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RESEARCH ARTICLE

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Assessing the effects of hydrological and chemical stressors on macroinvertebrate community in an Alpine river: The Adige River as a case study

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Abstract

In this study, the combined effects of hydrological and chemical stressors on benthic macroinvertebrates were evaluated in order to explore the response of the biological community to multiple stressors. The Adige River, located in the south-eastern Alps, was selected as a case study because representative of the situation of a large river in which the variety of stressors present in the Alpine region act simultaneously. As expected, streamflow showed a seasonal pattern, with high flows in the spring–summer period; however, locally, the natural hydrological regime was altered by the presence of hydropower systems, which chiefly affected low flows. Multivariate analysis showed seasonal and spatial patterns in both chemical and hydrological parameters with a clear gradient in the concentration of nitrate, personal care, and pharmaceutical products moving from headwaters to the main stem of the river. The macroinvertebrate community composition was significantly different in summer and winter and between up and downstream sites. Streamflow alteration chiefly due to water use by hydropower affected community composition but not richness or diversity. *Gammarus* sp., Hirudinea, and *Psychomyia* sp., were positively correlated with flow variability, increasing their densities in the sites with higher streamflow variability because of hydropeaking. The results obtained in this study show that the composition of the macroinvertebrate community responded to seasonality and to changes in the main stressors along the river and highlights the importance of the spatial and temporal variability of stressors in this Alpine river. Taking into account, this variability will help the decision-making process for improving basin management.

KEYWORDS

Adige River, benthic invertebrates, hydropower, multiple stressors, personal care products, pharmaceuticals, streamflow

1 | INTRODUCTION

Agricultural, industrial, and domestic activities exert pressures on freshwater ecosystems, in some cases, impairing their ability to provide essential services (EFSA, 2016). Threats to freshwater biodiversity are grouped under a number of interacting categories such as water overexploitation, water pollution, flow alteration, destruction or degradation of habitat, geomorphological alterations, land use changes, and invasion by exotic species and pathogens (Arthington, Naiman, McClain, & Nilsson, 2010; Dudgeon et al., 2006; Ormerod, Dobson, Hildrew, & Townsend, 2010; Vörösmarty et al., 2010). Diffuse (e.g., agricultural activities and intensive animal farming) and point (e.g., from urban areas due to the increase in the human population density) pollution are the main sources of contaminants entering freshwater ecosystems. In particular, concerns have been raised regarding pesticides (insecticides, herbicides, and fungicides), pharmaceutical products (PhACs), and personal care products (PCPs) (Ippolito, Carolli, Varolo, Villa, & Vighi, 2012).

Alpine rivers are part of the essential freshwater reservoir in Europe (Alpine Convention, 2009), since they provide freshwater for human consumption and for productive activities such as agriculture, livestock, and industry (Viviroli et al., 2011; Viviroli, Weingartner, & Messerli, 2003). In addition, the rough topography of their watersheds creates favourable conditions for hydropower production, which however alters the hydrological regime, thereby impacting the freshwater ecosystem (Liebig, Cereghino, Lim, Belaud, & Lek, 1999; Moog, 1993). Moreover, with the expected reduction of glacial runoff due to the retreat of Alpine glaciers, sediment loads will decrease, thereby driving potentially significant shifts in the biological communities of glacier-fed rivers (Ilg & Castella, 2006).

Studies conducted by Lencioni, Maiolini, Marziali, Lek, and Rossaro (2007); Lencioni, Marziali, and Rossaro (2011) provided basic knowledge on the structure and functional properties of Alpine invertebrate communities. Other studies focused on the effects of specific factors such as hydropeaking (Bruno, Siviglia, Carolli, & Maiolini, 2012; Carolli, Bruno, Maiolini, & Silveri, 2010), glacier retreat (Khamis, Hannah, Brown, Tiberti, & Milner, 2014), stream origin (Lencioni & Spitale, 2015), altitude, and water temperature (Lencioni & Rossaro, 2005). However, to the best of our knowledge, studies on the combined effects of a multiplicity of stressors are still lacking in the Alpine region. In this regard, the application of a comprehensive approach that allows the effects of multiple stressors to be investigated at the catchment level may provide essential information to better understand and assess biological responses to this multiplicity of stress factors.

Given the wide range of activities conducted in its catchment, resulting in a multiplicity of stressors, the Adige River was selected in the EU FP7 project GLOBAQUA (Navarro-Ortega et al., 2015) as a case study representative of the Alpine region. In the present work, specific attention was given to the middle course of the Adige River, in the province of Trento, and to one of its main tributaries, the Noce River. The predominant pressures affecting the Adige River are: (a) streamflow and water temperature alterations caused by hydropower production (Zolezzi, Bellin, Bruno, Maiolini, & Siviglia, 2009; Zolezzi, Siviglia, Toffolon, & Maiolini, 2011); (b) land use (mainly agriculture) and industrial activities (Cassiani et al., 2016), which relevance increases from upstream to downstream; and (c) nutrients and pollutants released by waste water treatment plants (WWTPs); that is, effluents, which are expected to show significant seasonal variations due to tourism (Chiogna et al., 2016). All these pressures may negatively impact the benthic invertebrate communities, which, thanks to their capacity to respond to both chemical and physical alterations, can be used as indicators for bioassessment.

