



Spatial Assessment of Water Quality Along a Collective Surface Irrigation Network Under Blending Water Reuse Conditions

Pietro Drei¹ · Giuseppe Maistrello¹ · Stevo Lavrnić¹ · Alberto Bavieri² · Michele Solmi² · Attilio Toscano¹

Received: 7 July 2026 / Accepted: 24 September 2026
© The Author(s) 2026

Abstract

Agricultural water reuse involving the blending of treated wastewater (TWW) with conventional irrigation water in open irrigation networks is not explicitly regulated under Regulation (EU) 2020/741, which may present exposure pathways comparable to unblended direct reuse. This study assessed water quality dynamics along a fully operational irrigation canal network in Northern Italy, under real-scale hydraulic and agronomic conditions characteristic of reuse systems involving blending, in which TWW discharged from a wastewater treatment plant (WWTP) accounted for 68% to 86% of total canal flow. Over two irrigation seasons (2023–2024), 11 physical, chemical, and microbiological parameters were monitored at five points: one at the WWTP outlet, one upstream representing the conventional irrigation source, and three downstream of the confluence across 30 campaign days. Data were analyzed using generalized linear mixed models with sampling point as a fixed effect and sampling date as a block-level random intercept. The contribution of TWW to conventional irrigation water was primarily reflected in nutrient enrichment, with concentrations downstream of the confluence exceeding those of the conventional source by 48% to 59% for total nitrogen and 139% to 195% for total phosphorus, confirming the WWTP as the main nutrient source. In contrast, the effluent introduced no additional load for the parameters directly regulated under Regulation (EU) 2020/741 (*E. coli*, biochemical oxygen demand (BOD₅), and total suspended solids (TSS)). TSS were significantly lower in TWW than in conventional irrigation water, leading to a 29% to 43% reduction in TSS after mixing, BOD₅ at the WWTP outlet did not differ significantly from that in conventional irrigation water, and for *E. coli* no significant effects were detected. However, BOD₅ increased significantly at one point after the confluence relative to the conventional source, from an input that could not be attributed to the effluent. Overall, these results demonstrate that although TWW accounted for the majority of canal flow in volumetric terms, it did not lead to generalized deterioration of irrigation water quality. Instead, it provided nutrient inputs that may be harnessed for agricultural reuse. This work presents one of the first systematic, real-scale assessments of TWW reuse through blending in a fully operational European irrigation district, illustrating the regulatory gap

Extended author information available on the last page of the article

in Regulation (EU) 2020/741 and informing risk-based monitoring frameworks for reuse systems where blending occurs.

Keywords Irrigation canals · Reclaimed water · Regulation (EU) 2020/741 · Risk-based monitoring · Treated wastewater

1 Introduction

Water scarcity is an intensifying global concern, driven by climate change, prolonged droughts, and increasing competition among urban, industrial, and agricultural demands (Ofori et al. 2021). Agriculture remains the largest consumer of freshwater globally, accounting for approximately 70% of total withdrawals, particularly in arid and semi-arid regions (Flörke et al. 2018; Christou et al. 2024). In this context, the reuse of treated wastewater (TWW) has emerged as a promising strategy to alleviate irrigation water shortages, especially in areas facing limited access to conventional water sources (Yalin et al. 2023; Christou et al. 2024; Valenti et al. 2026) or to supply non-food/energy crops (Barbagallo et al. 2014). However, despite its recognized potential, less than 20% of global wastewater is currently treated, and only 2–15% of that is reused for agriculture (UN-Water 2017; European Investment Bank 2022). Expanding the safe use of TWW for irrigation could substantially reduce dependence on freshwater and enhance the resilience of agricultural systems to water stress (Christou et al. 2024; Mancuso et al. 2024).

Regulation (EU) 2020/741 sets harmonized minimum requirements for the reuse of TWW in agricultural irrigation across EU Member States, including minimum water quality standards, monitoring protocols, and permitting procedures (Suman and Toscano 2021). A key provision of the regulation is the mandatory development of a water reuse risk management plan, which serves as site-specific tool to ensure safe operation of water reuse systems (Drei et al. 2025). Within this framework, the multi-barrier approach, as introduced by WHO (2006) is adopted as a management strategy to achieve tolerable levels of health risk. The regulation allows for the reuse of lower water quality classes when sufficient barriers are in place and properly validated (Maffettone et al. 2024).

Agricultural TWW reuse can occur in three configurations, distinguished by whether TWW is conveyed through engineered infrastructure or discharged into a natural water body. In unblended direct reuse, only TWW is conveyed to the point of use (Perulli et al. 2026); in blended direct reuse, TWW is blended with conventional irrigation water within a collective irrigation network before abstraction (Mancuso et al. 2022); in indirect reuse, TWW is discharged into a natural water body, such as a river, and abstracted downstream (Kesari et al. 2021). While Regulation (EU) 2020/741 is mainly based on the unblended direct paradigm, and indirect reuse is outside its scope, the blended direct configuration prevails in many collective irrigation networks. In fact, in many peri-urban and downstream agricultural regions, farmers frequently draw irrigation water from drains or canals that receive wastewater treatment plant (WWTP) effluents, often without formal regulation or control (Rice and Westerhoff 2015; Thebo et al. 2017). Thebo et al. (2017) estimated that over 35 million hectares of cropland are located within wastewater-dependent catchments, highlighting the global relevance and under-recognition of blended direct reuse practices. However, Regulation (EU) 2020/741 remains primarily focused on unblended direct reuse,

offering no explicit guidance for the blended configuration that prevails in collective irrigation networks, thereby leaving a significant regulatory gap.

From an agricultural and environmental management perspective, mixing TWW with freshwater within irrigation networks can function as a passive treatment barrier by diluting microbial and chemical contaminants (Assouline et al. 2020; Khandelwal et al. 2024). This practice has also been recommended to mitigate adverse effects on soil hydraulic properties and water quality, supporting its potential inclusion as a site-specific barrier in risk-based reuse strategies. Recognising such blending within irrigation networks as a potential component of the multi-barrier approach may help strengthen the safety and sustainability of blended direct reuse schemes (Drei et al. 2025; Allgaier et al. 2026).

Numerous studies have assessed the impacts of WWTP effluents on river systems, demonstrating their influence on water quality, introducing nutrients (e.g., nitrogen, phosphorus), and organic matter (BOD₅, COD) (Ngwira and Lakudzala 2018; Mandaric et al. 2019), as well as emerging contaminants such as pharmaceuticals, personal care products, endocrine-disrupting compounds, and disinfection by-products (Lin et al. 2020; Wolfand et al. 2023). These discharges have also been shown to affect microbial communities, possibly increasing the prevalence of bacteria associated with wastewater, nutrient cycling, and faecal sources, as well as enhancing the spread of antibiotic resistance genes (ARGs) and mobile genetic elements (Russo et al. 2019; Pascual-Benito et al. 2020; Lu et al. 2022; Álvarez-Fraga et al. 2026). However, despite this extensive body of research on rivers, the effects of WWTP effluents on irrigation water networks remain poorly addressed (Al Aukidy et al. 2012; Abugu et al. 2021). Recent work has begun to characterize the chemical and microbiological properties of irrigation canals at the district scale in Northern Italy, demonstrating that canals receiving WWTP effluents exhibit systematically higher nutrient loads and greater microbiological contamination than those supplied by surface water sources (Gaggia et al. 2026). Although such district-scale comparisons can give a good estimate of the overall irrigation water quality, they cannot precisely quantify and assess the processes occurring within a single canal downstream of a discharge point. On the other hand, these processes are particularly important for risk management planning under Regulation (EU) 2020/741: crediting blending as a barrier requires the effluent contribution and its attenuation to be resolved at the point of abstraction, which a comparison between canal categories cannot provide. The mixing dynamics at individual TWW confluence points, the net spatial attenuation of water quality parameters along the receiving reach, and the persistence of the effluent signal as far as the points of abstraction therefore remain unaddressed.

In this context, the Emilia-Romagna region of Northern Italy offers a representative case. It is an important national agricultural hub and is increasingly challenged by water scarcity and climate variability. The region's irrigation infrastructure, composed mainly of open surface canals, is of a multi-source nature, transporting irrigation water, surface runoff, and TWW through the same distribution infrastructure. This configuration inherently supports blended direct reuse through mixing with conventional sources, raising questions about its influence on irrigation water quality and reuse safety. Critically, these systems operate under fully real-scale conditions, where hydraulic management, water abstraction, and canal operations are driven by agronomic demand.

This study aimed to evaluate the impact of TWW discharge on irrigation water quality within a fully operational surface canal network in the Emilia-Romagna region, under the real-scale hydraulic and agronomic conditions characteristic of blended direct reuse. Spe-

cifically, by resolving a single confluence into the upstream conventional source, the effluent itself, and three sequential downstream stations, it quantified spatial gradients in physical, chemical, and microbiological parameters along the receiving reach, assessing the relative contribution of TWW to downstream water quality dynamics and evaluating the compliance of mixed irrigation water with the quality classes defined by Regulation (EU) 2020/741. The results inform risk-based monitoring of blended direct reuse and are consistent with the case for its explicit inclusion within the scope of Regulation (EU) 2020/741, where it is structurally embedded in irrigation infrastructure.

2 Materials and Methods

2.1 Study Area

The study was conducted in the irrigation network of the Metropolitan City of Bologna (Emilia-Romagna region, Northern Italy), an area characterized by intensive agricultural activities and increasing water stress. The selected irrigation district, managed by the Consorzio della Bonifica Renana, covers a total surface of 22,540 ha, with an average irrigated area of 9,860 ha. The water distribution infrastructure includes approximately 390 km of open surface canals and 16 km of pressurized pipelines. The open surface canals are compartmentalized by several irrigation structures (i.e., weir, sluice gate), which imply an in-line reservoir to allow water abstraction by farmers. These canals are artificial infrastructure constructed for irrigation supply and land drainage rather than natural watercourses, so TWW conveyed through them constitutes direct conveyance rather than discharge into a natural water body. Five WWTPs discharge TWW into this network, enabling reuse through blending with conventional irrigation water across the district.

The focus of this study was the Molinella canal, which is part of the irrigation network and is influenced by the discharge from the Molinella WWTP. The Molinella WWTP is based on a conventional activated sludge process, followed by disinfection with peracetic acid prior to discharge into the receiving canal. The case study was defined by several monitoring points located along a segment of the Molinella canal, upstream and downstream the confluence with TWW. The first section of the canal conveys only conventional irrigation water, that is water abstracted from the Po River and conveyed through the district network to the study reach. At the confluence, the Molinella canal intersects with the Cavaliera canal, which carries only TWW from the Molinella WWTP. The second section of the Molinella canal, after the confluence, conveys mixed irrigation water (Fig. 1).

2.2 Sampling Design

The sampling was structured as a multi-year, multi-point water quality monitoring program to capture the spatiotemporal variations in irrigation water quality influenced by TWW inflow. Five sampling points were selected along the canal network to characterize water quality conditions (Fig. 1):

- PA: Cavaliera canal, WWTP outlet, 859 m upstream of the confluence (TWW).
- P1: Molinella canal, 500 m upstream of the confluence (conventional irrigation water).

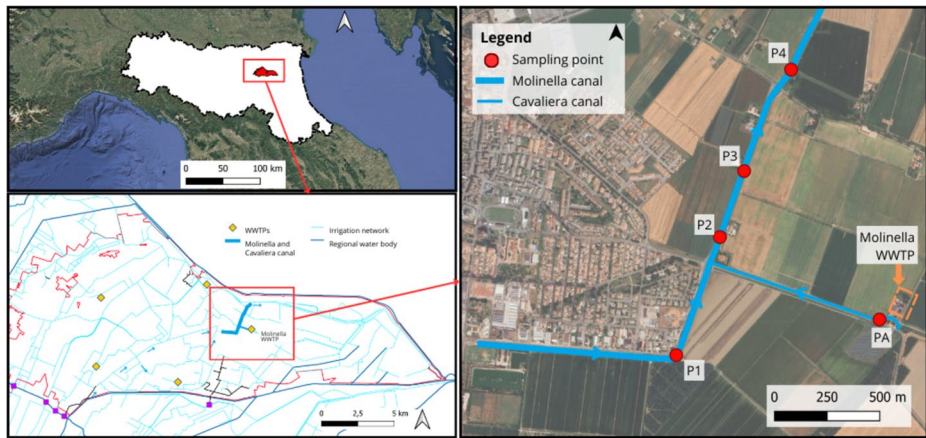


Fig. 1 Study area and sampling network in the Molinella canals. Sampling points: PA (TWW), P1 (conventional irrigation water), P2, P3 and P4 (mixed irrigation water)

- P2: Molinella canal, 50 m downstream of the confluence (mixed irrigation water).
- P3: Molinella canal, 332 m downstream of Point 2 (mixed irrigation water).
- P4: Molinella canal, 534 m downstream of Point 3 (mixed irrigation water).

