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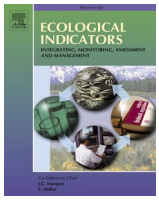

Microplastics and leaf litter decomposition dynamics: New insights from a lotic ecosystem (Northeastern Italy)

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ABSTRACT

Microplastics represent one of the main environmental concerns of our time and their presence is well known in all freshwater ecosystems. However, there is still a lack of knowledge about the interference with some environmental dynamics, such as the leaf litter decomposition, which represents a key process in freshwater ecosystems. The work presented herein analyzed the leaf litter decomposition in a lotic ecosystem, in relation to water physicochemical parameters, macrobenthic invertebrate functional feeding guilds (FFG) and, as a novelty, the microplastics as additional factor. Physicochemical features were monitored every 15 days for one year. *Phragmites australis* decomposition rates were investigated during four seasons (summer, autumn, winter, and spring) using the leaf bag technique. Microplastic items were also collected within the leaf bags (used as retaining tool) and within macrobenthic invertebrate colonizers. Shredders were the most contaminated FFG in summer and autumn, while scrapers showed high microplastics levels in autumn and winter. Decomposition rates significantly differed among seasons ($0.007 < k < 0.022$) and water temperature was the main driver of the decomposition dynamics (relative importance = 70.3 %), positively affecting the decay rates, followed by pH (9.7 %), which showed a negative contribution. Microplastics showed a negative effect (3.1 %), with a relative importance similar and opposite to that observed for the shredders (3.9 %), which value was similar to those recorded for scarpers (2.7 %). This study represents a field investigation regarding the microplastic effects on the organic matter decomposition rates in freshwater environments carried out directly on field. Our results provide new insights about the microplastic interference on environmental dynamics and could represent a starting point for further studies.

1. Introduction

Environmental contamination due to plastics is one of topmost environmental concerns of the Anthropocene (Waters et al., 2016; Akindele et al., 2019, 2020; Multisanti et al., 2022). Microplastics (MPs), are generally identified as plastic particles smaller than 5 mm (GESAMP, 2015; Alimba and Faggio, 2019), and have been found ubiquitously in all aquatic ecosystems from alpine areas to ocean floor sediments (Van Cauwenberghe et al., 2013; Free et al., 2014; Woodall et al., 2014; Pastorino et al., 2020, 2022) and from temperate areas to tropical zones (Mani et al., 2015; Horton et al., 2018; Nel et al., 2018; Akindele et al., 2019).

Riverine systems are particularly sensitive to MP pollution, which can increase in association with wrong waste management (Free et al., 2014), particularly if this impact is linked to high population densities (Baldwin et al., 2016). The occurrence of MP pollution in water is often associated with high industrial activity areas, such as urban centers (Dris et al., 2015) and harbors (Mathalon and Hill, 2014; Naidoo et al., 2015; Nel et al., 2017; Wang et al., 2017). Wrong waste management, combined with huge production, cause big plastics discharge into the oceans each year, via riverine systems (Lebreton et al., 2017). Moreover, discharge of MP polluted wastewater (such as brine) contributes to the

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degradation of water quality and thus water cannot be directly used for potable purposes (via desalination) and/or industrial applications (Panagopoulos 2022a, 2022b; Panagopoulos and Giannika, 2022).

Microplastics may also pose a chemical hazard. Indeed, a complex mixture of chemicals (i.e., additives), manufacturing byproducts, and chemical contaminants can accumulate on plastic when it becomes litter (Burgos-Aceves et al., 2021). Finally, one of the main sources of primary MP pollution is represented by cellulosic microfibers of clothes made with a blend of polyester/cellulose which are released through washing (De Falco et al., 2019).

Due to their small size, MPs can be available and mistaken for food by aquatic organisms, such as invertebrates and fishes. This issue causes cascading effects on trophic webs and increases the worry about influences on ecological and human health. Microplastic ingestion has been observed during field works (Bour et al., 2018; Windsor et al., 2019; Bertoli et al., 2021) and during laboratory experiments, which highlighted extremely negative effects (Imhof and Laforsch, 2016; Hurley et al., 2017; Ziajahromi et al., 2017; Guzzetti et al., 2018; Redondo-Hasselerharm et al., 2018; Scherer et al., 2018; Prokić et al., 2021; Silva et al., 2019, 2021a). Effects of ingestion can involve gastrointestinal systems (Derraik, 2002; Tourinho et al., 2010), growth dynamics (Redondo-Hasselerharm et al., 2018) and reproduction cycles (Sussarellu et al., 2016). Moreover, MP ingestion can lead to oxidative stress (Lu et al., 2016; Piccardo et al., 2021), uptake and bioaccumulation of harmful chemicals (Betts et al., 2008; Teuten et al., 2009; Lavers et al., 2014), and also to death (Teuten et al., 2009; Wright et al., 2013). Among the organisms that are highly susceptible to MPs contamination, macrobenthic invertebrates are particularly vulnerable (Silva et al., 2021b; Pagano et al., 2022). These organisms have a key role in freshwater ecosystems, covering all trophic functions (Cummins, 1974; Metcalfe Smith, 1994) and ecological niches (Voshell, 2002) and representing a pivotal trophic resource for other animals, such as fish and bird species (Bertoli et al., 2015). Moreover, they are among the main actors in the decomposition of the organic matter and especially in leaf litter breakdown (van Dokkum et al., 2002; Hieber and Gessner, 2002; Raposeiro et al., 2017; Bertoli et al., 2020, 2022). The leaf litter decomposition represents one of the main key processes in freshwater environments (Boulton and Brock, 1999; Gessner and Chauvet, 2002; Dinka et al., 2008; Dolinar et al., 2016; Gessner and Tlili, 2016; Seena et al., 2019) and, in leaf litter breakdown processes in streams respond unambiguously to anthropogenic stresses (Gessner and Chauvet, 2002). However, the effects of MP pollution on decomposition dynamics are still less studied. Macroinvertebrates can ingest available MPs in freshwater environments (Hurley et al., 2017; Nel et al., 2018; Windsor et al., 2019; Akindele et al., 2019, 2020; Bertoli et al., 2021) and this can lead to false satiation, reducing the feeding rates (Rist et al., 2017). Due to this reasons, Lòpez-Rojo et al. (2020) highlighted a reduction of leaf litter decomposition mediated by detritivore macrobenthic invertebrates (Trichoptera larvae) and Ockeden et al. (2022) found similar results. Changes in breakdown dynamics can be indirectly caused by disruption of the macroinvertebrate food supply, which could alter the ecosystem processes (Ockeden et al., 2022). This can depend on the alteration of microbial activities on the leave, which affect palatability and food quality for macrobenthic shredders (Yardy and Calaghan, 2020), causing a reduction in macroinvertebrate feeding. Microbes are the first detritus colonizers, starting the decay process and making leaves more palatable for macrobenthic invertebrate shredders (Gessner et al., 1999). Several studies analyzed the effects of nanoplastics on the fungal activities in relation of litter decomposition (Seena et al., 2019, 2022; Du et al., 2022), and the effects of microplastics on stream microbial decomposers in association to nanoparticles (Trabulo et al., 2022). Batista et al. (2022) also highlighted that micro(nano)plastic have direct effects on fungal activities and indirect consequences on shredders.

On the other hand, other studies did not record negative effects on decomposition due to MPs. For example, Kratina et al. (2019) found negative physiological responses in freshwater shredders (*Gammarus* spp.) but weak effects on decomposition. Silva et al. (2021a, 2021b, 2022) did not find effects on leaf litter decomposition due to MPs exposure but observed an abundance reduction of the benthic macroinvertebrates. Huang et al. (2021) showed that PE microparticle concentrations similar to those used in Silva et al. (2022) (but with a longer exposure period) affected the mediating role of benthic macroinvertebrates in the freshwater nitrogen cycle.

However, most of the studies regarding the relationship between leaf litter decomposition and microplastics were performed mainly in laboratory via microcosmos approaches and were not directly carried out on the field. Another point is that often these studies were carried out only considering target species and/or one trophic level (Rist et al., 2017; Kratina et al., 2019; Ockeden et al., 2022).

In this context, it was deemed of interest to study the leaf litter breakdown within a riverine stretch, investigating the contribution of biotic/abiotic features and MPs on the decomposition rates of the vegetal organic matter. Our aims were: i) analyze the main physicochemical water parameters searching for seasonal trends and characterize the macrobenthic invertebrate communities in the studied watercourse section; ii) verify the presence of MPs, within leaf litter and macrobenthic invertebrates; iii) investigate the contribution to seasonal decomposition rates due to biotic and abiotic predictors, including MPs retained by the leaf litter. Regarding the biotic factors, we focused on macrobenthic invertebrate communities. We expected that the MPs abundances in the leaf litter could significantly and negatively affect the decomposition dynamics.

2. Material and methods

2.1. Study area

The present work was carried out in the Italian portion of the Vipacco River (Friuli Venezia Giulia, Northeast Italy) (Fig. 1), which is the major left tributary of the Isonzo River (Mosetti, 1983). The area was formerly described by Bertoli et al. (2021) in a previous study that highlighted the presence of MPs in the same watercourse as well as in the macrobenthic invertebrates. The springs of the Vipacco River are located in Slovenia, within the karstic area of Mount San Lorenzo (1019 m a.s.L.). The watercourse flows for 45 km and then crosses the State border between Slovenia and Italy. The Italian stretch is 4.5 km long high plain watercourse, and it flows within the Savogna d'Isonzo municipality (Friuli Venezia Giulia Region), where it joins the waters of the Isonzo River. The Slovenian stretch upstream to the State border is characterized by the presence of natural woodland zones, surrounded by agricultural areas (arable fields, permanent crops, fruit trees and vineyards) and scarce urbanization. Sources of impact can be associated to small industrial activities, landfills, construction sites and railway/road networks close to the watercourse. The Italian Vipacco stretch is slightly modified, and it flows within a territory where urbanization is scarce, but agricultural/and waste-water discharges are present. The stretch is sensitive to anthropic impacts and therefore it is included in the ecological monitoring framework managed by local authorities (Regional Agency for Environmental Protection of Friuli Venezia Giulia, ARPA FVG; https://www.arpaweb.fvg.it).