This work aims to identify the relationships between multiple pressures and the response of the invertebrate community at the investigated sites, which are representative of a number of scenarios encountered in Alpine rivers. We hypothesised that (a) seasonal and spatial patterns of hydrological and chemical parameters are observed not only according to the natural seasonal hydrological regime and the different water uses (e.g., hydropeaking), but also according to the activities in the basin (e.g., tourist activities upstream in winter and agriculture downstream in spring-summer) (Hypothesis H1); (b) the richness, diversity, and invertebrate community composition change as a consequence of the temporal and spatial pattern of water pollution and hydrological alterations (Hypothesis H2).

2 | STUDY AREA

The Adige River, with a total length of about 410 km, is the second longest river in Italy after the Po River. It rises near Lake Resia at the elevation of 1,586 m a.s.l. (46.834444, 10.514722), and it then flows through the southern-east Alps, and reaches the Adriatic Sea at Rosolina Mare, south of Venice (45.149722, 12.320278; Autorità di bacino del Fiume Adige, 2008). Glaciers cover a total surface area of 128 km^2, although this extent is reducing at a relentless pace due to the observed trend for increasing temperature (Lutz et al., 2016). The flow regime has a typical Alpine character, with peaks in summer due to snow melting, and in autumn when cyclonic storms hits the catchment from the south. At the gauging station of Ponte San Lorenzo in Trento, the long-term mean annual streamflow is 203 m^3/s, with a contributing surface area of 9,763 km^2.

The majority (68.7%) of the territory of the Trento Province is covered by forest, and the remainder by rocks (11.5%), agriculture (16.5%), urban areas (2.8%), and water (lakes and rivers 0.05%; TERNA, 2011). Land use percentages for the study area are reported in Table S1. The main water use is for hydropower. For this purpose, 28 reservoirs, 15 in the Bolzano and 13 in the Trento provinces, are in operation with a total operational storage of 560.59×10^6 m^3. Another important activity is tourism, which leads to a larger increase of presences in both the winter and summer seasons, with the largest increment in winter.

3 | MATERIALS AND METHODS

3.1 | Sampling

Sampling was performed in two campaigns: The first (referred to as 1) was held in February, and the second (referred to as 2) in July 2015, in order to capture both low and high flow conditions occurring in the winter and summer seasons, respectively. Both winter and summer are tourist seasons, with the highest increase in population in the winter. Seven sites were sampled in each sampling campaign (Figure 1): five along the Noce River (sampling points from 1 to 5 in Figure 1),

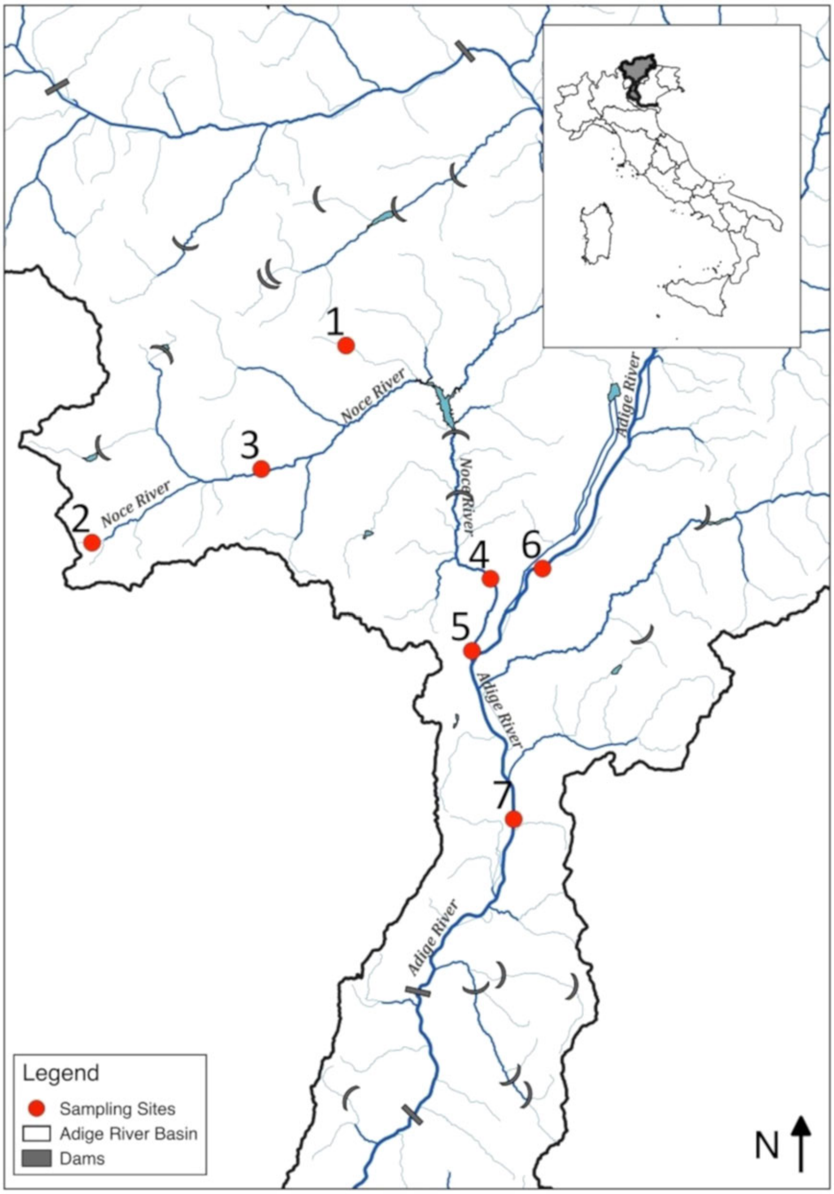

FIGURE 1 Map of the Adige River catchment, indicating the sampling sites [Colour figure can be viewed at wileyonlinelibrary.com]