The final sampling methodology was established through a two-phase design process. During the initial phase of the 2023 irrigation season, a denser network of sampling points was deployed across both canals, including additional points upstream of P1, around the confluence (25 m and 75 m downstream), and along the Cavaliera canal between PA and the confluence. Although no tracer test could be performed due to the technical and logistic reasons, the preliminary data analysis revealed no substantial differences in water quality between the auxiliary points and their adjacent fixed locations, confirming that P1 was representative of the conventional irrigation water source, PA of the TWW, and that adequate mixing conditions were present at P2. Based on these observations, and supported by the literature that microbial water quality is largely homogeneous along short canal stretches (Lothrop et al. 2018), the network was consolidated to the five sampling points. This approach is consistent with monitoring designs adopted in comparable canal systems receiving WWTP effluents (Al Aukidy et al. 2012; Pascual-Benito et al. 2020).

Two monitoring campaigns were carried out from June to October 2023 and from April to October 2024, with 12 and 18 sampling days, respectively. All five points were sampled on each occasion. The number of observations differs among parameters and is reported per parameter and sampling point in Table S1b. Sampling was planned at ten-day intervals; the median interval achieved was eight days.

2.3 Parameters Measured

Water samples were collected following the standard procedures described in APHA (2017) and Lothrop et al. (2018). At each point, three samples were taken from a depth of 0.15–0.30 m within a 2 m radius and combined into one 2-litre bottle. Each parameter was measured once on this composite. Microbiological samples were collected using sterile con-

tainers. All samples were transported in insulated coolers and stored at 4 °C until analysis. The following parameters were analyzed:

- Physical: total suspended solids (TSS) measured in accordance with standard methods (APHA 2017).
- Chemical: five-day biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), ammonium nitrogen (NH₄⁺-N), and nitrate nitrogen (NO₃⁻-N). All analyses were conducted following standard methods for the examination of water and wastewater (APHA 2017).
- Microbiological: Total coliforms (CT) and *E. coli* determined following the ISO 9308-1:2014 and faecal streptococci (SF) following ISO 7899-2:2000.

Dissolved oxygen (DO) was measured in situ using a portable multiparametric probe (Hanna Instruments, Model HI9829), which was also used to record water temperature, saturation and pH. Saturation and pH are reported in Table S1a but were not modelled.

2.4 Hydraulic Characterization

To evaluate the contribution of TWW to the canal's flow regime, a hydraulic assessment was performed. The Molinella canal was surveyed at 9 cross-sectional locations, producing 8 canal segments. Two bridge crossings served both as cross-sectional locations and as reference points for repeated water level measurements (Fig. 2), useful for the calculation of the water volume in each canal segment. Wetted cross-sectional areas and stored water volumes in each segment were calculated based on bed elevation data and water level measurements collected on each sampling day. The average water volume stored within the entire section of the Molinella canal (3,000 m long), both before and after the confluence, was estimated at approximately 5,400 m³ and it was rather constant during the irrigation season. It should be noted however that canal water levels and flow distribution were regulated throughout the monitoring period by weirs and sluice gates operated by the Consorzio della Bonifica Renana according to agronomic demand, with farmers actively abstracting water for crop irrigation at multiple points along the network. Such kind of management, characteristic of a fully functional irrigation district, resulted in continuously variable hydraulic conditions that were not subject to experimental control and are inherent to the real-scale nature of the study. Water sources contributing to the canal flow included:

- Conventional irrigation water: 1,720 m³ day⁻¹ (constant input from the Po River);
- TWW from the Molinella WWTP: seasonally variable discharge ranging from 3,700 to 10,600 m³ day⁻¹.

The conventional input was determined at the sluice gate located upstream of P1, which regulates the entry of water into the canal, keeping it constant throughout the irrigation season; the head was measured in the field and the discharge derived from the corresponding weir equation. This value was cross-checked against an independent estimate obtained from the channel cross-sectional area and the flow velocity measured at the same location with a portable current meter. TWW discharge was not measured but obtained from the operational

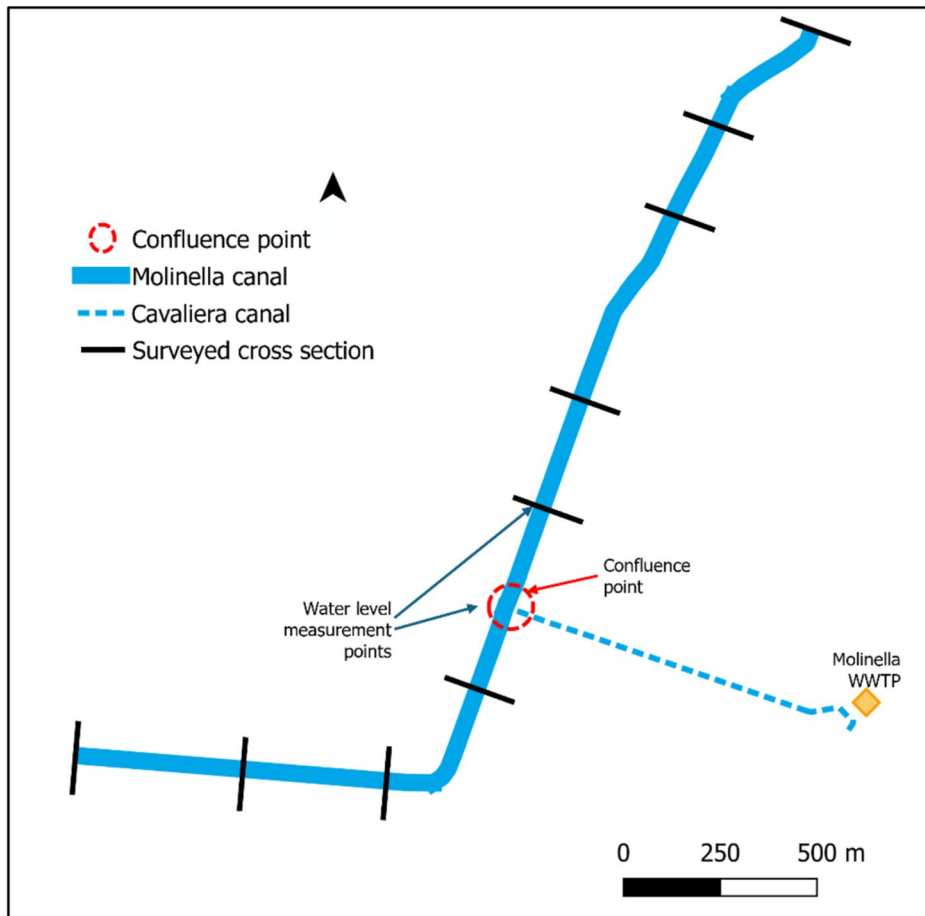


Fig. 2 Hydraulic characterisation of the study reach. Black bars mark the nine surveyed cross-sections delimiting the eight canal segments. Arrows indicate the two road bridges where water levels were recorded on each sampling day; both coincide with surveyed cross-sections, and no water level was recorded at the confluence. Sampling points are shown in Fig. 1

records of the water utility, available as monthly treated volumes and expressed as mean daily discharge.

Flow velocity was measured with a portable current meter at the two road bridges crossing the canal, the only points at which the instrument could be deployed. Therefore, the theoretical hydraulic residence time (HRT) of the post-confluence water reach containing mixed water, estimated as the ratio between the post-confluence reach length (916 m from the confluence to P4) and the mean flow velocity (0.04 m s^{-1}), was approximately 6.4 h. Based on water volumes of different water sources in the examined segment, a dilution ratio of TWW and conventional irrigation water was estimated for each sampling event. The resulting values ranged from 2.15 to above 6.16 across sampling events, corresponding to a TWW contribution of 68% to 86% of total canal discharge (median 74%), confirming that TWW represented the dominant fraction of total canal discharge throughout the

monitoring period. No formal propagation of uncertainty was applied to the dilution ratios or to the HRT. The conventional input derives from a single measured head, the TWW term from monthly treated volumes rather than same-day metering, and both are conditioned by the management regime described above. They are therefore reported as descriptors of the prevailing mixing regime rather than as measured values.

2.5 Statistical Analysis

An exploratory analysis was first conducted on raw data to examine distributions and identify outliers. Normality, homogeneity, and independence among sampling points were then verified to guide model selection and avoid pseudoreplication (Hurlbert 1984). Generalized linear mixed models (GLMMs) were fitted separately for each parameter, with sampling point as a fixed effect and sampling date as a random intercept (Zuur et al. 2007; Schreiber et al. 2022; Chaudhary et al. 2025). Each sampling date constitutes a block in which the five points were measured jointly, so the random intercept absorbs the among-date (block) variability shared by all points on a given occasion. Sampling spanned 30 campaign days. Spatial pseudoreplication is therefore addressed by the sampling design and by the date-block random intercept, which absorbs the conditions shared among points on each sampling occasion. Analyses were carried out in R (packages: *glmmTMB*, *DHARMA*, *emmeans*, *multcomp*).

For physical and chemical parameters, Gamma-distributed GLMMs with log-link and constant dispersion were initially tested. Model assumptions were evaluated with *DHARMA* using scaled residuals from a fixed-seed simulation (seed=123; $n=10000$): tests of uniformity (Kolmogorov–Smirnov), dispersion, outliers and for the microbiological models zero-inflation. Constant dispersion was used by default; a sampling-point-specific dispersion structure (`dispformula=~Sampling_point`) was tested for the parameters showing point-dependent residual variance (TP, TN, COD, TSS) and retained only where it lowered the AIC (TP, TN, TSS), but not COD. Model selection was based on AIC together with the residual diagnostics. Estimated marginal means (EMMs) with 95% confidence intervals were computed, and the compact-letter display of homogeneous groups was derived from Tukey-adjusted pairwise comparisons of the estimated marginal means ($\alpha=0.05$; *emmeans/multcomp*). Microbiological parameters (*E. coli*, total coliforms, faecal streptococci) were $\log_{10}$ -transformed and modelled with Gaussian GLMMs. Water temperature was included as a covariate in the microbiological models. No AIC comparison against models without the covariate was performed, since the two are fitted to different subsets of observations owing to missing temperature records on some sampling occasions. Results of microbiological analyses are presented separately due to their distinct scale and covariate structure. In cases of minor diagnostic deviations, the best-fitting model was retained and results interpreted with caution.

Model selection and diagnostics were performed to ensure reliable inference, including checks for residual distribution, variance homogeneity, overdispersion and outliers. The random intercept on sampling date is a block term and does not model correlation between dates. Temporal independence of the model residuals was therefore tested explicitly, and each model was refitted with an explicit temporal correlation structure and with sampling year as a fixed effect as sensitivity analyses. The models specified above were retained in all cases, since the contrasts among sampling points are estimated within a sampling occa-

sion and are therefore unaffected by correlation between dates. Procedures and results are reported in the Supplementary Materials.

Sampling was conducted exclusively under stable meteorological conditions, and to minimise the influence of surface runoff on water quality, samples collected within three days of a rainfall event were excluded, a criterion that removed one observation over the two seasons. Observations were further excluded on dates on which the WWTP was not operating normally, on the date of a solids overflow from the plant, and where analytical data were missing or invalidated. Since these are real monitoring data, no further values were removed, and potential outliers were retained. All numerical results, AIC comparisons, residual plots, and detailed diagnostic tests for each parameter are reported in the Supplementary Materials. Minor deviations from ideal assumptions were observed for a few parameters; however, the models were overall considered robust for inference. The GLMMs adopted here are descriptive and capture spatial patterns in water quality parameters but cannot identify their mechanistic causes.

The dilution ratio was not included as a covariate. It is a date-level quantity, constant across the five sampling points on any given occasion, and therefore operates at the same level as the random intercept on sampling date rather than as a predictor of differences among points. It is moreover derived from monthly treated volumes and referenced to a constant conventional input, so it does not resolve the mixing conditions prevailing on the sampling day itself. Sampling date was accordingly retained as a block term.