In the present study, we considered a 2 km watercourse section, from the State border to the middle of Italian stretch. The watercourse section included different mesohabitats, which were uniform flow areas (60 %), pools (20 %), glides (10 %) and riffles (10 %). Main substrates were coarse/fine gravel and cobbles. The riverbed vegetation cover was always low (range: 1–20 %). As reported in a previous study (Bertoli et al., 2021), three wastewater discharge spots were detected in the considered stretch, in association with small urban areas (Fig. 1): the first near the State border, the second in the middle of the studied section and the last in the middle of the Italian Vipacco River stretch. No other wastewater discharge spots were observed along the considered watercourse

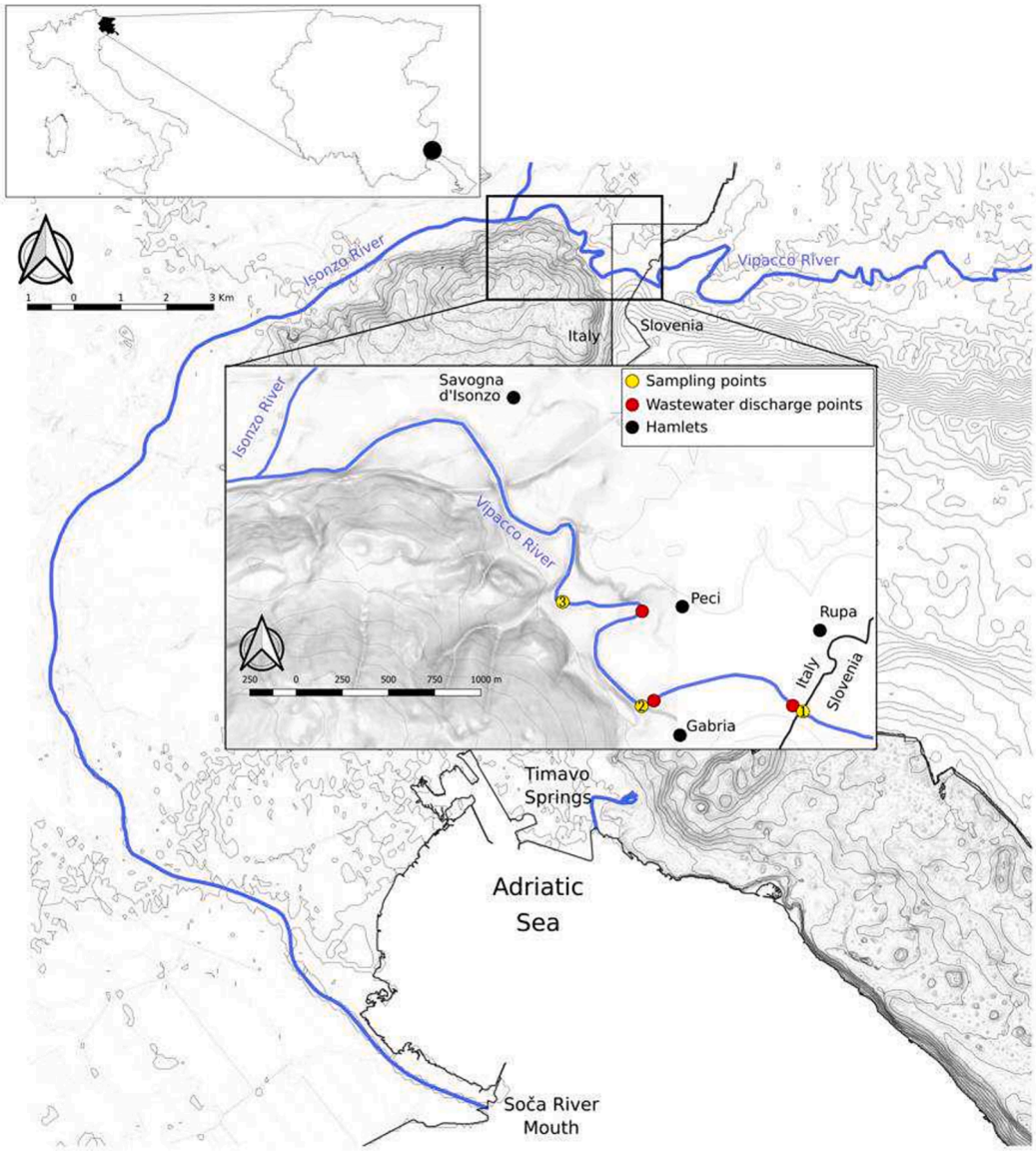

Fig. 1. Study area and sampling sites in the Vipacco River (source: Bertoli et al., 2021, modified).

section.

For the present study, three sampling sites were chosen to represent the hydrological characteristics and impacts of the investigated river section. Position and number of the sampling sites were strongly conditioned by the river accessibility, as the Vipacco River is a non-wadable watercourse for the most of its Italian stretch and it is subject to high variations of discharge values. As it was not possible to add other sampling points, we considered 2 km Vipacco section. The first site was located 10 m upstream of the first wastewater discharge point, few meters downstream of the State border; the second site was placed 20 m downstream of the second wastewater discharge point, near the Gabria hamlet; finally, the third point was located 400 m downstream of the third water discharge point. Characteristics and geographical coordinates of each site are reported in Table S1.

2.2. Physicochemical water parameters

Physicochemical water parameters were monitored every 15 days for one year (from the beginning of June 2021 to the end of May 2022). At each sampling site, values of water temperature (°C), pH (unit), conductivity (μS cm^{-1}), and dissolved oxygen (concentration, mg L^{-1}; saturation, %) were recorded using field probes (HI 9033 conductivity meter; HI 9125 pH/ORP meter; HI 9147 dissolved oxygen meter; Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Values were measured in the water column, approximately at mid-depth (20–50 cm) compatibly with the accessibility to the sampling points, as the Vipacco River is a non-wadable watercourse. During each sampling event, at each site water samples were also collected in sterile bottles and brought to the laboratory. Here, ammonia (NH_4^+) concentrations (mg L^{-1}) were measured via adaptation of the Nessler method, analyzing the absorbance at 420 nm (ASTM, 2015); nitrate (NO_3^-) concentrations (mg L^{-1}) were measured analyzing the absorbance at 525 nm, via adaptation of the cadmium reduction method (APHA, 1998); finally, the phosphates (PO_4^{3-}) concentrations (mg L^{-1}) were measured via adaptation of the ascorbic acid method (APHA, 1998), analyzing the absorbance at 610 nm. A multi-parameter spectrophotometer (HI83200-02, manufactured by Hanna Instruments Inc. Woonsocket, RI, USA) was used for the measures and three replicates were done for each parameter.

Data regarding the hydrometric level and water discharge of the Vipacco River were obtained from the Slovenian Environmental Agency website (ARSO, https://www.arso.gov.si/), consulting the "Miren I" hydrometric station, located a hundred meters upstream of the site 1 and

of the state border between Italy and Slovenia.

2.3. Microplastic sampling in water and sediment

Sediment and water samples were collected to verify and quantify the microplastic presence in the Vipacco River. Sediment samples were collected at the beginning of the study with a manual corer (250 cm^2 sampling surface) within the riverbed, few meters far from the banks, accordingly with the accessibility of the sampling sites. Three samples were placed in glass jars (1 L) to avoid external particle contamination and frozen at − 20 °C for storage. Samples were then thawed before extraction and determination of microplastic chemical composition (Pastorino et al., 2020; Bertoli et al., 2021). Water samples were collected at the same time of the leaf bag experiment (section 2.4.), every 15 days. For this purpose, an Apstein plankton net (opening 400 x1000 mm; mesh size 50 μm) was kept under water for 5, 10 and 15 min (Valine et al., 2020). Three replicates were collected (one for each time of submersion). The water samples were placed into glass jars (500 mL) and brought to the laboratory where they were conserved at 4 °C. Between each replicate, the net was cleaned with ultrapure water to avoid contamination of the following sample. Chemical types of MPs were identified both in sediment and water samples (section 2.5.).

2.4. Leaf bag technique and macrobenthic invertebrate samplings

The leaf bag technique (Petersen and Cummins, 1974) was chosen to study the organic matter decomposition in the Vipacco River and the assemblages of macrobenthic invertebrate colonizers. This technique simulates the natural accumulation of vegetal organic matter in freshwater environments, which represent both a trophic source and refugia for macroinvertebrates. However, leaf litter can act as retention structure for MPs transported by the stream, potentially affecting the decomposition process. The presence of MP items was separately investigated both in the macrobenthic invertebrate colonizers and within the leaf bags. Before the present study, a preliminary experiment was carried out in the same sampling sites during spring 2021, to check the capacity of the leaf bags to retain MPs, analyzing 12 leaf bags per site after 45 days of submersion. This preliminary experiment allowed to collect polypropylene, polyamide and polypropylene in all the bags placed at chosen sites and polystyrene in sites 2 and 3. After verifying that leaf bags can retain MPs, we proceed with the full experiment.

Leaf bags were prepared according to Basset et al. (2006) and in line with previous investigations carried out in freshwater water bodies located in the same geographical area (Bertoli et al., 2016, 2020, 2022). Leaves of common reed, *Phragmites australis* (Cav.) Trin. ex Steud was chosen for the study. Common reed is a cosmopolitan species, considered to be the world's most widespread angiosperm (Ridley 1923; Hutchinson 1975; Packer et al., 2017). Native populations grow naturally in temperate zones and on every continent except from Antarctica, and it could be found in wetlands, lakes, and watercourses. The species is present along the considered portion of the Vipacco before it meets the waters of the Isonzo River.

