



## Research article

# Impact of wastewater treatment and drought in an Alpine region: a multidisciplinary case study

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## ABSTRACT

In the context of global climate change, drought occurrence in streams of alpine origin is a recent phenomenon, whose impact is still poorly investigated. In this study, we adopted a three-disciplinary approach to investigate the response of an Alpine river (NW Italy) to severe drought conditions occurred in the year 2022. We hypothesised that the considerable loss in the water flow could exacerbate wastewater treatment plant (WWTP) discharge effects, lowering dilution capacity of the stream system and then increasing chemical/microbial pollution and altering benthic community characteristics. To assess river response to drought conditions of the considered year, the concentration of the main chemical variables, faecal indicator bacteria, pathogen presence and structural/functional organisation of benthic macroinvertebrates and diatom communities were measured monthly in the reaches located upstream and downstream of a WWTP (January–December 2022). Main environmental variables, such as flow velocity, water depth, and flow regime, were also considered. A multivariate analysis approach was then applied to emphasise correlations between selected variables and flow regime.

Comparing upstream and downstream sections over the considered year, a common behaviour of chemical/microbiological parameters was observed, with generally higher concentrations of nutrients and bacterial indicators downstream of the local WWTP. Moreover, a positive correlation between water scarcity and nutrients/bacterial concentrations was revealed. The macroinvertebrate communities responded accordingly, both in terms of density and biological metric shift.

Interestingly, differences between the two sections were strictly associated with hydrological conditions, with higher dissimilarities found in low-flow conditions. As the magnitude and duration of drought events are projected to increase in the years to come in different parts of Europe, this work can serve as a first building block and as a hint for future studies aimed at improving our knowledge about the consequences of these events that is pivotal for planning effective management strategies.

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## 1. Introduction

Climate change is causing significant alterations in natural ecosystems due to global rising temperatures and hydrological regime disruption. These have a profound impact on water resources, affecting both their quantity and quality. The IPCC's Climate Change 2022 Synthesis Report highlights the moderate level of confidence in the observed changes in precipitation patterns and glacier melting, which in turn influence water runoff and hydrological regimes [1].

Rivers represent the most dynamic, pivotal and biodiverse freshwater aquatic environments, but unfortunately, they are also among the most modified and threatened ecosystems in the world [2].

Previous studies highlighted the impact of climate change on river regimes, underlining how alterations in stream flow induced by climate change might vary regionally [3].

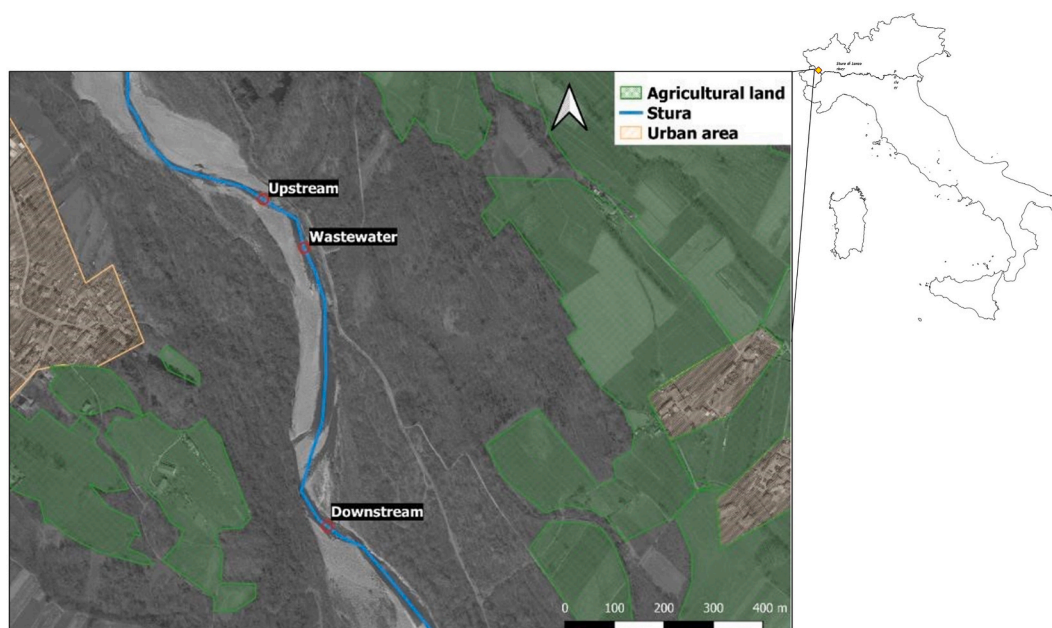
In particular, the biodiversity and integrity of riverine ecosystems depend on natural flow regimes [4]. Changes in flow regime can lead to dramatic consequences for stream ecosystems, such as biomass and biodiversity collapse, local extinction, and the invasion of exotic species, particularly for alpine aquatic environments [5]. Indeed, alpine aquatic environments are extremely fragile systems to multiple stressors [6]. Rivers of alpine origin are characterised by a typical hydrological regime in which the flow rate varies significantly throughout the year. During the warmer months, melting snow and ice contribute to increased flow, while in the winter months, the flow may decrease due to reduced melting and possible freezing. This natural regime could be altered by diminution of snowfalls or alteration of snowmelt, that are direct consequences of climate change.

In the context of this rapid climate change, point sources of contaminants can play an important role. In particular, the impact of wastewater treatment plants (WWTPs) may be exacerbated by climate change as a consequence of the lower water flow, resulting in a loss of dilution and self-purification capacity of the river.

As an example, in 2022 different European countries experienced a series of severe droughts, which were particularly evident in the Po basin, northern Italy [7]. The drought was exacerbated by a persistent sea level pressure anomaly dipole over north-western and south-eastern Europe from November 2021 to March 2022 which blocked the transit of Atlantic perturbations in the Mediterranean area and resulted in a negative rainfall anomaly in central and northern Italy.

In general, in literature it is known that there is a relation between water quality in river segments and its dilution capacity especially in Mediterranean rivers [8–10], but the Alpine region was poorly investigated. Flow reduction essentially means a collapse or loss of wet surface and fast-flowing water environments, with the consequent depletion or disappearance of many habitats and the species that populate them [11]. Much more neglected is the aspect linked to the impact of water shortages on water quality.

In this work, which is to be intended as a preliminary study, we hypothesised that water shortages may worsen the chemical and microbiological quality of rivers, leading to significant biodiversity loss. To test this hypothesis, we examined a river stretch affected by an anthropic discharge for one year, analysing changes in water quality and aquatic benthic communities both upstream and downstream of a WWTP. Our ultimate aim was to investigate, with an interdisciplinary approach, the modifications occurring in an alpine river during an important period of drought giving insights into the relationship between the chemical, microbiological, and ecological parameters and the flow conditions. To our knowledge, this is the first multidisciplinary study in which the impact of WWTP



**Fig. 1.** Location of the Stura River (NW Italy) and position of the three sampling sites. Upstream (U - coordinates 45°13'23.0" N, 7°33'42.2" E), WWTP discharge (W - coordinates 45°13'18.6" N, 7°33'43.7" E) and downstream (D - coordinates 45°13'01.0" N, 7°33'48.2" E).

effluent discharge is evaluated in relation to water shortage induced by climate change in an alpine river. It must be underlined that, being carried out on a single river and in the time span of the year 2022 only, this study stands out as a circumscribed description of the response of a stream subjected to water stress conditions, and is meant to be a first step in the understanding of a broader phenomenon requiring more time and more extensive datasets to be fully assessed.