3 Results

The selected GLMMs, along with their results, are summarized for each parameter in Tables 1 and 2. For each parameter, the estimated marginal means ($\pm 95\%$ CI) from the selected GLMM were systematically compared with the observed value based on the exploratory analysis of the raw data, which is detailed in Table S1a of the Supplementary Materials. The homogeneous groupings reported below were confirmed by the sensitivity analyses described in Sect. 2.5, with a single exception for TSS, where the alternative correlation structure resolved one additional pairwise difference without contradicting any of those reported here. Full results are given in Tables S2b and in the per-parameter sections of the Supplementary Materials.

3.1 Chemical and physical parameters

The effect of sampling point on TP concentrations was statistically significant ($p < 0.001$). Estimated marginal means ($\pm$ SE) revealed a spatial gradient, with PA showing the highest concentration ($1.84 \pm 0.19 \text{ mg L}^{-1}$) and P1 the lowest ($0.27 \pm 0.03 \text{ mg L}^{-1}$) (Table 1). Post-hoc pairwise comparisons (Tukey-adjusted) identified three statistically homogeneous groups: PA (a), P2-P3-P4 (b, mixed irrigation water), and P1 (c) (Fig. 3). Median concentrations ranged from 0.27 mg L^{-1} at P1 to 1.78 mg L^{-1} at PA; variability was lowest at P1 and highest at P4. Both raw data and GLMM estimates indicated a downstream gradient in TP concentrations, from P1 to P2–P4 (mixed irrigation water), with consistently higher concentrations at PA.

Table 1 Summary of the selected GLMMs for chemical and physical parameters, including model fit, chi-square p-values, estimated marginal means (EMMs), and homogeneous groups

Parameter	Selected GLMM	χ^2 (df)	p	Estimated Marginal Means – EMM ($\pm$ SE)	Statistical homogeneous groups (a, b, c, ab)
TP	<i>Value~Sampling_point + (1 Date); dispformula=~Sampling_point; Gamma distribution and log-link function</i>	363.27 (4)	<0.001	PA (1.84 ± 0.19 mg L ⁻¹) P1 (0.27 ± 0.03 mg L ⁻¹) P2 (0.75 ± 0.06 mg L ⁻¹) P3 (0.79 ± 0.08 mg L ⁻¹) P4 (0.64 ± 0.09 mg L ⁻¹)	a: PA b: P2 - P3 - P4 c: P1
TN	<i>Value~Sampling_point + (1 Date); dispformula=~Sampling_point; Gamma distribution and log-link function</i>	86.02 (4)	<0.001	PA (7.39 ± 0.96 mg L ⁻¹) P1 (3.60 ± 0.44 mg L ⁻¹) P2 (5.30 ± 0.60 mg L ⁻¹) P3 (5.71 ± 0.66 mg L ⁻¹) P4 (5.41 ± 0.64 mg L ⁻¹)	a: PA b: P2 - P3 - P4 c: P1
NO ₃ ⁻ -N	<i>Value~Sampling_point + (1 Date); Gamma distribution and log-link function</i>	61.94 (4)	<0.001	PA (3.46 ± 0.46 mg L ⁻¹) P1 (1.34 ± 0.18 mg L ⁻¹) P2 (2.34 ± 0.31 mg L ⁻¹) P3 (2.21 ± 0.29 mg L ⁻¹) P4 (1.93 ± 0.26 mg L ⁻¹)	a: PA b: P2 - P3 - P4 c: P1
NH ₄ ⁺ -N	<i>Value~Sampling_point + (1 Date); Gamma distribution and log-link function</i>	39.22 (4)	<0.001	PA (1.31 ± 0.22 mg L ⁻¹) P1 (0.55 ± 0.09 mg L ⁻¹) P2 (0.93 ± 0.15 mg L ⁻¹) P3 (1.11 ± 0.18 mg L ⁻¹) P4 (1.20 ± 0.20 mg L ⁻¹)	a: PA - P2 - P3 - P4 b: P1
COD	<i>Value~Sampling_point + (1 Date); Gamma distribution and log-link function</i>	6.86 (4)	0.143	PA (22.0 ± 1.87 mg L ⁻¹) P1 (22.1 ± 1.88 mg L ⁻¹) P2 (21.8 ± 1.85 mg L ⁻¹) P3 (22.8 ± 1.93 mg L ⁻¹) P4 (24.7 ± 2.10 mg L ⁻¹)	a: PA - P1 - P2 - P3 - P4
BOD ₅	<i>Value~Sampling_point + (1 Date); Gamma distribution and log-link function</i>	16.3 (4)	0.0026	PA (10.5 ± 0.93 mg L ⁻¹) P1 (10.7 ± 0.93 mg L ⁻¹) P2 (10.8 ± 0.94 mg L ⁻¹) P3 (13.5 ± 1.19 mg L ⁻¹) P4 (12.1 ± 1.05 mg L ⁻¹)	a: P3 b: PA - P1 - P2 ab: P4
TSS	<i>Value~Sampling_point + (1 Date); dispformula=~Sampling_point; Gamma distribution and log-link function</i>	170.04 (4)	<0.001	PA (19.8 ± 3.12 mg L ⁻¹) P1 (114.6 ± 8.31 mg L ⁻¹) P2 (80.9 ± 7.44 mg L ⁻¹) P3 (75.4 ± 7.30 mg L ⁻¹) P4 (65.5 ± 5.26 mg L ⁻¹)	a: P1 b: P2 - P3 - P4 c: PA
DO	<i>Value~Sampling_point + (1 Date); Gamma distribution and log-link function</i>	40.93 (4)	<0.001	PA (5.29 ± 0.58 mg L ⁻¹) P1 (4.09 ± 0.45 mg L ⁻¹) P2 (3.57 ± 0.39 mg L ⁻¹) P3 (3.24 ± 0.35 mg L ⁻¹) P4 (3.83 ± 0.42 mg L ⁻¹)	a: PA b: P1 c: P3 bc: P2 - P4

Similarly, the effect of sampling point on TN concentrations was statistically significant ($p < 0.001$). Estimated marginal means ($\pm$ SE) revealed a spatial gradient, with PA showing the highest concentration (7.39 ± 0.96 mg L⁻¹) and P1 the lowest (3.60 ± 0.44 mg L⁻¹) (Table 1). Post-hoc pairwise comparisons (Tukey-adjusted) identified three statistically homogeneous groups: PA (a), P2-P3-P4 (b, mixed irrigation water), and P1 (c) (Fig. 4).

Table 2 Summary of the selected GLMMs for microbiological parameters, including model fit, chi-square p-values, estimated marginal means (EMMs), and homogeneous groups

Parameter	Selected GLMM	Sampling point		Temperature		Estimated Marginal Means – EMM ($\pm$ SE)	Statistical homogeneous groups (a, b, c, ab)
		χ^2 (df)	p	χ^2 (df)	p		
<i>E. coli</i>	<i>Value</i> <i>log~Sam-</i> <i>pling_</i> <i>point + Tem-</i> <i>perature + (I</i> <i> Date);</i> <i>Gaussian dis-</i> <i>tribution with</i> <i>identity link</i>	4.97 (4)	0.290	4.50 (1)	0.034	PA ($3.04 \pm 0.15 \log_{10}$ CFU 100 mL ⁻¹) P1 ($3.09 \pm 0.14 \log_{10}$ CFU 100 mL ⁻¹) P2 ($3.32 \pm 0.16 \log_{10}$ CFU 100 mL ⁻¹) P3 ($3.39 \pm 0.16 \log_{10}$ CFU 100 mL ⁻¹) P4 ($3.29 \pm 0.15 \log_{10}$ CFU 100 mL ⁻¹)	a: PA - P1 - P2 - P3 - P4
Total coliform	<i>Value</i> <i>log~Sam-</i> <i>pling_</i> <i>point + Tem-</i> <i>perature + (I</i> <i> Date);</i> <i>Gaussian dis-</i> <i>tribution with</i> <i>identity link</i>	10.52 (4)	0.033	0.63 (1)	0.425	PA ($4.06 \pm 0.14 \log_{10}$ CFU 100 mL ⁻¹) P1 ($4.35 \pm 0.14 \log_{10}$ CFU 100 mL ⁻¹) P2 ($4.43 \pm 0.14 \log_{10}$ CFU 100 mL ⁻¹) P3 ($4.54 \pm 0.14 \log_{10}$ CFU 100 mL ⁻¹) P4 ($4.44 \pm 0.14 \log_{10}$ CFU 100 mL ⁻¹)	a: P3 b: PA ab: P1 - P2 - P4
Faecal streptococci	<i>Value</i> <i>log~Sam-</i> <i>pling_</i> <i>point + Tem-</i> <i>perature + (I</i> <i> Date);</i> <i>Gaussian dis-</i> <i>tribution with</i> <i>identity link</i>	4.49 (4)	0.343	0.56 (1)	0.452	PA ($3.18 \pm 0.13 \log_{10}$ CFU 100 mL ⁻¹) P1 ($3.27 \pm 0.13 \log_{10}$ CFU 100 mL ⁻¹) P2 ($3.16 \pm 0.13 \log_{10}$ CFU 100 mL ⁻¹) P3 ($3.11 \pm 0.13 \log_{10}$ CFU 100 mL ⁻¹) P4 ($2.94 \pm 0.13 \log_{10}$ CFU 100 mL ⁻¹)	a: PA - P1 - P2 - P3 - P4

Median concentrations ranged from 2.82 mg L⁻¹ at P1 to 6.00 mg L⁻¹ at PA, with the highest variability at PA (Fig. 4). Both raw data and GLMM estimates indicated a downstream gradient in TN concentrations, with P2–P4 showing intermediate values and consistently elevated concentrations at PA.

Similarly to TN, the effect of sampling point on NO₃⁻-N concentrations was statistically significant ($p < 0.001$). Estimated marginal means ($\pm$ SE) revealed a spatial gradient, with PA showing the highest concentration (3.46 ± 0.46 mg L⁻¹) and P1 the lowest (1.34 ± 0.18 mg L⁻¹) (Table 1). Post-hoc pairwise comparisons (Tukey-adjusted) identified three statistically homogeneous groups: PA (a), P2–P3–P4 (b, mixed irrigation water), and P1 (c) (Fig. 5). Median concentrations ranged from 0.94 mg L⁻¹ at P1 to 3.11 mg L⁻¹ at PA, with maximum concentrations above 15 mg L⁻¹ at P2 and P3. PA and P3 exhibited broader IQRs, indicating greater temporal variability (Fig. 5). Both raw data and GLMM estimates indicated a downstream gradient in NO₃⁻-N concentrations with consistently elevated values at PA compared to P1 and intermediate levels at P2–P4.

As for other nitrogen forms, the effect of sampling point on NH₄⁺-N concentrations was statistically significant ($p < 0.001$). Estimated marginal means ($\pm$ SE) showed P1 had the

