowering tendency until the recharge in April. This trend of the phreatic surface testifies the enormous amount of recharge provided for the agricultural needs, which hardly can be drained out from the aquifer until "natural" levels. Surprisingly, the groundwater flowing to the Mincio River, according to the data provided by AIPO and by the Mincio Consortium, does not alter its flow between upstream and downstream the investigated area. This fact will be better inspected in the last Paragraph comparing the hydraulic heads from the surrounding area and discharge and SiO_2 concentrations of the Mincio River.

The physico-chemical parameters measured in the field well-differentiated surface- and groundwater samples and provided further information on their source. This is particularly efficient with sample C5. Although sampled in a natural channel, the temperature, pH, and EC are like groundwater. Thus, also considering the small difference between the groundwater and ground level in both recession and recharge periods, the channel where C5 is sampled is fed by groundwater for all the hydrological year. Also water chemistry agrees with this finding. This case provides two pivotal pieces of information for the right bank of the Mincio River. First, it is an empirical manifestation of the potentiometric map goodness since the heterogeneous distribution of wells in the area could lead to errors in the hydraulic heads interpolation. Secondly, it testifies that during both recession and recharge periods there is a diffuse feeding of groundwater to the secondary channels network in the right bank flowing to the Mincio River. Moreover, this diffuse feeding of groundwater could also directly affect the Mincio River, and not only its right bank tributaries. In spring F2, an anomaly in temperature was found. Although the spring had EC and pH values comparable to groundwater, the temperature was similar to surface waters and highly influenced by the air temperature (measured in the near meteorological station, Figure S2). The underlying reason is that the spring is a so-called "*fontanile*" (Figure S4), which is a small, semi-artificial, pond. These ponds are also well known for transferring nitrogen from groundwater to surface waters (Balestrini et al., 2021). The relatively small dimensions of the spring F2 and the low discharge make it vulnerable to the seasonal variation of air temperature. Another similar spring has been investigated in the area (F4).

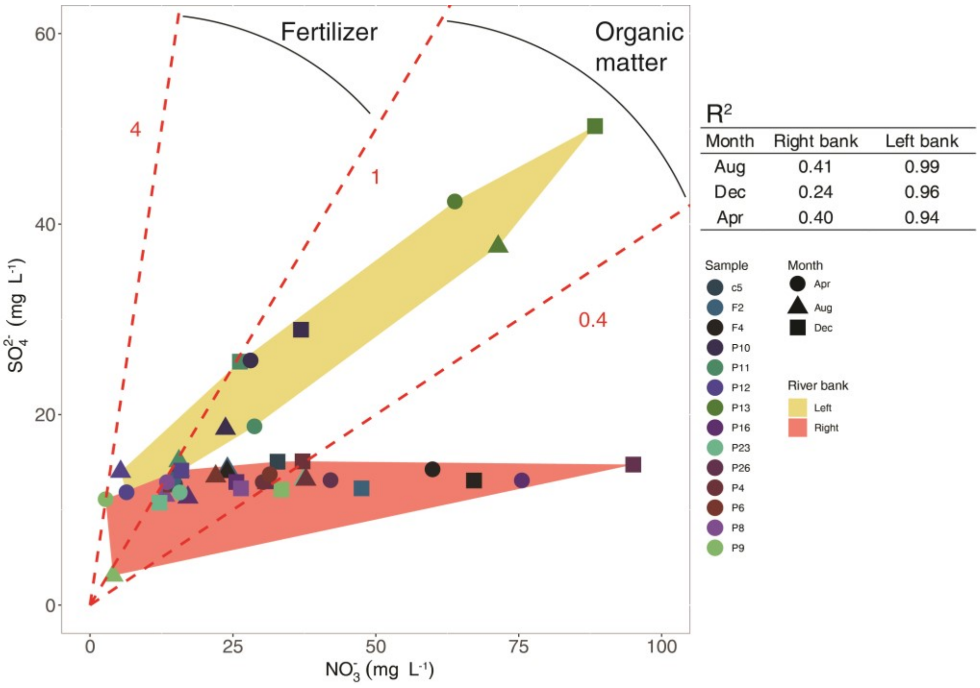
The chemical analyses during the hydrological year revealed trends over time and space like those previously reported for 2019 (Severini et al., 2021; Severini et al., 2020). NO_3^- concentration showed lower values in surface waters and higher in groundwater, up to 95 mg NO_3^- L^{-1} (Table S1). The timing of concentration rises is connected to the fertilization in the investigated area, performed in autumn and spring, in agreement with the preparation of the sowing season (Provolo & Riva, 2003) and concurrently with the reaching of the volumetric capacity of the slurry tanks, forcing farmers to spread it (Perego et al., 2012). Thus, the NO_3^- concentration increase measured in August and December is likely due to the same fertilization event. In August the slight increase relates to the fertilization and the concurrent irrigation in some portions of the area. The main leaching event occurs in December, when the abundant precipitation promotes leaching of all the manure spread, reaching the highest measured concentration of 95 mg NO_3^- L^{-1} (Table S1). Another NO_3^- increase is due to the spring fertilization. In general, the fast increase and decrease of NO_3^- concentration are due to biogeochemical processes of nitrification and denitrification in the aquifer. Isotopic analyses were not performed in the area, thus the above-mentioned process cannot be proven. Anyway, it was partially explored in Severini et al. (2020), where the chemical variation of recharge water percolating through the vadose and saturation zone was analysed. Summarising, the high and temporal limited NO_3^- concentrations could be limited to vertical zoning of physico-chemical features within the shallower saturated zone, as reported by other authors (Petrella & Celico, 2013; Petrella et al., 2009). The upper groundwater layer (where groundwater samples were collected through a bailer sampler) is mostly composed of rainwater or irrigation water and is characterized by oxic conditions. This entails that denitrification and/or other nitrate reduction processes cannot be carried out by the microbial community and NO_3^- concentrations remain high. After the recharge, the thickness of the upper and oxic groundwater layer becomes thinner and finally negligible, due to the mixing and diffusion of chemical compounds with the underlying groundwater. Only at this stage, the microbial communities can perform denitrification and/or nitrate reduction, fostering the reduction of NO_3^- concentration. In Figure S5, the two main source directions of NO_3^- can be identified upgradient with reference to the investigated area, whereas a clear contamination plume can be seen starting from the well P26, with relatively high upstream concentrations of NO_3^- too. The NO_3^- contamination in groundwater is also transferred to surface waters. The spring F2 and the channel C5 reached 47.50 and 32.8 mg NO_3^- L^{-1}, respectively. These values, although below the limits of the Water Framework Directive (WFD) of 50 mg NO_3^- L^{-1} ("EU Water Framework Directive," 2000) constitute a risk for the good ecological status requested by the WFD since such high NO_3^- concentrations in surface waters can unavoidably lead to eutrophication. A focus on the Mincio River concentrations will be introduced in the relative Paragraph. In the investigated area, NO_3^- and SiO_2 are deeply correlated, since both originate from manure and SiO_2 has been proposed as a tracer of fertilization (Severini et al., 2020). The temporal variation of SiO_2 is anyway different from NO_3^- since no biogeochemical processes can alter its values in groundwater. Given the constant values until December, the fertilization in August was quite light, as also NO_3^- and SO_4^{2-} showed a restrained increase of concentrations. On the contrary, starting from December, the leaching of manure-rich waters resulted in a constant increase of SiO_2

concentrations, testifying the abundant fertilization performed during the autumn. The concentration increase seems postponed by a few months after fertilization, as happened also in 2019 (Severini et al., 2020). Probably this is the reason why in April we see the effects of dilution given by recharge and the concentrations decrease instead of a raise due to spring fertilization. An increase would be expected after April, but the conspicuous and continuous recharge keeps the SiO_2 concentrations low by dilution, a phenomenon already reported for other analytes in a nearby area of the Po plain (Rotiroti et al., 2019). An interesting differentiation can be appreciated between surface- and groundwater from January to April. The surface waters have lower SiO_2 concentrations than groundwater. An exception is given by the spring F2, characterised by low SiO_2 values. From January, groundwater samples show increasing concentrations, whereas in surface waters they decrease (except C5) although diffusively fed by groundwater. This trend can be explained by comparing Figures 3 and 4(c). When in winter the groundwater level is still relatively high, the groundwater feeding surface waters makes the two categories have similar SiO_2 values. Below the groundwater level measured in January, the interaction between surface- and groundwater is less intense. The groundwater percentage in the total flow of the surface waters lowers, resulting in a dilution of the SiO_2 from groundwater, although during this period is more concentrated than during surface- groundwater maximum interaction. From April, the recharge provided by irrigation produces a rise of the groundwater level and the interaction between surface- and groundwater becomes stronger again. At this moment, the groundwater percentage in the total discharge of rivers and channels goes back to notable levels, resulting in an immediate increase of SiO_2 values, which are still relatively high in groundwater. This mechanism is the same regulating also the loads in the Mincio River but will be examined in depth in Paragraph 4.2. The spatial distribution of SiO_2 reported in Figure S5 confirms the interpretation proposed for NO_3^-. Two main contamination sources can be identified upgradient with reference the wells P4 and P13, with the last showing the highest concentrations. Unexpectedly, a clear dissimilarity between SiO_2 and NO_3^- is reported for the southern contamination plume near the well P26 and the spring F2. These points are in a portion of the aquifer with groundwater flowing from another direction compared to the northern right bank area. The hypothesis of the NO_3^- origin in synthetic fertilizers with low SiO_2 seems unlikely, due to the low SO_4^{2-}/NO_3^- ratio (discussed below). It is then plausible that this groundwater has different chemical parameters, especially among those not analysed, like other (usually in trace) metals. Few chemical compounds present in groundwater can decrease the silica concentrations. A plausible explanation could be related to the presence of higher Aluminium (Al) concentrations, not related to deep and enriched circuits but to anthropic sources instead, like Municipal Solid Waste (MSW) (Rapti-Caputo & Vaccaro, 2006). There are two recycling depots near F2 and P26, respectively 9.6 km N-W and 4.2 km N, but the absence of potentiometric maps of groundwater outside the investigated area doesn't allow a direct correlation. Anyway, the higher presence of Al in groundwater in this area could result in SiO_2 precipitation (Katsumi, 1956). Aluminium salts hydrolyse into aluminium hydroxide and coprecipitate the silica, decreasing its concentration. Such reaction has been employed to remove silica from boiler-feed water (Betz et al., 1940). Although the ratio between colloidal and dissolved silica is not known for our samples, 1 mg Al L^{-1} can precipitate about

40 mg of colloidal silica but at pH <5, whereas a larger amount of Al is required to precipitate dissolved silica (20 mg Al L^{-1} at pH 8 can precipitate 15 mg silica) (Okamoto et al., 1957). In addition, during some samplings the values of SiO_2 in F2 were drastically lower than P26, highlighting also the likelihood of illegal dumping in F2 (during autumn and winter). Moreover, this fact is worsened by cyclic freezing (Fig. S2, S4), which promotes the precipitation of amorphous silica (Dietzel, 2005) of the spring water during winter (Fig. S2, S4). These are anyway just hypotheses, for which additional investigations are required to explain the low SiO_2 concentrations between the spring F2 and well P26.

The less informative analyte is probably F^-. Its concentrations were constant along time (0.26 ± 0.11 mg F^- L^{-1}) and could be associated with the background level. The only temporal variation was observed in August, December, and April, i.e. during the greater recharge events, as testified by the hydraulic heads (Figure 3). Here, the recharge promoted the dilution of F^-. Cl^- showed generally low values (8.21 ± 2.82 mg Cl^- L^{-1}) but a slight constant increase of concentrations during the non-irrigation period, like SiO_2. This trend is only interrupted in February, when the brief but sufficient precipitation promotes the dilution, as partially happens to SiO_2. Higher concentrations were analysed in March and April, as for SO_4^{2-}, SiO_2, and NO_3^-, testifying a possible contribution from manure fertilization. Animal waste can contain high concentrations of Cl^-, e.g. up to 1980 mg L^{-1} (Panno et al., 2006). Since it does not undergo biogeochemical processes, it has been also proposed as a tracer extent and magnitude of contamination from livestock farms (Krapac et al., 2000; Krapac et al., 1998). Cl^- could also derive from synthetic fertilizers like potassium chloride (KCl), but these are poorly used in the study area (Italian National Institute of Statistics, 2010).

SO_4^{2-} peaked with the same timing and had a similar spatial distribution of NO_3^-, suggesting that fertilization is the SO_4^{2-} main source in the study area. Moreover, mineralogical data in a nearby area suggests the absence of sulfate in the mineralogical composition of the aquifer (Severini et al., 2021). Probably due to biogeochemical processes and its role as electron acceptors in sub-oxic conditions, the SO_4^{2-} concentration peaks are registered for a limited time after the fertilization. The common source of NO_3^- and SO_4^{2-} is testified by their significant correlation (R^2) (Cuoco et al., 2015) reported in Figure 6. This consideration is evident in the left bank of the Mincio River, where the highest concentration of SO_4^{2-} were measured and the correlation between NO_3^- and SO_4^{2-} is highly significant (average $R^2 = 0.963$). To identify this source of contamination, a plot of the SO_4^{2-}/NO_3^- ratio was performed during the three main fertilization samples (Figure 6). According to Federico et al. (2004), an SO_4^{2-}/NO_3^- ratio >4 or at least >1 (Busico et al., 2017) is related to synthetic fertilizers (e.g. N-rich calcium sulphates). In our study area, Ca^{2+} in groundwater is stable over time, thus the use of other fertilizers like the ammonium sulphate ($(NH_4)_2SO_4$) is more plausible. On the contrary, NO_3^- is much more concentrated in sewage, manure, and septic tanks, reaching an SO_4^{2-}/NO_3^- ratio of 0.14 (Panno et al., 2001; Pawar & Shaikh, 1995; Steinich et al., 1998). The samples with higher SO_4^{2-} and NO_3^- concentrations are in the eastern part of the investigated area (Figure 6), with the maximum concentrations upstream the groundwater flowpath (P13) and lower downstream (P11). Anyway, most of the samples have a low SO_4^{2-}/NO_3^- ratio testifying the use of organic matter as fertilizer (Figure 6), although some of them have values close to 1 and a small percentage of chemical fertilizer could be utilized in the area. Thus, the high SO_4^{2-} and NO_3^- concentrations measured in this zone like in P13 are not related to different fertilizers, but only to a more massive use of manure. Interviews with local farmers suggest that fertilization may occur every crop change, therefore multiple times per year. This could lead to a bigger enrichment of SO_4^{2-} and NO_3^- in the soil and vadose zone, which can massively percolate during the recharge phase (irrigation and precipitation). The higher an SO_4^{2-}/NO_3^- ratio (up to 2.62) is reached by the well P12 during the fertilizations in August and April. During these periods, the SO_4^{2-}/NO_3^- ratio suggests the use of chemical fertilizers instead of manure. In the western area, the SO_4^{2-}/NO_3^- ratio is less significant (average $R^2 = 0.35$), but similar to other areas where this approach was used (Busico et al., 2017). On the right bank of the Mincio River, the cultivated land is devoted to maize cultivation and permanent grasslands fertilized using manure (Severini et al., 2021). In fact, in Figure 6 all samples have usually an SO_4^{2-}/NO_3^- ratio between 0.4 and 1, suggesting the predominant use of manure as fertilizer. All SO_4^{2-}/NO_3^- ratios were lower than 1 but P9 in April ($SO_4^{2-}/NO_3^- = 4.09$), probably related to the massive use of chemical fertilizers in the area between the wells P9 and P4. Some samples showed extremely low SO_4^{2-}/NO_3^- ratios (P26= 0.16 in December). These values are similar to those sampled by Cuoco et al. (2015) in organic-rich waters from wastewater purification and could testify the use of a mixture of manure and liquids (urine and water), where the high amount of organic matter can't be easily mineralized. These mixtures are common in the area, as they are a common by-product of swine farms, where the solid and liquid phases are difficult to divide. A peculiar SO_4^{2-}/NO_3^- ratio must be reported for the well P9, which reached a ratio of 4.09 in April. This value is again relatable to the use of synthetic fertilizers, which seems spatially and temporally limited in the area. Regarding the contamination plume upstream P26, the low SO_4^{2-}/NO_3^- ratio excludes the contribution of chemical fertilizers.