## 2. Materials and methods

### 2.1. Study area and hydrological description

2022 has been one of the driest and hottest years ever recorded in the western Alpine area, resulting in severe runoff deficit in most of the lotic systems [7].

The study was conducted in the Stura di Lanzo (ST) river, a 3<sup>rd</sup> order lotic system and a tributary of the Po river in Piedmont region, NW Italy. The river has a torrential pluvial-nival hydrological regime and, according to the Regional Agency for the Environment Protection (ARPA) 2021 data, its average annual flow rate at the mouth is 23.36 m<sup>3</sup>/s. Like in many other Alpine basins, in the last years this stream experienced long periods of reduced flows alternating with short but often intense floods [12]. According to the data collected directly from ARPA Piemonte, in January the Stura di Lanzo showed a reduced runoff (6.17 m<sup>3</sup>/s), and the situation worsened in February and March where flow rates resulted markedly below the average, reaching a deficit of -60 % (4.88 m<sup>3</sup>/s). This trend continued over the year and characterised not only this lotic system, but the entire hydrographic network. In December the average inflow (5.53 m<sup>3</sup>/s) was far above the reference average conditions and the overall deficit of the largest river in this area, the Po, was 67 % lower than the historical average value.

We focused on a ST river reach characterised by the presence of 12,000 p.e. (population equivalents) WWTP which employs biological processes and secondary sedimentation and treats mainly urban wastewater.

### 2.2. Sampling

From January to December 2022, twelve sampling campaigns were carried out monthly.

On the studied section of the river (850 m long), three sites of sampling were defined (Fig. 1). The first one located 200 m upstream of the discharge (U), the second one located directly on the WWTP discharge (W) and the last one located 500 m downstream of the discharge (D).

### 2.3. Determination of chemical parameters

The details of the reagents used for the analytical determination, as well as those regarding the treatment of the samples, are fully reported in paragraph S1 of the Supplementary Material (hereafter SM).

Temperature (T, °C), electrical conductivity (EC,  $\mu\text{S cm}^{-1}$ ), dissolved oxygen concentration (DO, mg L<sup>-1</sup>), oxygen saturation (DO%) and pH were measured at each sampling point with a multiparametric probe (Scubla WTW Multi 3430). The concentrations of anions (Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>) and cations (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>) were determined by ion chromatography, while Total Carbon (TC), Inorganic Carbon (IC) and Total Nitrogen (TN) were determined using a TOC analyser. The instruments and the working conditions are listed in paragraph S2. The soluble reactive phosphorus (SRP) was measured spectrophotometrically, using the method described in a previous study [13]. Nitrites, ammonia, total phosphorus, silica and anionic surfactants were determined spectrophotometrically with a Cary 100 Scan spectrophotometer (quartz cells, pathlength 1 cm) according to the methods described in paragraph S3, and previously reported [14].

The volumetric contribution of the WWTP effluent to the stream flow (Y, in %) could not be directly measured, but it was estimated according to the calculations reported in paragraph S4.

### 2.4. Determination of microbiological parameters

For the microbiological analysis, 2 L from each sampling point were collected, transported refrigerated and processed within 24 h of sampling. All samples were evaluated for faecal indicator parameters (total coliform, enterococci, *Escherichia coli* and *Clostridium perfringens* spore counts) and pathogens (*Salmonella* spp. and verocytotoxin-producing *E. coli* - VTEC). Quanti-Tray<sup>TM</sup> 2000 (IDEXX Laboratories, Milan, Italy) was used for the quantification of coliforms, enterococci and *E. coli*, and the results were expressed as Log Most Probable Number (MPN)/100 mL. The enumeration of *C. perfringens* spores was performed using a membrane filtration method according to ISO 14189:2013 (International Standards Organisation, 2013) [15], and the results were reported as Log Colony-Forming Unit (CFU)/100 mL. The presence/absence of *Salmonella* spp. and VTEC DNA in water samples was evaluated using a PCR method with a previously reported protocol [16,17].

### 2.5. Determination of benthic macroinvertebrates and diatoms

To assess biological diversity and richness we focused on two benthic communities, i.e. macroinvertebrates and diatoms, because of their importance in ecological functional processes and diffuse use in biomonitoring methods. Regarding macroinvertebrates, five samples were collected monthly with a Surber net (600  $\mu\text{m}$  mesh size; 0.05 m<sup>2</sup> area) randomly in riffle (i.e. erosive) habitats far away

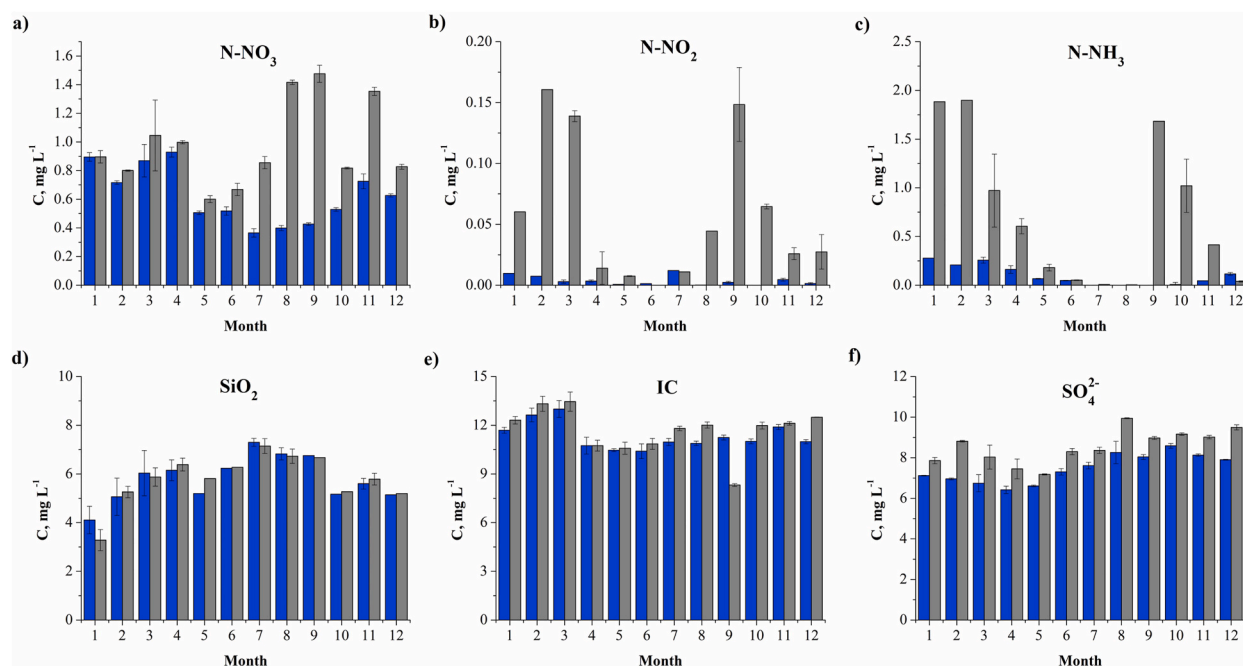
from each other approximately 5 m in both the U and D stretches, then preserved into plastic jars with 75 % ethanol until identification (see paragraph S5). For phytobenthos, samplings were carried out following the macrobenthic sampling schedule. Briefly, diatom samples were collected, treated and analysed following the standard procedures [18,19]. For each sample we calculated the Inter-calibration Common Metric index (ICMi) [20], the diatom quality index adopted at national scale in the framework of the WFD application, by using the OMNIDIA 6.1.5 software. Ecological guilds (i.e. low profile, high profile, motile and planktonic) were assigned to each taxon basing on the classification proposed in a previous study [21]. Details concerning sampling, treatment and analyses of diatom samples are reported in the Supplementary Material (see paragraph S5).