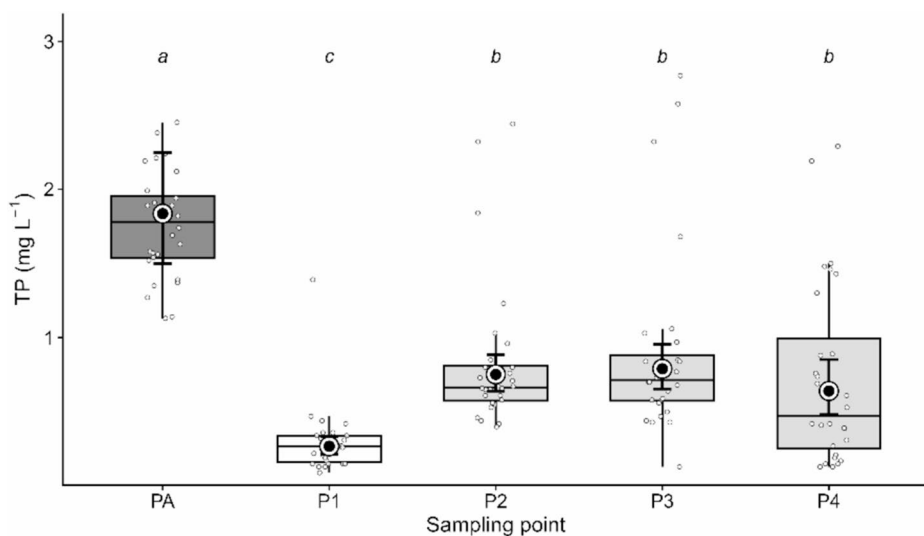


Fig. 3 TP concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

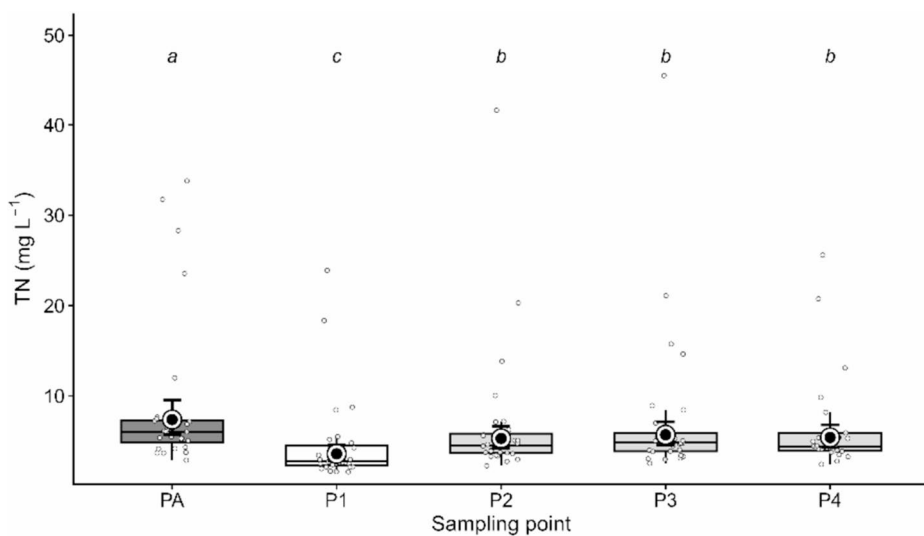


Fig. 4 TN concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

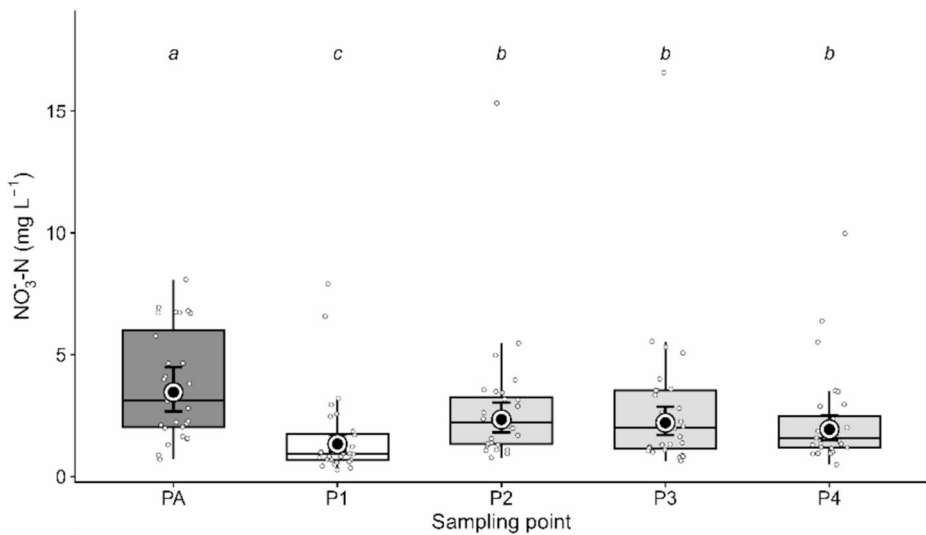


Fig. 5 $\text{NO}_3\text{-N}$ concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

lowest concentration ($0.55 \pm 0.09 \text{ mg L}^{-1}$), whereas PA had the highest ($1.31 \pm 0.22 \text{ mg L}^{-1}$), although PA did not differ from P2–P4 ($0.93\text{--}1.20 \text{ mg L}^{-1}$) (Table 1). Tukey-adjusted post-hoc comparisons identified two distinct groups: PA–P2–P3–P4 (a) and P1 (b) (Fig. 6). Median concentrations ranged from 0.44 mg L^{-1} at P1 to 1.23 mg L^{-1} at P3, with the broadest interquartile range (IQR) at PA and the narrowest at P1 (Fig. 6). Episodic peaks occurred at mixed-water points, reaching 12.4 mg L^{-1} at P4. Overall, both observed distributions and GLMM estimates indicated a clear separation between P1 and all other points, with consistently higher $\text{NH}_4^+\text{-N}$ concentrations at PA–P2–P3–P4, among which no significant differences were detected.

The effect of sampling point on COD concentrations was not statistically significant ($p=0.143$). Estimated marginal means ($\pm \text{SE}$) were similar across points, from $21.8 \pm 1.85 \text{ mg L}^{-1}$ at P2 to $24.7 \pm 2.10 \text{ mg L}^{-1}$ at P4; confidence intervals overlapped widely, and Tukey-adjusted comparisons assigned all points to a single homogeneous group (a) (Fig. 7). Observed medians varied only slightly from 19 mg L^{-1} at P1 to 23 mg L^{-1} at P4, with interquartile ranges being relatively consistent across points (Fig. 7). Episodic peaks $>60 \text{ mg L}^{-1}$ occurred at multiple locations (e.g., 68 mg L^{-1} at P1 and 65 mg L^{-1} at PA), indicating pronounced temporal variability. Overall, both model estimates and observed distributions showed no spatially structured differences in COD among sampling points during the study period.

The effect of sampling on BOD_5 concentrations was statistically significant ($p=0.0026$). Estimated marginal means ($\pm \text{SE}$) were lowest at PA ($10.5 \pm 0.93 \text{ mg L}^{-1}$) and highest at P3 ($13.5 \pm 1.19 \text{ mg L}^{-1}$), with P4 showing intermediate values ($12.1 \pm 1.05 \text{ mg L}^{-1}$) (Table 1). Tukey-adjusted post-hoc comparisons assigned P3 to a distinct group (a), P4 to an intermediate group (ab), and PA, P1, and P2 to group (b) (Fig. 8). Observed medians increased

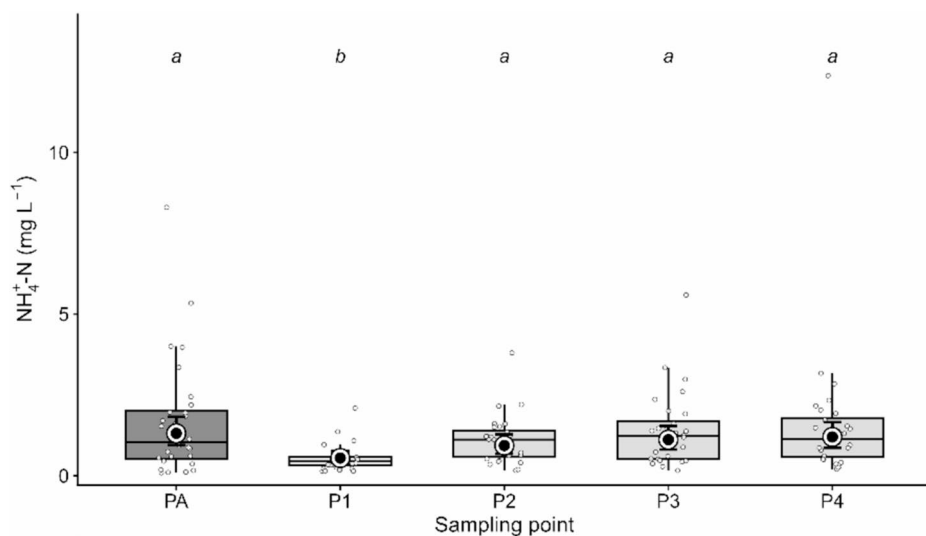


Fig. 6 $\text{NH}_4^+\text{-N}$ concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

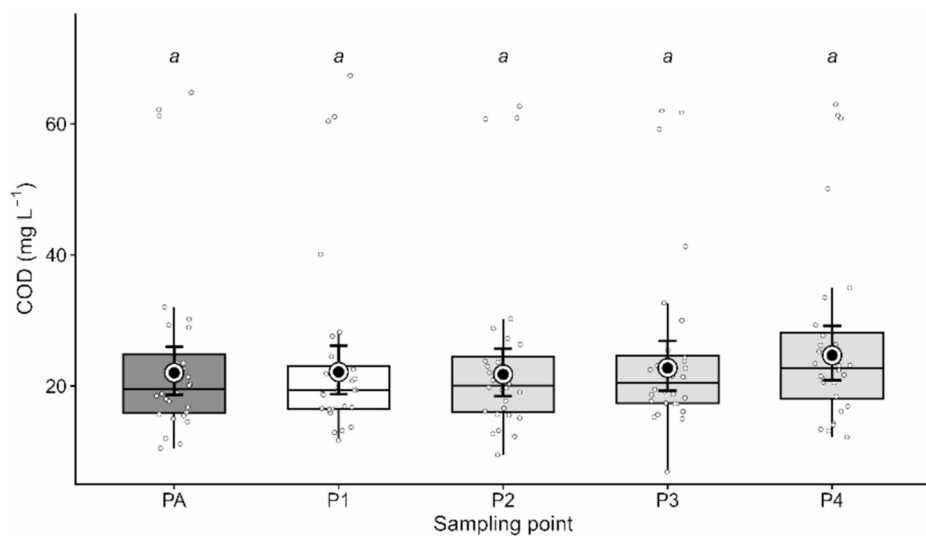


Fig. 7 COD concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

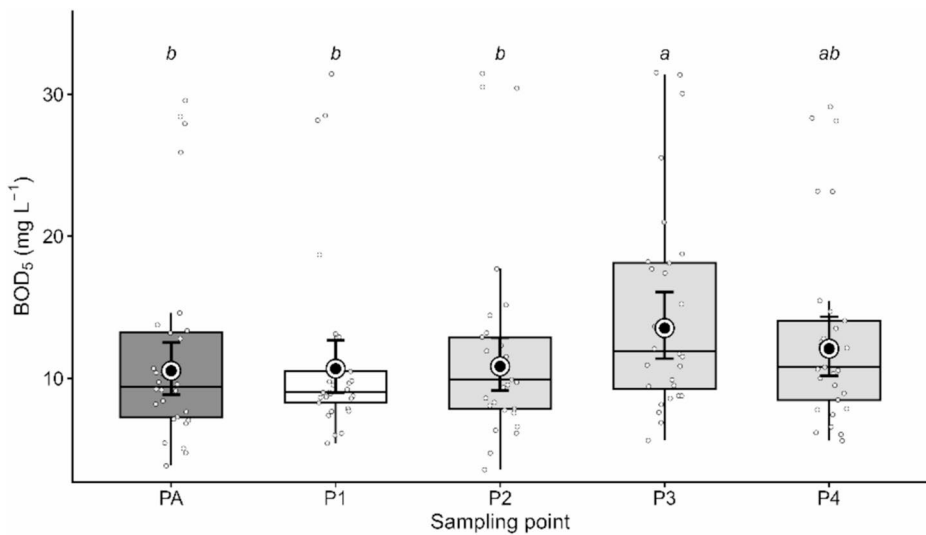


Fig. 8 BOD₅ concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

from 9.4 mg L^{-1} at PA to 11.9 mg L^{-1} at P3, with mean values peaking at P3 (14.7 mg L^{-1}) and P4 (13.0 mg L^{-1}). The interquartile range was widest at P3, indicating greater temporal variability at that point, while PA and P1 exhibited narrower distributions and more stable values (Fig. 8). Overall, BOD₅ showed moderate spatial differentiation, with higher variability at P3, and to a lesser extent at P4.

The effect of sampling point on TSS concentrations was highly significant ($p < 0.001$). Estimated marginal means ($\pm \text{SE}$) ranged from $19.8 \pm 3.1 \text{ mg L}^{-1}$ at PA to $114.6 \pm 8.3 \text{ mg L}^{-1}$ at P1 (Table 1). Tukey-adjusted post-hoc comparisons separated the sampling points into three groups: P1 (a), P2–P3–P4 (b), and PA (c) (Fig. 9). Observed medians showed a consistent pattern, from 13 mg L^{-1} at PA to 109 mg L^{-1} at P1, with maxima reaching 200 mg L^{-1} at P1 and 217 mg L^{-1} at P2. Variability was greatest at P1 and P2, which exhibited broad interquartile ranges, while PA displayed a much narrower distribution and more stable concentrations (Fig. 9). Overall, both raw data and GLMM estimates indicated a spatial gradient in TSS, with P1 statistically different from PA, and downstream points (P2–P3–P4).