R^2

Month	Right bank	Left bank
Aug	0.41	0.99
Dec	0.24	0.96
Apr	0.40	0.94

Figure 6. SO_4^{2-}/ NO_3^- plot from August 2020, December 2020, and April 2021. The side-table reports the single R^2 values. The different lines defining the source of pollution are derived from Busico et al. (2017) and Cuoco et al. (2015).

4.2 Transferring of NO_3^- Contamination from Groundwater to the Mincio River

The Mincio River loads showed important changes across the study area. As seen using SiO_2, temperature, and the potentiometric maps in Figure 2, the groundwater diffusively feeds the secondary channels and the Mincio River in its right bank. This fact is also testified by the different SiO_2 loads between C16 (lower) and C12 (higher). Since the SiO_2 values are higher in groundwater than surface water and the Mincio River is constantly fed by groundwater, its concentrations rise between the extremes of the study area (C16 and C12). The difference between the loads in these two points reaches the minimum values in February and March when the groundwater level is lowest. As explained for the channel in C5, on this occasion the groundwater volumes constitute a low proportion of the Mincio River flow, resulting in a conspicuous dilution of SiO_2. The remaining hydrological year showed loads at C12 always higher than those at C16, testifying the continuous feeding of the Mincio River by groundwater. The SiO_2 load peaks are a function of two factors: the high concentrations in groundwater and the higher or lower contribution of groundwater to the Mincio flow. In summer, a combination of high groundwater input but with low SiO_2 concentrations results in a high load. In winter, groundwater has a high SiO_2 concentration, but the low groundwater level produces a reduced input to the Mincio River. Anyway, the combination is still relevant and the highest load in C12 is measured. The spring load is an intermediate scenario, with rising groundwater levels but decreasing SiO_2 concentrations. The load at C12 results therefore moderate. Although the feeding mechanism was demonstrated using SiO_2, discharge data report no variation in the Mincio River flow among time between C16 and C12. Although no wells are available to reconstruct the potentiometric map in the S-E part of the study area, the chemical data and the absence of an increase in the discharge indicate that the southern part of the study area is a flow-through system (Woessner, 2000) with a gaining right bank and a losing left bank. The feeding groundwater doesn't result in an incremented discharge of the river but in an exchange between groundwater flowing from the right bank and river water flowing out through the left bank. This exchange is higher when the groundwater level is higher. In addition, NO_3^- is transported from groundwater to surface water through the same mechanism. Nevertheless, the biogeochemical processes and its higher variation in groundwater make its loads different. First, NO_3^- concentrations peak in groundwater in three moments under high recharge and fertilization rates. The higher NO_3^- concentrations in groundwater correspond to the maximum loads measured at C12, testifying also the rapid circulation between groundwater and river through surface-groundwater interaction. During October and March, the NO_3^- loads are higher at C16 than at C12. This unexpected result is probably due to the N uptake by macrophytes and denitrification happening in the 8.9 km of river course between C16 and C12. In fact, in this stretch of the Mincio River high denitrification and uptake rates, up to 2500 mmol N m^{-2} y^{-1}, were reported (Pinardi et al., 2009).

Multiplying the monthly loads for all the year, an overall amount of 911.47×10^3 kg NO_3^- y^{-1} are gained by the Mincio River in the investigated section, constituting a serious threat to the environmental quality of the downstream water bodies in the sense of the EU Water Framework Directive (2000), such as Mantua Lakes, the Po River (Tavernini et al., 2011; Viaroli et al., 2018) and the Po River Delta (Naldi & Viaroli, 2002; Viaroli et al., 1993). Among them, the Mantua Lakes, three hypereutrophic shallow fluvial lakes fed by the Mincio River and located ~ 12 km S-E the study area, are the more threatened (Bolpagni et al., 2014; Bresciani et al., 2013; Pinardi et al., 2018a) by the NO_3^- loads generated in the watershed and transported by the Mincio River.

4.3 The Effects of the Nitrate Directive Fertilization Limits Reintroduction in the Italian NVZ

2019 was the last year with a derogation granted to the Lombardy region regarding the use of N fertilizers inside the NVZs, which limit was raised from 170 kg N ha^{-1} y^{-1} to 250 kg N ha^{-1} y^{-1}. Since 2020, the limits are back to the value proposed in the Nitrate Directive ("Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources," 1991). Given the fast (<1 month) circulation of N among the soil, groundwater, and the Mincio River, a comparison between 2019 and 2020 can provide the first evidence of the short-term effects of the NVZ N fertilization limit. This case represents an example of how N limitation can affect an already contaminated alluvial aquifer over a short period. The comparison proposed below takes into account only one fertilization during the autumn of 2019 and 2020. This comparison is supported by the similar condition of the study area between the two periods, such as same land use (Severini et al., 2021), fertilizer typology (manure)

(Severini et al., 2020), and precipitation (as described previously). No data are available on the monthly use of fertilizer in the area, but since the above-mentioned conditions didn't change between 2019 and 2020, we suppose that the percentage of manure used in autumn with respect to the total annual amount remained the same.

Figure 7(b) shows that a constantly higher NO_3^- load was measured in C12 and the difference with C16 was like that measured in December 2020. Given the similar condition aforementioned, this difference can be attributed to the limit of 250 kg N ha^{-1} y^{-1} used for fertilization in 2019. Groundwater had on average a higher NO_3^- concentration in 2019 (25.77± 20.64 mg NO_3^- L^{-1}) than in 2020 (19.81± 18.56 mg NO_3^- L^{-1}). Also in 2019, some samples had NO_3^- concentrations higher than 50 mg L^{-1} (Severini et al., 2020) (Table S2). This small difference in groundwater concentrations leads to an enormous difference in the daily load of NO_3^- to the Mincio River. In 2020, the C12 NO_3^- load was closer to that from C16, underlying a significantly lower NO_3^- input to the river. This trend is constant along the autumn, with some moments in which a lower NO_3^- load in C12 than C16 was measured, due to nitrification and macrophytes uptake (Paragraph 4.2). These biogeochemical factors probably influence also the NO_3^- loads in 2019, but the difference between C12 and C16 is so massive that their values have a negligible effect. Only during the massive recharge in December (which promotes the percolation of all the NO_3^- stored in the soil and vadose zone), the difference between the load in C16 and C12 in 2020 is like 2019. Therefore, the total NO_3^- gained by the Mincio River during the autumn in these two periods is significantly different (Figure 7(c)). The higher and constant difference between the loads in C16 and C12 in 2019 resulted in a total of 22.4×10^3 kg NO_3^- gained by the Mincio River. After the reintroduction of the limit of 170 kg N ha^{-1} y^{-1} for fertilization in the NVZ, in the same period, the river gained only 9.2×10^3 kg NO_3^-, i.e. a reduction of 59% in the NO_3^- gained.

These findings point out two important issues regarding the transfer of NO_3^- to surficial water bodies. First, the reintroduction of the fertilization limit of 170 kg N ha^{-1} y^{-1} in the NVZs produces a significant effect also after one hydrological year. This is particularly true in areas like the one presented and others similar (e.g. Balderacchi et al., 2016), where the transfer of N from the soil to groundwater and finally to surface waters is fast. Therefore, stakeholders, authorities, and administrations aiming at reducing the effects of abundant N fertilization can consider that limiting their use in NVZs with fast hydrological cycles can be an effective solution also over short periods, not only years or decades after its introduction. Secondly, when the N contamination needs to be investigated to protect surface water bodies from eutrophication and nutrients surpluses, the loads exported should be considered as a better indicator of potential nutrients sources. As reported for this study area, a difference of few mg N L^{-1} in groundwater can determine a difference of metric tons (t) of N exported by the river, since this value is the expression of both N concentration and river discharge. These t of N are then transported to other water bodies, where their buffer, absorption, and uptake capacities could not be able to reduce N loads. This is the case of the previously mentioned Mantua Lakes, but also other water bodies which could receive high N input from feeding groundwater and contribute to eutrophication in Italy (Palmeri et al., 2005; Sacchi et al., 2013; Salvetti et al., 2006) and other areas worldwide (Benelli et al., 2018; Holman et al., 2008; Nakayama & Watanabe, 2008; Stuopis et al., 2010).

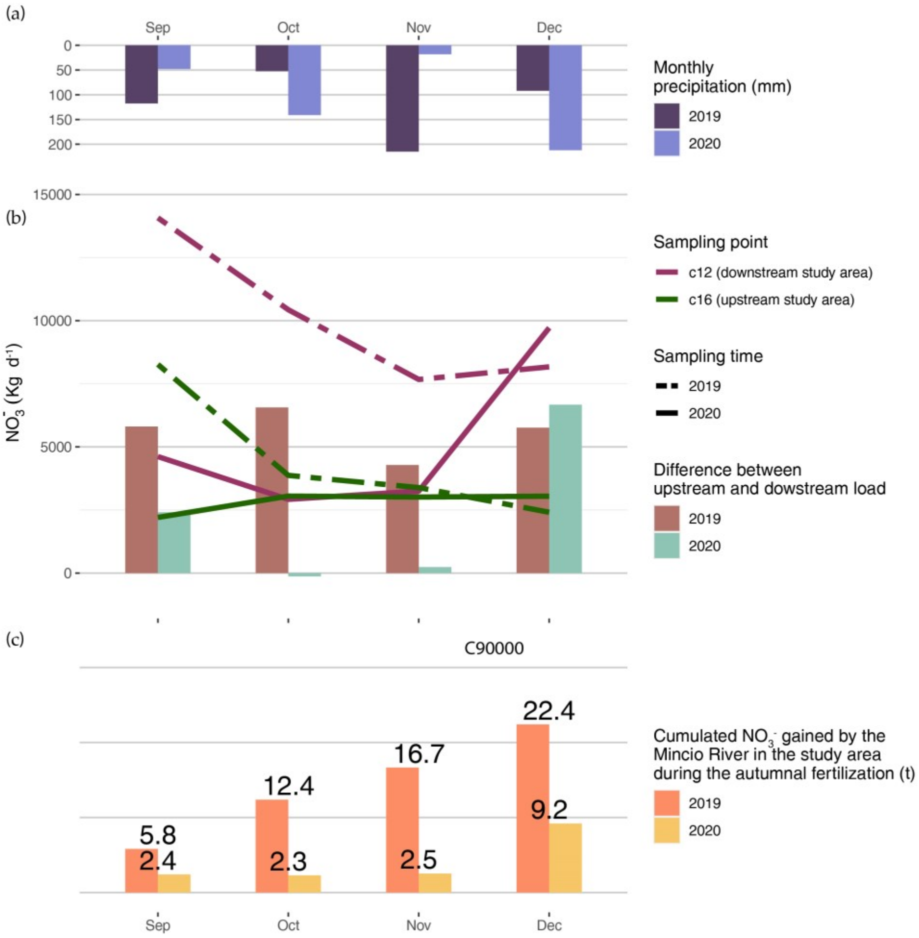


Figure 7. NO$_3^-$ loads in 2019 and 2020. (a) Monthly precipitation. (b) Loads comparison between C12 (purple) and C16 (green) in 2019 (dashed line) and 2020 (solid line). The difference in loads between them is reported in the bar plot. (c) Progressively cumulated loads differences between 2019 (orange) and 2020 (yellow).

5 Conclusions

This study pointed out how the reintroduction of the fertilization limit of 170 kg N ha^{-1} y^{-1} resulted in an improvement of the chemical quality of both groundwater and surface water, consistent with the objectives of the Nitrate and Water Framework Directives. The investigated alluvial aquifer is still contaminated right after the manure application and the abundant recharge events, but the average NO$_3^-$ concentration decreased by 23% from 2019 to 2020. According to flow and chemical data, the cross-river sector of the Mincio River analysed in this study is a flow-through system, gaining groundwater from its right bank and losing stream water through its left bank. As a result, loads of manure tracer (SiO$_2$) are always higher downstream of this interaction area. Also the NO_3^- loads are higher downstream the interaction area, but their values depend also on other biogeochemical processes either amplifying or reducing the effects of fertilization. In a comparison between the autumn fertilization of 2019 and 2020, a 59% reduction of the NO_3^- gained daily via the river-groundwater interaction in the Mincio River was observed. This is an interesting output of this study, supporting a fast and significant response of the coupled riverine-groundwater system to reduced fertilization rates. Moreover, this result constitutes a potential red flag for the authorities and stakeholders interested in the surface- and groundwater quality of the Po plain. Although the Mincio River is one of the most important left tributaries of the Po River, this area is still poorly investigated from the hydrogeological perspective, notwithstanding its role in the alteration of the N loads in the Mincio River. Although the hydrogeological conceptual model proposed well explains the transport of N from manure to groundwater and the Mincio River, some aspects are still unknown. In fact, in this area, no isotopic investigations have been previously performed, and the biogeochemical processes occurring in groundwater are only partially explained. The origin of the N pollution, although some inferences can be done with the SO_4^{2-}/NO_3^- ratio, is still not investigated in detail. Moreover, the strength of the biogeochemical processes modifying the NO_3^- concentrations in groundwater in the study area are little known. These concerns will be the focus of the next investigations in the study area.

Supplementary Materials: The following materials are available online at www.mdpi.com/xxx/s1, Table S1: NO_3^- concentrations (mg L^{-1}) during the investigated period, from June 2020 to May 2021. Table S2: NO_3^- concentrations (mg L^{-1}) during autumn 2019. Figure S1: ephemeral physico-chemical parameters. Figure S2: air temperature. Figure S3: Piper plot. Figure S4: the spring F2. Figure S5: NO_3^- and SiO_2 concentrations in the study area during December 2020.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Article

River-groundwater interaction and recharge effects on microplastics contamination of groundwater in confined alluvial aquifers: a test study in northern Italy

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Abstract: Although microplastics are much studied in marine, freshwater, and terrestrial ecosystems, groundwater is still little studied, despite constituting around one-third of total freshwater and providing almost half of all drinking water worldwide. Literature provides only a few examples of microplastics contamination in groundwater and are mainly characterised by a chemical approach, focusing on the main polymers and sources of microplastics. This approach, although highly efficient, requires expensive instruments for chemical characterization. Moreover, little importance is given to the hydrogeological processes able to affect the contamination, such as river-groundwater interaction and aquifer recharge. This study was carried out with two aims. The first aim is the formulation of a method of operation based on the hydrogeological aspects of the investigated area and characterised by a high result : cost ratio. Microplastics were extracted from samples through filtration and flotation methods, and successively counted and characterized morphologically (area, circularity, and Feret diameter) through staining and automated microscopy analysis. The second aim is to apply this methodology and investigate the microplastics presence in some portions of an alluvial aquifer with losing rivers, before and after a recharge event. Microplastics in groundwater had a higher circularity than those in surface water, but similar area and Feret diameter, pointing out that in porous (mainly gravels) aquifers the transport is more influenced by the shape than the size of microplastics. The recharge promotes the migration of smaller microplastics with a more rounded shape. These findings provide new pieces of evidence on the presence and transport of microplastics in groundwater and underline how the hydrogeological characteristics of the area can be one of the main drivers of microplastics contamination in groundwater.