Leaves were previously collected during the senescent phase, choosing only intact ones at least 30 cm above the water surface. Apical leaves and those that met water were avoided. After air drying, the leaves were cut into fragments about 10 cm-long. Basal and apical portions were not considered. The obtained fragments were oven-dried to constant weight (60° C for 72 h), in order to obtain standardized samples and to get initial conditions as uniform as possible (Bärlocher, 1997). Lots of 3.0000 ± 0.0001 g (dry weight) were put inside tube-shaped bags made with cotton net (5 mm mesh). The chosen mesh size allows macrobenthic invertebrate colonization and reduces leaf material loss (Basset et al., 2006). Sampling was performed during four seasons: summer (June-August 2021), autumn (October-December 2021) winter (February-March 2022) and spring (April-May 2022). During each season, 144 leaf bags were gently put underwater (36 at each sampling site), close to the bottom and tethered with strings to stones and/or trees to avoid sample loss. Twelve leaf bags were taken from each sampling site after 15, 30, and 45 days of submersion, placing them in separate glass boxes for transportation. Eight leaf bags were used to measure the decomposition rates and were collected in the boxes with river water. In laboratory, the leaves were softly washed to separate them from sediments and macrobenthic invertebrates. Then, leaf material was oven-dried at 60 °C for 72 h and weighed ($x \pm 0.0001$ g dry weight) to obtain the remaining leaf mass (M_t) after $t = 15$, 30, and 45 days of submersion. M_t was expressed in percentage and the initial weight ($t = 0$ days, $M_t = 3.0000 \pm 0.0001$ g dry weight) was considered equal to 100 %.

Four leaf bags were used to investigate the microplastic accumulation in leaf litter and in the macrobenthic invertebrate colonizers. Samples were stored in glass jars filled with 70 % ethanol to prevent gut content excretion from the invertebrates (Windsor et al., 2019). In the laboratory, macrobenthic invertebrates were separated from the leaves using nets with 50 μm mesh size, in order to avoid microplastic loss. Samples were processed avoiding the use of plastic tools, using glass Petri dishes and steel tweezers. Leaf fragments and detritus were stored in 70 % ethanol and then analyzed to detect the presence of microplastic in the leaf bags material. Invertebrates associated to these leaf bags were stored at − 20 °C and then analysed to investigate the microplastic presence in the macrobenthic invertebrate community. When it was possible, taxonomical classification of the macrobenthic invertebrates was led at least to the genus level and each identified taxon was assigned to a Functional Feeding Guild (FFG), following indications provided by Merritt and Cummins (2017).

2.5. Microplastic analyses

The extraction of sediment samples was achieved with a prefiltered saturated NaCl solution by mechanical agitation (20 min, 100 rpm); 6-μm pore paper disk filters were used to filter the supernatant. The procedure was repeated three times. 6-μm pore paper disk were also used to filter the water samples by vacuum on (Whatman®, Sigma-Aldrich, St. Louis, MO, USA). The filters were then set on a glass Petri disk to prevent contamination during the oven drying phase (35 °C). Dried samples were examined under a stereomicroscopy and MP particles were collected for chemical analyses.

Macroinvertebrates were pre-treated by direct tissues digestion using a Creon enzyme (37 °C; TRIS-buffered pH) and sonicated for 1 h. The procedure was performed to remove the tissues avoiding damages to the plastic polymers for subsequent chemical identification (von Friesen et al., 2019). Digested samples were filtered using tools equipped with 6-μm pore paper disk, stored in glass Petri dishes, and dried overnight at 40 °C (Ziajahromi et al., 2017). Air exposure was reduced during the described activities, in order to minimize potential aerial contamination while filtering the samples under a HEPA-filtered laminar-flow fume hood. Positive and negative controls ($n = 3$) were performed for each batch to guarantee quality control of the whole analytical process. Finally, the samples were then sorted using a stereomicroscopy at 10–80× (SMZ-800 N; software NIS-elements D, Nikon, Tokyo, Japan). Chemical composition of target-items was investigated by microscopy associated with Fourier transform infrared spectroscopy (μFT-IR; Nicolet iN10 MX, ThermoFischer Scientific, Waltham, MA, USA) armed with an MCT-A detector (spectral range, 7.800–650 cm^{-1}) cooled with liquid nitrogen and operating in reflection mode. Identification was performed by determining the target-items spectral match (%) which was compared to the spectral libraries of normal and aged microplastics (OMNIC™ Picta™ software libraries, ThermoFisher Scientific) integrated with spectral libraries of our laboratory. A threshold for spectra back-recognition > 80 % of match was imposed. Particle size detection limit (LOD) was equal to 10 μm. The collected items were finally classified in agreement with criteria provided by Galgani et al. (2014).

2.6. Statistical analysis

The presence of seasonal and/or spatial differences in physico-chemical parameters were investigated using two-way ANOVA and honestly significant difference (HSD) (factors "Site" and "Season"), while one-way ANOVA was applied to check for significant variations in seasonal discharge values. Normality assumptions were checked with a Shapiro-Wilks test and the data were transformed if required ($\log(x + 1)$). Variance homogeneity was investigated via Bartlett test. Then, Principal component analysis (PCA) was applied on a previously standardized dataset to identify potential seasonal trends for abiotic features. Pearson correlation coefficient (r) was used to check for correlation between abiotic variables. Two-way PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001) was performed to check significant seasonal and/or spatial differences in observed macrobenthic invertebrate communities. Multivariate homogeneity of group dispersions was checked using PERMDISP (Anderson, 2006) before PERMANOVA application. SIMPER analysis (Clarke, 1993) was applied to the data matrix to identify the main taxa contributing the most to observed significant differences highlighted by the PERMANOVA.

Differences in seasonal trends regarding the number of microplastic items collected in the leaf bags were investigated using the non-parametric Kruskal-Wallis test and the Conover–Iman post-hoc test (Conover and Iman 1979; Conover, 1999). The same tests were also used to detect significant differences among the number of microplastic items observed in the macrobenthic invertebrates FFG.

The remaining leaf mass M_t (%) under submersion (at $t = 15$, 30, and 45 days) was calculated and seasonal decomposition rates were obtained using a negative exponential decay function (Olson, 1963; Petersen and Cummins, 1974):

$$M_t = M_0 e^{-kt}$$

where M_0 is the percent mass at the beginning of the submersion (100 %) and k is the decomposition rate. Data were then transformed ($\log(M_t + 1)$) and the ANCOVA was used to assess for differences between seasonal decomposition rates, where k is the slope of linear regression equations; time was considered as covariate (Zar, 1984; Bärlocher, 2005).

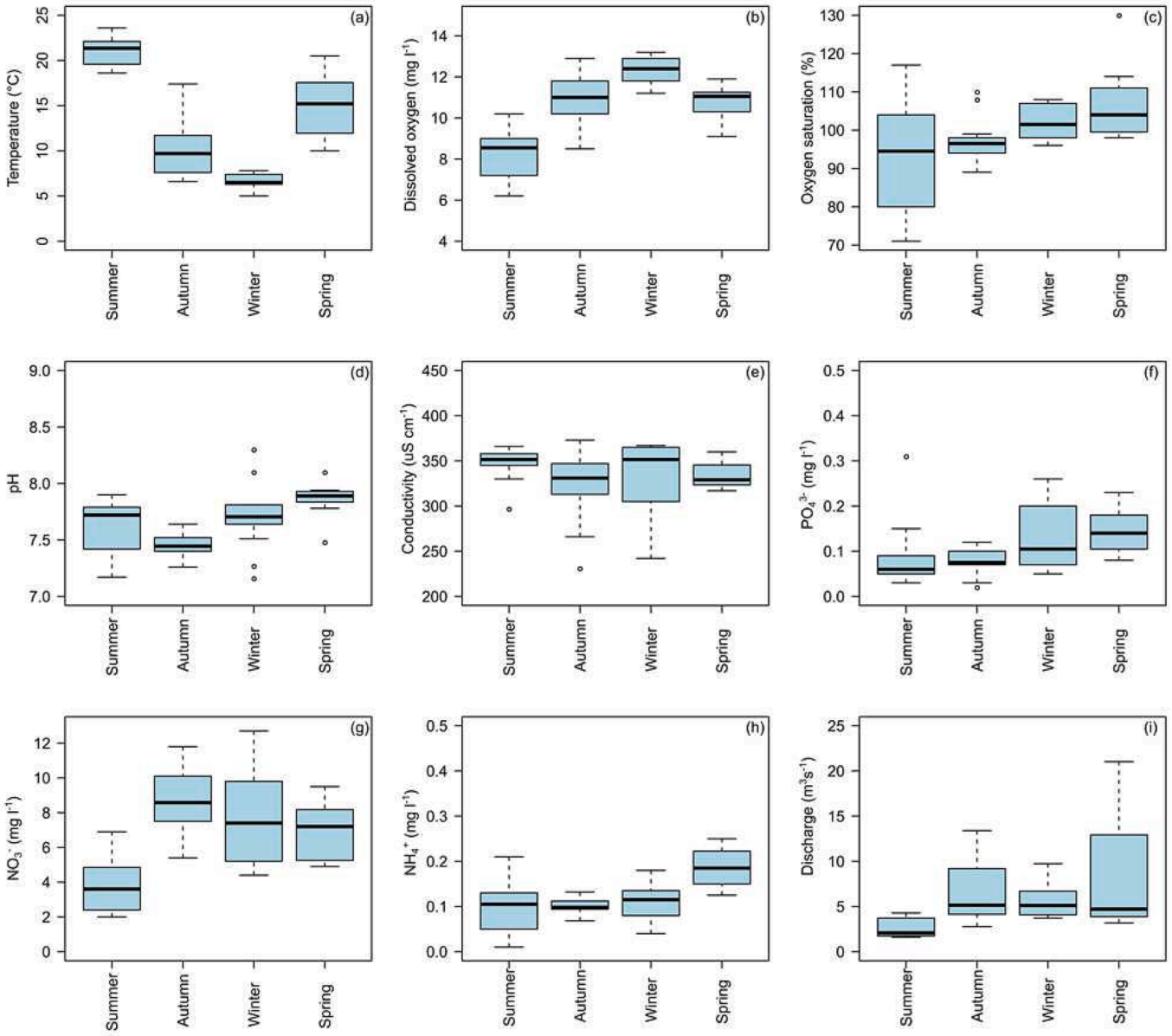

Fig. 2. Seasonal trends of physicochemical parameters monitored during the study period in the Vipacco River.