The effect of sampling point on DO concentrations was statistically significant ($p < 0.001$). Estimated marginal means ($\pm \text{SE}$) ranged from $5.29 \pm 0.58 \text{ mg L}^{-1}$ at PA to $3.24 \pm 0.35 \text{ mg L}^{-1}$ at P3, with intermediate values at P1 ($4.09 \pm 0.45 \text{ mg L}^{-1}$), P2 ($3.57 \pm 0.39 \text{ mg L}^{-1}$), and P4 ($3.83 \pm 0.42 \text{ mg L}^{-1}$) (Table 1). Tukey-adjusted post-hoc comparisons delineated four partially overlapping groups: PA (a), P1 (b), P3 (c), and P2–P4 (bc) (Fig. 10). Observed median concentrations decreased from 6.0 mg L^{-1} at PA to 3.4 mg L^{-1} at P3, with a slight increase at P4 (3.5 mg L^{-1}). Variability was greatest at PA and P1, where broad interquartile ranges indicated marked temporal fluctuations, while narrower distributions at P2–P4 suggested more stable conditions (Fig. 10).

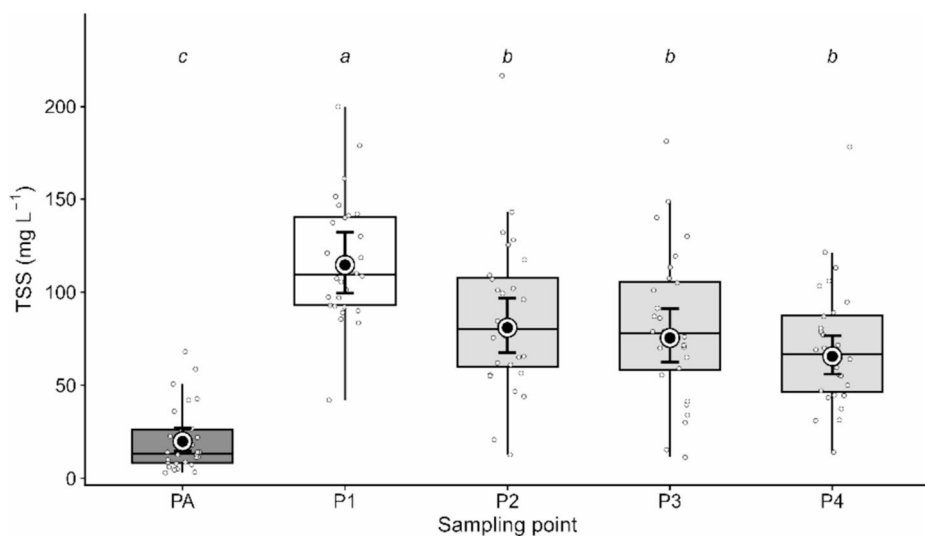


Fig. 9 TSS concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

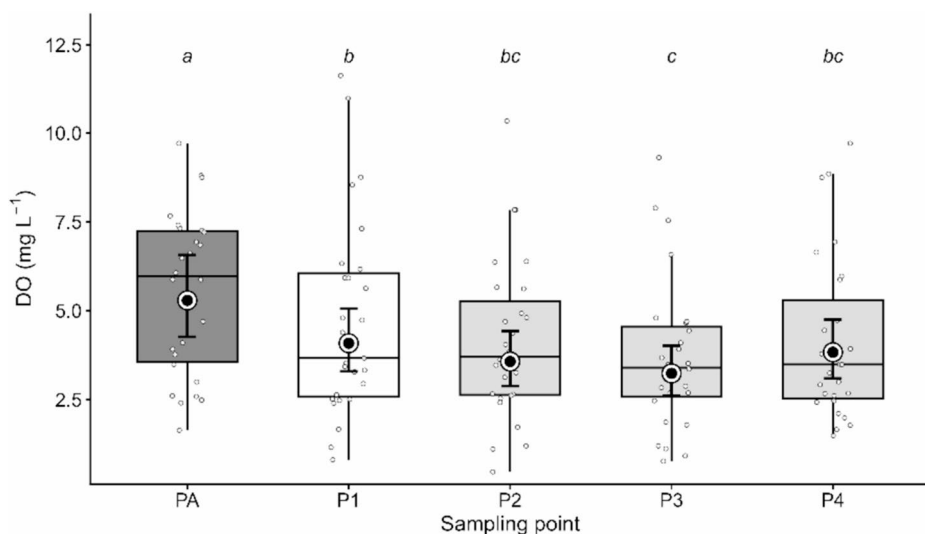


Fig. 10 DO concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. For this parameter a high value is favourable, so the letters do not rank water quality in the same direction as for the other parameters. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

3.2 Microbiological Parameters

The effect of sampling point on *E. coli* concentrations was not statistically significant on the log₁₀-transformed scale ($p=0.290$), whereas water temperature had a significant positive effect ($p=0.034$). Estimated marginal means ($\pm$ SE) ranged from 3.04 ± 0.15 to 3.39 ± 0.16 log₁₀ CFU 100 mL⁻¹ across points, with wide confidence intervals overlapping substantially and post-hoc tests assigning all sampling points to a single homogeneous group (a) (Table 2; Fig. 11). Observed data distributions, however, highlighted strong heterogeneity and episodic peaks. Median concentrations ranged from 300 CFU 100 mL⁻¹ at PA to 3,000 CFU 100 mL⁻¹ at P3, with maxima reaching 80,000 CFU 100 mL⁻¹ at PA and 45,000 CFU 100 mL⁻¹ at P3. PA showed the widest spread of values, from 20 to 80,000 CFU 100 mL⁻¹, and the broadest interquartile range (100–17,800 CFU 100 mL⁻¹). Median concentrations were higher at P3 and P4 than at P1 and P2. Overall, both raw and model-based results indicated no statistically significant differences in *E. coli* concentrations among sampling points. However, large temporal fluctuations and episodic peaks were evident across the system.

Contrary to *E. coli*, the effect of sampling point on total coliform concentrations was statistically significant ($p=0.033$), whereas water temperature was not a significant predictor ($p=0.425$). Estimated marginal means ($\pm$ SE) ranged from 4.06 ± 0.14 to 4.54 ± 0.14 log₁₀ CFU 100 mL⁻¹ across points, with Tukey-adjusted post-hoc comparisons assigning P3 to group (a), PA to group (b), and P1, P2, and P4 to an intermediate group (ab) (Table 2; Fig. 12). Observed median concentrations ranged from 9,400 CFU 100 mL⁻¹ at PA to 30,000 CFU 100 mL⁻¹ at P3. Maximum concentrations reached 5.0×10^5 CFU 100 mL⁻¹ at P3 and 5.6×10^5 CFU 100 mL⁻¹ at P4. Interquartile ranges were relatively narrow across

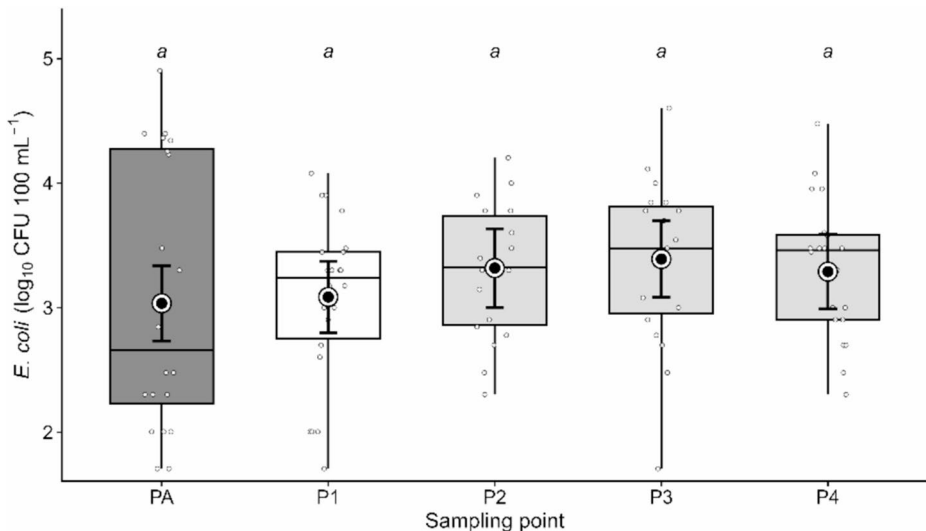


Fig. 11 *E. coli* concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

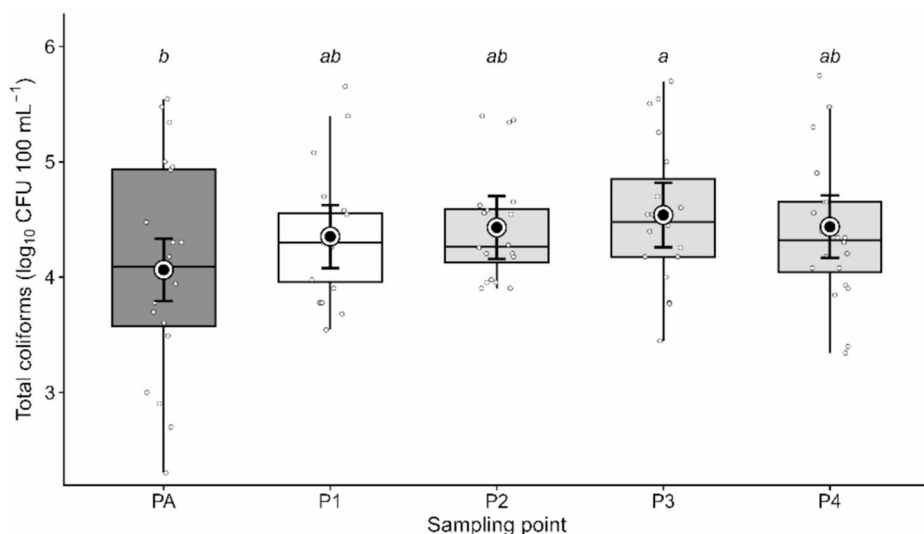


Fig. 12 Total coliform concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration ‘a’ to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

points, indicating moderate variability compared with other microbiological parameters. Overall, both raw and model-based results indicated significant differences among sampling points, with higher values at P3 and lower values at PA.

The effect of sampling point on faecal streptococci concentrations was not statistically significant ($p=0.343$), and water temperature was also not a significant predictor ($p=0.452$). Estimated marginal means ($\pm$ SE) ranged from 2.94 ± 0.13 to 3.27 ± 0.13 log₁₀ CFU 100 mL⁻¹ across points, with Tukey-adjusted post-hoc comparisons assigning all sampling points to a single homogeneous group (a) (Table 2; Fig. 13). Median concentrations were relatively stable across locations, ranging from 1,100 CFU 100 mL⁻¹ at P4 to 1,850 CFU 100 mL⁻¹ at PA. Maximum values reached 26,000 CFU 100 mL⁻¹ at PA and 60,000 CFU 100 mL⁻¹ at P1. Variability was moderate, with PA and P1 exhibiting wider ranges compared to downstream points. These distributions are shown in Fig. 13, where observed means align with model estimates.