## 2.6. Statistical analysis

For chemical parameters, linear and non-linear fits were carried out with the Origin(Pro) 8.5 software package (OriginLab Corporation, Northampton, MA, USA). The dataset was centred, autoscaled and then subjected to PCA to get insights into possible correlations among the measured chemical parameters in the U and in the D sites, the chemical-physical parameters (pH, T, EC, %DO, ppm DO) and the daily flow regimes (Qday). The Principal Components analysis (PCA) was carried out using the software Chemometric Agile Tool - CAT [22] and the significance of the correlations was assessed using the Pearson's test.

For microbiological parameters, the presence/absence of pathogens and concentration of bacterial indicators (log conversion) were statistically analysed with IBM SPSS Statistics version 28.0 for Windows. The data distribution was evaluated using the Shapiro-Wilk's test. According to non-gaussian distribution, to analyse the differences in microbial indicators between U, W and D samples, a Kruskal Wallis' test was applied. The correlation between microbial indicators and river flow was analysed with Spearman's correlation. The relationship between presence/absence of pathogens and river flow was evaluated with binary logistic regression. Significance was evaluated with 95 % confidence intervals ( $p < 0.05$ ).

Regarding macroinvertebrates and diatoms, analyses of abundance data were conducted in the R Environment (R Development Core Team, 2020) [23]. Concerning benthic macroinvertebrates, each Surber sample was considered as a replicate, and prior to performing regression models, outliers were removed using the method for data exploration reported in a previous study [24]. Differences in the community composition between sites (U, D) were investigated by using non-metric multidimensional scaling (NMDS), multivariate analysis of variance (PERMANOVA) and PCoA (see details in the supplementary material). Statistical differences in community composition associated with variation of flow in different months (Q), site (U, D and also W for diatoms) and their interaction were tested via permutational multivariate analysis of variance (PERMANOVA), with Bray-Curtis distance, using the 'ADONIS' function in the vegan package [25]. Analysis on specific taxa importance was quantified using the similarity percentage procedure (SIMPER) [26]. Moreover, a generalised additive model (GAMs) was used to assess the influence of flow variations on selected biological parameters. Further information on the statistics applied is reported in paragraph S5.



**Fig. 2.** Concentrations (C) of some of the chemical parameters measured in the Stura river through the year 2022. Upstream values are reported in blue, downstream values in grey. The waste values have been omitted to appreciate the marked or less marked differences between the U and D concentrations. IC = inorganic carbon. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 3. Results and discussion

#### 3.1. Chemical parameters

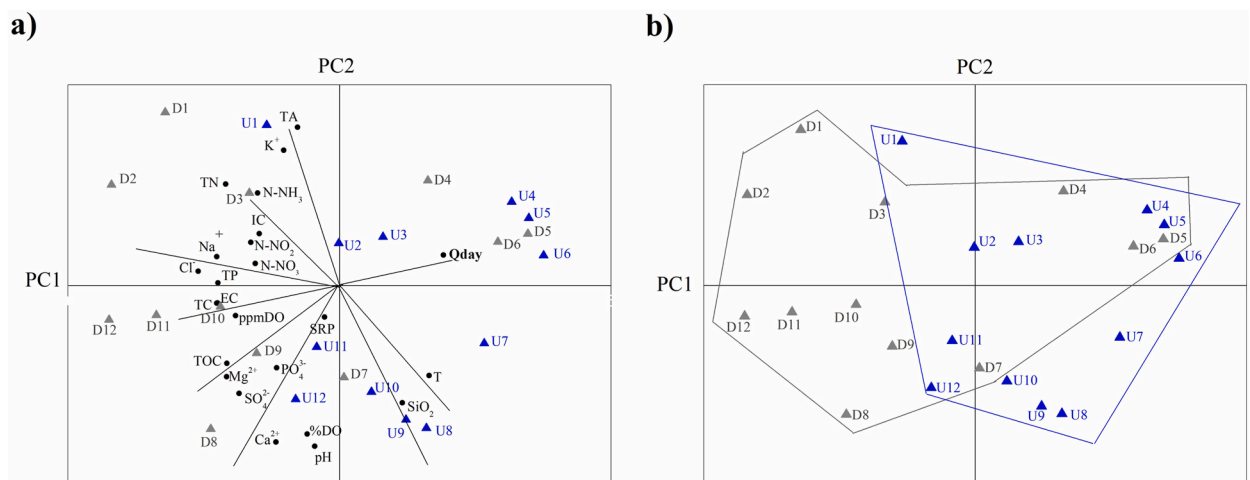
The water temperatures ranged from 4–5 °C in winter to 21–26 °C in summer (see SM, Fig. S1). Stream flow (Q) varied with seasons: in spring months, the registered values were up to three times higher than in winter and two times higher than in summer. EC followed the inverse pattern of Q, falling to lower values in spring and early summer.

As shown in Fig. S2, the concentrations of all the measured parameters in the downstream samples were generally higher than the concentrations in the upstream ones, pointing out the role of the inputs from the WWTP in delivering extra-nutrient loads in the stream. Concentrations in the effluents were remarkably higher for almost all the measured parameters, with the few exceptions of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{SiO}_2$  and for  $\text{N-NO}_3$  in the period from January to June. In some cases, the differences in the nutrient loads U vs D were consistent (e.g.  $\text{N-NO}_2$ ,  $\text{N-NO}_3$ ,  $\text{N-NH}_3$ , Fig. 2a-b-c). Though seasonal trends could not be observed, the differences between U and D tended to become more pronounced in the months associated with lower flow regimes (January–February and July–September), as a consequence of the less dilution power of the water body. Exceptions to this were represented by  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{SiO}_2$  and IC (Fig. 2d–e), whose difference between U and D ( $\Delta\text{UD}$ ) remained negligible through the year, and by species as  $\text{SO}_4^{2-}$  (Fig. 2f),  $\text{K}^+$  and  $\text{Cl}^-$ , whose  $\Delta\text{UD}$  kept constant month by month. Stream and effluent may differ in nutrient composition, for example in the case of nitrogen: the nitrogen content was mainly represented by  $\text{N-NO}_3$  in the stream and by  $\text{N-NH}_3$  in the effluent. This data is coherent with a previous study on a pool of streams in the region of Catalonia, Spain [27], which, being dominated by Mediterranean climate, are typically subjected to summer and winter low flows and even flow intermittency. Furthermore, it is worth noting that  $\text{N-NH}_3$  concentration in the stream tends to fall in the spring months (April–June) until reaching 0 in summer (July–August); meanwhile,  $\text{N-NO}_3$  remains well present in the waters.