Keywords: microplastics; river-groundwater interaction; aquifer recharge; surface waters; method

1 Introduction

Microplastics are intensively studied pollutants, defined as solid and water-insoluble polymer particles smaller than 5 mm (Arthur et al., 2008; Bergmann et al., 2015) and larger than the so-called nanoplastics, whose upper limit is usually set between 100 and 1000 nm (Hartmann et al., 2019). They can be typified as primary if they are originally produced as microplastics, such as for nurdles, fibres, and particles used in cosmetics (Cole et al., 2011; Gregory, 1996; Zitko & Hanlon, 1991) or as secondary, which result from plastic fragmentation by physical, biological, and chemical processes (Browne et al., 2007; Thompson et al., 2004). Their origin can be attributed to the enormous production of plastics worldwide, which according to Geyer et al. (2017), reached 380 Mt in 2015. In addition, most of the monomers used to produce plastics (e.g. polyethylene, polypropylene, polystyrene, and polyethylene terephthalate) are derived from fossil hydrocarbons, resulting in non- or scarcely biodegradable final products (Geyer et al., 2017; Iwata, 2015). Biodegradable plastics are also produced using both renewable and fossil sources, but in lower quantities than non-biodegradable plastics and they can anyway contribute to microplastic pollution (Wei et al., 2021). Consequently, plastic waste accumulates in landfills or, worse, in the environment (Barnes et al., 2009), where it takes centuries for breakdown and decomposition (Barnes et al., 2009; Moore, 2008). Therefore, the presence of this pollutant in different environmental matrices worldwide has been ascertained by the scientific community, threatening both human health and biosphere.

Plastic pollution was initially recognized and studied in marine environments, where investigations started in the 1970s (e.g. Carpenter et al., 1972; Colton Jr et al., 1974). Comparatively, the study of microplastic pollution in freshwater ecosystems started much later and produced many fewer studies (Eerkes-Medrano et al., 2015; Li et al., 2018; Wagner et al., 2014), possibly due to difficulty in sampling uniformity in non-large-surface water bodies. Paradoxically, other important aquatic compartments such as groundwater, which is the single most important supply for drinking water in many areas of the world (Schmoll et al., 2006), have received almost no attention to date. The first case of microplastics contamination in groundwater was reported in a fractured medium, with karst crevices and conduits reportedly allowing the transfer of microplastics (Panno et al., 2019). In other aquifers without conduits and/or with lower porosity, soil can be assumed to work as a barrier for microplastics, probably dissuading researchers from further investigations. This hypothesis has recently been challenged, with two recent articles reporting microplastic contamination in alluvial aquifers, raising new questions on microplastics contamination and migration processes, especially from the hydrogeological perspective. Goeppert and Goldscheider (2021) demonstrated the possible microplastics transport in alluvial aquifer using tracer tests (uranine and microplastic tracer particles), thus invalidating the role of the aquifer (and soil) as *a priori* barrier for microplastics. Samandra et al. (2022) were the first and, to the best of our knowledge, so far the only to report on microplastics contamination in an alluvial unconfined aquifer. They detected eight different microplastic polymers in groundwater and evaluated microplastics abundance.

Microplastics can contaminate groundwater through different contamination pathways, according to hydrogeological factors such as the groundwater recharge source (e.g. vadose zone or surface water) and timing (e.g. recharge or recession periods), the diffuse (e.g. losing river) or point (e.g. leaks of the drainage system) microplastic source, the aquifer characteristics (e.g. hydraulic conductivity), and the possible interactions between surface water and groundwater (e.g. feeding or losing river). Moreover, different from solute contaminants, microplastics percolation and transport in the aquifer are also influenced by the relationships between microplastics dimensions and pore diameters.

The present work has two aims. The first aim is to set up a protocol for the separation, quantitative analysis, and geometrical characterization of microplastics in water samples. The second aim is to test the protocol in two sub-areas of a confined alluvial aquifer and verify the presence of microplastics in groundwater and the possible effects given by a recharge event. We hypothesize that the recharge and recession entity and timing of an area play a pivotal role in driving groundwater contamination by microplastics. Although this is easily expected, few qualitative and quantitative empirical data are available about these dynamics on microplastics. In addition, we hypothesize that microplastics in groundwater have different geometrical properties (e.g. area) compared with microplastics from the feeding surface waters, due to different interactions between microplastics and the environment (river or aquifer) during the transport processes. Together with the particles' surface charge, we also hypothesize that the geometrical characteristics of microplastics are pivotal in controlling their distribution in porous media. Results from this works provide a method of operation based on a hydrogeological perspective. This, along with the chemical characterization of polymers of microplastics and their transport of pollutants, should be central in the process of standardization of microplastics analysis methods, which are now strongly asked by policy-makers, e.g. the Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption ("Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) (Text with EEA relevance)," 2020).

2 Material and Methods

2.1 Study area

This study was performed in an alluvial aquifer surrounding the city of Parma (Po Plain, Northern Italy, Figure 1). This area was selected due to its peculiar hydrogeological and anthropic characteristics. Intensive agriculture (mostly hay, tomatoes, and maize) results in numerous recognised sources of microplastics contamination (Qi et al., 2020). The city of Parma is located in the centre of the study area. It represents another possible important microplastics source for surface waters (Mani et al., 2015; Peng et al., 2018) and groundwater due to leaks of the drainage system (Chen et al., 2020; Ngo et al., 2019). Finally, the area has many large industrial complexes, another possible source of microplastics contamination (Karbalaei et al., 2018) and is close to the so-called *packaging valley*, composed of more than 300 firms working in packaging and packaging machinery manufacturing and providing the biggest amount of industrial plastic wastes in the region (Foschi et al., 2021). All these factors can be expected to contribute to microplastic production and pollution (Karbalaei et al., 2018). Three rivers cross the area, namely the Taro, Baganza, and Parma River. The last two merge within the Parma city area and all of them flow into the River Po, the largest Italian river. Further, to support the intense agricultural activity, a vast network of irrigation channels is present (Figure 1), which are mostly natural, hence porous.

From the (hydro)geological perspective, the area has already been characterized by Zanini et al. (2019), while several Master Thesis of the University of Parma investigated some phenomena at small scale (e.g. Lancini, 2019; Viani, 2017). The area is characterized by the Emilia–Romagna Supersynthem (Lower Pleistocene, about 800ky BP to present), made up of fan and alluvial plain deposits, together with intra-valley and terrace deposits. The grain size of the sediments is highly variable, resulting in the juxtaposition of more permeable layers (gravel and sand) with low-permeability layers (clay and silt) (Zanini et al., 2019). They tested the hydraulic conductivity of these layers in the wells of the Parma University Campus (which includes the piezometer LDCAMPUS used within this study). A pumping test performed in the more permeable layer resulted in transmissivity and storativity of 3×10^{-4} m^2/s and 1.9×10^{-4}, respectively. In the low-permeability layer, the hydraulic conductivity was lower (in the order of $10^{-7}/10^{-9}$ m/s) and estimated using a Lefranc test. Granulometric data of a litholog near LDCAMPUS (3.8 km N-E, Figure S1, Table S1-S2) is reported in the supporting information and provides another piece of evidence of the low permeability of the confining unit.

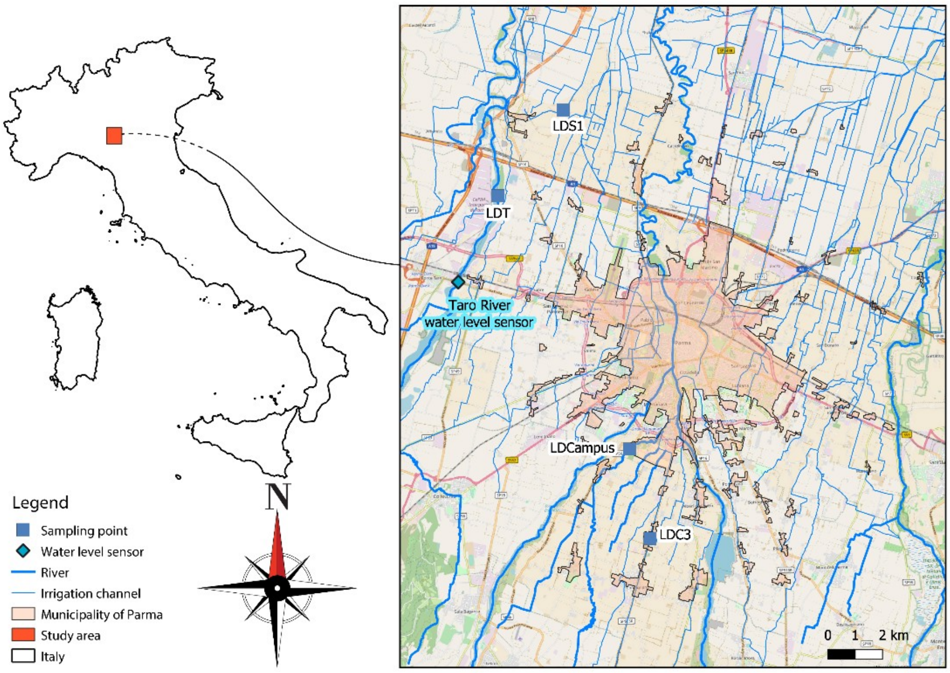

Figure 1. Study area. Basemap from OpenStreetMap (https://www.openstreetmap.org, 2021).

2.2 Data acquisition and microplastics sampling

The geological contextualization performed by Zanini et al. (2019) was spatially expanded, using stratigraphic data from the "Geognostic tests database of the Emilia-Romagna Region" ("Geognostic tests database of the Emilia-Romagna Region" 2020) and from Petrucci et al. (1975), together with outcropping data from the "Geological map of the Emilia-Romagna Region, 1:25.000 scale" ("Geological map of the Emilia-Romagna Region" 2004). Near the sampling points, a detailed reconstruction of outcropping materials and shallow aquifer architecture was performed using geophysical investigations (i.e. resistivity imaging) from Francese et al. (2016). These helped to verify the confined or unconfined conditions of the aquifer. Finally, these data were used to identify two sub-areas were evaluate the effect of river-groundwater interaction and aquifer recharge on the microplastics contamination in groundwater.

Water samples were collected from four stations during a small recession phase (25 March 2021), interrupted by a recharge event (13 April 2021) (Figure A1). A complete representation of hydraulic head variations during the hydrologic year in LDCAMPUS is reported in Figure A2. During both sampling periods, two samples were of surface waters, namely LDT (sampling point in the Taro River) and LDC3 (sampling point in an irrigation channel named La Riana Channel). The other two samples were groundwater, namely LDCAMPUS (piezometer) and LDS1 (natural spring). The sampling time was established using precipitation data (Parma meteorological station) and data of the Taro River flow (Taro River water level sensor, Figure 1), obtained from the Regional Environmental Protection Agency of Emilia-Romagna (ARPAE)"Hydrometric levels dataset") website. In addition, piezometric heads in LDCAMPUS were measured using a pressure transducer with a data-logger (STS DL.OCS/N/RS485). The LDCAMPUS piezometer is composed of a 3" polyvinyl chloride (PVC) tube reaching 30 m bgl (below ground level) and screened between 23.80 and 26.80 m bgl. The piezometer stratigraphy data begins with deposits made by silt and clay from 0 to 4.90 m bgl. Below this horizon is found the confined "shallow aquifer" (from 4.90 to 26.70 m bgl), which is mainly made of gravels with rare and thin clay lenses. Below 26.70 m bgl, other deposits made by silt and clay are found, from 26.70 to 30.00 m bgl, where the piezometer ends.

All water samples were taken and processed with glass or metallic tools and instruments to avoid contamination, except for the hydrogen peroxide (H_2O_2) container which was made of plastic (High-density polyethylene). In surface (and spring) water, 5 L samples were taken using a glass beaker at different depths (surface, medium, bottom) near the riverbank and stored in glass bottles with cork tops. All material was rinsed with Milli-Q water before sampling. In the piezometer, a metallic Bailer (250 mL) with a metallic wire was used. Groundwater was not purged before sampling to avoid contamination from the plastic tube of the water pump. The bailer sampler was used to sample groundwater at groundwater/air interface and at medium height, but not at the piezometer bottom to avoid contamination from the piezometer tube made of PVC, which fragments (with density up to 1.37 g/cm^3) fall to the bottom of the water column. 5 L of groundwater were stored and processed like surface waters. The sample amount was decided based on different necessities. The first is to maximise the number of possible microplastics found sampling a higher volume, which is usually between 0.5 and 2 L (Mintenig et al., 2019; Panno et al., 2019; Samandra et al., 2022). The second is to sample a large volume but feasible to transport and store. Thus, the final sample volume was set to 5 L for both surface and groundwater, making them also more comparable. Although final values are reported as microplastics per litre (microplastics/L), the treatment of different volumes entails the use of a different amount of reagents, making the comparison between surface water, groundwater, and analytical samples (5 L of Milli-Q) senseless.

Together with microplastics, other samples were taken for isotopic analysis of tritium in LDS1 to evaluate the effect of a recharge event. The analyses for the determination of the tritium activity were carried out according to the procedures provided by the International Atomic Energy Agency (1998). The analytical prediction uncertainty was ± 0.5 TU for ^{3}H. In the field, temperature, dissolved oxygen (O_2 and O_2%), electrical conductivity (EC), Oxidation Reduction Potential (ORP), and pH were measured using a multi-parameter probe (HI9829 HANNA Instruments, Woonsocket, RI, USA), calibrated the day before sampling. These measurements were not performed in LDT (Taro River) due to logistic problems with the probe.

2.3 Microplastics extraction and processing

The 5 L samples were processed after 24 h, to allow the sedimentation of suspended materials. After that, the supernatant and suspended solids were filtered on polycarbonate track-etched (PCTE) filter membranes (47 mm diameter and 10 µm pore diameter, Steriltech Corporation). Although PC is plastic, the hydrophilic surface of the filters prevents the staining of the filter, also avoiding false positives from filter particles. Between 2 and 7 membranes were used per sample, according to the suspended solid load. Bottles and vacuuming gear were rinsed thoroughly with Milli-Q water (Millipore, Bedford, MA, USA) before and after every sample to avoid contamination. The filter membranes were left covered in an oven until dry (Erni-Cassola et al., 2017). The digestion of organic material was performed after drying by placing the membranes in 20 ml of 30% H_2O_2, followed by further oven drying (Erni-Cassola et al., 2017). Once dried, the membranes were carefully washed with Milli-Q water using in the same beaker and checked using backlight for possible residuals. Filters were again placed in the oven until dry. After desiccation, the solid sample was rinsed with a high-density floating solution (5.57 mol/L) of K_2CO_3 (≥99.0%, VWR Chemicals) for microplastic isolation (Gohla et al., 2021). The samples were poured in glass density-separation funnels for the separation of sediments (heavier) from microplastics (lighter). The lower sediments were eliminated from the funnel and the K_2CO_3 solution was retrieved for future use (Gohla et al., 2021). The upper solution of K_2CO_3 and microplastics were filtered over a single PCTE filter, which was dried at ambient temperature in a covered glass Petri dish. Milli-Q water was used to carefully wash the remaining microplastics from the funnels. In addition, washing the filters with Milli-Q water minimizes the formation of K_2CO_3 crystals, which could affect the quality of successive staining protocol.