4 Discussion

4.1 Spatial Variability of Water Quality Parameters and Drivers of Downstream Dynamics

The analysis of spatial variability in water quality builds upon the hydraulic characterization of the Molinella canal, which was carried out to quantify the relative contribution of TWW and conventional irrigation water to the overall flow regime. The hydraulic survey,

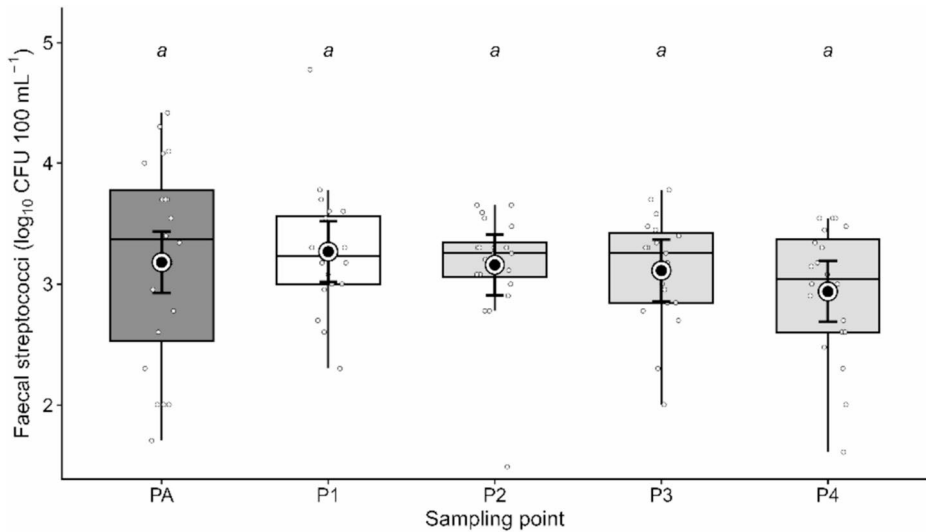


Fig. 13 Faecal streptococci concentrations by sampling point. Boxplots show the observed data; overlaid points and bars show the estimated marginal mean and its 95% confidence interval from the selected GLMM. Letters denote homogeneous groups from Tukey-adjusted post-hoc comparisons, from the highest concentration 'a' to the lowest. PA is the WWTP outlet on the Cavaliera canal; P1 is the conventional irrigation source upstream of the confluence; P2, P3 and P4 are mixed irrigation water at increasing distance downstream

performed across nine cross-sections over a 3 km reach, indicated stable flow conditions during the monitoring period, with an average stored volume of approximately 5,400 m³. TWW represented the dominant inflow, ranging from 3,700 m³ day⁻¹ to episodic peaks with 10,600 m³ day⁻¹, compared with a constant 1,720 m³ day⁻¹ contribution from conventional irrigation water. Consequently, the ratio between TWW and conventional irrigation water volumes (TWW/conventional irrigation water) remained consistently above 2, confirming that TWW accounted for a substantial and often prevailing portion of the total canal discharge. Because the five sampling points were measured jointly on each occasion, the date random intercept absorbs the conditions shared among them and the contrasts among points are estimated within a sampling occasion. The spatial patterns discussed below are therefore not confounded by variation in the TWW contribution between dates, although the design does not resolve the effect of the mixing ratio itself (Sect. 2.5).

For TP, TN, and NO₃⁻-N, the GLMMs identified highly significant effects of sampling point, with PA consistently exhibiting the highest concentrations and P1 the lowest, showing that TWW had higher concentration of these elements than conventional irrigation water. Moreover, post-hoc tests distinguished P1 from P2–P3–P4, indicating that nutrient concentrations were higher downstream of the confluence where TWW entered the canal. NH₄⁺-N followed a different pattern: concentrations at PA were significantly higher than at P1, but no differences were detected between PA and P2–P4. In agreement with Gikas (2017), who identified WWTP effluents as dominant sources of TN and TP in Mediterranean rivers, our results confirm the WWTP discharge as the primary contributor of nutrients to the canal system. This interpretation is corroborated at the district scale by Gaggia et al. (2026), who reported mean TN and NO₃⁻-N concentrations of 15.65 and 6.76 mg L⁻¹, respectively, in

canals of the same irrigation district receiving WWTP effluents, values within which the concentrations observed at PA in the present study fall. Along the mixed reach, however, the two nitrogen species diverge: both observed means and model estimates show $\text{NH}_4^+\text{-N}$ rising and $\text{NO}_3^-\text{-N}$ falling between P2 and P4. This is not the pattern that net nitrification would produce, and the low dissolved oxygen levels discussed below would constrain it in any case. The design does not resolve the processes behind these trends.

Considering the mixed irrigation water downstream of the confluence (P2–P3–P4), which are those most representative of irrigation conditions, nutrient concentrations ranged approximately, based on the estimated marginal means from the GLMM, 5.3–5.7 mg L^{-1} for TN and 0.6–0.8 mg L^{-1} for TP. These values are substantially higher than those reported for natural surface waters in Europe, where ecological targets under the Water Framework Directive typically range from $<3.5 \text{ mg L}^{-1}$ for TN to $<0.12 \text{ mg L}^{-1}$ for TP (Nikolaidis et al. 2022; Erba et al. 2022). However, it is important to note that these thresholds were designed for ecological surface waters under the Water Framework Directive (WFD), whereas irrigation canals are managed systems with different functional goals. Therefore, although nutrient levels in mixed irrigation waters exceed ecological targets, they should mainly be understood from an agronomic reuse perspective. In fact, these concentrations fall within the range that can supply a relevant fraction of crop N and P demand under fertigation practices, particularly for high-demand crops such as maize and vegetables commonly grown in the Emilia-Romagna (Odone et al. 2024), and TWW, therefore, can be seen as an additional input that decreases farmers' reliance on mineral fertilizers. As highlighted by (Mainardis et al. 2022), when managed within integrated reuse schemes, fertigation with TWW represents a practical opportunity for circular economy application, supporting both irrigation and nutrient supply while minimizing external inputs.

A significant decline in DO was observed along the irrigation canal, with EMMs decreasing from 4.1 mg L^{-1} at P1 to 3.2 mg L^{-1} at P3, followed by a partial recovery to 3.8 mg L^{-1} at P4. These values correspond to median saturations of 38–44% at P1–P4 (Table S1a). This trend, coupled with a peak in BOD_5 at P3 (13.5 mg L^{-1}), suggests oxygen depletion attributable to biochemical oxidation of organic matter. BOD_5 at PA (10.5 mg L^{-1}) did not differ significantly from P1 (10.7 mg L^{-1}), indicating that TWW did not increase the biodegradable organic load relative to conventional irrigation water, suggesting that external factors rather than the WWTP effluent are responsible for the localised organic enrichment and oxygen depletion observed at P3. The origin of this signal remains unclear and likely reflects the complexity of a fully operational irrigation network, where minor uncharacterised inputs along the canal reach cannot be excluded (Barroso et al. 2024). In contrast, COD did not vary significantly among sampling points, with EMMs ranging narrowly between 21.8 mg L^{-1} and 24.7 mg L^{-1} , suggesting that the pool of non-biodegradable organic matter was relatively invariant and was not strongly affected by mixing of water sources. Similar patterns were reported by Aly et al. (2025) in the Ismailia Canal (Egypt), where TWW input caused localized decreases in DO and increases in BOD_5 . In contrast, COD rose at the discharge point but declined downstream due to dilution (Aly et al. 2025). Comparable findings were also described by Almeida et al. (2021) in the Ramalhoso River (Portugal), where BOD_5 increased immediately after WWTP discharges before declining downstream. In the case presented in this paper however, the absence of significant difference in COD and the non-significant contrast between PA and P1 for BOD_5 emphasize that TWW did not exacerbate organic pollution.

TSS exhibited an inverse gradient compared to nutrients: concentrations were significantly lowest at PA (19.8 mg L⁻¹) and significantly highest at P1 (114.6 mg L⁻¹), while P2, P3, and P4 (80.9, 75.4, and 65.5 mg L⁻¹, respectively) formed a single homogeneous group, with no significant differences among them. This pattern between P1 and P2–P3–P4 can be explained by two concurrent mechanisms: (i) the low TSS concentrations of TWW at PA, which contributed between 68% and 86% of canal flow depending on TWW discharge and thus exerted a positive effect after the confluence, and (ii) progressive sedimentation and settling of suspended solids along the canal due to the low water velocity. This combined effect resulted in downstream stabilization at intermediate values. This pattern contrasts with Wolfand et al. (2022), who, using a modelling approach for the effluent-dominated Los Angeles River, predicted increased TSS concentrations under TWW reuse scenarios, highlighting how local hydrology and mixing regimes critically determine suspended solids dynamics. This was consistent with An et al. (2023), who also reported that the mixing of TWW with surface waters can improve water quality in receiving systems. Our results indicate that TWW was not a driver of increased suspended solids. On the contrary, the introduction of TWW with lower TSS than conventional irrigation water improved downstream water quality, underscoring its potential as a positive contributor in reuse scenarios.

Among microbiological parameters, only total coliforms exhibited a significant difference, with EMMs increasing from 4.06 log₁₀ CFU 100 mL⁻¹ at PA to 4.54 log₁₀ CFU 100 mL⁻¹ at P3, while intermediate values were observed at P1, P2, and P4 (4.35–4.44 log₁₀ CFU 100 mL⁻¹). This localized increase at P3 may be related to the concurrent BOD₅ peak, since higher organic matter availability could have supported microbial regrowth under the hydraulic conditions of the canal (Barroso et al. 2024). Neither *E. coli* nor faecal streptococci showed significant variation among sampling points. These findings indicate that the TWW discharge did not exacerbate faecal contamination within the irrigation canal. The high variability observed for *E. coli*, particularly the episodic peaks up to 4.9 log₁₀ CFU 100 mL⁻¹ at PA and 4.7 log₁₀ CFU 100 mL⁻¹ at P3, highlights the stochastic nature of microbial contamination in open canals. Water temperature was a significant covariate only for *E. coli*, reinforcing the influence of environmental drivers on bacterial persistence and decay. Overall, faecal indicators showed limited persistence downstream under the prevailing canal conditions, although episodic spikes in contamination remain a potential risk. Comparable evidence was reported by Pascual-Benito et al. (2020), who found that WWTP effluents consistently increased faecal indicator concentrations in a Mediterranean stream, but that subsequent decay was strongly modulated by flow and temperature. Similarly, Lu et al. (2022) demonstrated that WWTP effluents substantially altered bacterial community composition in both water and sediment immediately after the discharge point, though recovery to the previous values occurred within short distances. Taken together, these results highlight that microbial responses to TWW inputs can be highly variable and context-dependent, shaped by both hydraulic conditions and the makeup of microbial communities. This supports the idea that microbial risks in irrigation reuse schemes are highly site-specific and modulated by environmental factors. At the district scale, Gaggia et al. (2026) demonstrated across 27 irrigation canals in the same district that urban influence, rather than chemical water composition, was the primary driver of faecal indicator concentrations, with *E. coli* in WWTP-fed canals showing weaker sector-level association than other indicators, attributed to the partial mitigation provided by plant-level disinfection. This supports the interpretation that the *E. coli* levels observed along the Molinella canal

Table 3 Summary of statistically significant spatial effects for water quality parameters based on GLMM analyses. The table reports whether each parameter showed an overall significant effect of sampling point ($p < 0.05$), and the direction of significant pair

Parameter	Significant effect ($p < 0.05$)	PA vs. P1	P1 vs. P2–P4*
Total Phosphorus (TP)	Yes	Yes (PA > P1)	Yes (P1 < P2–P4)
Total Nitrogen (TN)	Yes	Yes (PA > P1)	Yes (P1 < P2–P4)
Nitrate-N (NO_3^- -N)	Yes	Yes (PA > P1)	Yes (P1 < P2–P4)
Ammonium-N (NH_4^+ -N)	Yes	Yes (PA > P1)	Yes (P1 < P2–P4)
Chemical Oxygen Demand (COD)	No	No	No
Biochemical Oxygen Demand (BOD ₅)	Yes	No	Yes (P1 < P3)
Total Suspended Solids (TSS)	Yes	Yes (PA < P1)	Yes (P1 > P2–P4)
Dissolved Oxygen (DO)	Yes	Yes (PA > P1)	Yes (P1 > P3)
<i>E. coli</i>	No	No	No
Total coliforms	Yes	No	No
Faecal streptococci	No	No	No

PA (TWW); P1 (conventional irrigation water); P2–P3–P4 (mixed irrigation water)*Significant pairwise differences downstream of P1

reflect background contamination characteristic of the broader district, and that episodic peaks at PA are more appropriately attributed to treatment performance fluctuations than to a structural microbiological hazard introduced by the effluent.

4.2 Overall Effects of TWW on Irrigation Water Quality

The spatial analysis revealed clear, parameter-specific differences in water quality along the canal system, confirming the role of TWW discharge as the primary source of nutrient enrichment and a driver of downstream biogeochemical processes. Despite accounting for 68–86% of the canal discharge, TWW did not produce systematic deterioration of conventional irrigation water quality. A synthesis of significant pairwise differences between PA vs. P1 and P1 vs. P2–P4 is presented in Table 3.

For parameters directly regulated under Regulation (EU) 2020/741, the effluent introduced no additional load relative to the conventional irrigation source. TSS were significantly lower in TWW than in conventional irrigation water, leading to a reduction in suspended solids downstream of the confluence by 29 to 43%. BOD₅ at PA did not differ significantly from P1, indicating that TWW did not introduce additional biodegradable organic load. Downstream, however, BOD₅ at P3 was significantly higher than at P1 (Sect. 4.1): this load is not attributable to the effluent, but from the perspective of the irrigator, and of the risk-based framework advocated here, it is present in the water actually abstracted. For *E. coli*, no significant spatial differences were detected, confirming that TWW discharge did not exacerbate faecal contamination within the canal under mean operating conditions.