and the remaining two along the main stem of the Adige River, respectively, upstream of the confluence of the Noce itself and the Avisio (sampling Point 6 in Figure 1), and downstream the city of Trento (sampling Point 7, Figure 1).

Locations were selected according to the objectives of the GLOBAQUA project (Navarro-Ortega et al., 2015), and their main characteristics are described in Table S1. Water temperature, pH, dissolved oxygen (DO), and electrical conductivity were measured using a multiparameter probe (Aquatroll 200), while turbidity was measured using an optical turbidimeter (Ponsel IR). River velocity was measured using a radar gun (Decatur Electronics Europe Inc., Welber et al., 2016), except at Sites 2 and 3 where mean water velocity was determined by tracer tests using bromine (in February 2015) and NaCl (in July 2016).

At each site, water samples were collected at 50 cm depth at three points (left, right, and center of the river section) and mixed immediately after sampling. Water samples for the analysis of PhACs, PCPs, and pesticides were stored in 1 L grey PE bottles and within a few hours were transported to the laboratory in a refrigerated isothermal container and stored at −20°C until extraction and analysis. Water samples for ion analyses were collected in triplicate. The samples were filtered immediately through glass fibre filters (Whatman GF/F) and frozen at −20°C until analysis.

Macroinvertebrate communities were sampled using a pond net (0.32 m width and 500-μm mesh size) along the wadable zone of the river. Six samples were randomly collected at each site after disturbing the streambed 1-m upstream of the net by kicking. More than 90% of the river bed was mainly stones and cobbles in all sites. We used the same number of sampling actions at each site, six times, approximately 0.32 m^2 of surface sampled and a duration of 3 min each action. This procedure provides semiquantitative data; however, as we always used the same procedure, patterns between sites were comparable. Samples were preserved with 4% formaldehyde.

3.2 | Determination of hydrological stressors

The hydrological regime was characterised by means of suitable statistical indicators of water discharge variation: annual mean, standard deviation, and coefficient of variation (FCV), 10th, 25th, 75th, and 90th quantiles

(Q10, Q25, Q75, and Q90, respectively). Streamflow records (both daily and hourly) were obtained from the Ufficio Dighe of the Province of Trento (www.floods.it). As streamflow measurements were not available at Sites 1, 2, and 4, reliable estimates were extracted from the simulations performed by Bellin, Majone, Cainelli, Alberici, and Villa (2016). The natural regime (i.e., in the absence of water use) was reconstructed by excluding all water uses within the catchment (Bellin et al., 2016). Statistics were also computed for the time series of streamflow (Q) increments between two successive time periods, t_{i+1} and t_i, defined as follows:

$$\Delta Q = Q\,(t_{i+1}) - Q\,(t_i). \tag{1}$$

3.3 | Chemical analyses

An offline solid phase extraction (SPE) preceded the determination of PhAC concentrations by ultra-high performance liquid chromatography coupled to triple quadrupole linear ion trap tandem mass spectrometry (UHPLC-QqLIT-MS^2) (Gros, Rodríguez-Mozaz, & Barceló, 2012). For PCPs, the analyses were carried out using a method based on isotope dilution and online solid phase extraction–high performance liquid chromatography–tandem mass spectrometry (on line SPE–HPLC–MS^2) (Gago-Ferrero, Mastroianni, Díaz-Cruz, & Barceló, 2013). Analyses of the target pesticides were performed using a method based on isotope dilution online solid phase extraction–liquid chromatography–tandem mass spectrometry (SPE–LC–MS/MS) as described in Palma et al. (2014). Nitrate, sulfate, chloride, sodium, potassium, and calcium were determined by ion chromatography (761 Compact IC, Metrohm).

3.4 | Macroinvertebrate analysis

In the laboratory, samples were sieved through a 500-μm mesh, and macroinvertebrates were sorted, counted, and identified under a dissecting microscope (Leica Stereomicroscope). Identification was at the genera or species level for nearly all groups of taxa with the exception of the Oligochaeta and Diptera, which were identified at the family level. For each site, taxonomic richness (S), Shannon diversity (H), and percentage of Ephemeroptera, Plecoptera, and Trichoptera (EPT %) were determined. Moreover, in order to assess the biological status, the extended biotic index (IBE; Italian biotic index, Hilsenhoff, 1982) was calculated. The IBE is based on the presence of invertebrates representative of groups of varying sensitivity to pollution and number of taxa (Ghetti, 1997).