A 1 mg/L solution of Nile Red (Fisher Scientific) and methanol (≥99.8%, VWR Chemicals) was prepared just before the microscope analysis, according to Erni-Cassola et al. (2017). After applying a few drops (3 to 4) to cover the filter, filters were cut in a half using a lancet (previously washed with Milli-Q) to fit over standard microscope slides and stage, covered with coverslips, and fixed with tape. The samples were stored at 60° C for 10 min in the dark. A stereomicroscope (Leica S8AP0) was equipped with a camera (Leica DFC295) and an external fluorescence light source (excitation 470 nm, royal blue), and an orange photography filter was used for imaging (Erni-Cassola et al., 2017; Labbe et al., 2020). Microscopy images were taken using exposure times between 0.7 s and 1 s. Several images were taken for every filter, usually overlapping by around 30% to enable reconstruction of the panoramic view of the filter, using the demo version of Autostitch (Maes et al., 2017). A minimum background signal (e.g. fine sediments on the filter) must be provided, for the program to reconstruct the image correctly and to avoid distorted outputs. After the reconstruction, ImageJ (version 1.53e, Schneider et al., 2012), was used to perform automated particle recognition and quantification based on the fluorescent particles using parameters similar to Erni-Cassola et al. (2017) and Prata et al. (2020). After the automated quantification of microplastics, an output is produced by ImageJ, in which the microplastics selected in the red channel are highlighted in yellow. This final image is inspected for false-positive or errors (see section 4.1). A more detailed description of the protocol and the specifications used in both Autostitch and ImageJ are reported in the Appendix. Simultaneously, three replicates of 5 L Milli-Q water were analysed as control samples (blanks), using the same procedure described above. The resulting analytical contamination was subtracted from the values observed in the field samples (Frei et al., 2019; Samandra et al., 2022) to avoid overestimation given by laboratory contamination, and negative values were rounded to zero (e.g. LDC3 and LDS1 from 25-03-2021). Besides the count of the fluorescent microplastics, the used ImageJ protocol allows to quantify important features as the microplastics area (μm^2), Feret diameter (μm), and circularity, which is expressed in a number between 0 (low) and 1 (perfect circle). Statistical analysis of these results was performed using the statistics software R (R Core Team, 2020). The statistical approach was chosen to verify possible differences in the above-mentioned geometrical parameters between the samples and sampling time (i.e. after the recharge event). Differences among controls (Ctrl), groundwater (GW), and surface water samples (SW) were tested using the Kruskal-Wallis Test (Kruskal & Wallis, 1952) from the package "Rstatix" (R Core Team, 2020). If significant, Dunn's post hoc test was performed (David, 2019) through the R package "FSA" (Ogle et al., 2021), using the Benjamini–Hochberg method for adjusted *p* values. Differences between sampling times were tested using the Wilcoxon rank sum test (Helsel et al., 2020). Both statistical tests were chosen based on the sampling design, together with verified and non-verified assumptions.

3 Results

3.1 Hydrogeological features of the study area

A detailed hydrogeological characterization of the investigated area is presented in Figure 2. The outcropping sediments are related to the Ravenna Subsynthem (AES8) and the Modena Unit (AES8a) (Calabrese & Ceriani, 2009). The Ravenna Subsynthem (originated between the upper Pleistocene and Holocene) primarily comprises sandy gravel, sand, and stratified silt, with a discontinuous cover of clayey silt. The Modena Unit (originated during the Holocene) primarily comprises sands with gravel lenses, covered by a discontinuous layer of clayey silt (Calabrese & Ceriani, 2009). The outcropping materials were grouped based on their relative permeability. More permeable outcropping (gravel and sand) is found in the S-W part of Figure 2 (near the foothill) and nearby the main watercourses, while the less permeable outcroppings (clay and silt) are reported in the N-E part of Figure 2. Thus, the aquifer is identified as unconfined at S-W and near the Taro River, which dissected the clay and silt cover. On the contrary, it is identified as confined in the remaining area (N-E), where the confining (clay and silt) layer can reach 15 m of thickness (litholog 181120P501).

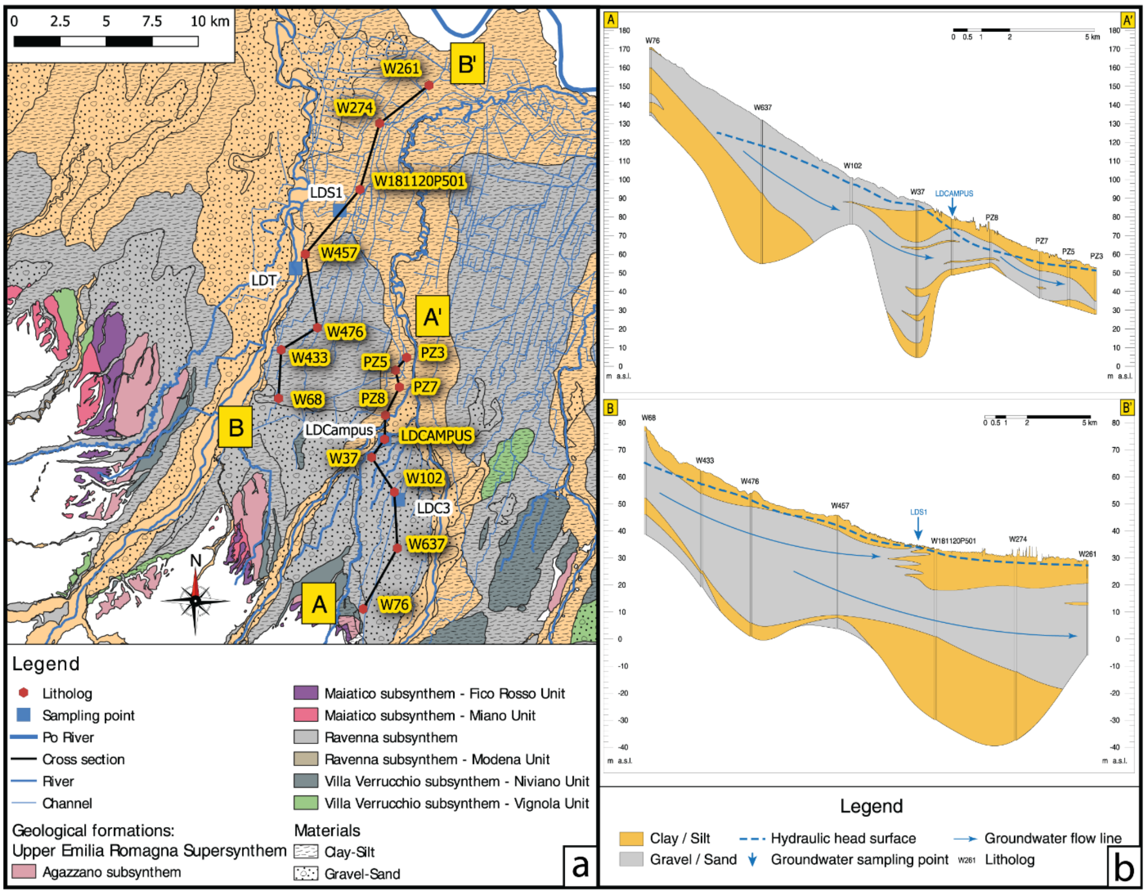

Figure 2. Geological and hydrogeological settings of the investigated area at large scale. (a) Geological chart of the region including the two sub-areas, with geological Formations and outcropping materials, modified from the Geological map of the Emilia-Romagna Region, 1:25.000 scale ("Geological map of the Emilia-Romagna Region" 2004). (b) Hydrogeological cross-sections are reported in panel (a); the hydraulic head values were derived from (Zanini et al., 2019).

The identified sub-areas where sampling was performed are also reported in Figure 2. Each one is composed of a sampling point for surface waters and another one for groundwater. The first sub-area is in the central part of Figure 2. It is composed of a losing channel (namely LDC3) and a piezometer downgradient (LDCAMPUS, Figure 3). Between these two points, the aquifer changes from unconfined to confined. The hydraulic head of the pressure transducer in the LDCAMPUS piezometer showed a recession period of groundwater interrupted by a recharge event (Figure A1). The aquifer recharge supplied by rain results in a small rise of hydraulic head. This phenomenon, although of small entities, is appreciable also in the previous (small) recharge events (Figure A1). The fast response of the system provides other pieces of evidence to the high permeability of the surrounding area. Without the recharge event, the hydraulic head would keep lowering with a regression coefficient of 0.015 d^{-1}. The recharge resulted also in a variation of EC (Table 1). In both LDC3 and LDCAMPUS, the EC values were lower after the recharge given by precipitation. This evidence is consistent with the conceptualized river-groundwater interaction, with LDC3 feeding LDCAMPUS.

The water level of the Taro River rapidly increased after rainy days. Therefore, the discharge of LDS1 increased, due to the high groundwater recharge provided by the Taro River (Figure 3). The spring discharge was not measured during the first sampling due to the low flow and incompatibility with the current meter (Small Current Meter C2, OTT, Kempten, Germany). After the recharge event consequent to the flow rise in the Taro River, the spring discharge raised from non-measurable to 4.8 L/s. The recharge effect on LDS1 is evident also in tritium content (Table 1). After the recharge event, the tritium content of the spring increased by 31.7% (+ 2.24 TU) and was closer to the tritium content of precipitation. Thus, the tritium content of LDS1 over time is consistent with the proposed river-groundwater interaction, with LDT feeding LDS1. These data also testify that the groundwater flow nets reconstructed with data from Viani (2017), Lancini (2019), and Zanini et al. (2019) are still valid, at least in the investigated sub-areas. Unlike for surroundings of LDCAMPUS, no data about hydraulic conductivity and storativity are available for this sub-area. Nevertheless, given the fast response of the spring to the recharge, and the comparable grain size of the aquifer, we assumed that hydraulic conductivity is like that of the area near LDCAMPUS, in the order of 10^{-4} m/s.

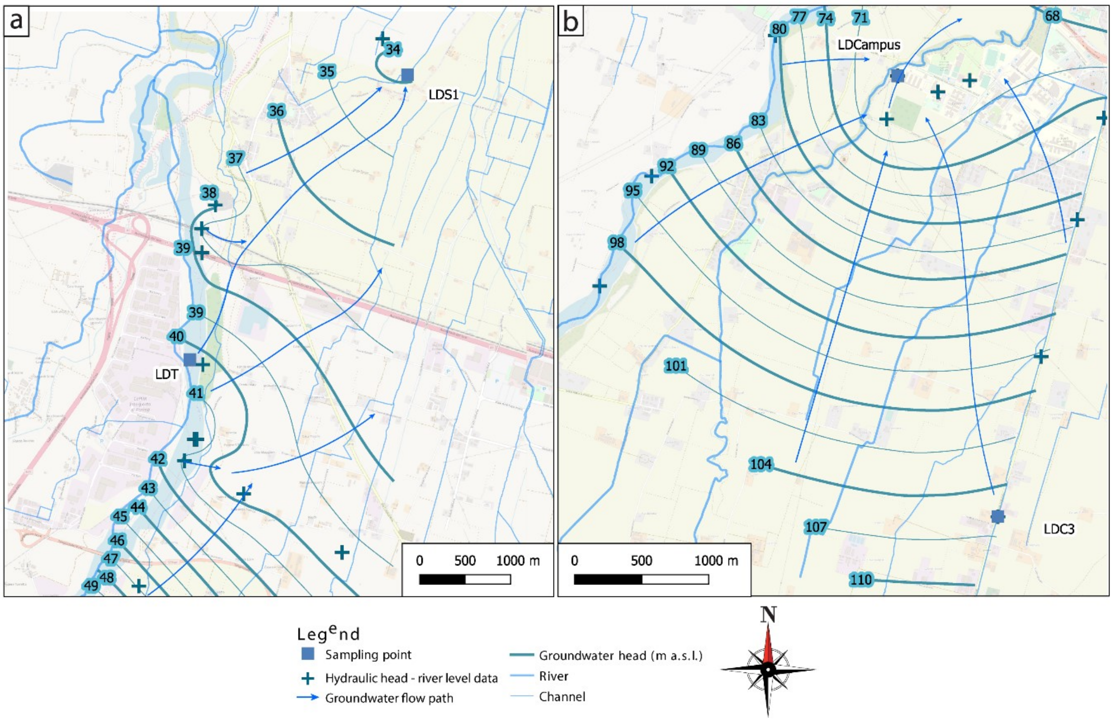

Figure 3. Groundwater net for the sub-areas. (a) Groundwater flowpath between LDT and LDS1, hydraulic head data from Lancini (2019); (b) Groundwater flowpath between LDC3 and LDCAMPUS, hydraulic head data from Viani (2017).

Table 1. Isotopic and field data. The dot represents not analysed samples.

Date	25/03/2021	25/03/2021	25/03/2021	13/04/2021	13/04/2021	13/04/2021	04/21
Sample	LDS1	LDCAMPUS	LDC3	LDS1	LDC3	LDCAMPUS	Precipitation
t (° C)	14.40	16.00	6.40	14.40	7.70	14.00	-
O_2 (mg/L)	2.99	8.51	8.96	2.08	9.22	7.67	-
O_2 %	29.30	86.50	72.80	20.40	77.30	81.00	-
EC (µS/cm)	586.10	893.00	608.70	577.50	467.70	308.40	-
ORP (mV)	89.80	201.50	143.10	154.90	139.50	190.20	-
pH	7.56	7.28	7.96	7.29	7.96	6.92	-
^{3}H (TU)	7.06	-	-	9.30	-	-	10.00
δD (vs V-SMOW)	-45.10	-	-	-43.80	-	-	-
δ^{18}O (vs V-SMOW)	-7.44	-	-	-7.42	-	-	-13.20

3.2 Microplastics quantification and geometric characterization

To reconstruct the whole panoramic view of the filters, typically 45 ± 11.4 (from here, average ± standard deviation) photos were taken for every microscope slide, *i.e.* half-filter. The maximum number of photos taken was 76, while the least was 29. In total, 989 photos were taken. Although the use of Milli-Q water and accurate cleaning of instruments, the three control samples had a mean concentration of 36.9 microplastics/L, which will be contextualised in Section 4.1. The microplastics presence in both surface and groundwater was highly heterogeneous. Surface water samples had a mean concentration of 43.9 ± 49.7 microplastics/L, while groundwater samples' mean was 45.78 ± 86.1 (Figure 4). The concentration of analytical and field samples is reported in Table A1. Only a few sampling points showed a higher microplastics concentration after the recharge event, like LDS1, where also a discharge increase is appreciable.

Regarding the geometrical characterization, control and environmental samples showed interesting and peculiar characteristics. A statistical difference was reported between microplastics in all the three samples' typology, not only in the area (Chi square = 144.18, df = 2, p *value* = 2.2E-16), but also in circularity (Chi square = 105.41, df = 2, p *value* = 2.2E-16) and in the Feret diameter (Chi square = 161.41, df = 2, p *value* = 2.2E-16). These differences were further tested using the Dunn's test for pairwise comparison, showing that microplastics in control samples had a smaller area (median values: Ctrl=2430 µm^2, SW=4375 µm^2, GW=4691 µm^2) and Feret diameter (median values: Ctrl=74.5 µm, SW=105 µm, GW=111 µm) than surface and groundwater samples, while the circularity was higher (median values: Ctrl=0.952, SW=0.877, GW=0.895) in control samples (Table A1).