The TWW contribution was primarily reflected in nutrient enrichment, with TN, TP, and NO_3^- -N consistently higher at PA than at P1 and intermediate at P2–P4. Concentrations in the mixed irrigation water (TN of 5.3–5.7 mg L⁻¹ and TP of 0.6–0.8 mg L⁻¹) fall within

ranges that can supply a relevant fraction of crop nitrogen and phosphorus demand, representing a potential agronomic benefit through reduced reliance on fertilizers and supporting circular economy principles in agricultural water management (Mainardis et al. 2022; Odone et al. 2024).

However, these agronomic benefits must be considered alongside the associated risks. From a public health perspective, the episodic *E. coli* peaks reaching 80,000 CFU 100 mL⁻¹ at PA and 45,000 CFU 100 mL⁻¹ at P3 represent a critical concern. These values reflect real-scale observations collected under actual operating conditions, with all samples retained to capture the full variability of the system. Such peaks are likely attributable to transient fluctuations in WWTP treatment performance driven by influent load variability or hydraulic peaks, inherent characteristics of full-scale operations, and could pose significant health risks if irrigation water is applied to crops intended for raw consumption. From an environmental perspective, TN and TP values substantially exceeding WFD ecological targets indicate a potential risk of in-channel eutrophication and non-point nutrient export to downstream receiving water bodies, particularly under conditions of low crop uptake or irrigation mismanagement.

Taken together, these findings demonstrate that TWW reuse through blending in open irrigation networks delivers measurable agronomic benefits but simultaneously introduces public health and environmental risks that cannot be adequately captured by mean-condition assessments alone. This underscores the need for risk-based monitoring frameworks that explicitly account for worst-case contamination scenarios and nutrient dynamics in the context of blended reuse management.

4.3 Implications for Risk Management within Blended Direct Reuse

To further contextualise the regulatory implications of this study, field measurements at PA were compared with compliance monitoring data provided by the water utility for the Molinella WWTP. These compliance data are unpublished and outside the scope of this article; they are included exclusively to inform the regulatory discussion, and a formal statistical comparison between the two datasets is not appropriate given their different origins and sampling protocols. The point of compliance, located inside the WWTP immediately after the disinfection stage, and PA are physically separated by approximately 1 m, yet occupy conceptually distinct regulatory positions: the former is subject to the full monitoring and quality requirements of Regulation (EU) 2020/741, while the latter, situated immediately after the discharge point into the Cavaliera canal, falls entirely outside its scope. Compliance assessment during 2023 revealed an asymmetric quality profile: BOD₅ and TSS met Class A requirements in 97% and 92% of samples respectively ($n=36$), with no exceedances beyond Class B. *E. coli* achieved only Class D, with a mean of 5,309 CFU 100 mL⁻¹, a maximum of 45,000 CFU 100 mL⁻¹, and only 20% of samples meeting the Class A threshold ($n=10$); the multi-year dataset ($n=28$, 2021–2022–2023) confirms this as a structural characteristic of the system. Median *E. coli* concentrations at PA in the present study (300 CFU 100 mL⁻¹; $n=22$, June–October 2023 and April–October 2024) were higher than those recorded at the point of compliance by the water utility (161 CFU 100 mL⁻¹; $n=10$, 2023). Given the different sampling frequencies, protocols, and dataset origins, this comparison is illustrative rather than statistically inferential; nonetheless, it suggests that a further increase in *E. coli* concentrations may occur across the unmonitored transition between the end of the

disinfection process and the receiving canal. This finding highlights a gap in the point-based compliance framework and reinforces the need to transition to a risk-based approach that extends monitoring and quality requirements beyond the WWTP outlet.

The case for this regulatory gap is structural and does not rest on the present dataset: blending reuse occurs wherever WWTP effluents enter collective conveyance infrastructure, a configuration documented at continental and global scale (Rice and Westerhoff 2015; Thebo et al. 2017). The results reported here corroborate that argument with field evidence from one such system, aiming to start constituting a solid empirical foundation. Regulation (EU) 2020/741 was designed for unblended direct TWW reuse and provides no framework for the blended direct configuration that prevails in open canal networks, despite its documented widespread occurrence in peri-urban agricultural systems (Rice and Westerhoff 2015; Kesari et al. 2021). In the Molinella canal, TWW dominated total inflow throughout the monitoring period, shaping the chemical and microbiological quality of water abstracted for irrigation across an entire agricultural district. This system operates without any regulatory oversight of water quality at the point of abstraction, a condition that is neither exceptional nor confined to Italy, but representative of many irrigation systems across Europe and beyond (Christou et al. 2024). Overcoming this paradigm requires shifting from a compliance-based to a risk-based governance framework that treats the entire water reuse system (comprising WWTP, the distribution network, and the farm irrigation system) as an integrated, interconnected system. Regulation (EU) 2020/741 already introduced site-specific risk assessment as a foundational provision, and the subsequent adoption of Delegated Regulation (EU 2024/1765 2024) operationalised its technical specifications, together implicitly acknowledging that fixed quality thresholds assessed at a single point are insufficient for the diversity of real-world reuse configurations. This provision must be extended and formalised for water reuse through blending, with risk assessment encompassing the full pathway from treatment to field (Drei et al. 2025; Allgaier et al. 2026).

The implications of this shift extend to a deeper reconceptualisation of water safety in agricultural systems. The One Health framework recognises the structural interdependence of human, animal, and environmental health across shared environments, and its application to reclaimed water reuse requires the integrated assessment of all interconnected compartments (i.e., groundwater, surface water, soil, crops, and human populations) rather than the isolated evaluation of individual water sources or treatment steps (Penserini et al. 2024). The present study captures water quality dynamics across TWW, conventional irrigation water, and mixed irrigation water reaches for physical, chemical, and microbiological parameters, and demonstrates that contamination and enrichment processes cannot be attributed to any single source in isolation. This multi-compartment evidence base is consistent with a One Health approach to reuse assessment. The findings also expose a particularly consequential regulatory asymmetry: the WWTP effluent, despite its Class D microbiological compliance, is subject to rigorous regulatory oversight, whereas conventional irrigation water, sourced from the Po River and entering the system at P1, is structurally excluded from the regulatory scope, despite TSS concentrations averaging 114.6 mg L^{-1} and *E. coli* median concentrations of $2,000 \text{ CFU } 100 \text{ mL}^{-1}$, both exceeding Class A and Class C thresholds respectively under Regulation (EU) 2020/741. This asymmetry reveals a contradiction: risk is assigned to the regulated source, while an unregulated source with potential contamination remains outside governance (Mateo-Sagasta et al. 2018). Formal inclusion of blended direct reuse within Regulation (EU) 2020/741, underpinned by site-specific risk management plans

encompassing the full water reuse pathway from treatment to field abstraction, would represent a coherent development of European water reuse governance, one guided by the integrated One Health principles (Allgaier et al. 2026).

4.4 Limitations of the Study

Several constraints delimit the scope of the findings reported here. The study describes a single irrigation district and influence of one WWTP. The spatial patterns observed are therefore specific to this system, and their extension to other blending configurations requires confirmation under different conditions. The GLMMs adopted are descriptive rather than mechanistic, but they were deemed the best choice for this research. While sampling point acts as a proxy for the cumulative effect of mixing, in-stream biogeochemical processes, lateral exchanges and abstraction were not considered due to the technical and logistical difficulties, and a future study could have this objective. The reach was not exhaustively characterised hydraulically and minor uncharacterised inputs along the canal cannot be excluded, and the localised organic enrichment and oxygen depletion observed at P3 remain unattributed. Dissolved oxygen was measured during the sampling events, so the night-time minimum was not recorded. Systematic surveys of lateral inputs would be required to resolve this signal.

Future research could extend the current approach to contaminants of emerging concern, particularly pharmaceutical residues, as well as to the occurrence and dissemination of antibiotic resistance. Examining these parameters across the wastewater–canal interface would provide a broader understanding of the chemical and microbiological implications of water reuse under operational conditions, identifying additional priorities for the safe management of reclaimed water in irrigation systems.

5 Conclusions

This study provides robust evidence of spatial variability in water quality parameters along an irrigation canal receiving TWW discharge from a WWTP, assessed under the real-scale operational conditions characteristic of water reuse practices in Northern Italy. By combining GLMMs with monitoring across 11 physical, chemical, and microbiological parameters, this research addressed both regulated parameters under Regulation (EU) 2020/741 (*E. coli*, BOD₅, TSS) and additional indicators of environmental and agronomic relevance, providing an assessment that extends beyond the minimum legal requirements.

The analysis revealed clear spatial gradients for nutrients and suspended solids, while organic and microbiological parameters showed more stable or site-specific dynamics. Importantly, TWW inputs did not systematically deteriorate irrigation water quality; rather, they contributed to nutrient enrichment that can be reframed as a resource for fertiligation. Taken together, these findings emphasize that reuse systems involving blending can buffer water quality variability and, at the same time, deliver agronomic benefits. However, monitoring must remain targeted to parameters with inherently higher variability, particularly ammonium and faecal indicators, where episodic peaks represent the primary risk.

From a regulatory standpoint, a compliance framework focused on the WWTP outlet does not adequately capture the quality that water abstracted by farmers downstream has.

In practical terms, the point of compliance and the point of use are not the same water, and the operational change these results support is to assess quality where abstraction occurs rather than only where the effluent is discharged. Monitoring protocols for reuse systems involving blending must be designed to reflect the specific hydraulic and agronomic conditions of each system, accounting for the full range of variability rather than relying solely on mean-condition assessments. The effect of blending was also found to be parameter-specific, marked for suspended solids and absent for the faecal indicators, so its recognition as a barrier within a water reuse risk management plan requires site-specific verification rather than assumption. Moreover, the case for extending Regulation (EU) 2020/741 to explicitly address blending direct reuse is supported by these findings, which point towards harmonized water safety standards across EU Member States where such practices are structurally embedded in irrigation infrastructure. These conclusions are bounded by the constraints set out in Sect. 4.4, in particular the single-site scope and the limited resolution of hydraulic inputs in an open canal network.

This work provides a methodological and empirical reference point for further research on blended direct reuse systems. Extending monitoring to longer time series, diverse hydrological conditions, and additional pollutants and pathogens will be essential to fully assess the long-term sustainability and safety of water reuse systems. In particular, extending the GLMM-based approach to larger datasets, different canal systems, and varying TWW-to-freshwater ratios would enhance its capacity to disentangle site-specific effects from generalizable patterns, thereby providing a stronger scientific basis for the management of blended direct reuse. Finally, monitoring should be expanded to contaminants of emerging concern (e.g., pharmaceuticals, pesticides, antimicrobial resistance determinants) and to the potential industrial contributions to the influent, to quantify interactions with nutrients and microbial dynamics, and to further characterize the conditions under which TWW can be safely and sustainably used in agricultural irrigation.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40710-026-00876-5>.

Acknowledgements The authors express their sincere gratitude to Lorenzo Cavallina, and Roberto Nigrelli from the Consorzio della Bonifica Renana, as well as Liliana Petrotto, Giovanni Urso, Francesca Gaggia, and Elia Pagliarini from Alma Mater Studiorum - University of Bologna, for their technical support and valuable collaboration.

Author Contributions PD: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualisation. GM: Writing – review & editing, Methodology, Investigation, Data curation, Conceptualisation. SL: Writing – review & editing, Methodology, Investigation, Conceptualisation. AB: Validation, Resources, Data curation. MS: Validation, Resources, Data curation. AT: Writing – review & editing, Methodology, Investigation, Conceptualisation, Supervision, Funding acquisition.