3.5 | Statistical analysis

Organic pollutants included in the analysis were grouped into three families, based on their mode of action: pesticides (including herbicides and insecticides), PCPs, and PhACs. If the concentration was below the detection limit (mLOD), a value equal to one-half of the limit was assigned (Clarke, 1998), while the average of mLOD and quantification limit (mLOQ) was assigned when the concentration was in between these two values. Principal component analysis (PCA) was applied to the hydrological and environmental data. To diagnose autocorrelation and colinearity between environmental data, draftsman plots were used. When the determination coefficient was higher than 0.90, one of the variables forming the pair was removed. Variables included in the dataset analysed by PCA were standardised (the variable values were divided by the total for that variable) and inspected for normality, and when necessary log transformed using decimal logarithms. This resulted in the selection of the coefficient of variation of water discharge (FCV), water temperature (temp), nitrate concentration, water conductivity (cond), water turbidity (turb), urban and agricultural land use percentages (% urb, % agr), PCPs, PhACs, and pesticides ("Pest") as variables to be used in the PCA analysis.

With the aim of finding temporal and spatial patterns in the community, composition and density data (individual/m^2) were used. Taxa present at less than 1% of the total density or only present at one site were excluded. Taxa densities were log transformed to reduce the influence of extreme observations on the subsequent ordination procedure (Siddon, Duffy-Anderson, & Mueter, 2011). Species richness (S) and Shannon diversity were calculated for each site and sampling period. These measures were contrasted between samplings and between up and downstream sites using a general linear model (GLM, sampling and site group as fixed factors).

A non-parametric distance-based redundancy analysis (dbRDA) was performed to determine the correlation between taxa composition and the environmental variables. RDA is a direct ordination analysis that selects a set of variables (predictors) that best explains the variability of a biological community (Borcard, 1992). Additionally, a PERMANOVA test was used to analyse differences in the macroinvertebrate community between samplings and site groups. Spearman correlations between some biological parameters and environmental characteristics were also calculated. Analyses were performed using PRIMER 6 (version 6.1.6, Primer-E Ltd, Plymouth U.K.) and SPSS (IBM) for the GLM.

4 | RESULTS AND DISCUSSION

4.1 | Hydrological characteristics

At all sampling locations, water discharge was higher in the summer (July) than in the winter (February) sampling campaign (Figure S1), except at Site 5, where the natural hydrological regime is altered by hydropower, this section being located downstream, and at short distance from the restitution of the Mezzocorona hydropower plant. Based on the analyses of the time series and their statistics, greater variations in discharge between summer and winter seasons were observed for small streamflows (i.e., the 10th and 25th quantiles, Q10 and Q25) compared with high streamflows (Table 1). This was due to the alterations caused by hydropower, which are particularly evident at low flow (seee.g., Zolezzi et al., 2009).

Figure 2 shows the streamflow (first row) and the duration curves (second row) at Sites 3, 4, and 5. Site 4, which is located between the Mollaro reservoir and the restitution of the Mezzocorona power station, showed a general reduction in streamflow with respect to the natural regime and was not impacted by hydropeaking. Downstream from the reservoir and before the restitution of the Mezzocorona power station, the river is fed by the constant release of about 2 m^3/s from the reservoir (Provincia Autonoma di Trento, 2006) to guarantee the minimum ecological flow (MEF), which supplements the natural contribution of the residual catchment. The other sites showed no observable alterations in the duration curves with respect

TABLE 1 Main hydrological characteristics and variables calculated at each sampling site

Site	Annual max Q (m^3/s)	Month when max Q occurs	Annual min Q (m3/s)	Month when min Q occurs	Annual mean flow (m^3/s)	FCV	Q10	Q25	Q75
1	7.56	May	0.25	April	0.59	0.35	0.25	0.32	0.63
2	21.76	September	0.79	February	0.14	0.08	0.05	0.06	0.12
3	88.16	October	5.92	February	11.19	73.05	3.87	5.25	14.81
4	203.98	November	5.04	February	9.71	90.51	5.06	6.54	10.34
5	246.43	November	42.49	April	35.7	434.96	10.63	18.22	49.25
6	1135.2	June	68.36	February	133.6	7581.40	55.46	70.75	171.18
7	1542.6	June	115.20	February	209.9	16687.9	92.80	121.63	261.64

Note. FCV: coefficient of variation; Q10: 10th quantiles; Q25: 25th quantiles; Q75: 75th quantiles.

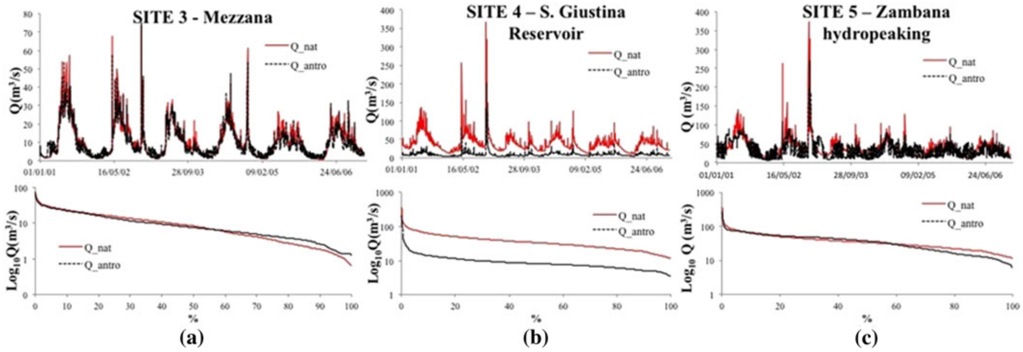

FIGURE 2 Streamflow time series (first row) and flow duration curves (second row) for Sites 3 (first column), 4 (second column), and 5 (third column). In the top row, the black lines indicate water discharge (recorded or computed using the model by Bellin et al. (2016)) in the presence of utilisations, and the red lines indicate the reconstructed natural water discharge (in the absence of utilisations). Similarly, in the second row, the black lines indicate the flow duration curves obtained in the presence of utilisations, and the red lines indicate the flow duration curves of the reconstructed natural flow regime [Colour figure can be viewed at wileyonlinelibrary.com]