Forward multiple stepwise regression analysis was used to investigate the importance of abiotic and biotic parameters as sources of variation for decomposition rates (k), which was considered as the dependent variable. A reduced set of predictors was obtained evaluating the results of PCA and correlation analyses, to prevent multicollinearity. For this purpose, strongly correlated variables were excluded if $r>|0.4|$ and $p < 0.001$ (Raposeiro et al., 2017; Bertoli et al., 2020, 2022). The number of shredders and scrapers observed in the leaf bag were used as a biotic variable. Abiotic and biotic data were transformed ($\log(x+1)$) to meet normality assumptions, which were tested as described above. After the multiple stepwise regression, the relative importance was quantified as percentage for significant predictors, using the LMG method (Grömping, 2007).

All analyses were performed using RStudio version 2021.9.0.351 (R Core Team, 2021; RStudio Team, 2021). Figures were produced with RStudio and processed with software Inkscape version 0.92.

3. Results

3.1. Physicochemical water parameters

Trends of the main Vipacco River physicochemical parameters are shown in Fig. 2.

All the considered features significantly differed among seasons, except conductivity and phosphates, which did not show significant variations during the study period. The ANOVA did not allow to highlight significant spatial variations for all the considered features (Table S2). Mean values (±standard deviation) of water temperature ranged between 6.6 ± 0.9 °C in winter and 21.7 ± 1.5 °C in summer. Values were significantly higher in summer, decrease in autumn and winter and then increased in spring as expected (Fig. 2a). Waters of the Vipacco River were generally well oxygenated, with dissolved oxygen concentrations ranging between 10.3 ± 0.8 mg L^{-1} (spring) and 12.7 ± 0.5 mg L^{-1} (winter) (Fig. 2b). Oversaturation condition was often recorded (Fig. 2c), except during the summer, when oxygenation levels were significantly lower than the other seasons and values lower than 75 % were recorded. pH showed remained between 7.5 ± 0.1 and 7.9 ± 0.1 (Fig. 2d), while conductivity showed values ranging from 318 ± 45 µS cm^{-1} (in autumn) e 382 ± 139 µS cm^{-1} (in winter) (Fig. 2e). Regarding the nutrient concentrations, values observed for PO_4^{3-} ranged between 0.06 ± 0.02 mg L^{-1} and 0.23 ± 0.40 mg L^{-1}, but no significant differences were recorded, nor spatially or seasonally (Fig. 2f). Concentrations of NO_3^- showed mean values between 3.53 ± 1.08 mg L^{-1} and 9.28 ± 1.84 mg L^{-1}, with lower levels in summer than in other seasons, while NH_4^+ reached the highest level in spring (Fig. 2g, h). During the study, discharge values ranged between 1.6 and 54.4 m^3 s^{-1} and it was significantly lower during summer, as expected, than in the other seasons. The highest variability in discharge values was observed

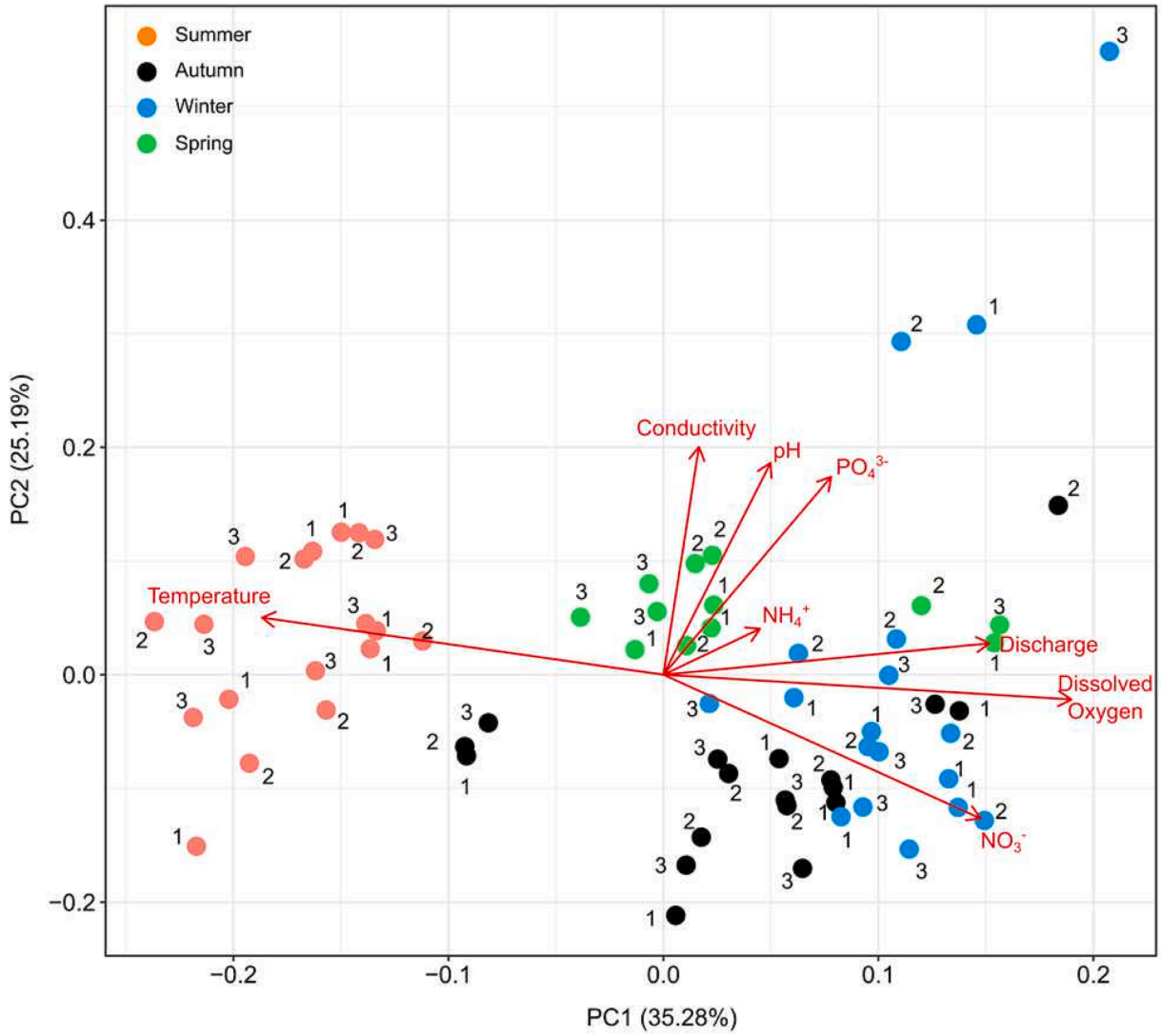

Fig. 3. Principal component analysis (PCA) applied to physicochemical parameters monitored in the Vipacco River during the present study.

in autumn and spring (Fig. 2i).

Regarding the PCA ordination, the first two axes explained 60.5 % of observed variability (Fig. 3), while the first three axes explained 77.7 % (Table S3). Discharge and concentration of dissolved oxygen and nitrates were positively related to the first axis PC1, while water temperature showed a strong negative correlation with the PC1 axis. Conductivity, pH, and phosphate concentrations were positively related to the second axis PC2, while ammonia was mostly related to the third axis (Table S3). Summer loads take place in the left side of the plot, associated to high temperatures, low discharge, and less oxygenated waters. Winter and autumn samples take place in the right side of the plot, associated to high nitrate levels, high discharges, and oxygenated

Table 1
Percentage frequencies of the macrobenthic invertebrate taxa observed in the Vipacco River through leaf bag technique in the present study. Each taxon in associated to a Functional Feeding Guild, *sensu* Merritt and Cummins (2017) (FFG: P = Predators; CG = Collector-gatherers; CF: Collector-filterers; SH = Shredders; SC = Scrapers: L = larvae).