The contribution of the WWTP effluent to the flow of the receiving stream was variable, from <6 % to >35 % (Fig. S3), with no strict correlation to the period of the year, contrarily to what was previously observed in other studies [28,29]. This may be due to the fact that this stream, regardless of the severe drought conditions of 2022 and though displaying a highly variable hydrologic regime, is not an intermittent water course. Yet, it is true that the highest % values are shown in the driest months, with an average of 25.3 %: this is coherent with the increased nutrient concentration observed in the same period at U and D. The 79 % of June is likely to be biased by an extra addition of water in the WWTP effluent from a side canal, observed during this sampling and not present in the previous and following ones. For this reason, the estimated value for that month has been excluded from Fig. S3. Thus, the WWTP inputs become more impactful during low flows as the dilution capacity of the stream decreases, while during higher flows a major role in shaping the final chemistry of the receiving stream may be played by the upstream conditions.

In order to investigate possible correlations between the measured concentration data and  $Q_{\text{day}}$ , we performed a PCA on the U and D data. The W samples were excluded from the analysis, since the highest concentration values flattened the informativity (i.e., the relative differences) of the upstream and downstream.

Fig. 3a shows the scores and the loading plot for PC1 vs. PC2, which explain the 39.07 % and the 19.6 % of the total variance respectively. PC1 displays positive loadings for the daily flow and negative loadings for all the nutrients, except for  $\text{SiO}_2$ : this suggests a negative correlation between the hydrological and the chemical variables for the stream, implying that lower values of  $Q_{\text{day}}$  positively affect the concentration of the nutrients in water at the downstream. This is particularly evident in those months associated with lower  $Q_{\text{day}}$  values, January–March and November–December. On the other hand, the grouping of spring months in the same position as the



**Fig. 3.** Principal Component Analysis (PC1 vs. PC2) of the data. a. Biplot with the scores of the samples U (in blue) and D (in grey) and loadings of the measured parameters. b. Scores plot of U samples and D samples with highlights on the two groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

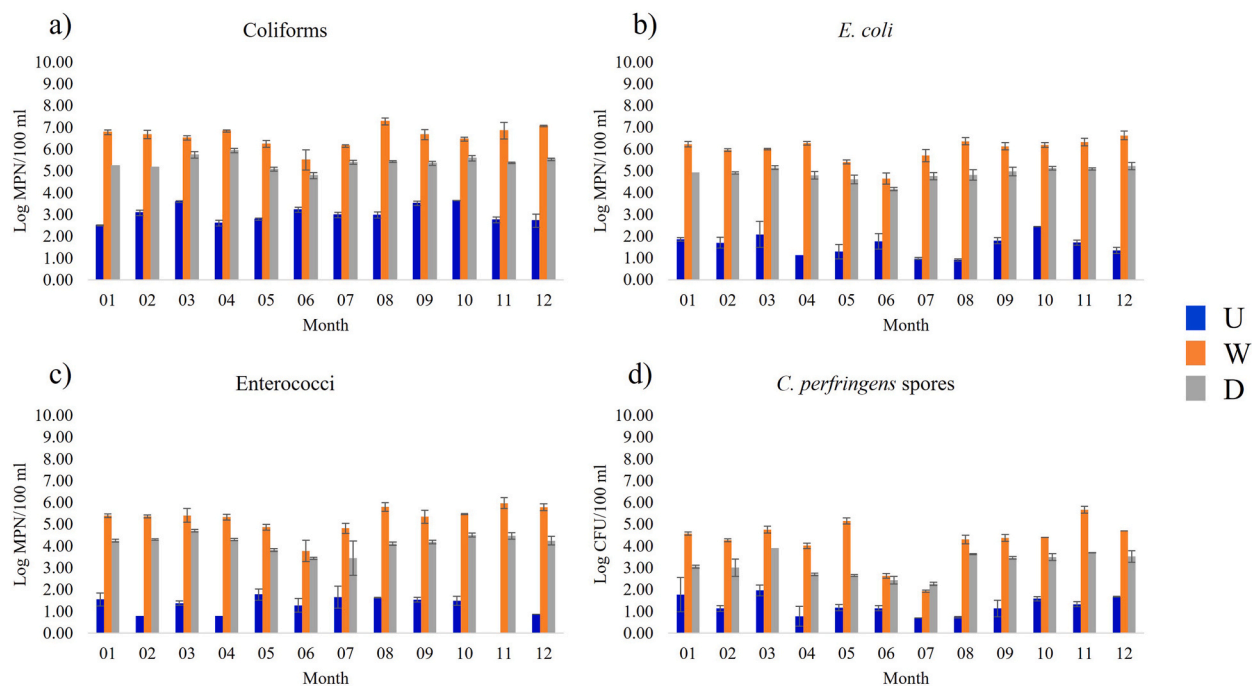
Qday loading is evident. Thus, the PC1 axis emphasises the role of the flow regimes (and, as a consequence, of the dilution capacity of the stream) in contrasting the effect of WWTP effluents as point sources of pollution and chemical discontinuity. As further evidence, most of the measured parameters appear to be negatively correlated with Qday in the loading plot. In the case of TP, TC, TOC, IC,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  the linear negative correlations are statistically significant (Pearson's test at 95 % confidence level, see Fig. S4). This result is consistent with a previous study [29] assessing the influence of WWTPs over nutrient uptake in intermittent watercourses.

Moreover, as reported in Fig. 3b, the plot of PC1 vs. PC2 showed a clear differentiation between the upstream and the downstream samples, which are distinctly clustered for positive and negative scores of PC1, respectively. The sample scores on PC1 support the hypothesis of the contribution of the WWTP effluents to the water quality downstream, which display higher nutrient loads. A positive correlation between Qday and the sampling sites is observed in the high-flow seasons, when the difference between U and D tends to be levelled.