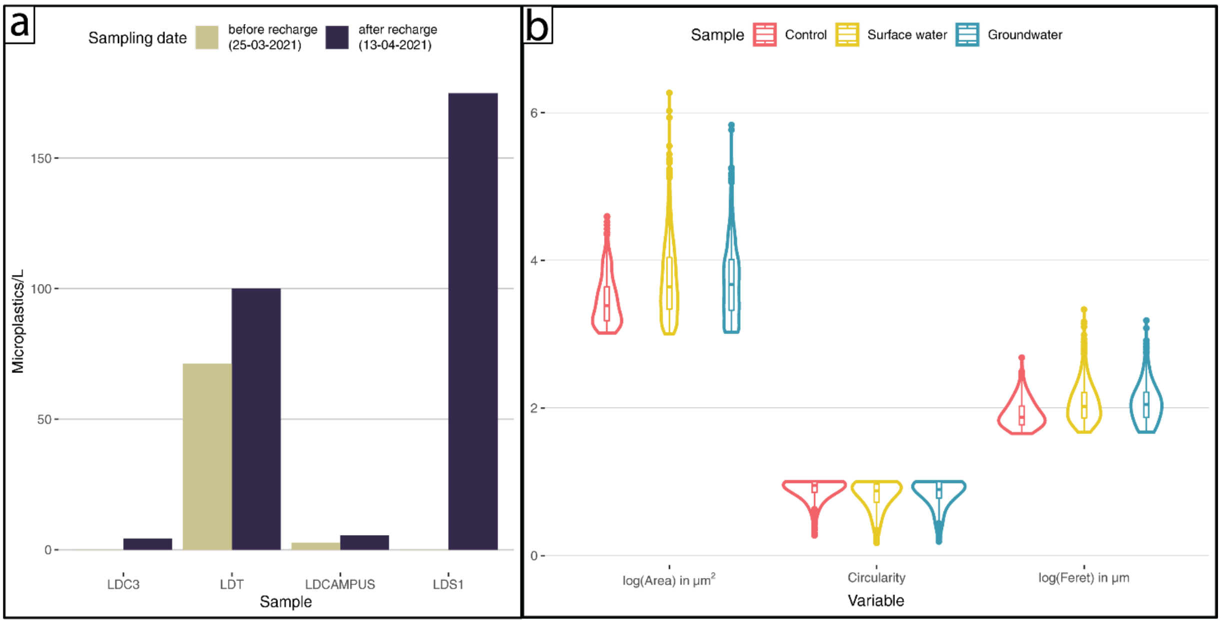

Figure 4. Microplastics characterization. (a) Microplastics occurrence (particles/L). (b) Microplastics geometric attributes in controls (3), surface and groundwater samples; Inside the violins, boxplots indicate the minimum and maximum values, lower and upper quartile and median. Area and Feret diameter were log-transformed for graphical reasons.

Between surface waters and groundwater, the pairwise comparison using Dunn's test revealed a higher circularity in groundwater than surface waters, whereas no differences were reported for area and Feret diameter (Table A1). The geometrical features of microplastics were also tested between the two sampling times (before and after recharge). As a result, the Wilcoxon Rank Sum Test highlighted a significant difference between the sampling times in all the parameters, except for circularity in surface waters, where the test was not performed due to assumptions violation. In detail, before the recharge event, microplastics had a larger area and Feret diameter in both surface- (test statistic= 264753 and test statistic= 271315, respectively; Table A1) and groundwater (test statistic= 143738 and test statistic= 146741, respectively; Table A1). In groundwater, circularity was higher after the recharge event (test statistic= 190540, Figure 5, Table A1).

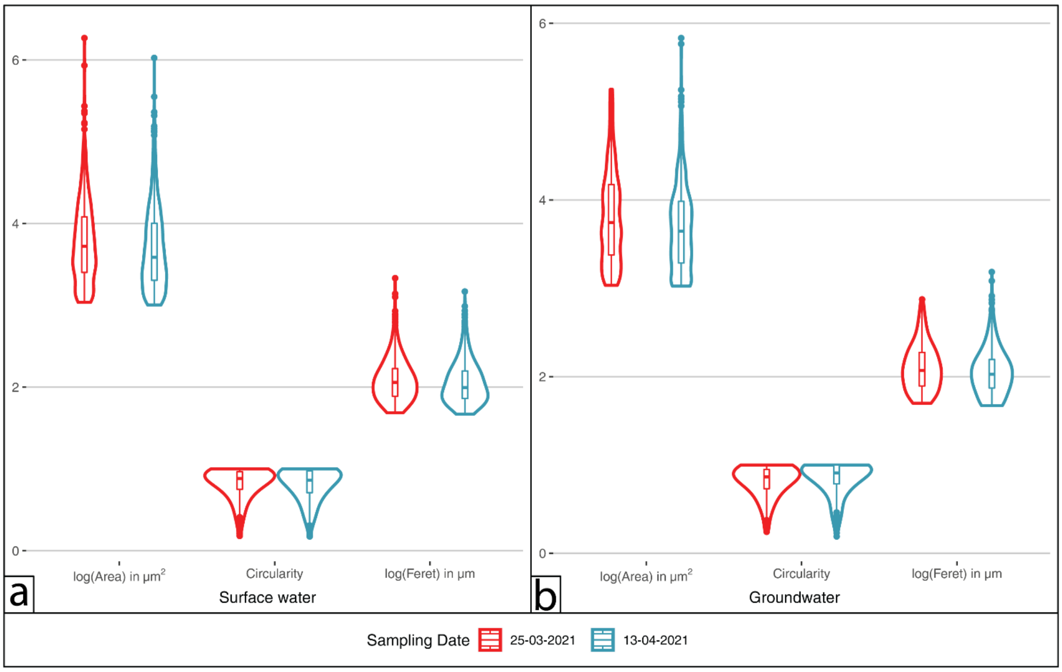

Figure 5. Microplastics characterization between sampling times. (a) Microplastics geometric attributes in surface waters. (b) Microplastics geometric attributes in groundwater. Inside the violins, boxplots indicate the minimum and maximum values, lower and upper quartile, and median. Area and Feret diameter were log-transformed for graphical reasons.

4 Discussion

4.1 Assets and disadvantages of the extraction protocol

The proposed sampling strategy aimed to avoid any form of contamination during the sampling using only metallic or glass material. The sampling with the metallic bailer can be more time-consuming than the direct sampling with a baker. Anyway, this is dependent on the depth of piezometric level and the Bailer volume.

The methodology used for microplastics extraction, derived from the integration of previously derived methods, allowed a good characterization of the microplastics contamination through an inexpensive and easily performable procedure. Although microplastics investigation through fluorescence had already been reported as a successful method (Shim et al., 2017), some improvements were adopted. Starting from the protocol proposed in Erni-Cassola et al. (2017), a higher number of filters was utilized for every sample during the first filtration step. This is due to the high load of suspended solids found in surface waters during the recharge event, which happened to obstruct the filter and possibly cover microplastics with smaller solids such as silt and clay, which were difficult to remove from the final processed sample. The high amounts of suspended solids also made the use of a floating solution for microplastic unavoidable. To allow the isolation of also denser microplastics like PVC, a solution of potassium carbonate (K_2CO_3) was prepared, with a density of 1.54 g/cm^3. This particular floating solution was preferred to others like ZnCl and NaI as K_2CO_3 is cheaper, recyclable, and more environmentally friendly (Gohla et al., 2021), but has a higher density than the NaCl solutions (up to 1.2 g/cm^3). However, the high density of K_2CO_3 solution also does not allow the sedimentation of smaller sediments like silt and clays, which were found in the final filter and are responsible for a light background signal of the dye during the fluorescence analysis (Figure 6).

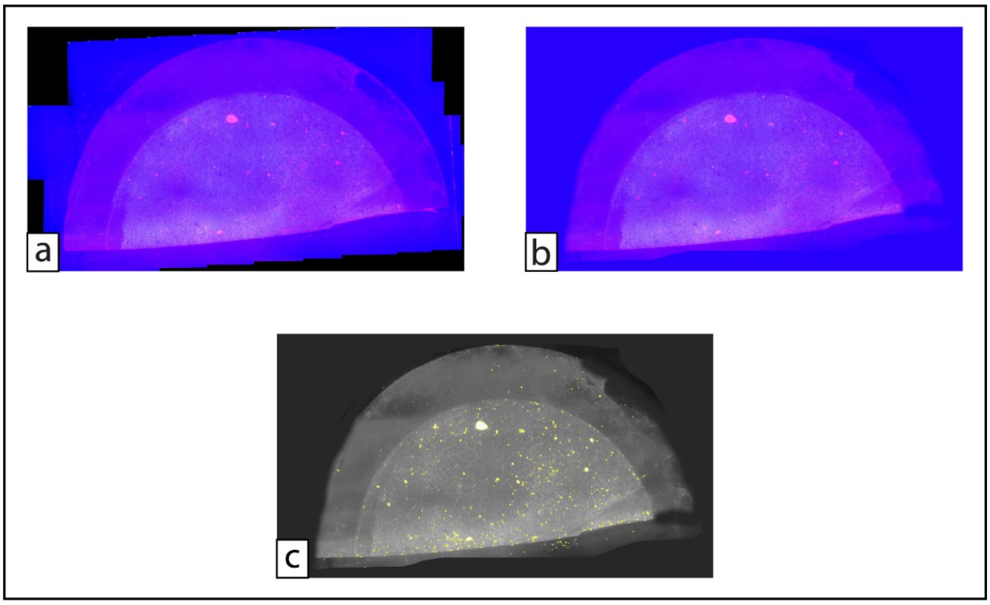

Figure 6. Filter membranes reconstructed and processed. (a) The image output from Autostitch. Only the central area of the filter is the actual filtering area, whose pink background could be due to the presence of silt and clay stained by the dye; on the top side of the image, it is still possible to see the original scale of the individual photos taken. (b) The brush tool with blue colour was used to correct filter imperfections. (c) The final output from ImageJ (red channel), in which the identified microplastics are underlined in yellow, allows the post-analysis search of errors or false positives.

In addition, high-density solutions of K_2CO_3 can easily precipitate (Gohla et al., 2021), forming crystals in the final processed sample. Despite that, these crystals had no consequences during the fluorescence analyses (Figure 7), since they have no red colour and were well cancelled from the image during the colour splitting in ImageJ (see detailed analysis protocol in the Appendix). These macroscopic precipitates were associated with K_2CO_3 since they were not present in the previous stages of the analysis.

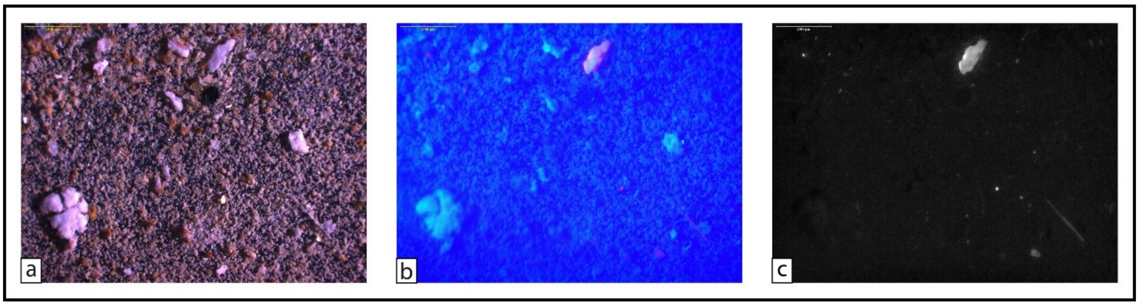

Figure 7 | High magnification image of an analysed filter membrane. (a) Original image under normal light; the white object in the bottom-left corner is supposed to be K_2CO_3

precipitate. **(b)** Same filter portion under fluorescence conditions. **(c)** The final output from ImageJ (red channel).

The filters were clipped in half as in Erni-Cassola et al. (2017), but the cutting attempts resulted in the loss of material from the filter. As a solution, filters were cut immediately after the Nile red solution application, which wetted the material over the filter and avoided its loss during the cutting phase. If used in a larger volume than necessary, the Nile red solutions could dye the filter borders. In this case, the filter image was modified in a raster graphics editor using a blue brush to correct these defects (Figure 8), which would otherwise be considered as microplastics by ImageJ. The blue colour was preferred since it cannot result in false positives in ImageJ due to channel separation (see detailed analysis protocol in the Appendix).

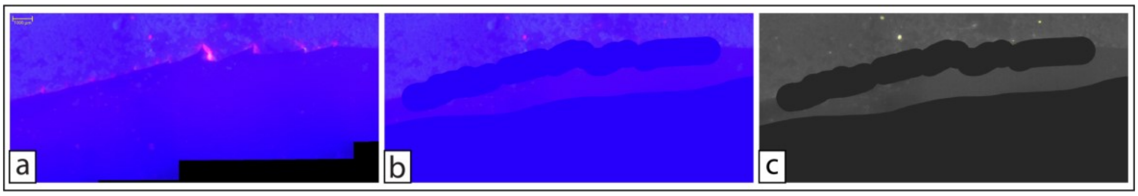

Figure 8. Filter correction using the brush tool. (a) Original image from Autostitch; the pink spots are filter portions accidentally dyed by the Nile red solutions and are quite common in the clipped segment of the filter (b) Colour image after correction. (c) The final output from ImageJ (red channel).

The use of microscope slides and coverslips was preferred to simply uncovered Petri dishes for two reasons: firstly, they avoid laboratory contamination without lowering the image quality. Secondly, they allowed the inspection of the whole (half-)filter, conversely to Petri dishes whose edges would interfere with the microscope objective. The use of Petri dishes larger than the filter (47mm diameter) was discouraged as they could not steadily hold the filter during sample handling. The necessity to analyse the whole filter, instead of some random parts of it, derived from the microplastics distribution in the filter, which is not homogeneous. In fact, during the filtration process, microplastics stick to the internal side of the filtration instrument. After rinsing, they were more concentrated on the external area of the filter. In addition, more microplastics were also found in the central part of the filter, where the first spilled sample was filtered. For these reasons, the use of randomly taken images was avoided and an accurate, but more time-consuming, reconstruction of the whole filter image was performed using the demo version of Autostitch, as reported in Maes et al. (2017), using images taken through a microscope and resulting in a higher resolution with a theoretical smaller minimum dimension of particles for detection.

The microscope adaptations proposed by Labbe et al. (2020) were successfully replicated and utilized for the analyses presented in this study. Although the obtained images were characterized by lower quality in comparison to those obtained using originally-made fluorescence microscopes, their resolution was sufficient to characterize the contamination. Although a high number of microplastics was detected, the comparison between normal light and final output of the identification procedure (Figure 7) showed a lower sensibility for microplastics of elongated and thin shape such as fibers, which were not detected for their whole length but only for portions of it. Last but not least, the microscope adaptation was realized with an incredibly low budget (< 100 €).

If compared to other similar studies (Erni-Cassola et al., 2017; Samandra et al., 2022) the three controls had a high number of microplastics, but similar to Shim et al. (2016). This high number affected the microplastics count in environmental samples. Nevertheless, a meticulous rinse of all laboratory instruments with Milli-Q water was performed between each sample and a white cotton laboratory coat was always worn in the laboratory. Thus, the contamination came from unfiltered laboratory solutions and airflow (although AC was turned off down during analyses), due to geometric traits of microplastics compared to environmental samples (see Section 4.2) rather than sample intra-contamination, confirming the validity of a rinsing protocol of the instruments. The material (high purity grade) bought for the laboratory solutions was sold in plastic packages, except for Nile red. Hence, a pre-filtration of all the used solutions should be performed before use (Maes et al., 2017; Prata et al., 2020; Samandra et al., 2022). A control test using three ulterior analytical blanks of 5 L, but with (re-)filtered Milli-Q and reagents showed significantly lower contamination (respectively 13.4, 6.4 and, 35.6 microplastics/L), with a mean value of 18.4 microplastics/L. Anyway, since not present in Erni-Cassola et al. (2017), the re-filtration was not performed before the analysis of the presented samples. From the geometrical perspective, microplastics from control samples were characterized by a statistically higher circularity, but a smaller area, and Feret diameter than those from environmental samples. From a graphical point of view, the violin plot (Figure 4b) produced interesting information. This graphical output was preferred to a classic boxplot since it allows a deeper understanding of the distribution of a numeric variable, with the violin's shape representing the density estimate of the variable (Figure A3). It can be inferred from Figure 4b that both area, circularity, and Feret diameter had a restricted distribution and were more condensed in the lower (upper for circularity) part of the range if compared to environmental samples, which were characterized by a sensibly different violin shape. This consideration is consistent with the afterward tests on filtered analytical blanks and supports the partial origin of microplastics contamination in unfiltered laboratory solutions, which are expected to possibly contain smaller microplastics than those in environmental solutions (e.g. Frei et al., 2019; Prata et al., 2020), with a possible more rounded shape due to the abrasive and smoothing effect of the used salts (i.e. K_2CO_3) in plastics packaging.