			Summer			Autumn			Winter			Spring		
			1	2	3	1	2	3	1	2	3	1	2	3
Class/order	**Family/genus**	**FFG**												
Tricladida	*Dugesia*	PR	0.02	0.70	1.43									
	Dendrocoelum	PR		0.06	0.09				0.09					
	Planaria	PR		0.00	0.12									
Hirudinea	*Erpobdella*	PR	0.20	0.27	0.76		0.29	0.16	0.18	0.24	0.11	0.08	0.15	0.11
	Glossiphonia	PR	0.04	0.04	0.27			0.08						0.11
	Hemiclepsis	PR	0.04	0.06	0.79		0.14	0.08	0.09		0.23	0.13		0.14
Oligochaeta	*Eiseniella tetraedra*	CG					0.14		0.09			0.05		
Arachnida	Hydracarina	PR	2.58	1.22	0.40	0.11		0.23	0.18	0.16	0.11	8.14	4.31	0.84
Malacostraca	*Asellus*	SH	1.36	5.92	6.28	2.54	5.65	4.83	3.55	10.61	3.75	7.82	11.71	11.75
	Gammarus	SH	0.04	0.04	0.09				0.18	0.24		0.08	0.69	0.56
Gastropoda	*Bithynia*	SC	9.15	7.03	7.89		0.43	0.08	0.09	0.97	0.57	0.24	4.18	1.23
	Gyraulus	SC	0.07		0.21			0.08			1.25	0.11	0.10	0.98
	Lymnaea	SC	0.60			0.16			0.09		0.00	0.08	0.03	0.25
	Physa	SC	0.09	0.02	0.03	0.22	1.01	2.26	0.09		0.11	0.16		0.14
	Planorbis	SC												
	Theodoxus	SC	11.80	0.12	0.94	0.43			0.09			2.52	0.05	
	Valvata	SC	0.76	1.36	0.94	0.00	0.14						0.03	
Hexapoda														
Coleoptera	Dytiscidae (L)	PR	0.45	0.31	0.27									
	Elmis	CG	0.29	0.25	0.03	0.27	1.59	0.16	0.09			0.11	0.13	0.04
	Gyrinidae (L)	PR	0.20			0.16	0.14	0.16	0.00	0.16	0.11	0.03	0.03	
	Haliplidae	SH		0.02	0.06		0.14						0.05	
	Helicus substriatus	SH	0.02	0.02	0.09		1.16					0.40	0.10	0.88
	Hydraenidae	CG												0.07
	Hydrophilidae (L)	PR			0.03									
	Limnius	CG											0.08	
	Oulimnius	CG	17.72	16.01	33.24	2.64	6.52	4.98	2.22	4.05	17.71	10.52	13.43	30.62
	Stenelmis	CG	0.27	0.00	0.03							0.24		0.04
Ephemeroptera	*Caenis*	CG	4.43	0.10	0.24	4.53	1.59	0.93	1.15			3.21	0.33	0.35
	Ecdyonurus	SC		0.02										
	Ephemera	CG				0.05								
	Ephemerella	CG	0.09									1.18	0.51	0.11
	Paraleptophlebia	CG					0.14		0.09					
	Potamanthus	CG				0.05					0.11	0.08	0.10	0.42
Diptera	*Atherix ibis*	PR	0.05			0.11				0.08				
	Ceratopogonidae	PR				0.11		0.08	0.53		4.54	0.37	0.13	0.46
	Chironominae	CG	43.29	64.51	42.38	33.33	52.03	68.54	10.75	39.11	33.37	27.06	25.58	26.97
	Empididae	PR				0.11	0.14	0.16	0.09				0.08	
	Limoniidae	PR						0.08						
	Orthocladiinae	CG	0.49	0.39	0.58	51.62	22.03	13.94	76.73	39.19	31.10	34.29	36.06	21.15
	Psychodidae	CG	0.05			0.11		0.23						
	Simuliidae	CF				0.49	0.14	0.00	0.44					
	Tabanidae	PR						0.00	0.09					0.04
	Tanypodinae	PR	2.27	0.60	0.24	0.54		1.25	0.27	0.49	2.16	0.70	0.21	0.56
	Tipula	PR	0.02	0.08	0.03							0.11	0.03	
Heteroptera	*Aphelocheirus*	PR	0.22			0.27					0.11	0.11		
	Hydrometra	PR		0.08										
	Nepa	PR		0.02										
Odonata	*Calopteryx*	PR	0.09		0.03	0.16	1.01	0.16	0.44	0.57	0.34	0.16	0.05	0.00
	Coenagrionidae	PR	0.20	0.29	0.09	0.32	0.43	0.16	0.62	0.40	0.68	0.75	0.33	0.60
	Onychogomphus	PR									0.11			
Plecoptera	*Leuctra*	SH	0.24	0.06	0.00	0.05								
Trichoptera	*Hydropsiche*	CF	1.91		0.00	0.97	2.03	0.23	0.27	0.24		0.03		
	Hydroptila	SC	0.33		0.00	0.05			0.09		0.11	0.19	0.26	0.21
	Lepidostoma	SH	0.09		0.00	0.22	2.61	0.08	0.44	2.11	1.48	0.29	0.87	0.28
	Leptocerus	CG	0.11	0.19	0.43	0.11	0.29	0.70	0.18		1.14	0.48	0.23	1.02
	Limnephilidae	SH			0.03				0.53	1.21	0.57	0.08	0.08	0.00
	Mystacides	CG	0.34	0.14	0.49	0.27		0.23	0.18		0.23	0.11	0.03	0.04
	Oecetis	CG	0.09	0.10	1.43		0.14	0.08						
	Polycentropodidae	CF						0.08						
	Rhyacophila	PR						0.00	0.09	0.16		0.11	0.08	0.07

waters. Finally, spring loads are in the middle of the plot and are related to intermediate conditions. The correlation analysis performed via calculation of the Pearson coefficient (r) highlighted that water temperature was negatively related to dissolved oxygen ($r = -0.83$, $n = 60$, $p < 0.001$), nitrate ($r = -0.62$, $n = 60$, $p < 0.001$), and discharge ($r = -0.57$, $n = 60$, $p < 0.001$). pH was positively correlated to conductivity, phosphate, and ammonia ($r > -0.42$, $n = 60$, $p < 0.01$, for all comparisons). In order to avoid multicollinearity, the dissolved oxygen and NO_3^- concentrations were excluded from the multiple regression analysis, due to the strong correlation with temperature and discharge. Among the other nutrients, NH_4^+ was kept, due to the lower correlation, despite the r values was higher than the chosen threshold. We also choose to keep conductivity and pH in the model, due to the important contribution in the decomposition processes. Finally, discharge was kept due to the high contribution to the riverine dynamics, especially in a watercourse such as the Vipacco River.

3.2. Macrobenthic invertebrates

During the present study 30,410 macrobenthic invertebrate specimens were identified, belonging to 43 genera. 12,851 individuals were collected during summer, 3828 in autumn, 3242 in winter and the remaining 10,489 in spring. Composition of communities sampled via leaf bag technique is reported in Table 1.

Insects were always the dominant group, with frequencies ranging between 73.3 % and 96.6 % in the samples. Diptera was the most abundant taxon, mostly represented by the subfamily Chironominae (10.7–68.5 % of the samples), especially in summer and winter. Coleoptera was the second taxon in terms of frequencies (2.4–42.3 %), especially in summer and spring. The genus *Oulimnius* was the most frequent in the samples. Ephemeroptera and Trichoptera showed similar frequencies (0.1–6.2 % and 0.6–5.5 % respectively). Ephemeroptera were mostly recovered in spring (especially in site 1) and autumn, while Trichoptera were observed during the whole monitoring year. Among Ephemeroptera, genus *Caenis* was the most frequent (0.0–4.5 %), except during winter. Among the Trichoptera, *Lepidostoma* (0.0–2.6 %), *Hydropsyche* (0.0–1.9 %) and *Oecetis* (0.0–1.4 %) were the most abundant genera. Other insects were observed with percentages frequencies lower than 1.4 %. Gastropoda and Malacostraca were also abundant in the samples (0.4–22.5 % and 1.4–12.4 % respectively). Among Gastropoda, *Theodoxus* (0.0–11.8 %), *Bithynia* (0.0–9.1 %) and *Valvata* (0.0–2.6 %) were the most abundant genera, while among crustaceans *Asellus* (1.4–11.8 %) was the most frequent in the samples. The two-way PERMANOVA highlighted significant differences in community composition were observed both seasonally and among sites (Table S4) and the SIMPER test showed that the contribution to observed variability was mainly related to Chironomidae subfamilies (29.9 %), the coleopteran *Oulimnius* (10.9 %) and some Gastropoda (*Theodoxus*, *Bithynia*, *Valvata,* together 12.0 %). Variability was also related to the crustacean *Asellus*, to Hydracarina and to the mayfly *Caenis* (contributions respectively equal to 5.7 %, 4.3 % and 3.48 %), while contribution of other taxa was<2 %.

In terms of FFG, the communities were mostly represented by collector-gatherers (67.2–93.0 %), followed by shredders (1.7–14.2 %), and scrapers (0.9–22.8 %). Collector-gatherers were dominant in all the seasons and were mainly represented by Diptera Chironomidae, Trichoptera *Hydropsyche* and Coleoptera *Oulimnius*. The main taxa of shredders were crustaceans, especially *Asellus*. Shredders progressively increase their abundances in autumn, winter, and spring.

Scrapers increased their abundance in summer and included all the Gastropoda genera. Some taxa could act both as gatherers and scrapers, such as families Elmidae and Leptoceridae. Predators showed almost the same frequencies during the whole monitoring year (1.9–10.7 %). Finally, collector-filterers were the less abundant FFG (0.0–1.9 %), with only few specimens observed. *Hydropsyche* could be considered both as gatherer and/or filterer, but in the present study it was considered as a collector-gatherer. Due to these results, we decided to exclude collector-filterers from further analyses regarding decomposition rates.

3.3. Microplastics in sediment and water

Microplastics were detected both in sediment and water of the Vipacco River. Chemical types observed in the sediment samples were polypropylene (PP) polyurethane (PU), polyethylene terephthalate (PET), polyvinyl chloride (PVC) and polyethylene (PE) (Fig. 4a). PE and PET were the most abundant (30.6 %) followed by PP (16.7 %) and PVC (13.9 %), while PU showed the lowest frequency (8.3 %). Mean MP density in sediment samples was 300 ± 098.1 items kg^{-1}, while the mean size of microplastic particles was microplastics particles was 140 ± 250 μm.

A higher number of polymers was observed in the water than in sediment, as polystyrene (PS) and polyamide (PA) were also detected. PP and PET were the most abundant polymers in the water samples (35.5 % and 25.5 % respectively), followed by PA (16.0 %). Other chemical forms showed frequencies between 2.2 and 10.1 % (Fig. 4b). The mean density of microplastics in the waters of the Vipacco River was 0.313 ± 0.251 items m^{-3}, while the mean size of microplastic particles was microplastics particles was 460.2 ± 10.5 μm.

3.4. Microplastics in leaf bags and in FFG

An amount of 157 MP items were collected through leaf bags and the most abundant polymers were PP (24.5–36.4 %) and PET (15.8–29.7 %). PE and PA showed similar frequencies (10.2–21.2 % and 10.8–20.4 %, respectively), despite PA was not observed in the spring sampling campaign. Frequencies of PVC and PS were lower than 15 %, while PU items were observed only in summer, with lower occurrences than the other polymers (4.1 %) (Fig. 5a). The mean size of microplastic particles within the leaf bags was 344.7 ± 123.4 μm. The number of MP in the leaf bag did not differ significantly through the seasons, despite spring values showed a decreasing trend (Kruskal Wallis test: $H = 3.919$, $d.f. = 3$, $p = 0.27$) (Fig. 5b).