to the reconstructed natural streamflow. However, the streamflow record at Site 5 (third column, first row) reflects the regularisation effect of the upstream reservoirs (Mollaro and S. Giustina) with a significant reduction of high flows, which was also reflected in the flow duration curve.

The cumulative distribution functions (CDFs) of daily streamflow variation (ΔQ) at Sites 4, 5 and 7 are shown in Figure 3. All the CDFs were rather steep at ΔQ = 0, suggesting the more frequent occurrence of small or no changes in water discharge between two successive time periods. Subdaily variations (green line) were steeper than daily changes (red line), particularly at Site 7, revealing that small variations were more frequent at the subdaily scale, as expected. Subdaily variations are not presented for Site 4 since no measurements were available at this site, and streamflow was calculated using the hydrological model at the daily scale. The largest alteration in the CDF as a result of anthropogenic pressure (i.e., hydropower) was observed at Site 5, with the daily variations in the natural (reconstructed) streamflow being steeper around zero with respect to the observed (altered) streamflow. For simplicity, only sites with significant differences are reported in Figure 3; the others showed a behaviour similar to that of Site 7.

This analysis showed that hydropower acts differently according to the location where the impact is observed. Downstream the reservoir and upstream the restitution (seee.g., Site 4), the regularisation effect of the reservoir not only makes streamflow smaller but also less variable in time than under natural conditions, while the opposite is observed

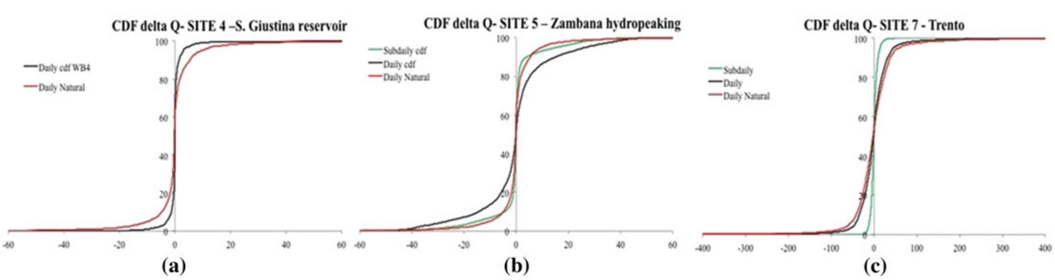

FIGURE 3 Cumulative distribution functions of water discharge increments ΔQ at Sites 4 (a), 5 (b), and 7 (c). Black lines refer to the observed (recorded or computed by the model) daily streamflow, the red ones to the reconstructed natural daily streamflow, and the green ones to the actual (only recorded) hourly streamflow. Note that at Site 4, streamflow was obtained using the model by Bellin et al. (2016), which operates at the daily time scale, and thereby hourly streamflow increments were not available [Colour figure can be viewed at wileyonlinelibrary.com]

downstream the restitution (e.g., Site 5), where hydropeaking makes small variation less frequent than under natural conditions.

4.2 | Physical and chemical parameters

As expected, water temperature was higher in summer than in winter (Table 2). In both sampling campaigns, a similar spatial gradient of water electrical conductivity and turbidity was observed, with higher values observed at downstream sites (Table 2). Turbidity was higher in summer with the highest value (172 FNU) observed at Site 7. On the other hand, nitrate and chlorine (Cl) concentrations were higher in winter than in summer with the highest concentrations observed at Site 2 (17.9 mg/L and 13.07 mg/L, respectively) downstream of the Tonale WWTP. A similar behaviour was observed for SO_4, with the highest value (38.24 mg/L) observed in winter at Site 6.

For the three groups of chemicals considered in the analysis, the concentrations were higher in winter than in summer at all sampling sites. The concentrations of PCPs and PhACs detected during the two sampling campaigns were reported in a recent paper by Mandaric et al. (2017). The most abundant PCP was octyl-dimethyl-*p*-aminobenzoic acid (ODPABA), with concentrations reaching up to 748 ng L^{-1} (Mandaric et al., 2017) at Site 4. Diclofenac was the most abundant among PhACs, reaching concentrations up to 675 ng L^{-1} at Site 2. Pesticide concentrations were lower than for the other two families of chemicals. The total concentration of pesticides (included herbicides and insecticides) in winter was 97.1 ng/L, with the highest detected concentration at Site 7; in summer, it declined to 61.1 ng/L, and the site with the highest concentration was Site 5.