### 3.2. Microbiological parameters

The results of faecal indicator bacteria monitored in the different samplings are reported in Fig. 4. The concentration of coliforms (Fig. 4a), *E. coli* (Fig. 4b), enterococci (Fig. 4c) and *C. perfringens* spores (Fig. 4d) is strongly influenced by the discharge of the WWTP (W) since the concentration of all the microbial indicators increases significantly between the U and D sample (SM, Table S1). Moreover, there is also a statistically significant difference between U and W samples for all the microbial indicators analysed, indicating the ability of the WWTP to induce a detectable faecal contamination with the discharge of its sewage (SM, Table S1). Moreover, the concentration of indicators in the W sample was higher with respect to other studies [17,30,31] that analysed wastewater effluent. Furthermore, a statistically significant difference was observed for coliforms, *E. coli* and enterococci between W and D samples (SM, Table S1) even if with lower intensity with respect to the difference between U and D. These results highlight the possibility that the river is not able to restore completely the microbial indicator concentrations present before the discharge of the sewage. The same trend was observed in other studies [32,33], in which they observed an increase of coliforms, *E. coli* and enterococci counts just downstream of the WWTP discharge. The presence of faecal indicator bacteria in rivers is of great interest to public health authorities since their presence is associated with the presence of human pathogens [34] implicated in waterborne diseases, such as *Salmonella* spp., pathogenic *E. coli* strains and *Shigella* spp. [35]. The presence of faecal indicator bacteria is an indication of the status of the receiving water body since their presence in high quantities could indicate an alteration in the WWTP operation and/or a decrease of water flow in the receiving river.

The results of the correlation analysis between the faecal indicator bacteria concentration and the stream flow data (used as an indicator of water shortage) are reported in Table 1 (graphical representation reported in Fig. S5). The upstream and downstream sample points were analysed separately, to determine the impact of WWTP in association with the water quantity variation. In



**Fig. 4.** Mean concentration ( $\pm$  standard deviation) (log CFU/100 mL or log MPN/100 mL) of microbial indicators in water samples of 12 samplings. U: upstream of WWTP discharge; W: WWTP discharge; D: downstream of WWTP discharge.

**Table 1**

Spearman's correlation between faecal indicator bacteria from upstream (U) and downstream (D) sampling points and stream flow. Bold indicates statistically significant results.

	Stream flow (m <sup>3</sup> /s)
Log MPN Coliforms (U)	-0.291 p = 0.385
Log MPN <i>E. coli</i> (U)	-0.455 p = 0.160
Log MPN Enterococci (U)	0.456 p = 0.159
Log CFU <i>C. perfringens</i> spores (U)	-0.433 p = 0.184
Log MPN Coliforms (D)	-0.400 p = 0.223
Log MPN <i>E. coli</i> (D)	<b>-0.800 p = 0.003</b>
Log MPN Enterococci (D)	<b>-0.700 p = 0.016</b>
Log CFU <i>C. perfringens</i> spores (D)	<b>-0.664 p = 0.026</b>

particular, in the U sample no statistically significant correlation was observed but, interestingly, in the D sample the counts of faecal indicator bacteria (i.e., *E. coli*, enterococci and *C. perfringens*) were inversely correlated with stream flow variation, emphasising the negative impact of the WWTP as the flow rate decreases.

In literature, it is well reported the assumption that the faecal indicator bacteria quantity in surface waters can be influenced by floods and drought conditions in relation to climate change [36,37]. As aforementioned, although different studies that considered rivers of different origin are published, no data on faecal contamination in alpine rivers were reported. Conversely to our findings, in other studies [38,39], MPN of *E. coli* in river (montane areas of South-East Asia and Brazil, respectively) was found higher during the wet season with respect to dry season. This could be explained not only by the different sources of contamination, e.g. animal and human dejections at the soil surface and the overflowing of latrines, but also - and mainly - by the different location and typology of the river. Moreover, in a river situated in Spain (a Mediterranean stream) [40], the authors found the highest stream recovery capacity during the dry season (the period with the highest temperature).

In alpine rivers, it is possible to observe the opposite trend; i.e., the period with the highest stream flow should be the period with increasing temperature, as late spring/early summer, since it is due to the release of water from alpine glaciers and snow melting. To the best of our knowledge, this is the first study in which the impact of a WWTP on an Alpine river has been investigated in relation to climate change and especially during a prolonged severe drought period, so it is not possible to directly compare the results with previous reports. The different results reported here could be explained by the different origin of the river, in which the dilution factor of the stream flow plays a pivotal role in the dispersion of faecal indicator bacteria with respect to other factors, such as the temperature, and highlights the importance to preserve these natural environments from the water shortage, direct consequence of the climate change.

The results of pathogen (i.e., *Salmonella* spp. and VTEC) detection performed with conventional PCR are shown in SM, Table S2. The presence of *Salmonella* spp. was detected in 50 % (6/12) of the U, in 83.3 % (10/12) of the W and in 75 % (9/12) of D, highlighting the impact of WWTP on the river. A similar result was obtained in another study (Morocco) [33] in which the presence of *Salmonella* was detected only in the downstream sample and only in the summer season, due also to the flow reduction that is characteristic of the hottest period. Conversely, the frequency of positive water samples for *Salmonella* reported in another study [16], in different rivers, was lower than the value revealed in the present study.

As reported in Table S2, *E. coli* O157:H7 and Shiga-like toxin (I and II) were never found in the samples analysed. The only genes detected were the one encoding for the flagellar antigen H7 (U: 50 % (6/12); W: 100 % (12/12) and D: 75 % (9/12)) and the one encoding for intimin protein (U: 0 % (0/12); W: 8.3 % (1/12) and D: 8.3 % (1/12)), necessary for the infection since it is responsible for bacterial adhesion to the host intestinal epithelium.

In this regard, it should be emphasised that the flagellar antigen H7 is not directly associated with the presence of pathogenic strains [41] and that its detection in the present study is not correlated with the presence of the pathogenic strain O157:H7, as no positivity for the somatic antigen O157 was ever detected for any of the samples analysed.

The presence of pathogens in rivers poses a direct threat to human health, especially when the water is used for recreational and irrigation purposes. In our study, despite the absence of *E. coli* O157:H7 in all the sampling points, *Salmonella* spp. was found upstream and increased along with the sewage discharge highlighting the need to carefully monitor the WWTP activities and to develop mitigation strategies to reduce the effect of water shortage.

Conversely to indicator bacteria, the pathogen presence, even if increased after the WWTP discharge, is not associated with stream flow variation.

### 3.3. Benthic macroinvertebrates

We collected 120 macroinvertebrate samples (12 months × 5 samples × two sites, U and D). A total of 62.465 individuals were counted and identified, out of which 45.424 upstream and 17.041 downstream. We identified 61 taxa upstream and 57 taxa downstream. The decrease of invertebrate densities in the downstream stretch suggests an evident impact of WWTP discharge, as the morphometry of stream didn't change between U and D and the flow velocity, shear stress, shading are the same on both sample sites. The upstream communities were primarily dominated by Simuliidae (56.58 %) and *Baetis* sp. (20.25 %), while downstream communities were predominantly dominated by Naididae (31.54 %) and *Baetis* sp. (19.71 %). Taxa richness was higher upstream (mean

16.66 ± 3.19 SD) than downstream (mean 12.58 ± 2.84 SD). Ephemeroptera, Plecoptera, and Trichoptera (EPT) together amounted to 26527 individuals, with 18388 specimens collected upstream and 8139 downstream. The average taxonomic richness of EPT (EPT\_S) per surber was higher upstream (10.8 ± 2.12 SD) compared to downstream (6.74 ± 1.16 SD). In the downstream section, a small number of pollutant-resistant groups (e.g., Oligochaeta and Chironomidae) became dominant, while sensitive taxa (represented by EPT) decreased in abundance and richness or disappeared. Regarding the Functional Feeding Groups, upstream samples were predominantly composed of filter feeders (53.15 %), in downstream ones, collectors dominated the communities, with an elevated presence of gatherers (81.99 %). In downstream sites, scrapers and filterers tended to decrease, while collector abundance increased probably because of the increased organic matter loads [42].