In macrobenthic invertebrates PP, PA, PET, PE, PS and PVC were found (Fig. 6a). PP (29.4–40.5 %) and PET (13.6–35.3 %) were the most abundant. PE and PS were observed in all samples but showed lower frequencies (7.1–23.5 % and 5.9–14.3 % respectively) than PP and PET while PVC (2.4–11.8 %) and PA (4.8–13.6 %) were less abundant than the other polymers. The mean size of microplastic particles within the leaf bags was 1016.1 ± 498.7.4 μm. Regarding the FFG, in summer and autumn the mean number of MP items per individual was significantly higher in the shredders than the other groups (Fig. 6b), while collectors-gatherers showed levels of contamination slightly but significantly higher than scrapers and predators (Kruskal Wallis test: $H = 11.258$, $d.f. = 2$, $p < 0.01$, Conover test: $p < 0.01$ for all comparisons). In autumn, levels of MP increased for scrapers. It was not possible to detect significant differences in spring among the considered FFG.

3.5. Decomposition rates

Decomposition of *Phragmites australis* in the Vipacco River fitted with negative exponential seasonal models (Fig. 7). Significant differences were highlighted for decomposition rates k ($days^{-1}$) between all the monitored seasons (ANCOVA: $F_{1,190} > 13.940$, $p < 0.001$ for all comparisons). The highest value was detected in summer ($k = 0.022$ $days^{-1}$) when decomposition was faster than in the other seasons. The organic matter breakdown became slower in autumn and winter ($k = 0.009$ and $k = 0.007$ $days^{-1}$, respectively). Then, the k value increased in spring ($k = 0.014$ $days^{-1}$).

Results of the stepwise multiple regression are showed in Table 2. In particular, 63.4 % of the observed variability in decomposition rates was related to seven significant factors: five were abiotic (water temperature, pH, conductivity, discharge, and number of MP items in the leaf

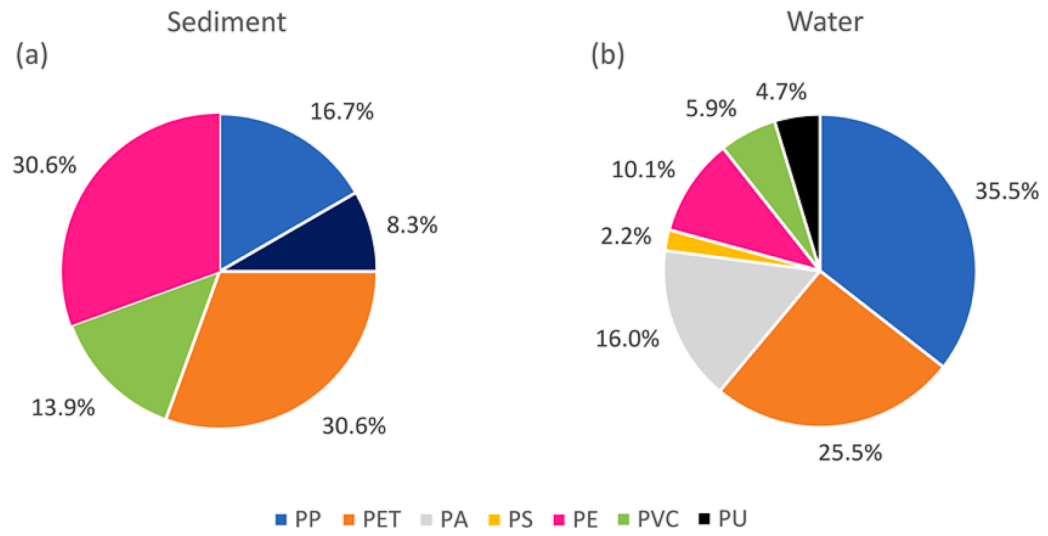

Fig. 4. Microplastic chemical composition observed in sediment (a) and water (b) samples of the Vipacco River during the present study (PP = polypropylene; PU = polyurethane; PET = polyethylene terephthalate; PVC = Polyvinyl chloride; PE = polyethylene, PA = polyamide; PS = polystyrene).

bags) and two were biotic (abundances of shredders and scrapers) (multiple stepwise regression: $r^2 = 0.634$; $F_{8,279} = 60.40$; $p < 0.001$). Water temperature showed the highest relative importance (73.0 %), positively affecting the decomposition rates, followed by pH (9.7 %), which showed a negative contribution. Another factor which contributed to increase the *k*-values was shredder abundance (3.9 %). Factors that showed a negative effect on decay rates were discharge (3.7 %), number of MP items in the leaf bags (3.1 %) and the abundance of scrapers (2.7 %). Concentration of NH_4^+ did not show significant contribution to the decomposition in the investigated watercourse (Table 2).

4. Discussion

The present study reports seasonal data regarding the decomposition process of *Phragmites australis* in a freshwater riverine ecosystem, in relation to the main biotic/abiotic features, also considering MP particles. These data highlight the negative effect due to MPs on riverine system dynamics through an experiment carried out directly in nature.

4.1. Physicochemical features

Values of water discharge observed in the Vipacco River were in line with those reported by Mosetti (1983), who indicated low levels with discharges equal to 1 $m^3 s^{-1}$ and flood events with peaks equal to 300 $m^3 s^{-1}$. These wide ranges are related to the great variability characterizing the karstic zones where the Vipacco River originates. Moreover, the Vipacco riverbed is formed by least permeable sediments that do not allow underground water losses and, therefore, the river is heavily affected by atmospheric regimes. In fact, as rainfall decrease during the summer, discharge is lower than in other seasons, affecting other water physiochemical parameters, as highlighted by the application of the PCA. Oxygen levels drop down in summer, according to decreasing discharge trends and to increasing temperatures. The condition characterized by of low water level, low oxygenation and increasing temperature could favor the algal blooming, especially in sites where water discharge points are present and higher nutrient concentration could be detected. However, hydrological regime of the Vipacco River, characterized by the occurrence of rapid discharge increasing, plays a fundamental role in maintaining a good auto-depuration dynamics of the watercourse.

Despite the Vipacco River flows within a scarce urbanized territory, where intensive agricultural and wastewater discharge point are present, conductivity values were related to pH, phosphate, and ammonia, and were in line with levels indicated by Forneris and Perosino (1995) for calcareous lowland areas. Conductivity and pH may also depend on carbonate transportation due to discharge, as the Vipacco springs are located in a karstic territory, as observed in some karstic water bodies in the same area (Bertoli et al., 2020, 2022). However, the pH values were in line with those reported by the same authors for lowland watercourse sections with macrophyte presence, characterized by weak or medium alkalinity. Water physicochemical features are in line with values reported by the Regional Agency for the Environmental Protection for the decade 2009–2019 (https://www.dati.friuliveneziagiulia.it). The main features that affect the investigated system appeared to be water temperature and discharge, as highlighted by the application of the PCA.

4.2. Microplastics in water and sediment samples

Concentrations of MP polymers observed in the samples are in line with ranges reported in relation to MP contamination in riverine ecosystems (Table S5). Despite our analysis was related to 2 km stretch of the watercourse, MP levels observed in water samples of the Vipacco River seem to be less dramatic than other freshwater watercourses of the Italian territory, such as Ticino, (Winkler et al., 2022), Ofanto (Campanale et al., 2020), Ombrone (Guerranti et al., 2017) and Po (Piehl et al., 2019). Moreover, the number of MP items observed in the water is lower than those reported for other European rivers as Seine (Dris et al., 2018), Rhone (Faure et al., 2015), Thames (Rowley et al., 2020), and for other great rivers (Yangtze and Hanjiang) in China (Wang et al, 2017). Concentrations of MPs observed in the Vipacco stretch similar to those reported for Danube in Austria (Lechner et al., 2014), and for the hydrological network of the Oregon State (Valine et al., 2020). On the other hand, MP concentrations in the Vipacco sediments were similar to those reported for rivers Thames (Horton et al., 2017) and Ottawa (Vermaire et al., 2017). It is important to point out that comparisons are very difficult and must be considered with great caution, because many factors are involved in MPs quantification, with special regard to sampling/extraction techniques and for the expression of the results (Kumar et al., 2021). Sampling strategies in riverine ecosystems are often difficult, and require different tools (Razeghi et al., 2021) such as manta nets (Moore et al., 2011) drift nets (Lechner et al., 2014), plankton nets (Dris et al., 2018) and MP traps (Lahens et al., 2018). For sediments, trawl nets and box corers are usually employed, but also shovels or trowels can be also used (Rocha-Santos and Duarte, 2015). Finally, the discharge