The result of the PCA analysis for the hydrological and chemical data is shown in Figure 4, and the loading scores for each variable are reported in the Table S3. The first two components explain a total variance of 54.8%. The first axis (abscissa) was positively correlated with the coefficient of variation of streamflow, temperature, turbidity, PCP concentration, and agricultural land uses. Summer samples at Sites 6 and 7 showed the highest correlations. PhACs and nitrate were on the negative side of this axis, as were winter samples at Site 2. Axis 2 (ordinate) showed a positive correlation with conductivity, pesticides, and urban and agricultural land uses. Winter samples at Site 7 showed the highest positive correlation, and concentrations observed in the summer at Sites 1 and 3 were on the negative side. Most of the sites (2, 3, 4, 6, and 7) moved downwards in the PCA in the summer sampling, reflecting a reduction in the concentration of most chemical compounds and higher river discharge. By adding the third axis (not shown in Figure 4), the explained variance increased to 77.3% of the total variance and confirmed the strength of the correlation between nitrate and PhACs, and Site 2 in winter on one side; and PCP and pesticides and Site 7 on the other. As shown by Mandaric et al. (2017), the joint effect of low streamflow and higher tourist presences during winter resulted in an overall higher concentration of PPCPs (pharmaceuticals and personal care products). The concentrations of pesticides were also higher in winter, although they are applied to crops in spring–summer. Higher water discharge in summer caused a global reduction of all pollutants due to higher dilution. Unfortunately, few studies are available on the concentration of pesticides in the Adige River. Benfenati et al. (1990) performed a simultaneous analysis of 50 pesticides in water samples from the Adige River and revealed low levels of dichlobenil, lindane, atrazine, simetryne, and metholachlor. A recently published national report on the levels of pesticides in samples collected in 2013–2014, (ISPRA, 2016) confirmed the diffusion of these pollutants into the river in the Province of Trento. Of the 33 substances analysed, boscalid, dimetomorf, fluopicolide, and chlorpyrifos were the most frequently found in surface waters.

In summary, our data showed a spatial pattern of chemicals (upstream, Site 2, urban pollution, downstream pesticides), and, as suggested by Hypothesis 1, hydrological seasonality determines the level of dilution at the most polluted sites.

TABLE 2 Values for the different physical and chemical variables, richness, diversity, and IBE measured in the Adige River basin

Parameters	1.1*	2.1	3.1	4.1	5.1	6.1	7.1	1.2**	2.2	3.2	4.2	5.2	6.2	7.2
Temp (°C)	1.3	4.1	3.9	6.4	5.7	5.8	7.7	13.7	12.8	11.4	14.7	13.7	15.2	15.7
CE (µS/cm)	67.0	77.3	87.7	201.6	202.5	182	231.5	125	132	68.4	180	173	160	72.5
Turb (FNU)	0.01	3.5	2.15	3.5	6.2	2.6	3.15	4.6	2.7	62.5	4.0	4.2	70	172
NO3 (ppm)	2.1	17.9	4.4	4.4	3.0	3.8	2.9	0.4	2.7	1.2	2.1	2.3	1.7	1.9
SO4 (ppm)	15.4	13.5	27.6	28.2	8.10	38.2	21.9	34.5	9.8	25.8	12.7	12.9	32.9	33.2
Cl (ppm)	2.4	13.0	6.01	6.1	3.82	6.5	4.8	1.1	1.9	1.3	1.37	1.4	2.6	3.3
PhACs (ng L^{-1})	447.8	10051.3	2313.8	938.7	1283.4	1443.6	6014.77	291.04	3157.0	417.7	292.6	343.2	434.5	1263.6
PCP (ng L^{-1})	33.1	993.9	350.8	2417.2	501.8	44.6	553.06	43.1	270.4	175.1	168.4	51.9	208.4	11549.9
Pest (ng L^{-1})	3.4	3	22.5	17.6	13.4	6.6	33.6	2.1	5.1	3	9.7	25.8	4.7	10.7
Species richness (S)	35	29	19	30	24	26	26	20	15	14	18	15	15	16
Diversity shannon (H)	2.3	2.0	1.8	2.0	1.3	2.4	1.4	1.7	1.6	2.2	2.3	2.2	1.8	1.6
IBE	12	12	10	10	8	11	11	10	9	9	10	9	9	9
Classification IBE	Class I	Class I	Class I	Class I	Class II	Class I	Class I	Class I	Class II	Class II	Class I	Class II	Class II	Class II

Note. IBE: extended biotic index; PCP: personal care products; PhACs: pharmaceutical products; Cl: chlorine.

*First sampling campaign.