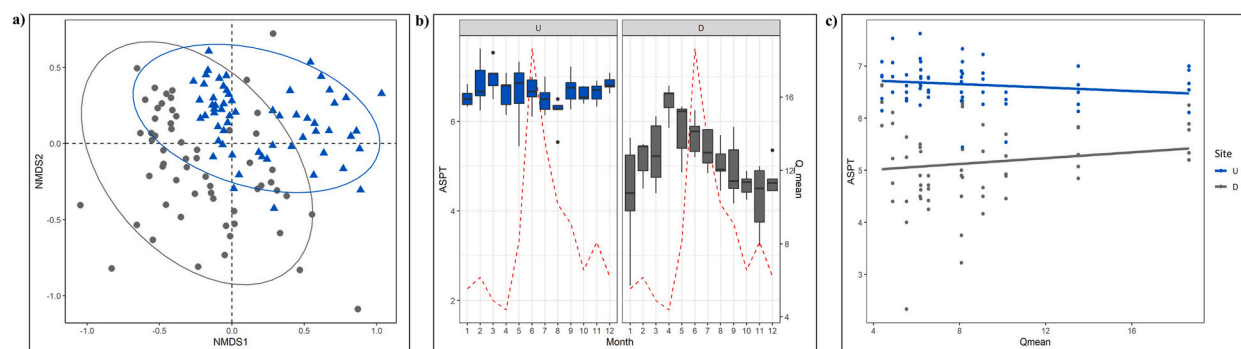
To depict differences in the taxonomic composition of benthic communities between sites, an NMDS ordination and a PERMANOVA (Fig. 5a) were performed, yielding significant differences ( $F = 0.17$ ;  $p = 0.001$ ) and the dispersion with beta dispersion. We observed that the downstream site community was more heterogeneous along the sampling period, compared to the upstream site community, indicative of a response to multiple stressors caused by the wastewater treatment plant and water scarcity. Most likely, during the months when the flow increased, differences between the two communities (U and D) were less evident.

The SIMPER analysis highlighted those families contributing the most to the differences between sampling sites. In particular, D sites were characterised by a higher relative abundance (specimens/surber) of Naididae (D mean = 141 vs. U mean = 7), Lumbriculidae (D mean = 27 vs. U mean = 6) and Hydroptilidae (D mean = 12 vs. U mean = 4) and lower relative abundance of Hydropsychidae (D mean = 45 vs. U mean = 135), *Caenis* sp. (D mean = 31 vs. U mean = 57) and *Habrophlebia* sp. (D mean = 0 vs. U mean = 7).

ADONIS showed a significant difference between sites, with a stress of 22 % ( $df = 1$ ,  $SS = 3,29$ ,  $R^2 = 0,16$ ,  $F = 41,26$ ,  $P < 0.001$ ) and a significant difference compared to flow conditions ( $df = 1$ ,  $SS = 1,023$ ,  $R^2 = 0,051$ ,  $F = 12,83$ ,  $P < 0.001$ ).

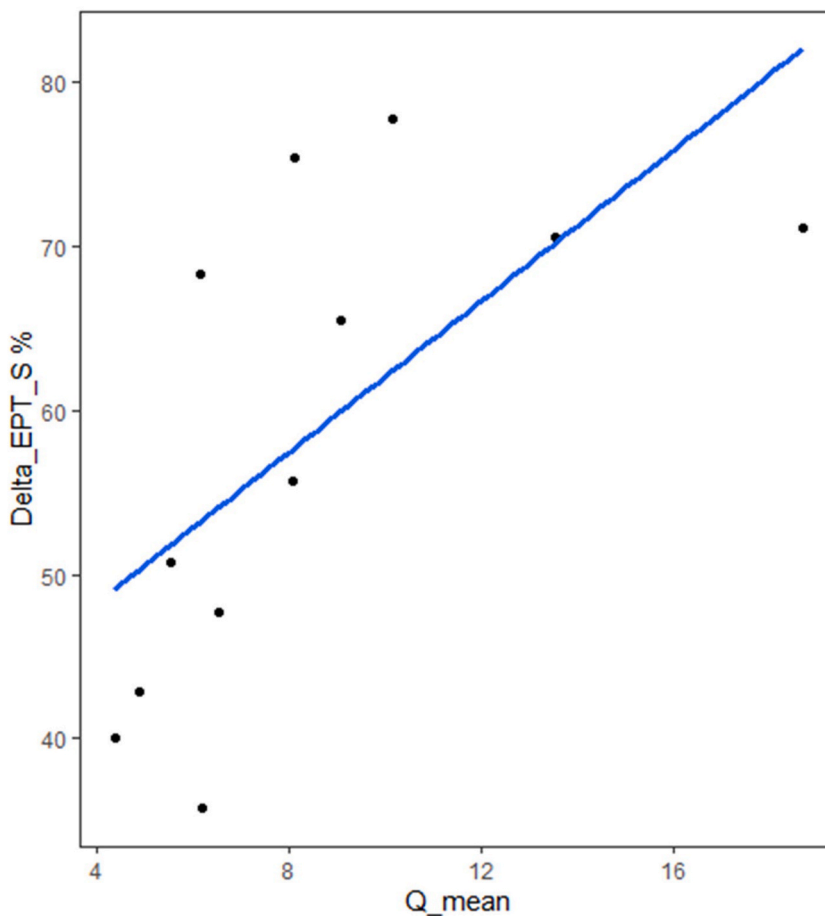
We registered significant nonlinear relationships of the attributes of the benthic macroinvertebrate community in relation to the river flow. In particular, for ASPT (Average Score Per Taxon), the patterns suggested a hump-shaped relationship, with the flow as a good predictor of the community attributes of the macroinvertebrate community, especially in D sampling sites. In general, higher values of ASPT were recorded in the upstream site, which remained quite constant throughout the studied year, (Fig. 5b). On the contrary, in the downstream section we detected an evident variation in ASPT values, with the lowest values registered when the flow achieved the lowest range ( $F = 24.14$ ;  $R^2 = 67\%$ ;  $P < 0.001$ , Fig. 5c) and an increase of ASPT values as the flow rate increases. The analysis of the difference in metrics, such as EPT\_S, between the upstream and downstream sites revealed a significant relation ( $F = 5.14$ ;  $R^2 = 65.5\%$ ;  $P = 0.004$ ) and correlation (Spearman's  $\rho$ -value  $< 0.05$ ) with the decrease in flow. Specifically, it was observed that at higher flow rates, the percentage similarity between EPT\_S in U and D sites was highly evident whereas the opposite occurred at reduced flow rates, where the difference increased significantly (Fig. 6).