4.2 Aquifer recharge and river-groundwater interaction as drivers of the microplastic contamination

Microplastics were present in all of the analysed samples. Nevertheless, the subtraction of the analytical samples' concentrations resulted in some samples without microplastics (LDC3 and LDS1 from 25 March 2021). This data provides ulterior pieces of evidence of microplastics contamination in groundwater, which studies are still rare. Groundwater samples had a mean concentration of 45.78 ± 86.1 microplastics/L whereas surface waters had a concentration of 43.9 ± 49.7 microplastics/L, similar to Samandra et al. (2022). Comparing all the samples, the difference in concentrations between before and after the recharge event was not significant. Nevertheless, some sampling points showed an undeniably higher number of microplastics after the recharge event. This is especially true in LDS1 and LDC3 before the recharge event, which samples did not contain microplastics (after control correction). This discrepancy can be owed to the different entities of groundwater recharge or surface water contributions and their initial concentration. Firstly, the comparison between the two phases should be performed only in a portion of the aquifer with a similar recharge condition or, better, along the same groundwater flowpath. Comparing LDS1 and LDCAMPUS microplastics concentrations leads to improper conclusions, as they are characterized by different recharge areas and contamination of recharging water. Concerning the comparison of single sampling points in two different periods, higher microplastics concentration was found during the recharge event, due to a higher hydraulic head given by aquifer recharge. This process was investigated at laboratory scale in porous media by Bizmark et al. (2020). They found that a higher pressure in the medium is responsible for a chaotic transport (i.e. simultaneous and continuous deposition and erosion) of colloidal particles such as microplastics, forcing them to be transported for longer distances. On the contrary, lower hydraulic pressures in the aquifer cause the localization of microplastics just near the input area. According to the presented data, this process can be upscaled also to the investigated area. The recharge event caused a higher hydraulic pressure (i.e. hydraulic head) that fostered the transport of microplastics farther from the source (i.e. the losing rivers). As result, more microplastics are found in the sampling groundwater points. When the hydraulic heads are lower, less microplastics were found in the groundwater sampling points, which are few km downgradient.

Within the same sampling period, the two groundwater samples highlighted a remarkable difference in microplastics concentration. This fact can be related again to the different recharge conditions, responsible for the mechanism described above. The entity of recharge in the Taro River (LDT recharging the aquifer near LDS1) was inevitably higher than in LDC3 (recharging the aquifer near LDCAMPUS). This is due to the different discharges of the rivers, with a mean annual value of 61.9 m^3/s (Taro River, i.e. LDT) and <1 m^3/s (La Riana Channel, i.e. LDC3). Finally, looking at the concentrations of those watercourses (Figure 4), LDT had a higher microplastics concentration than LDC3, resulting in higher microplastics concentration of groundwater recharged by these rivers, namely LDS1 and LDCAMPUS, respectively.

Another important result deals with the differences among the geometrical attributes of microplastics found in surface and groundwater. Besides allowing the discrimination between microplastics in control and environmental samples, these features provided interesting information regarding the dynamics of contamination and transport between surface and groundwater. The only difference between surface and groundwater microplastics geometry was observed in circularity, underlying that microplastics in groundwater had a more rounded shape than in surface waters.

This difference could be ascribed to the presence of secondary microplastics in surface waters with heterogeneous aspects, due to their variable original shape, myriad degradation processes, and chemical composition. On the contrary, in porous media their transport can be facilitated by a more rounded shape, as reported by Keller et al. (2020) and by further authors for bacteria (Weiss et al., 1995) and other colloidal materials (Liu et al., 2010; Seymour et al., 2013), resulting in the presence in groundwater of only the more rounded microplastics. The area and Feret diameter did not show differences between surface and groundwater samples.

The only difference appears to be given by the microplastics' circularity, which is higher in groundwater due to the preferential transport of rounded microplastics and lower in surface water due to the simultaneous presence of sharp-cornered and rounded microplastics. These preliminary results established new questions and encourage further investigations about the effect of microplastics' shapes during transport in porous media. In fact, to the author's best knowledge, this is one of the few characterizations for microplastics contamination in a confined alluvial aquifer, also with regards to their shape.

Between the two sampling times, the geometric features of microplastics showed unexpected variations. In both surface and groundwater, microplastics had a smaller area and Feret diameter after the recharge event. Regarding surface waters, these findings are consistent with the enhanced transport processes in rivers during the passage of a flood wave (Heidel, 1956). These events lead to a highly unsteady flow regime, resulting in resuspension and transport of deposited bottom sediments, the release of contaminants from the interstitial water of the sediments, and cause land erosion (De Sutter et al., 2001). During the recharge events, this high transport of sediments and suspended solids can consequently affect also microplastics that are sedimented at the bottom or on the riverbanks. Although larger microplastics were present, this effect must have affected more the smaller microplastics, which driven the decrease of the average microplastics size (area and Feret diameter). This fact can be also detected from Figure A3, where violins of the recharge period sampling have a higher density of points in the lower part of the violin range, especially in surface waters. In groundwater, circularity was higher during the recharge period (Table A1). This discrepancy is attributable again to the increased pressure in the porous medium, which seems to promote the transport in the aquifer of smaller and more rounded microplastics. The overall result seems indeed an increased abundance of microplastics after the recharge event in both surface- and groundwater, characterized also by a smaller area and Feret diameter and a more rounded shape (only in groundwater). These are just preliminary results and more samples are necessary to better characterize the effects of aquifer recharge and surface-groundwater interactions as drivers of microplastics contamination in the aquifer. Nevertheless, they point out important understandings of the effects of small recharge events, making these data fundamental for the future sampling timings. The contamination shows important variations not only between the recharge and recession periods, i.e. after a significant variation of hydraulic heads, but also after small recharge events. Thus, microplastics samplings cannot be performed only seasonally or many days after the recharge event, but they require a dynamic sampling timing strictly connected to the precipitation regime.

No chemical characterization was performed to characterize microplastics. Although this seems to be a preferred key in other articles (e.g. Samandra et al., 2022), we focused on the geometrical aspect of the contaminating microplastics, trying to correlate them with the transport in the aquifer due to the river-groundwater interaction and recharge effects. Nevertheless, the chemical characterization of microplastics is the natural consequence of this study. The identification of main polymers will allow to investigate the microplastics' origin. We hypothesise that, when investigating the microplastics contamination in groundwater, a jointed geometrical and chemical characterization of microplastics is essential for a reliable definition of the contamination. The presented results raise new questions on the methods and investigations on microplastics. To answer these questions, the method is being already implemented, starting from an already sensibly lower analytical contamination (18.4 microplastics/L) after reagents and Milli-Q filtration. It will be applied to a smaller area, aiming to characterise the microplastics migration from surface waters to groundwater under more controlled conditions (e.g. detailed hydrogeological numerical modelling and calculation of efficient porosity), focusing on the processes at small scale that in large areas like those here presented are difficult to control.

5 Conclusion

The investigation was performed using a protocol based on a self-made fluorescence microscopy setup and an open-source image processing program, resulting in a low cost : results ratio. Such a technique allows even those who lack access to a specialized analytic chemistry laboratory to conduct research in this field. This will be particularly useful in developing countries, where the research in this field is less advanced than that in developed countries and complicated by potentially limited access to expensive equipment, e.g. micro-FTIR or micro-Raman (Zhang et al., 2020).

In addition, the presented results shed new light on the interaction of the microplastics abundance and geometrical attributes with the hydrogeological characteristics of the aquifer. Although not characterized by a chemical perspective, observing microplastics contamination provided interesting information regarding their presence and transport in a porous material, i.e. alluvial aquifer. Furthermore, these findings raise new and urgent questions about microplastics in groundwater. For example, which mechanisms influence the transport of microplastics in alluvial aquifers? How do the geometrical features of microplastics, coupled to their electro-chemical characteristics, affect their transport in the aquifer? Extensive research is still required in this field, which is poorly explored and demands further attention by the scientific community.

CRediT authorship contribution statement

Edoardo Severini: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Laura Ducci**: Investigation, Validation. **Alessandra Sutti**: Methodology, Writing – review & editing. **Stuart Robottom**: Methodology, Writing – review & editing. **Sandro Sutti**: Methodology, Writing – review & editing. **Fulvio Celico**: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration.

Declarations of interests

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

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Appendix

SAMPLING PROTOCOL, MICROPLASTICS EXTRACTION, AND ANALYSES

This protocol was formulated for the preparation of environmental samples to detect and quantify small microplastics using fluorescence microscopy. It was derived from already published methods.

Before starting

- All the laboratory material and instruments must be washed with filtered water (e.g. Milli-Q to avoid contamination) before and after every sample handling. Laboratory members should wear white cotton laboratory coats.
- Microplastics extraction should be performed in a dedicated space, to avoid contamination during other laboratory activities.

Microplastics extraction

1. Sample 5L in a glass bottle using a glass beaker or a metallic Bayler sampler. This large volume is recommended to maximize microplastics identification in low concentrated environmental samples. If sampling is performed in wells or piezometers, a metallic bailer sampler and a metallic wire must be used.
2. Due to the possible high amount of suspended solids, the sample must be left to sediment for at least 24h.

3. The sample is filtered on PCTE filters (47 mm diameter and 10 µm pore diameter). Start with the liquid part and lastly proceed with the sedimented material. The bottle and vacuuming gears must be carefully rinsed with Milli-Q, as microplastics tend to attach to the borders.
4. Filters are placed in a 250 ml glass beaker, tapped with aluminium foil, and left dry in hoven at 60° C for 24 hours.
5. Perform the digestion of organic material was performed with 20 ml of 30%H_2O_2 every filter, to a maximum of 100 ml.
6. In the same beaker, filters are placed in hoven for 1h at 60° C and successively at 100° C for 7h.
7. Wash filters carefully in the same beaker with milli-Q water and check backlight for possible residuals.
8. The beaker must be capped with aluminium foil and left drying in a hoven at 60° C.
9. Rinse samples with a solution of K_2CO_3 with a density of 1.54 g/cm^3.
10. Left the high-density floating solution for 15 minutes over a magnetic stirrer to foster the dissolution of the dried sample, which stuck to the glass border during desiccation.
11. Pour the solution in glass density-separation funnels, tap with aluminium foils, and let rest for 12 h, promoting the separation of sediments (heavier) from microplastics (lighter).
12. Pour the lower part of the solution from the funnel, filter it on a paper filter, and recycle the K_2CO_3 solution.
13. The upper part of the solution is filtered on a new PCTE filter and let dry at normal temperature in a covered petri dish.
14. The vacuuming gear must be carefully rinsed with ulterior K_2CO_3 solution to retrieve all the microplastics attached to the borders.

Microscope identification

15. Arrange the microscope set-up for fluorescence imaging, using an external light source and orange photographic filters. If the light source is working with batteries as power source, continuously check the light intensity, as a lower light can affect the microplastics fluorescence with Nile red.
16. Prepare a 1 mg/L solution of Nile Red and methanol before the microscope analyses.
17. Apply 3-4 drops of solution and cut the filter membrane in half while still wet.
18. Put the half filter in a standard microscope slide (previously washed with Milli-Q), covered with a coverslip, and fix it with tape.
19. Store in the dark at 60° C for 10 min.
20. Perform the image acquisition, using the same light intensity and exposure time of the camera for all the photos, since the subsequent ImageJ characterization is based on pixel brightness.
21. Acquire images accordingly to the Autostitch requirements, e.g. 30% overlapping of the photos

Microplastics identification and characterization

22. Import images in Autostitch and set the program as follow:

- Width 4000 px.
- Rendering options: Multi-Band. If the reconstruction of the panoramic image can't be completed, a Linear blending method could resolve this issue.
- System Memory: 2.00.
- JPEG Quality: 100.
- Ransac parameters: 5000 Max iterations.

23. Import the output image in a raster graphics editor and correct filter defects using a blue brush.
24. Import the image in ImageJ and set the image scale according to your microscope and modify the script below for every image (i.e. use a different scale).
25. Perform the following script for detection and quantification of microplastics:

```
filename = getTitle();

run("Split Channels");
selectWindow(filename + " (green)");
close();
selectWindow(filename + " (blue)");
close();
selectWindow(filename + " (red)");

run("Subtract Background...", "rolling=50");

run("Window/Level...");
setMinAndMax(20, 220);
run("Apply LUT");
run("Close");


run("Auto Threshold...", "method=RenyiEntropy ignore_white white");

setOption("BlackBackground", false);
run("Convert to Mask");


run("Set Scale...", "distance= Ø Ø Ø known= Ø Ø Ø unit=unit");
\\inserth in the above row the values of the settled scale before running the script

run("Analyze Particles...", "size=1000-Infinity show=Outlines display add");
```

26. Save the Results table and close all the windows except the ROI Manager (necessary for the next script).
27. Open again the image and perform the results verification using the following script:

```
filename = getTitle();

run("Split Channels");
selectWindow(filename + " (green)");
close();
selectWindow(filename + " (blue)");
close();
selectWindow(filename + " (red)");
roiManager("Show All without labels");
```

28. Save the verification image in the same folder with all the other documents.
29. Aggregate the .csv files from all the filters in a single file to perform statistical analyses.

Among the above-mentioned parameters in the ImageJ macro, the fixed parameters were selected according to other published works and adapted to our filter images. In detail, the *rolling* value was lowered from 1500 (Erni-Cassola et al., 2017) to 50, producing better results with the final images, probably due to the different resolution of the elaborated images. A linear enhancement of brightness and contrast was performed using the *Window/Level* tool, modifying the values as reported above. In the *Autotreshold*, the RenyiEntropy method was preferred to others, according to Prata et al. (2020) who reported better results in presence of impurities, such as the suspended solids. Finally, during the particle analyses, a minimum object size of 1000 μm^2 was preferred to the inferior limit of 400 μm^2 set by Erni-Cassola et al. (2017) to ulteriorly prevent possible overestimations given by sediments. Together with laboratory blanks and the final check for false positives, the ImageJ script was also tested using artificially made filter images (Figure S2), resulting in good recovery rates, with an overestimation of +0.46% for microplastics of large dimensions (Figure S2a) and +0.23% for microplastics of small dimensions (Figure S2b).