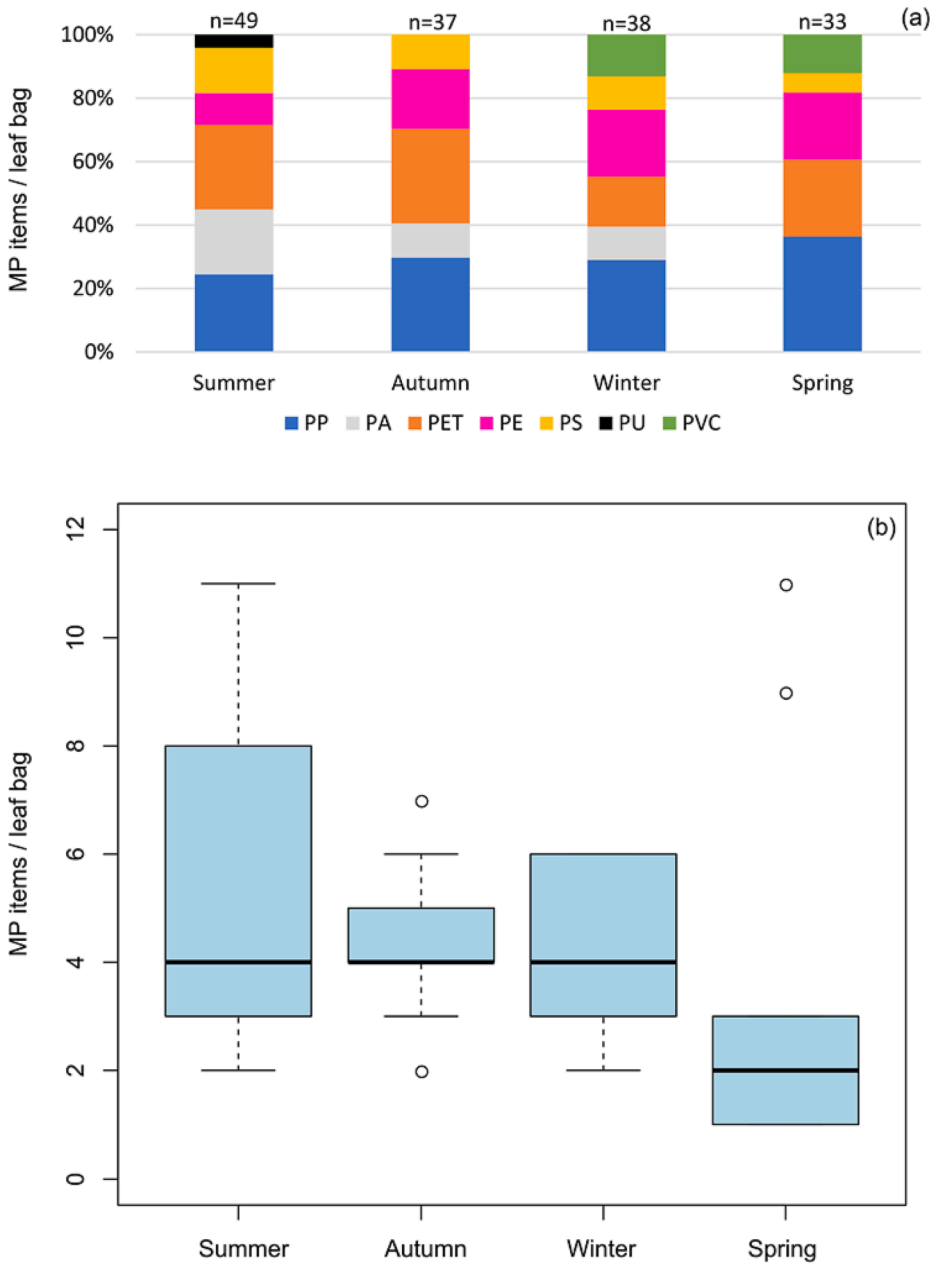

Fig. 5. Seasonal chemical composition (a) and seasonal microplastic accumulation (b) for microplastic observed in leaf bags (PP = polypropylene; PA = polyamide; PET = polyethylene terephthalate; PE = polyethylene, PS = polystyrene; PU = polyurethane; PVC = Polyvinyl chloride).

regimes must be considered, especially in watercourses with high level fluctuations such as the Vipacco River.

The most frequent polymers detected in the Vipacco samples were PET, PE and PP. PE and PP are used for used for packaging, containers, pipes, agricultural films, automotive parts, fishing gear and household items (Mishra et al., 2022). PET fibers are mainly used in clothes and represent the highest volume of artificial fibers produced worldwide (Alvarez Troncoso et al., 2022). However, PET is also a signal of high fibers dispersion from clothes through washing (Wang et al., 2019) and represent one of the most abundant microplastic recovered in riverine environments placed in urban areas and in industrial zones (Su et al., 2016; Wang et al., 2017), sewers (Talvitie et al., 2015) and atmosphere (Dris et al., 2016; Pastorino et al., 2020). The Italian stretch of the Vipacco River is placed in a scarcely urbanized territory, but wastewater discharge points are present (Fig. 1). This fact suggests that part of the observed MPs could be originated by clothes washing, as this is one of the major sources of plastic fibers in riverine ecosystems and in seas (Boucher and Friot, 2017; De Falco et al., 2019). On the other hand, PVC, PE, and PS can derive from industrial raw materials, waste from production processes or can be released into the environment during transport (Wang et al., 2019). The presence in the samples can be related to the land use in the Vipacco River area. Indeed, small industrial spots are present in the areas close to the watercourse, such as a production plant near the Rupa hamlet (Fig. 1) where PU, PVC and other polymers suitable for the production of synthetic leathers and fabrics are produced. Moreover, in the Slovenian territory upstream the State border near the Miren hamlet, two small plastic production areas exist.

4.3. *Macrobenthic invertebrates, leaf bags and microplastics*

The composition of macrobenthic invertebrate community is in line with those reported by ARSO (www.arso.gov.si) during routine environmental monitoring activities carried out in the Slovenian territory, upstream to the State border (Miren hamlet). Differences highlighted by

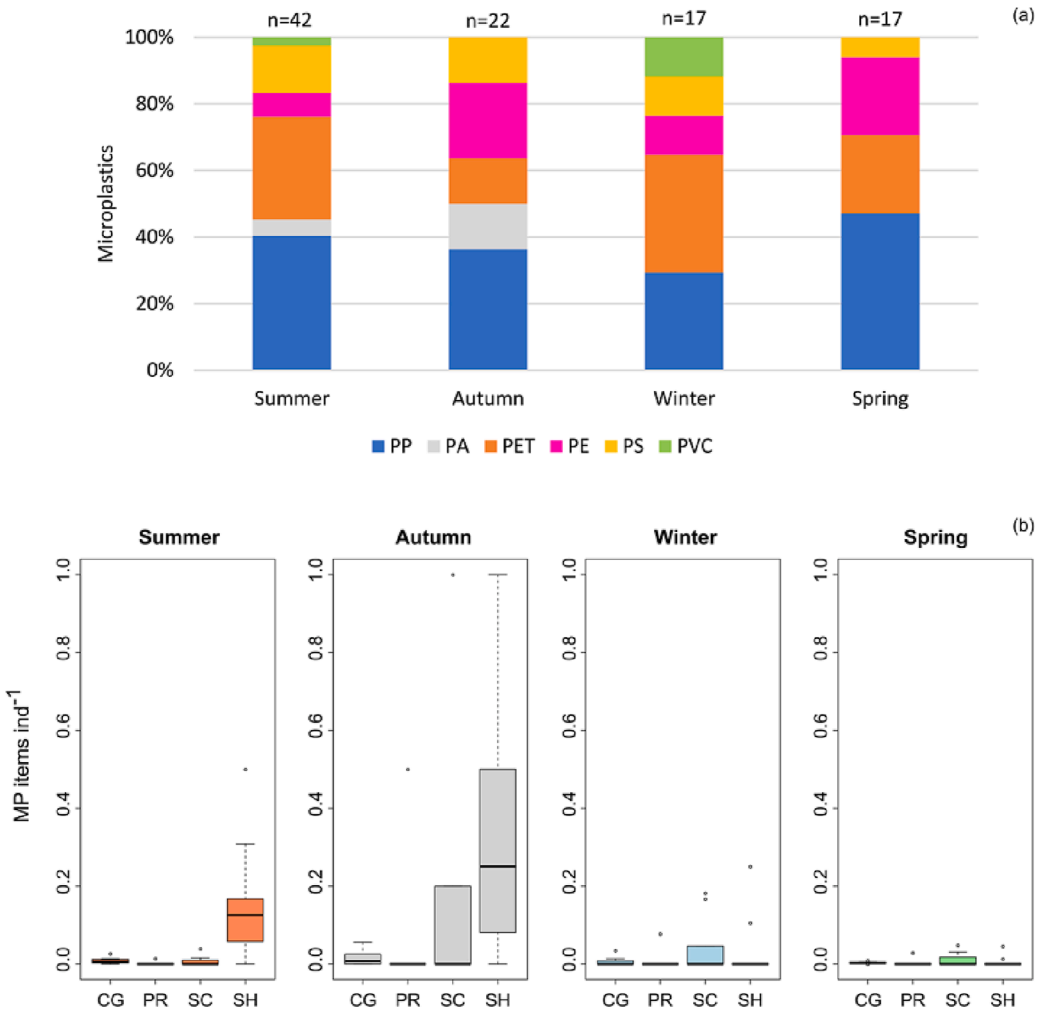

Fig. 6. Seasonal chemical composition for microplastic observed in macrobenthic invertebrates (a) and seasonal microplastic accumulation per individual in relation to the most abundant functional feeding guilds collected through leaf bag technique (FFG legend: P = Predators; CG = Collector-gatherers; SH = Shredders; SC = Scrapers; microplastic legend: PP = polypropylene; PA = polyamide; PET = polyethylene terephthalate; PS = polystyrene; PVC = Polyvinyl chloride).

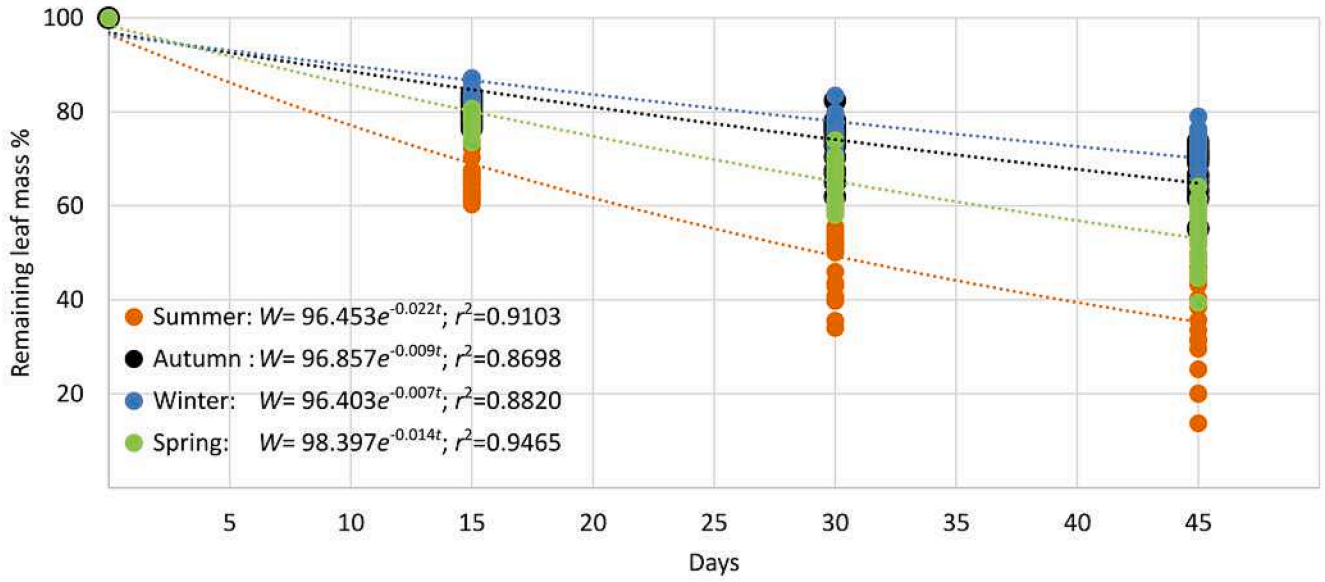

Fig. 7. Seasonal comparison of *P. australis* leaf mass decay calculated in the present study for the Italian stretch of the Vipacco River. The initial weight at $t = 0$ days (3.0000 ± 0.0001 g dry weight) was set at 100 %.