With this study it has been possible to find some indicator taxa and community metrics for water quality-quantity assessment. For instance, most species of Chironomidae are very tolerant to water pollution [43]. Moreover, high amounts of Oligochaeta and Gastropoda are often indicators of nutrient pollution [44]. However benthic macroinvertebrates are also susceptible to changes in flow regimes [45]. In particular, invertebrate community composition often changes in response to low or reduced discharge. These changes are probably a result of increased habitat suitability for some species and decreased suitability for others [46]. The responses of certain invertebrate taxa to flow reduction suggest that invertebrates might be useful indicators in assessments of reduced-flow impacts. It should be possible to identify taxa that are sensitive to flow reduction, such as those that drift when flows change, and are affected by sedimentation or have specific velocity requirements. This is underscored by the trend of certain indices, such as ASPT, which exhibited a negative trend, consequently indicating lower ecological quality, determined by the presence of high diversity also in terms of sensitive taxa such as EPT, as river discharge decreases. In the end, it was possible to highlight that the differences between

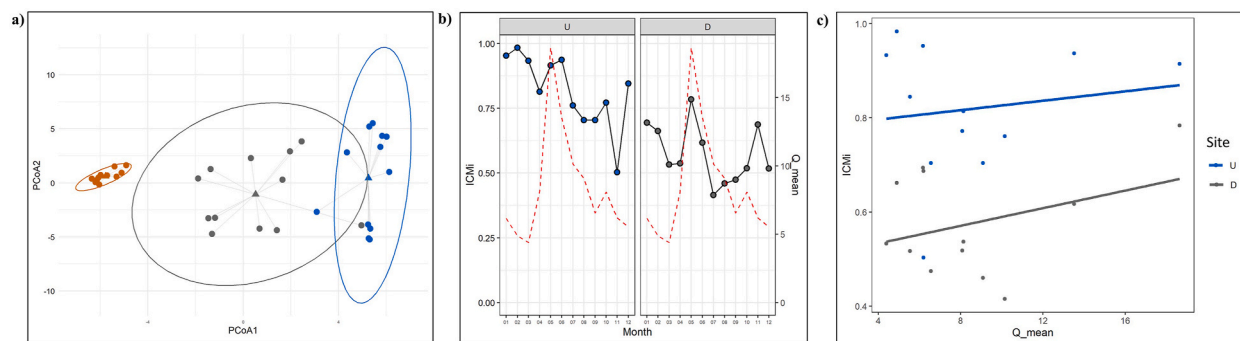


**Fig. 5.** Benthic macroinvertebrates. a) NMDS ordination plot depicting differences in the taxonomic composition of benthic invertebrate communities between U (blue triangles) and D (grey points) sites. Ellipses represent standard deviations around the centroids of the two groups. b) time series (x axis, Month) of flow (Q\_mean) and Average Score Per Taxon (ASPT, y axis) boxplot with box = 25th–75th percentiles; line = median; whiskers = 1.5 IQR (interquartile range), for U and D sites. c) Representation of the GAM smoother for flow (Q\_mean) interacting with ASPT. The lines (blue for U site and grey for D site) represent the smoothed function, while the dots indicate the distribution of all samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)





**Fig. 6.** Variation of EPT\_S upstream and downstream the WWTP discharge in different flow conditions ( $Q_{mean}$ ): Delta means the similarity percentage richness of Ephemeroptera, Plecoptera and Trichoptera in the D site comparing to the U site for each sampling date.



**Fig. 7.** Benthic diatoms. a) PcoA ordination plot based on Bray-Curtis dissimilarity matrices depicting differences in the taxonomic composition of benthic communities between U (blue points), W (orange points) and D (grey points) sites. Ellipses represent standard deviations around the centroids of the two groups. b) time series (x axis, Month) of flow ( $Q_{mean}$ ) and Intercalibration Common Metric Index (ICMi, y axis) points, for U and D sites. c) Representation of the GAM smoother for flow ( $Q_{mean}$ ) interacting with ICMi. The lines (blue for U site and grey for D site) represent the smoothed function, while the dots indicate the distribution of all samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

macrobenthos communities, especially for sensitive taxa such as EPT, increase with low flow.

### 3.4. Diatoms

In total, we collected 36 diatom samples, 12 in the U site, 12 in the D site and 12 in the W site, and we identified 78 species, 49 upstream, 63 downstream and 43 in wastewater. Taxa richness was significantly higher in D compared to U and W ( $22.41 D \pm 3.65$ ,  $17.33 U \pm 4.51$  and  $16.16 W \pm 3.51$ ); the same pattern was observed in terms of Shannon diversity index ( $2.96 D$ ,  $2.07 U$  and  $2.46 W$ ) and Evenness ( $0.66 D$ ,  $0.50 U$  and  $0.61 W$ ). Our results are partially in agreement with those highlighted in another study [47] where authors found higher diatom diversity (Shannon index) in a site located downstream a WWTP in comparison to the upstream section, while lower diatom richness downstream in comparison to the upstream site. Likely, in our study, communities developing in the D section received a contribution of taxa from both U and W sites, which led to the formation of richer and more diversified assemblages.