FIGURES AND TABLES

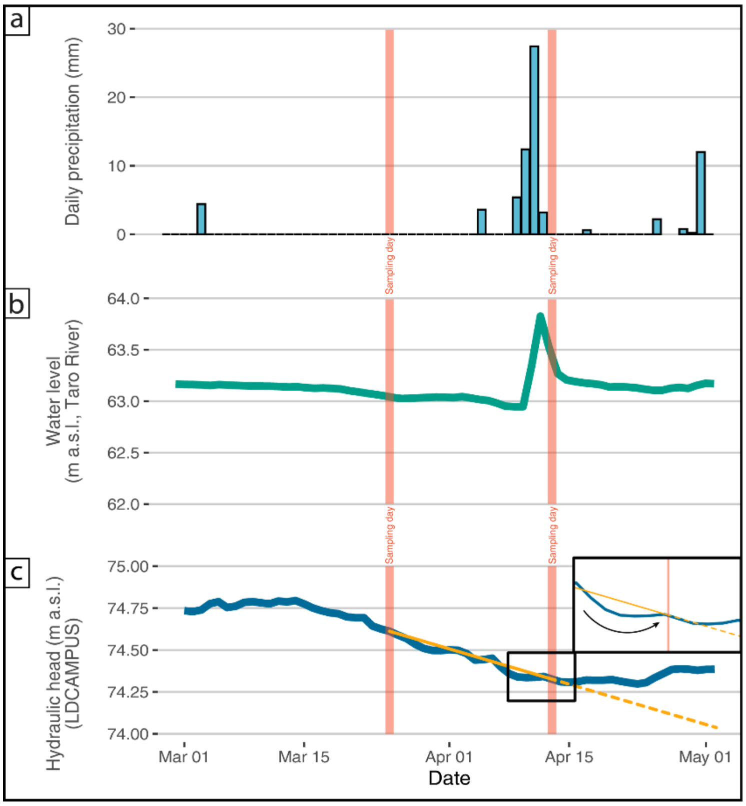

Figure A1. Hydrological data measured in surface and groundwater. (a) Precipitation data were measured in the Parma meteorological station. (b) Water level measured in the Taro River (c) Hydraulic heads measured in the LDCAMPUS piezometer using a pressure transducer with data-logger; the orange line represents the measured (continuous) and projected (dashed) discharge of groundwater, with a regression coefficient of 0.015 d^{-1}. A zoomed frame of the recharge event is also presented. Red bars indicate the sampling day.

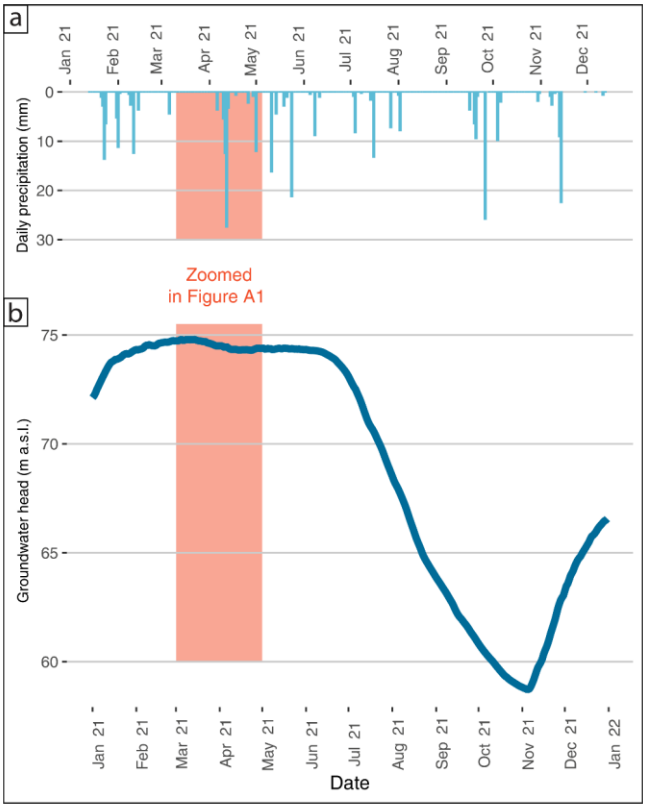

Figure A2. Hydraulic head and precipitation during 2021 in LDCAMPUS. (a) Cumulated daily precipitation data. (b) Groundwater head. The red area is presented more detailly in Figure A1.

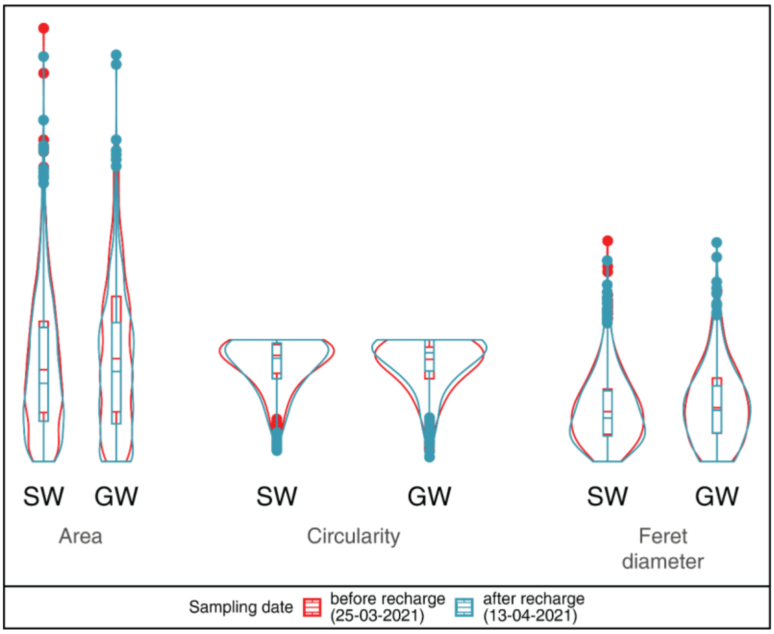

Figure A3. Comparison of geometrical attributes through violin plot. Comparison of the violins shapes from Figure 5. Violins are aligned, although the original graph has a lower base value for the area and Feret diameter in GW violins. SW represents samples from surface waters and GW samples from groundwater. Although not representative from a numerical perspective, this comparison grants a better understanding of the density estimate of a variable, i.e. the abundance of various microplastics dimensions for the investigated variables between the two sampling periods.

Table A1. Microplastics abundance, statistical analyses results, and median values. Values between brackets represent abundances before correction with analytical blanks. When the Kruskal-Wallis test is significant, the p *values* reported are those of the Dunn's post hoc test, adjusted using the Benjamini–Hochberg method. The Wilcoxon Rank Sum Test for circularity comparing different sampling times in surface water was not performed since the assumptions of normality and equality of variance were not met. The before and after recharge conditions are abbreviated as B and A, respectively.

Date	Sample	Abundance (microplastics/L)	Parameter	Sample	Test	p *value* (adjusted)	Sample	Median value
13-04-2021	LDC3	4.27 (41.20)	**Area**	Ctrl - GW	(Kruskal Wallis test) Dunn's post hoc test	2.25E-27	Ctrl	2430 (μm^{2})
13-04-2021	LDCAMPUS	5.57 (42.50)		Ctrl - SW	(Kruskal Wallis test) Dunn's post hoc test	1.17E-29	SW	4375 (μm^{2})
13-04-2021	LDS1	174.87 (211.80)		GW - SW	(Kruskal Wallis test) Dunn's post hoc test	0.581	GW	4691 (μm^{2})
13-04-2021	LDT	100.07 (137.00)		B - A recharge (SW)	Wilcoxon Rank Sum Test	0.000467	B recharge (SW)	5627 (μm^{2})
25-03-2021	LDC3	0 (24.4)		B - A recharge (GW)	Wilcoxon Rank Sum Test	0.000597	A recharge (SW)	3869 (μm^{2})
25-03-2021	LDCAMPUS	2.67 (39.60)					B recharge (GW)	5563 (μm^{2})
25-03-2021	LDS1	0 (18.20)					A recharge (GW)	4444 (μm^{2})
25-03-2021	LDT	71.27 (180.20)	**Circularity**	Ctrl - GW	(Kruskal Wallis test) Dunn's post hoc test	4.1E-11	Ctrl	0.952
	Blank 1	35		Ctrl - SW	(Kruskal Wallis test) Dunn's post hoc test	5.62E-24	SW	0.877
	Blank 2	55.8		GW - SW	(Kruskal Wallis test) Dunn's post hoc test	0.00000286	GW	0.895
	Blank 3	20		B - A recharge (SW)	Wilcoxon Rank Sum Test	×	B recharge (SW)	0.883
				B - A recharge (GW)	Wilcoxon Rank Sum Test	0.0000479	A recharge (SW)	0.863
							B recharge (GW)	0.865
							A recharge (GW)	0.91

Feret diameter	Ctrl - GW	(Kruskal Wallis test) Dunn's post hoc test	1.39E-31	Ctrl	74.5 (µm)
	Ctrl - SW	(Kruskal Wallis test) Dunn's post hoc test	4.08E-32	SW	105 (µm)
	GW - SW	(Kruskal Wallis test) Dunn's post hoc test	0.966	GW	111 (µm)
	B - A recharge (SW)	Wilcoxon Rank Sum Test	0.00598	B recharge (SW)	114 (µm)
	B - A recharge (GW)	Wilcoxon Rank Sum Test	0.00313	A recharge (SW)	98.8 (µm)
				B recharge (GW)	118 (µm)
				A recharge (GW)	106 (µm)

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSIONS

This doctoral thesis investigated trends of contaminants in alluvial aquifers, in environmental contexts where the river-groundwater interactions and the aquifer recharge are pivotal in the contaminants' migration. To maximize the explanatory capacity of the results, a multidisciplinary approach was adopted, integrating biogeochemical, hydrogeological, agronomical, and chemical methodologies. Such integration allowed to improve the comprehension of the mechanisms underlying the transfer of contaminants and to calculate the rate of transport. The general results highlight how, while the recent literature suggests a general improvement of the European groundwater quality, there are still several and relatively small areas where various contaminations are still a matter of concern.

4.1. CHARACTERIZATION OF THE NO_3^- CONTAMINATION

4.1.1. Groundwater contamination

The groundwater in the investigated area is characterized by an average NO_3^- concentration below the Italian and European concentration limit of 50 mg NO_3^- L^{-1} limit (25.77± 20.64 mg NO_3^- L^{-1}). Anyway, these values are not stable along time and they constitute an important threat to the quality of surface waters, which will be detailed exposed in the Section 4.1.2.

In the area, during the two main fertilization periods (autumn and spring) measured nitrate concentrations exceed 50 mg NO_3^- L^{-1}, peaking at 95 mg NO_3^- L^{-1}. Evidently, in the investigated area the use of organic manure as fertilizer is overabundant, in agreement with the calculations and outputs reported in Pinardi et al. (2018b) and Racchetti et al. (2019), where the authors calculated an N surplus of 132 kg N ha^{-1} y^{-1} and 136 kg N ha^{-1} y^{-1}, respectively. In addition to the N surplus, the migration of nitrate from the vadose zone to the Mincio River is fostered by precipitation and the gigantic recharge volume provided during the irrigation period. This is a peculiar output, since in the nearby and hydrogeologically similar Oglio basin watershed, the irrigation results in the dilution of NO_3^- groundwater concentrations (Rotiroti et al., 2019). The contamination, during both the high and low concentrations phases, can be traced using SiO_2. Its use can be promoted also in other areas with a similar hydrogeological, geochemical, and agricultural context, like most of the Po Plain. Although no isotopic analyses were performed in the study area, it has been possible to associate the NO_3^- in groundwater mostly to manure. Only a few examples of synthetic fertilizers input in the study area were reported. Moreover, the denitrification was evaluated from both laboratory tests of vadose and saturation zones and from the comparison of NO_3^- and SiO_2 concentration curves in the study area. Both are affected by hydrodynamic dispersion, but the faster decrease of NO_3^- along the flowpath is also a function of the microbial denitrification.

As reported more specifically in the presented articles, some investigations are still required in the study area to fully understand the seasonal nitrate transfer and transformation dynamics. Those will be performed with the aim to quantify the amount of irrigation water provided every month, not only seasonally or annually. Finally, the data from $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{11}B$ will be used to better characterize the origin and biogeochemical processes affecting NO_3^- in groundwater, whereas ^{222}Rn will be used to characterize the river-groundwater interactions in the study area. This isotope was already used to characterize the river-groundwater interactions in flow-through systems (Stellato et al., 2008).

4.1.2. The Mincio River NO_3^- contamination and loads

In the Mincio River, a steep increase in NO_3^- concentrations can be observed in the investigated area. Usually during autumn and spring, the contemporaneity of high NO_3^- concentrations in groundwater and relatively-high groundwater level can lead to a diffuse input of NO_3^- rich groundwater to the Mincio River and the nearby secondary channels. Given the similar river flow between upstream and downstream the investigated area, the interaction between the groundwater and the Mincio River can be assimilated to a flow-through system. In other words, groundwater feeds the river in the right bank and the river feeds the groundwater in the left bank. As a result, part of the Mincio River water is displaced by the groundwater inputs, without a significant increase of downstream discharge but large chemical differences.

The variations of the NO_3^- loads in the Mincio River are the results of three main factors: the nitrate groundwater concentrations, the entity of the river-groundwater interaction, and the river discharge. The three main periods of groundwater contamination lead to three moments of high NO_3^- loads in the Mincio River, which can be explained as follows:

- Late Summer: groundwater has a relatively high NO_3^- concentration and the highest hydraulic heads were measured in this period. Moreover, the Mincio River discharge is at its highest. As a result, the high NO_3^- concentrations are function of groundwater nitrate levels, volume exchange between groundwater and river and, finally, river discharge.
- Winter: the groundwater level is the lowest of the investigated period and the river discharge averages seasonal variations. The high NO_3^- load is given by the NO_3^- concentrations in groundwater, which are the highest detected along the hydrological year.
- Spring: the start of irrigation results in a steep rise of groundwater level, which is recharged with N-rich water. Although the Mincio River discharge is still relatively low, a high NO_3^- load is measured, due to high NO_3^- concentration in groundwater and high groundwater level.

During these periods, the Mincio River receives from the diffuse input of groundwater an enormous amount of N, up to nearly 7 tons NO_3^- d^{-1}. Summed to the NO_3^- load coming from upstream the investigated area, the total Mincio River NO_3^- load can reach nearly 10 tons NO_3^- d^{-1}. These values are unusually high for a river with an average discharge of 10.6 m^3 s^{-1}.

4.1.3. The NO_3^- migration from groundwater to rivers and its role in the eutrophication of downstream water bodies

In the presented articles, it has been demonstrated how the increase of NO_3^- concentrations and loads in the Mincio River is regulated by the river-groundwater interaction. In the investigated area, only in some moments with peculiarly low NO_3^- loads, the reduction of NO_3^- concentrations given by denitrification processes and N uptake by macrophytes and phytoplankton can contrast the NO_3^- concentrations increase in the Mincio River. Thus, although effective in some situations, these processes cannot remove the large N input from groundwater. This is verified not only in the investigated area but along all the Mincio River course. Pinardi et al. (2018b) found that in the periods 1990-2000 and 2001-2010, the increase of nutrients concentrations between the 20th and 30th km of the Mincio River is not neutralized by the previously-mentioned processes (Figure 5). It can be concluded that the river-groundwater interaction in these few kms leads to an unbalanced nutrient stoichiometry affecting all the downstream Mincio course, favouring harmful algal blooms and further impacting the functioning of benthic and pelagic compartments. This is particularly true for the Mantua Lakes, three hypereutrophic shallow fluvial lakes fed by the Mincio River. Since most of the N input to the river derives from the investigated area (Figure 5), it is possible that the river-groundwater interaction has a pivotal role in the nutrient loads and stoichiometry of the downstream lakes.