**Second sampling campaign.

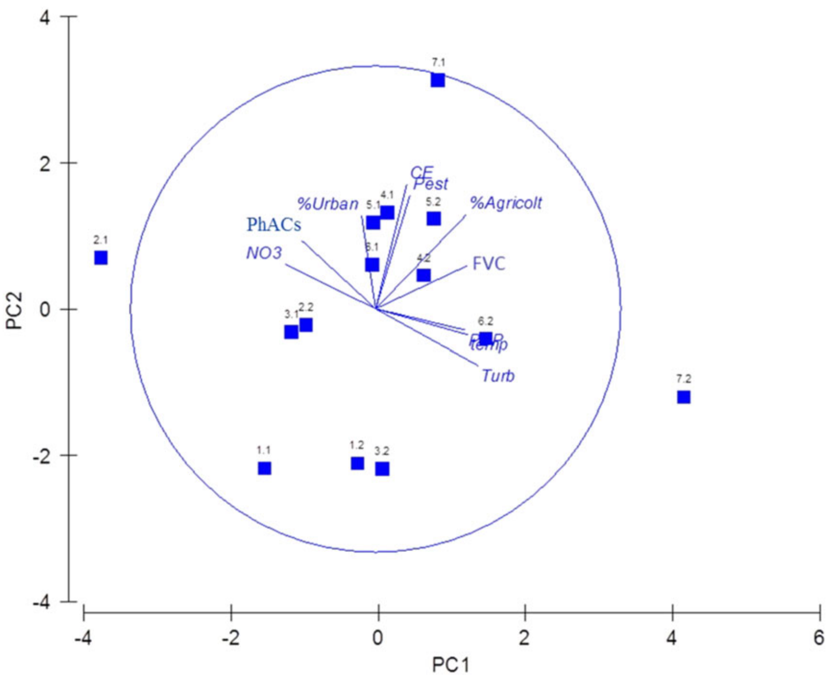

FIGURE 4 Principal component analysis analysis for environmental variables. Concentrations are represented with symbols and are labelled with two numbers, the first referring to the site, and the second to the season, with 1 indicating winter and 2 summer (e.g., 3.2 indicates the sample taken at Site 3 in the summer campaign) [Colour figure can be viewed at wileyonlinelibrary.com]

4.3 | Macroinvertebrate community

The highest species richness was detected at Site 1 in both sampling campaigns, while a gradual decrease was observed at Sites 2 and 3, corresponding to the absence of several sensitive species (Plecoptera, Trichoptera, and Coleoptera groups) and an increase in other taxa (e.g., Chironomidae). At all sites, richness was significantly lower in the summer with respect to the winter sampling campaign (GLM, $F = 24.63$, $P = 0.001$; Table 2). The Shannon diversity index ranged from 1.3 to 2.4, and the most obvious decrease between winter and summer was observed at Sites 1 and 6; however, seasonal differences were not significant at all sites (GLM, $P > 0.05$). None of the two metrics showed significant differences between upstream (Sites 1, 2, 3, and 4) and downstream sites (Sites 5, 6, and 7), where differences in flow variability and chemical concentration were observed. Higher richness and diversity relative to its upstream site were observed only at Site 4 in both samplings. This site located between the Mollaro reservoir and the restitution of the Mezzocorona hydropower plant is affected by a significant alteration in the natural streamflow, since the reservoir discharges a constant amount of water without any seasonal modulation, but it is not affected by hydropeaking, which instead impacts Site 5. In addition, the constant release of water reduces seasonal temperature variations (the release causes warming in winter and cooling in summer) that may favour the presence of some species (Maiolini, Silveri, & Lencioni, 2007; Ward, 1994). Accordingly, we found higher densities of some taxa, such as *Baetis*, Simuliidae, Chironomidae, and some species of Coleoptera, Trichoptera, and Gasteropoda, while other species (e.g., *Capnia* sp. and *Capnioneura* sp.) that are adapted to colder waters were less abundant. The mean densities of the most abundant species are reported in Table S2.

The first principal component of the dbRDA analysis (Figure 5a,b) separated most of the headwater sites (on the right) from low water (on the left) sites. Only Sites 4 and 5 showed a different correlation with this axis according to the sampling period. Lower conductivity, turbidity, flow variability, and pesticide pollution were observed in headwaters. These sites (from 1 to 3) were characterised by a higher number of taxa with the presence of Plecoptera (*Capnia* sp., *Perlodes* sp., *Isoperla* sp.) and Trichoptera (*Sericostoma* sp., *Micrasema* sp., *Hyporhyacophila* sp., *Psychomyia* sp, *Limnephilus* sp.), which were the taxa most sensitive to pollution among those detected in the two sampling campaigns. In summer, the most abundant taxa at Sites 4 and 5 were Coleoptera (*Helodidae* sp.) and Ephemeroptera *(Serratella* sp.*)*. Higher densities of *Gammarus* sp., *Hirudinea*., *Psychomyia* sp., *Hydropsyche* sp., *Baetis* sp., and the Dipteran families Chironomidae and Simulidae were present at the downstream sites, which are characterised by a higher percentage of agricultural and urban land uses, and a higher concentration of some of the related pollutants: pesticides and PCPs. The hydrological indicator included in the analyses (i.e., the coefficient of variation of the daily water discharge) was positively correlated with the presence of *Gammarus* sp. (Spearman correlation, $R = 0.60$, $P = 0.02$), *Hirudinea* ($R = 0.74$, $P = 0.003$), and *Psychomyia* sp. ($R = 0.85$, $P < 0.001$; Figure 5b). A clear seasonal pattern in the composition of the biological community was indicated by Axis 2. In particular, most sites occupied the upper part of the graph in summer and were characterised by poorer community composition (less taxa) compared with the winter sampling (located in the lower portion of the axis). This axis was positively correlated with water temperature and negatively with PhAcs and nitrate concentrations, which were both higher in winter at Site 2. These changes related to human perturbation at headwaters have been observed in previous studies in other Alpine rivers (Lencioni & Rossaro, 2005). Discharge from the WWTP just upstream Site 2 increased the nutrient and urban contaminant concentrations (mainly PhACs); however, the IBE was unable to detect any changes in community composition at this site, with respect to reference Site 1. This confirms some of the limitations of biotic indices described in other studies (seee.g., Clarke, 2013), and the interest to have multimetric indicators to detect effects.