Concerning ecological guilds, we observed a prevalence of low-profile taxa (72.83 %) in the upstream site, according to the definition of this guild (i.e. resource-stressed but disturbance-free [48]) which is generally associated to nutrient-poor environments [49] and high-flow velocity conditions [21,48]. According to previous studies, downstream and wastewater assemblages were dominated by motile taxa (respectively 61.60 % and 66.10 %), generally considered as successful competitors for nutrients in nutrient-rich environments [48] thanks to their ability of synthesising extracellular enzymes to consume macromolecules [50]. Moreover, motile diatoms have a competitive advantage compared to sessile ones, since they can actively move towards more suitable conditions [51]. In our study, this guild was more abundant during the warmest months; in this context, the warm water temperatures which can be reached downstream of a WWTP could enhance the ability of motile taxa to move, through a decrease of the cytoplasm viscosity in the raphe slit [47,52]. To visually inspect for differences in terms of taxonomical composition among diatom assemblages collected in the three sampling sites, we performed a PCoA. The analysis highlighted a clear difference in terms of diatom species composition between sampling sites; Fig. 7a displays that samples of the U site are mostly placed on the right side of the diagram, while those collected in the W site are mainly distributed on the left side, with a clear separation of the two spider graphs. The communities in D site are positioned halfway between those upstream and those in the wastewater, once more confirming previous results published in literature [47]. Fig. 7a displays an apparent taxonomic homogenization of the diatom communities inhabiting W, whose ellipse appears strongly gathered around the centroid. Communities belonging to the W site appeared, indeed, simpler and less heterogeneous than those characterising U and D sections. This could be explained by the conjoint chemical and hydrological pressures affecting the assemblages, confirming the results of previous studies [53,54]. The SIMPER analysis highlighted the diatom taxa significantly contributing to differences between the site assemblages. In particular, downstream (D) assemblages were mainly characterised by  $\alpha$ -meso polisaprobic taxa; for instance, *Fistulifera saprophila* (D mean = 68 vs. U mean = 0 and W mean = 14), *Nitzschia costei* (D mean = 48 vs. U mean = 0 and W mean = 8) and *Nitzschia fonticola* (D mean = 54 vs. U mean = 28 and W mean = 3) displayed higher abundance means in D compared to U site and W site. On the contrary, a lower relative abundance of  $\beta$ -mesosaprobic taxa, compared to the upstream site, such as *Achnanthyidium delmontii* (D mean = 28 vs. U mean = 110 and W = 49), *Achnanthyidium pyrenaicum* (D mean = 42 vs. U mean = 127), *Achnanthyidium minutissimum* (D mean = 6 vs. U mean = 23) was observed. In the wastewater (W) diatom communities were mainly defined by polysaprobic taxa as *Achnanthyidium saprophilum* (W mean = 119 vs. D mean = 13 and U mean = 0), *Mayamaea permitis* (W mean = 86 vs. D mean = 21 and U mean = 1), *Nitzschia soratensis* (W mean = 73 vs. D mean = 11 and U mean = 1), *Sellaphora nigri* (W mean = 43 vs. D mean = 3 and U mean = 0). The taxonomic and functional changes observed in our communities are consistent with those detected in another study [47], highlighting a shift in diatom assemblages from oligosaprobic/oligotrophic groups belonging to the low- and high-profile guilds upstream of a WWTP, to polysaprobic motile groups in the impacted sites. ADONIS, with a stress of 17 %, furtherly confirmed a significant difference between sites ( $df = 1$ ,  $SS = 0.89$ ,  $R^2 = 0.30$ ,  $F = 15.03$ ,  $P = 0.005$ ) and with changes in river flow ( $df = 1$ ,  $SS = 0.16$ ,  $R^2 = 0.41$ ,  $F = 2.82$ ,  $P = 0.005$ ).

Despite the importance of diatoms as bioindicators in lotic environments and the importance of their inclusion in the river monitoring programs [55], species composition and structural dynamics in WWTPs have rarely been documented [47,56]. In our study, diatom quality index ICMi displayed significantly ( $p < 0.05$ ) higher values in U in comparison to D and W sampling sites (0.81 U, 0.57 D and 0.42 W; i.e., corresponding respectively to “good”, “moderate” and “poor” ecological status), thus responding to this high anthropogenic pressure, as already observed in another study [47] for BDI index.

We registered significant nonlinear influences of the attributes of the benthic diatom community in relation to the river-flow. In particular the flow resulted as a good predictor of the community attributes measured ICMi. In addition, the elevated values of  $R^2$  indicated that the benthic diatom attributes had a good fit with the flow. Higher values of ICMi were recorded in the upstream site, especially in sampling periods where the river flow reached the range  $16 \text{ m}^3/\text{sec} - 18 \text{ m}^3/\text{s}$  (Fig. 7b). The lowest values of ICMi were registered in the downstream site when the flow achieved the less range  $5 \text{ m}^3/\text{s} - 7 \text{ m}^3/\text{s}$  ( $F = 5.96$ ;  $R^2 = 83 \%$ ;  $P < 0.001$ , Fig. 7c). In both U and D sections, the ICMi increased as the flow rate increased (Fig. 7c).

## 4. Conclusions

The study highlights the complex interplay between climate change-induced water shortages, wastewater treatment plant effluents, and their impact on river ecosystems and water quality. The hydrological description revealed the severe runoff deficit in the western Alpine area during the whole 2022, with a significant reduction in flow rates. Normally, when people think about the impact of climate change on rivers, they always consider the ‘quantity’ of water and not its ‘quality’.

This study takes the case of one river to do a first evaluation of the impact of water shortage on water quality, attempting to provide some preliminary suggestions on how reduced flows may exacerbate the influence of anthropogenic discharges. In the case of Stura di

Lanzo, these alterations had cascading effects on various water parameters, including nutrient concentrations and microbiological indicators. The analysis of the chemical parameters showed that, even if WWTP effluents have a low contribution in terms of final in-stream flow, they can heavily influence the final nutrient load in the downstream waters. The correlations found with the Qday values supported the hypothesis that the investigated Alpine river, already naturally subjected to seasonal flow regime shifts, became more vulnerable to WWTP effluents when drought conditions were pressing and its dilution capacity was threatened. To determine whether this is a characteristic pattern of rivers facing drought situations, the repetition of these analyses in the years to come and on comparable basins is essential.

Following the same trend of nutrients, microbiological parameters, including faecal indicator bacteria, revealed a significant impact of the WWTP on downstream water quality. The presence of pathogens, such as *Salmonella*, raised concerns about potential health risks associated with water use during drought conditions.

Macroinvertebrates and diatom communities responded sensitively to altered flow conditions, underscoring the vulnerability of the alpine river in the sampled year. The decrease in invertebrate densities downstream indicated habitat loss and changes in food availability. The shift in diatom community composition, with the increase in motile taxa downstream, reflected the influence of wastewater treatment plant effluents and reduced flow.

The study also explored the potential of using specific taxa and community metrics as indicators of water quality and quantity. For instance, the analysis of macroinvertebrates and diatoms suggested that certain taxa, particularly those associated with sensitive ecological conditions, could serve as indicators of reduced-flow impacts.

Beside climate-related effects, waters are heavily subjected to local human pressure, which affects their features on multiple sides. Manipulation of channels, building or modification of drainage basins etc., alter the natural flows, while pollution and land use may overturn their quality. It is therefore fundamental to consider local factors, such as wastewater treatment plant discharges, in conjunction with climate-induced changes. At the same time, the role of flow regimes in shaping the response of chemical and biological components in the river ecosystems emphasises the need for integrated monitoring and management strategies that account for both climate change and local stressors.

In conclusion, this research provides preliminary insights into the complex interactions between climate change-induced water shortage, local impacts and river ecosystem health in alpine regions. The findings underscore the importance of holistic approaches in water resource management, considering both quantity and quality aspects, especially in the context of changing climate conditions. Studies like the presented one may offer insights for policymakers, environmental managers, and researchers working towards sustainable river ecosystem conservation and management in the face of global environmental challenges. For this reason, the next steps may involve replicating the same experiment on other rivers with wastewater treatment plants of different origins, to assess whether a consistent response pattern of the examined variables can be observed.

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## Data availability statement

Data will be made available on request.

## CRediT authorship contribution statement

**Anna Marino:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Silvia Bertolotti:** Writing – original draft, Visualization, Validation, Investigation, Formal Analysis, Data curation, Conceptualization. **Manuela Macri:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Francesca Bona:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Silvia Bonetta:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Elisa Falasco:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Marco Minella:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Stefano Fenoglio:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

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 A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e35290>.

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