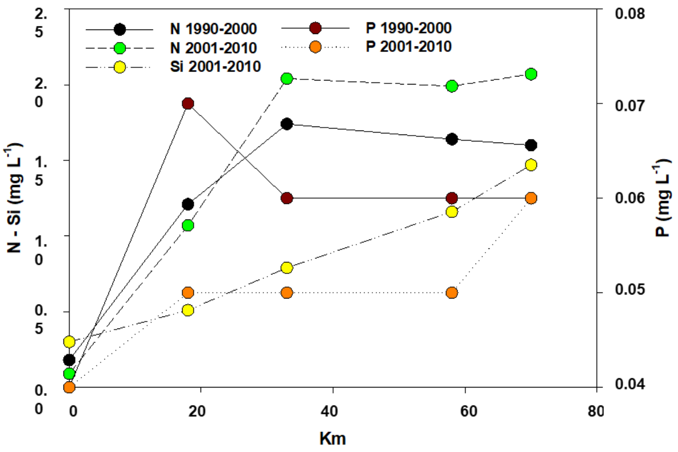

Figure 5. Nutrient concentrations (median values of NO_3-N, PO_4-P, DSi) along the Mincio River course: Peschiera del Garda (km = 0), Pozzolo (km = 18), Goito (km = 33), Formigosa (km = 58) and Governolo (km = 70). From Pinardi et al. (2018b).

This concept can be extended to all the Po Plain. Here, different studies with different approaches characterized the N input to the Po River, but never explicitly considering surface-groundwater N mass transfer. As an example, Palmeri et al. (2005) estimated a contribution of groundwater of around 36% to the N load of the Po River, while Viaroli et al. (2018) calculated an increased N input to the Po River comparing its N loadings and the soil system budgets of the watershed. Here, the N loadings increased in the 1990s and remained at high levels till today. It is possible that dynamics like those of the Mincio River can be found in other watersheds at the transition between the high and the low plain. In this belt, the shift from coarse to fine sediments and the consequent decrease of permeability can promote a diffuse interaction between river and groundwater and cause the presence of the “fontanili” (lowland springs), which promote the fast migration of contaminants like nitrate from groundwater to surface waters (Balestrini et al., 2021).

First results of systems similar to the investigated area of the Mincio River are already reported. Racchetti et al. (2019) calculated the soil system budgets of the Adda and Oglio rivers, finding a steep increase in NO_3^- concentrations in surface waters along the transition between high and low plain in these areas as well. Thus, it is undeniable that the approach used in this doctoral thesis should be expanded to other areas nearby the Mincio River to understand the real contribution of groundwater to the variation of NO_3^- concentrations along temporal and spatial scales.

4.1.4. Remediation practices and prospects

In light of this, the contamination (and periodical pollution) of groundwater can be ascribed to the contemporaneity of fertilization and aquifer recharge, which is the result of a lack of balance between the agricultural practices and the characteristics of the cultivated area. Starting from the irrigation practices, the flooding irrigation is possible only due to the extraordinary large amount of water available in the area, thanks to the Garda Lake and the Mincio River. Anyway, the area is characterised by highly permeable soils, vadose, and saturation zone. Thus, the use of flooding irrigation in this area is highly inefficient and higher volumes of water are necessary to increase the water absorption by the roots systems of plants.

As a matter of fact, in the area the maize is the most cultivated plant, which is a high profitable cultivar, but with high water needs, aggravating the irrigation problem. The solution must then start from a more efficient irrigation practice of less water-demanding plants. There are only few sprinklers in the area, and even fewer drip irrigation systems, although no official data are available for the area, but only at municipality level from the Italian National Institute of Statistics (2010). These systems are more abundant in the lower plain, where concrete channels to abstract water from the Mincio River are scarce.

Together with volumes, the problem is also related to the irrigation timing. The flooding irrigation consists in submerging soil with large water volumes around three times per week. As a result, during irrigation, a high recharge rate promotes the percolation of all the N preserved in the vadose zone in the form of NO_3^-, raising its concentration in groundwater. On the contrary, a more frequent irrigation but with lower volumes could generate various benefits in the study area. First, a higher denitrification rate in the vadose zone could be promoted, due to the more frequent favourable condition for the microbial community (higher humidity, i.e. occurrence of multiple oxic-anoxic niches due to capillary water). Although not specifically investigated, the fast decrease of NO_3^- concentrations in groundwater demonstrates the high denitrification potential of the microbial community in the investigated area, which also keeps the NO_3^- concentrations in groundwater low in periods far from fertilization. Secondly, given the river-groundwater interaction reported in Severini et al. (2022), a lower irrigation volume could also result in a lower intensity of interaction between groundwater and surface waters, safeguarding the latter from NO_3^- contamination.

With regards to manure fertilization, the problem is given by its overabundant use, caused by two factors: the high permeability reported above and the necessity of manure disposal. The high nutrients necessities of maize force the farmers to use a large amount of manure as fertilizer, which is inexorably lost due to the recharge (irrigation or precipitation) over the high permeable soil and aquifer. A question arises: is it economically efficient to use a large amount of fertilizer and lose most of it? Where does such cheap fertilizer come from? The answer is related to the high density of animal farms in the area. They provide an enormous amount of manure to farmers, not only preventing farmers from paying for the manure disposal in other structures but allowing them to earn from its sale.

On the other hand, farmers have a practically unlimited stock of N available all around the year, and only the Italian laws and European directives regulate the maximum amount usably. Some alternatives are available for both parts. Farmers could take advantage from using synthetic fertilizers such as slow-release fertilizer (SRF) or controlled-release fertilizer (CRF), like organic-N low-solubility compounds (e.g. urea-formaldehyde, UF), fertilizers with a physical barrier controlling the release (e.g. tablets coated by plastics) and inorganic compounds with low-solubility (e.g. magnesium ammonium phosphate, $MgNH_4PO_4$) (Trenkel, 2010). Animal farms should instead divert manure to other uses, the most suitable of which seems the biomethane (CH_4) production plant, a resource considered fundamental in the optic of energetic transition from fossil fuels to renewable energies. As a matter of fact, this is a highly encouraged sector in Europe, where its production increased from 752 Gigawatt hours (GWh) in 2011 to 17,264 GWh in 2016 (European Biogas Association, 2017).

The combined diversification of manure use and irrigation practices can certainly lead to an improvement of the groundwater quality and quantity in the investigated area. Here the conditions are particularly suitable for a step in this direction. Given the fast groundwater circulation (Severini et al., 2022), the area does not seem subject to the long term effects of high NO_3^- concentrations in groundwater (Hansen et al., 2017) and represents an optimal test site to evaluate these changes over time.

In surface waters, common solutions to high N inputs involve the dilution (e.g. from point sources) or the creation of buffer areas fostering the N uptake or denitrification in bare and vegetated sediments (Pinardi et al., 2009). This principle was applied in the Mincio River watershed, estimating the potential N removal (Pinardi et al., 2020). As a result, approximately all the N excess produced in the agricultural land of the Mincio watershed can be retained or removed by internal processes. This encouraging output provides useful suggestions to the possible remediation practices in the investigated area. Here, many irrigation channels have a highly variable discharge during the irrigation period and are dry during the non-irrigation period. Moreover, they are constantly depleted of the vegetation inside and around them, which have a high N uptake potential. It is reasonable to hypothesize that, keeping a minimum discharge in all these channels and permitting the presence of natural vegetation could lead to a significant N input reduction to the Mincio River. The high N concentrations in groundwater diffusively feeding the surficial channels before the Mincio River could be lowered by the previously described processes (Figure 6). These processes, cumulated with the denitrification in the vadose zone, could significantly reduce the total N input from groundwater to the Mincio River.

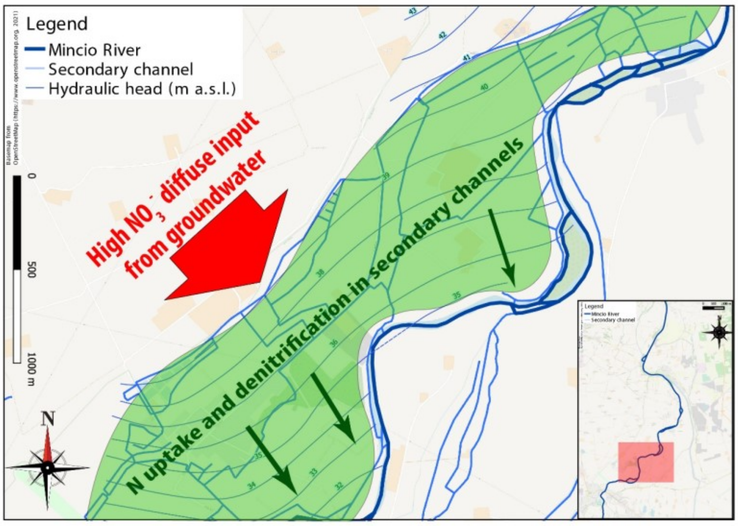

Figure 6. Conceptual scheme of the potential denitrification nearby the Mincio River.

Given that the N contamination of groundwater and the N concentrations in the Mincio River are the result of a lack of balance between the agricultural practices and the characteristics of the cultivated area, the impact that climate change could have on these practices and the investigated area must be forecasted. According to the report from the United Nations Intergovernmental Panel on Climate Change (IPCC, 2021), the global temperature will rise by 2.5-4 °C by the end of the 21st century, with an intensification of extreme events like heatwaves, precipitation, and droughts. The described scenarios will inevitably lead to a worsening of the situation described in the Mincio River and Goito aquifer. Higher temperatures will lead to higher evapotranspiration, forcing farmers to use higher volumes of irrigation water. These, coupled with extreme precipitation events, will boost the aquifer recharge and N leaching in the vadose zone. Two scenarios are possible at this point. If the system remains unchanged, the Mincio River and groundwater NO_3^- concentrations and loads will increase due to a higher recharge with N-rich waters. If the irrigation system will shift from flooding to drip irrigation, the lowering of groundwater level in the area could prevent the migration of N from groundwater to the Mincio River. Anyway, N could accumulate in groundwater with an increase of the average concentration, even though a more frequent irrigation could foster denitrification in the vadose zone. Regardless of the severity of climate change impact according to the IPCC, it is clear that its effects must be limited through a different use of both manure and irrigation water.

4.2. THE EFFECTS OF THE NITRATE DIRECTIVE FERTILIZATION LIMITS

As previously mentioned, a derogation to the Nitrate Directive allows farmers to use up to 250 kg N ha^{-1} y^{-1} instead of 170 kg N ha^{-1} y^{-1} inside the NVZs in some areas of the Po Plain. The derogation was successively not extended and from 2020, in the

NVZs like the investigated area, the amount of manure applied as fertilizer returned to 170 kg N ha^{-1} y^{-1}.

During the PhD, it was possible with continuous hydrogeological sampling campaigns to characterize in detail the changes in surface and groundwater quality after the reintroduction of the lower fertilization limit. The results demonstrated how the application of a European Directive can lead to significant environmental changes not only after decades, but also after one hydrological year. Certainly, in the investigated area the process is fostered by the fast circulation of groundwater and contaminant transport. The comparison of the effects in the Mincio River of the new fertilization limit was performed between autumn of 2019 and 2020. The results underline a drastic reduction of the NO_3^- input to surface waters, which decreased by 58%. These data provide fundamental information to the policy-makers and stakeholders, since they can also consider these actions as measures providing both immediate and long-term benefits. In the Mincio River watershed, there are numerous animal farms and the manure has been always abundant. However, the new limit of N fertilization will result in thousands of Kg of manure left unused. Thus, to hinder its illegal use as fertilizer above the maximum usable amount, policy-makers must also provide other applications of the additional manure. Once again, the best choice seems to redirect this resource to the biogas production, leading to different benefits like: i) hindering illegal amounts of fertilizers use; ii) providing another profit source to farmers; iii) creating new infrastructures helping during the energetic transition from fossil fuels to renewable energies; iv) protect surface and groundwater quality and quantity.

4.3. MICROPLASTICS CONTAMINATION IN GROUNDWATER: FIRST RESULTS AND OPEN QUESTIONS

Microplastics are a ubiquitous contaminant, which is found anywhere, from the bottoms of the oceans to the top of the mountains. During this PhD project, their presence was also characterised in a porous medium like the shallow alluvial aquifer of the Parma province, the first Italian case. The aim of this research was not primarily to demonstrate the presence of microplastics in groundwater, but to investigate them using a hydrogeological approach, namely conducting a research based on the hydrogeological characteristics of the aquifer and surrounding area rather than just characterize their presence. In addition, an extraction and analysis protocol was developed, also considering those who do not benefit from the proper facilities, i. e. a laboratory with analytical chemistry instruments (e.g. μFTIR) usually used in microplastics characterization but high-priced. Moreover, the presented extraction and analysis protocol takes into account the characteristics of surface and groundwater samples, like the possibly high load of suspended solids and organic matter.

The presented protocol is an initial version and many improvements can still be done. From its initial development in the study presented above, some improvements have been already performed. The blank contamination was the most problematic source of error, with an average contamination of 36.9 microplastics/L (35, 55.8, 20 microplastics/L per filter). This high value was probably connected to the use of unfiltered reagents and MilliQ water (which can present microplastics due to filters release). Thus, using a prior filtration with a 0.22 µm filter of reagents and water (in this case deionised water), the analytical contamination was lowered to 18.5 microplastics/L (13.4, 6.4, 35.6 microplastics/L per filter). To ulteriorly improve the protocol, more tests are needed and have already started.

However, the protocol provides interesting information on microplastics and their transport in groundwater. The hydrogeological approach allowed to visualize the effects of the aquifer recharge and river-groundwater interaction (main themes of the PhD project) on microplastics contamination. Although the results are just preliminary, they were worthy to be shared with the scientific community. The natural continuance of these results is the analysis of more recharge and recession periods to provide a solid interpretation of their effects in the Parma alluvial aquifer, or in another test site where the groundwater flowpath is already characterised. AS a matter of fact, other studies on microplastics have already started in two different test sites: the Mount Prinzera and the recharge area of the alluvial aquifer 20 Km N-W the Goito aquifer. Here, the presence and characteristics of microplastics in groundwater will be investigated through isotopic, chemical, and geometrical perspectives, leading to further improvements of the presented protocol.

4.4. OVERALL CONCLUSIONS

The research carried out and the results obtained during this PhD project proved how, to characterize complex processes like the contaminant migration from agricultural soil to groundwater and surface water, a multidisciplinary approach is necessary and useful. The main processes investigated during the PhD are the aquifer recharge and the river-groundwater interaction. In the Mincio River and Goito aquifer, different methodologies from different fields were combined to corroborate the interpretation of the site test problematics. Here, methods like soil system budget (agronomy), manure laboratory tests (biogeochemistry), and the use of tracers (hydrogeology) lead to identifying the sources of high NO_3^- concentrations in the Mincio River and provided alarming information on groundwater quality in an area not monitored by the environmental agencies (ARPA Lombardia and Veneto). In the Parma alluvial aquifer, the combined use of fluorescence microscopy (microbiology), microplastics characterization (environmental chemistry), and sampling along the groundwater flowpath (hydrogeology) provided some of the first pieces of evidence of microplastics contamination in groundwater, using a low cost-to-results ratio protocol.

In addition, these results highlight that, together with different methodologies, multidisciplinary approaches must be a key component of the research conceptualization from the start. The professional figure consequent to this approach can be summarized as a hydrogeologist capable to conceptualize a research line ranging between different fields and use their methods, with the final aim to perform studies in the environment where the problematics can be faced with a holistic approach.

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Edoardo

When you open your eyes
When you gaze at the sky
When you look to the stars
As they shut down the night
You know this story ain't over

Avantasia – The story ain't over
(Lost In Space Part 1) 2007