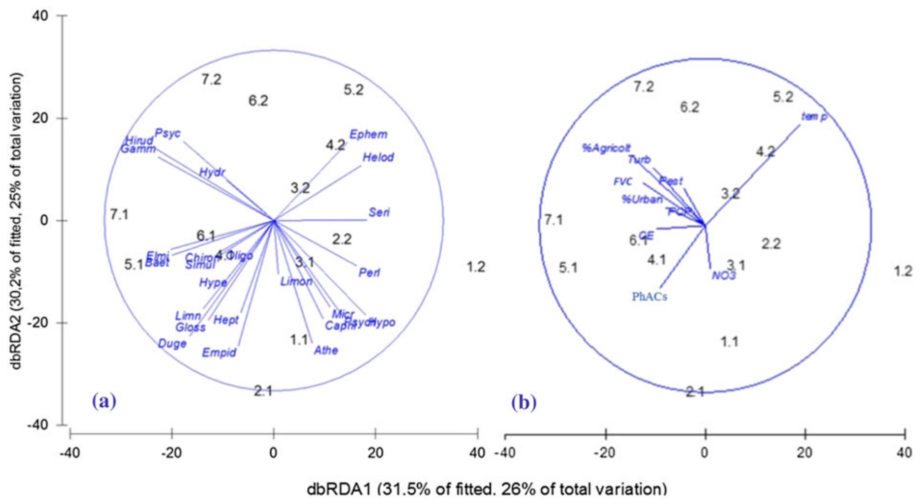

FIGURE 5 Distance-based redundancy analysis between biological and environmental variables [Colour figure can be viewed at wileyonlinelibrary.com]

The macroinvertebrate community composition was significantly different among samplings (PERMANOVA, pseudo F = 4.48, P = 0.001) and between up and downstream sites (pseudo-F = 3.90, P = 0.006) confirming the results of the multivariate analysis. Streamflow alteration due to hydropower seems to have an effect on community composition at Site 5, which is the site most affected by hydropeaking. A shift in community is observed from Site 5 downstream, but it does not affect significantly richness nor diversity (GLM results, see above). However, diversity is negatively correlated with the coefficient of variation of streamflow (Spearman coefficient = −0.40, P < 0.05). Here, the abundance of some species (e.g., *Gammarus*) increased while others (i.e., *Baetis* and Dipterans) declined. Because of its ability to enter into the sediment for refuge (Dole-Olivier, Marmonier, & Beffy, 1997), *Gammarus* has an advantage, with respect to other species, in tolerating rapid and periodic changes in the river flow due to hydropeaking (Mondy, Muñoz, & Dolédec, 2016).

As suggested by Hypothesis 2, the present study provides evidence for the seasonality in invertebrate community composition. The two samplings show differences according to taxonomical community composition and density. A general decrease in richness and abundance was observed in the summer season, although some taxa (e.g., *Serratella* and *Helodidae*) showed higher densities in this period.

Seasonal distribution of invertebrates was also identified in Apennine rivers (Bottazzi et al., 2011; Fenoglio, Bo, Cammarata, López-Rodríguez, & de Figueroa, 2014). These works suggest that the major forces shaping invertebrate communities seemed to be related to the Alpine climate and especially to snow accumulation and melting with the consequent substantial discharge variations.

In addition, a number of studies on glacial river ecosystems highlighted that water temperature is a key factor influencing biological communities (Brown & Milner, 2012; Milner, Brown, & Hannah, 2009). Therefore, most of the seasonal changes in taxa abundance observed in this study would be strictly related to species life cycle (Maitland, 1965; Milner & Petts, 2006), while the spatial patter is most likely related to stressors. Hydropeaking increased flow variability and determined a shift in the community at the downstream sites, but not in the diversity, partially according with our hypothesis. Dickson, Carrivick, and Brown (2012) highlighted that regulated flows may exert stronger effects on Alpine catchments than natural changes because they are active during winter, when river discharge and temperatures vary little. Pollution effects in the studied river appeared pointwise, were closely related with specific activities (i.e., urban and agricultural pollution) and were more evident in winter with lower flow. Such disturbances (i.e., hydropeaking and chemical inputs) produce discontinuities along the river, which influence the spatial distribution of organisms such as in this, as well as in other studies concerning glacial rivers (Knispel & Castella, 2003).

5 | CONCLUSIONS

This study shows that the composition of the macroinvertebrate community responded to seasonality and to changes in the main stressors along the Adige River. The inputs from WWTPs (already detected in headwaters) and a general increase in pollution downstream were the factors associated with chemical stressors, and these had more influence in winter when river discharge was lower. Water flow variability due to hydropower seemed to favour some taxa (e.g., *Gammarus*) at sites located downstream, the restitution of a large hydropower plant. Richness and diversity did not change significantly between upstream and downstream sites. This research also highlights the importance of the spatial and temporal patterns of stressors in this Alpine river. The ecological status of impacted Alpine rivers cannot be improved further without considering the combined effect of these drivers, as discussed in the present work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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