Funding Open access funding provided by Alma Mater Studiorum - Università di Bologna within the CRUI-CARE Agreement. This study was funded by the PRIMA Programme, as part of the BONEX project (Boosting Nexus Framework Implementation in the Mediterranean, <https://bonex-prima.eu/>), grant agreement number 2141, financed by Horizon 2020. This study was also partially funded by the EU Horizon Europe funding programme for research and innovation under the project BOOST-IN (grant agreement no. 101135239, <https://boostin.eu/>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abugu HO, Egwuonwu PF, Ihedioha JN, Ekere NR (2021) Hydrochemical evaluation of river Ajali water for irrigational application in agricultural farmland. *Appl Water Sci* 11. <https://doi.org/10.1007/s13201-021-01395-4>
- Al Aukidy M, Verlicchi P, Jelic A et al (2012) Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. *Sci Total Environ* 438:15–25. <https://doi.org/10.1016/j.scitotenv.2012.08.061>
- Allgaier P, Maffettone R, Grecksch K (2026) Challenges in Implementing European Regulation on Water Reuse for Sustainable Agriculture. *Environ Policy Gov*. <https://doi.org/10.1002/eet.70082>
- Almeida P, Albuquerque T, Antunes M et al (2021) Effects of wastewater treatment plant's discharges on a freshwater ecosystem—a case study on the Ramalhoso River (Portugal). *Water Air Soil Pollut* 232. <https://doi.org/10.1007/s11270-021-05131-1>
- Álvarez-Fraga L, Amarasiri M, Arnal C et al (2026) A Framework for Antimicrobial Resistance Risk Assessment, Monitoring, and Risk-Reduction Practices along the Reused Water–Soil–Crop–Human Continuum. *Environ Sci Technol* 60:19722–19745. <https://doi.org/10.1021/acs.est.6c03391>
- Aly MM, Zayed MSM, Salem MS, Mostafa MK (2025) Evaluating the impacts of discharging the effluent of Al-Gabal Al-Asfar wastewater treatment plant on Ismailia Canal in Egypt to maximize revenue and ensure sustainability. *Air Soil Water Res* 18. <https://doi.org/10.1177/11786221251315058>
- An S, Song Y, Fu Q et al (2023) Reclaimed water use improved polluted water's self-purification capacity –Evidenced by water quality factors and bacterial community structure. *J Clean Prod* 386. <https://doi.org/10.1016/j.jclepro.2022.135736>
- APHA (2017) Standard methods for the examination of water and wastewater, 23rd edn. American Public Health Association
- Assouline S, Kamai T, Šimůnek J et al (2020) Mitigating the impact of irrigation with effluent water: mixing with freshwater and/or adjusting irrigation management and design. *Water Resour Res* 56. <https://doi.org/10.1029/2020WR027781>
- Barbagallo S, Barbera AC, Cirelli GL et al (2014) Reuse of constructed wetland effluents for irrigation of energy crops. *Water Sci Technol* 70:1465–1472. <https://doi.org/10.2166/wst.2014.383>
- Barroso GR, Pinto CC, Gomes LNL, Oliveira SC (2024) Assessment of water quality based on statistical analysis of physical-chemical, biomonitoring and land use data: Manso River supply reservoir. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2023.169554>. 912:
- Chaudhary S, Siwach C, Anand S, Mishra VK (2025) A bibliometric analysis of statistical methods used for river water quality assessment & monitoring during the last three decades (1992–2023). *Water Environ J* 39:419–433. <https://doi.org/10.1111/wej.12984>
- Christou A, Beretsou VG, Iakovides IC et al (2024) Sustainable wastewater reuse for agriculture. *Nat Rev Earth Environ* 5:504–521. <https://doi.org/10.1038/s43017-024-00560-y>
- Drei P, Mancuso G, Lavrić S et al (2025) A novel methodology to develop EU water reuse risk management plan in agricultural water distribution networks and crop irrigation systems. *Water Res X* 28:100395. <https://doi.org/10.1016/j.wroa.2025.100395>
- Erba S, Buffagni A, Cazzola M, Balestrini R (2022) Italian reference rivers under the water framework directive umbrella: do natural factors actually depict the observed nutrient conditions? *Environ Sci Eur* 34. <https://doi.org/10.1186/s12302-022-00642-y>

- EU 2024/1765 (2024) Delegated Regulation (EU) 2024/1765 of 11 March 2024, supplementing Regulation (EU) 2020/741 of the European Parliament and of the Council with regard to technical specifications of the key elements of risk management
- European Investment Bank (2022) Wastewater as a resource. EIB Projects Directorate
- Flörke M, Schneider C, McDonald RI (2018) Water competition between cities and agriculture driven by climate change and urban growth. *Nat Sustain* 1:51–58. <https://doi.org/10.1038/s41893-017-0006-8>
- Gaggia F, De Feudis M, Pagliarini E et al (2026) Water quality of artificial canals used for agricultural purposes affected by urban and agricultural activities through a chemical and microbial perspective. *Agric Water Manag* 324. <https://doi.org/10.1016/j.agwat.2025.110116>
- Gikas GD (2017) Water quantity and hydrochemical quality monitoring of Laspias River, North Greece. *J Environ Sci Health Tox Hazard Subst Environ Eng* 52:1312–1321. <https://doi.org/10.1080/10934529.2017.1357408>
- Hurlbert SH (1984) Pseudoreplication and the Design of Ecological Field Experiments. *Ecol Monogr* 54:187–211
- Kesari KK, Soni R, Jamal QMS et al (2021) Wastewater Treatment and Reuse: a Review of its Applications and Health Implications. *Water Air Soil Pollut* 232:208. <https://doi.org/10.1007/s11270-021-05154-8>
- Khandelwal A, Castillo T, González-Pinzón R (2024) Evidence of deviations between experimental and empirical mixing lengths: Multi-discharge field tests in an arid river system. *Water Res* 256. <https://doi.org/10.1016/j.watres.2024.121629>
- Lin X, Xu J, Keller AA et al (2020) Occurrence and risk assessment of emerging contaminants in a water reclamation and ecological reuse project. *Sci Total Environ* 744. <https://doi.org/10.1016/j.scitotenv.2020.140977>
- Lothrop N, Bright KR, Sexton J et al (2018) Optimal strategies for monitoring irrigation water quality. *Agric Water Manag* 199:86–92. <https://doi.org/10.1016/J.AGWAT.2017.12.018>
- Lu Q, Mao J, Xia H et al (2022) Effect of wastewater treatment plant discharge on the bacterial community in a receiving river. *Ecotoxicol Environ Saf* 239. <https://doi.org/10.1016/j.ecoenv.2022.113641>
- Maffettone R, Manoli K, Drei P et al (2024) Water Reuse in the European Union: Risk Management Approach According to the Regulation (EU) 2020/741. In: Minella M, Bianco Prevot A, Maurino V (eds) *Water Reuse and Unconventional Water Resources: A Multidisciplinary Perspective*. Springer Nature Switzerland, Cham, pp 413–442
- Mainardis M, Ceconet D, Moretti A et al (2022) Wastewater fertigation in agriculture: Issues and opportunities for improved water management and circular economy. *Environ Pollut* 296:118755. <https://doi.org/10.1016/j.envpol.2021.118755>
- Mancuso G, Parlato MCM, Lavrić S et al (2022) GIS-Based Assessment of the Potential for Treated Wastewater Reuse in Agricultural Irrigation: A Case Study in Northern Italy. *Sustain (Switzerland)* 14. <https://doi.org/10.3390/su14159364>
- Mancuso G, Foglia A, Chioggia F et al (2024) Demo-scale up-flow anaerobic sludge blanket reactor coupled with hybrid constructed wetlands for energy-carbon efficient agricultural wastewater reuse in decentralized scenarios. *J Environ Manage* 359. <https://doi.org/10.1016/j.jenvman.2024.121109>
- Mandarić L, Kalogianni E, Skoulikidis N et al (2019) Contamination patterns and attenuation of pharmaceuticals in a temporary Mediterranean river. *Sci Total Environ* 647:561–569. <https://doi.org/10.1016/j.scitotenv.2018.07.308>
- Mateo-Sagasta J, Marjani Zadeh S, Turrall H (2018) More people, more food, worse water? A global review of water pollution from agriculture. FAO, Rome, and International Water Management Institute (IWMI)
- Ngwira L, Lakudzala D (2018) Assessment of the quality of SOBO industrial wastewater and its impact on water quality in Nankhaka River. *Phys Chem Earth* 108:9–12. <https://doi.org/10.1016/j.pce.2018.04.002>
- Nikolaïdis NP, Phillips G, Poikane S et al (2022) River and lake nutrient targets that support ecological status: European scale gap analysis and strategies for the implementation of the Water Framework Directive. *Sci Total Environ* 813. <https://doi.org/10.1016/j.scitotenv.2021.151898>
- Odone G, Perulli GD, Mancuso G et al (2024) A novel smart fertigation system for irrigation with treated wastewater: Effects on nutrient recovery, crop and soil. *Agric Water Manag* 297. <https://doi.org/10.1016/j.agwat.2024.108832>
- Ofori S, Puškáčová A, Růžicková I, Wanner J (2021) Treated wastewater reuse for irrigation: Pros and cons. *Sci Total Environ* 760. <https://doi.org/10.1016/j.scitotenv.2020.144026>
- Pascual-Benito M, Ballesté E, Monleón-Getino T et al (2020) Impact of treated sewage effluent on the bacterial community composition in an intermittent mediterranean stream. *Environ Pollut* 266. <https://doi.org/10.1016/j.envpol.2020.115254>
- Penseri L, Cantoni B, Antonelli M (2024) Modelling the impacts generated by reclaimed wastewater reuse in agriculture: From literature gaps to an integrated risk assessment in a One Health perspective. *J Environ Manage* 371. <https://doi.org/10.1016/j.jenvman.2024.122715>

- Perulli GD, Odone G, Mancuso G et al (2026) Impact of treated wastewater agricultural reuse on orchards and irrigation systems. *Irrig Sci* 44:11. <https://doi.org/10.1007/s00271-025-01063-4>
- Regulation EU (2020) /741 (2020) Regulation of the European Parliament and of the Council of 25 May 2020, on minimum requirements for water reuse
- Rice J, Westerhoff P (2015) Spatial and temporal variation in de facto wastewater reuse in drinking water systems across the U.S.A. *Environ Sci Technol* 49:982–989. <https://doi.org/10.1021/es5048057>
- Russo N, Pino A, Toscano A et al (2019) Occurrence, diversity, and persistence of antibiotic resistant enterococci in full-scale constructed wetlands treating urban wastewater in Sicily. *Bioresour Technol* 274:468–478. <https://doi.org/10.1016/j.biortech.2018.12.017>
- Schreiber SG, Schreiber S, Tanna RN et al (2022) Statistical tools for water quality assessment and monitoring in river ecosystems – a scoping review and recommendations for data analysis. *Water Qual Res J* 57:40–57. <https://doi.org/10.2166/wqrj.2022.028>
- Suman AB, Toscano A (2021) Public acceptance of water reuse for agriculture in the wake of the new EU regulation: Early reflections. *J Eur Environ Plann Law* 18:225–255. <https://doi.org/10.1163/18760104-18030001>
- Thebo AL, Drechsel P, Lambin EF, Nelson KL (2017) A global, spatially-explicit assessment of irrigated croplands influenced by urban wastewater flows. *Environ Res Lett* 12. <https://doi.org/10.1088/1748-9326/aa75d1>
- UN-Water (2017) The United Nations World Water Development Report 2017. Wastewater: The untapped resource. UNESCO
- Valenti F, Bustamante M, Mancuso G et al (2026) Exploring a food-energy-water nexus solution towards a sustainable and resilient Europe. *Resour Conserv Recycl* 225. <https://doi.org/10.1016/j.resconrec.2025.108618>
- WHO (2006) Guidelines for the safe use of wastewater, excreta and greywater: Volume 2 Wastewater use in agriculture/. World Health Organization. WHO
- Wolfand JM, Taniguchi-Quan KT, Abdi R et al (2022) Balancing water reuse and ecological support goals in an effluent dominated river. *J Hydrol X* 15. <https://doi.org/10.1016/j.hydroa.2022.100124>
- Wolfand JM, Sytsma A, Taniguchi-Quan KT et al (2023) Impact of wastewater reuse on contaminants of emerging concern in an effluent-dominated river. *Front Environ Sci* 11. <https://doi.org/10.3389/fenvs.2023.1091229>
- Yalin D, Craddock HA, Assouline S et al (2023) Mitigating risks and maximizing sustainability of treated wastewater reuse for irrigation. *Water Res X* 21. <https://doi.org/10.1016/j.wroa.2023.100203>
- Zuur A, Ieno E, Smith G (2007) Analysing Ecological Data. Springer

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Pietro Drei¹ · Giuseppe Maistrello¹ · Stevo Lavrnić¹ · Alberto Bavieri² · Michele Solmi² · Attilio Toscano¹

✉ Stevo Lavrnić
stevo.lavrnica@unibo.it

¹ Department of Agricultural and Food Sciences, Alma Mater Studiorum - University of Bologna, viale Giuseppe Fanin 50, Bologna 40127, Italy

² Consorzio della Bonifica Renana, Via Santo Stefano 56, Bologna 40125, Italy