Table 2
Stepwise multiple regression analysis between organic matter decomposition rates (k) and biotic/abiotic features. Only significant regressors are reported.

	β	Estimate	St. error	t value	p-level	r^2	F	p-level	Relative importance (%)
Intercept		$6.007 \times e^{-02}$	$1.112 \times e^{-02}$	5.400	<0.001			<0.001	
Temperature	0.664	$7.505 \times e^{-04}$	$6.394 \times e^{-05}$	11.738	<0.001	0.573	372.741	<0.001	73.0
pH	−0.174	$-5.464 \times e^{-03}$	$1.278 \times e^{-03}$	−4.277	<0.001	0.591	200.602	<0.001	9.7
Scrapers	−0.086	$-8.935 \times e^{-05}$	$3.946 \times e^{-05}$	−2.264	0.024	0.603	109.797	<0.001	2.7
Shredders	0.123	$6.084 \times e^{-05}$	$2.251 \times e^{-05}$	2.702	0.007	0.613	141.028	<0.001	3.9
Microplastics	−0.111	$-1.102 \times e^{-03}$	$4.518 \times e^{-04}$	2.439	0.015	0.619	89.724	<0.001	3.1
Discharge	−0.133	$-4.839 \times e^{-04}$	$1.824 \times e^{-04}$	−2.652	0.008	0.626	77.134	<0.001	3.7
Conductivity	−0.079	$-3.094 \times e^{-05}$	$1.513 \times e^{-05}$	−2.044	0.042	0.630	68.065	<0.001	2.2
NH_4^+	0.072	$3.137 \times e^{-03}$	$1.771 \times e^{-03}$	1.771	0.078	0.634	8.279	>0.050	1.7

PERMANOVA, and SIMPER test could be related to life-cycle of the organisms and to their feeding habits. In all seasons the communities are dominated by Diptera Chironomidae, mostly represented by the Chironominae subfamily, which are collector-gatherers. The great abundance of Chironomidae could be related to the presence of fine organic matter deriving from leaf decomposition. This organic matter is one of the main trophic resources for chironomids colonizing the leaf bags and it could favor their colonization (Grubbs et al., 1995; Mathuriau and Chauvet, 2002; Ligeiro et al., 2010). These invertebrates can indirectly affect decomposition and microbial activities, as they modify the abiotic and trophic condition within the bags through teguments breakdown, fecal excretion or building tubes and cases (Ligeiro et al., 2010; Ágoston-Szabó et al., 2016). However, leaf bags also attract shredders and scrapers, which have a pivotal role in leaf fragmentation. In fact, observed variability depend also on Gastropoda (i.e., *Theodoxus*, *Bithynia* e *Valvata*). These scrapers increased their abundances during summer, as high-water temperatures favor periphyton growth, which is a food resource for Gastropoda. Some taxa can use the bags also as a refugia (Karàdi-Kovàcs et al., 2015), while the presence of predators within the leaf bags could be related to the high number of preys within the bags (Mutshekwa et al., 2020).

Microplastics were found both in macrobenthic invertebrates and in leaf bags matrices. Due to small size, MP fragments are easily ingested by benthic macroinvertebrates who mistake them for food fragments (Scherer et al., 2018), leading to contamination of a large part of the community (Windsor et al., 2019; Akindele et al., 2020). A former analysis carried out in the same river (Bertoli et al., 2021) reported that 48.5 % of the macrobenthic invertebrate taxa were contaminated by MPs, especially collector-gatherers, in contrast with results reported in the present study, where shredders were the most contaminated FFG. These different results are likely related to the sampling methods, as the previous study focused on a community collected with a Surber net. In addition, shredders are attracted by leaf bag as a trophic resource, giving a high contribution in the first phases of decomposition, when the highest mass loss is recorded (Bertoli et al., 2016; Baert, 2017). This is more evident in autumn, when the natural accumulation of vegetal debris is usually recorded in rivers. Presence of MPs in scrapers is also likely related to the decomposition processes. The detection of MP fragments in the scrapers agrees with results provided by Akindele et al. (2019), which reported high MP content for different Gastropoda species in riverine ecosystems from Nigeria.

PP, PET and PE were the most abundant polymers found in the leaf bags, considered as retention structure. These polymers correspond to the most frequent MPs recovered in water and sediment samples. Regarding the polymer types, a higher number was observed in water than in the sediment, in agreement with observation reported by Bertoli et al. (2021), who observed a similar trend for the same river. This could be related to the coarse grain size of the Vipacco riverbed, which is mainly coarse gravel, cobbles and sometimes boulders. Fine sediment particles settle gradually at low energy zones, e.g., stagnant water and high MP concentrations could be detected in fine sediments (Tibbetts et al., 2018; Corcoran et al., 2019; Hoellein et al., 2019; Kumar et al., 2021). Hydrological characteristics of the river, with high variability in water discharge, can also hinder the MPs sedimentation, favoring transportation and allowing more variability in the water column. On the other hand, all polymer types recorded in water samples were observed in the leaf bags. In this context litter accumulation, occurring in zones with low energy and reduced current velocity, seem to be very efficient to capture all MP types observed in water column. This finding seems to suggest that leaf bags could be considered as a tool to quantify and analyze the microplastic accumulation in leaf litter within riverine ecosystems. However, further analyses need to be addressed to investigate factors that can affect MPs accumulation.

4.4. Decomposition rates

Decomposition rates measured for the Vipacco River in the present study (0.007–0.022 $days^{-1}$) are in line with those reported for the *P. australis* leaf litter breakdown in the Mediterranean area, from studies carried out in other freshwater ecosystems (Table S6). Following the categorization provided by Petersen and Cummins (1974), the observed values allow to classify the Vipacco River as a “fast decomposition” system ($k > 0.010$) in summer and spring and “medium decomposition system” in autumn and winter ($0.005 < k < 0.010$).

The main driver of Vipacco decomposition dynamics is the seasonal temperature variation of riverine waters, that showed the highest contribution in terms of relative importance after the stepwise multiple regression (Table 2). This result was expected, as in many freshwater environments temperature is one of the most important features that positively affect leaf litter breakdown (Hanson et al., 1984; Webster and Benfield, 1986; Bedford, 2005; Sangiorgio et al., 2008; Eid et al., 2014; Bertoli et al., 2016, 2020, 2022): despite we did not study the microbial contribution to decay rates, it is known that increasing water temperatures determine an intensification of microbial processes conditioning the leaves, that served as main energy source for macrobenthic invertebrates in aquatic environments (Dudgeon, 1982; Bertoli et al., 2016). Microorganisms affect the palatability of the leaves, attracting macrobenthic invertebrates (Gessner et al., 1999) such as shredders. The combined effect of high temperatures and low water levels observed in summer (as showed by the PCA and correlation analyses results) could enhance these dynamics. Another factor that can usually give a positive contribution to decay rates is pH, which showed the second contribution in terms of relative importance. This parameter is generally associated to high decay rates, as it affects the fungal community composition (Webster and Benfield, 1986; Thompson and Bärlocher, 1989; Sangiorgio et al., 2008). However, the contribution observed in the presence study was negative. This fact could be also related to the discharge decreasing as the Vipacco River origins by karstic springs (alkaline water). With reduced discharges, the importance of water temperature increases its importance in system dynamics, while discharge decrease. Low pH levels could also lead to negative contribution to decomposition dynamics, as observed in the present study. Among other abiotic features, conductivity showed also a negative contribution, as generally observed in other studies (Webster and Benfield, 1986; van Dokkum et al., 2002; Bertoli et al., 2020), but in the present work the relative importance was<3 %, indicating a reduced role in the decomposition

dynamic. This could be again related to reduced discharge levels and few flooding events. As opposite, flooding events could carry high concentrations of carbonates from the springs to the downstream areas. Similar trends were observed in karstic springs in Italy and Slovenia (Dolinar et al., 2016; Bertoli et al., 2020, 2022). In the Vipacco River, conductivity become a less important driver for decomposition, compared to other factors. Among the biotic ones, shredders, showed a significant contribution to leaf litter decay rates. They could have a pivotal role in litter breakdown, positively affecting leaf decomposition through fragmentation (van Dokkum et al., 2002; Hieber and Gessner, 2002; Cornut et al., 2010; Raposeiro et al., 2017; Bertoli et al., 2020, 2022). Interestingly, the relative importance values observed for shredders was similar to the value recorded for MPs within the leaf bags (Table 2), which have a negative effect on the decomposition rates. Moreover, MPs detected in shredders were more abundant than in other FFG. It is reasonable to think that MP items retained by leaf litter could have a negative contribution to decomposition dynamic, contrasting the action of other factors, such as macrobenthic invertebrate decomposers, as observed by Lòpez-Rojo et al. (2020). The synergic action of water temperature could also interfere, as it can alter the impact of microplastic on metabolism and feeding rates, which normally increase with the temperature (Kratina et al., 2019). As macrobenthic invertebrates feed MPs, this could lead to damages to of the digestion tract and/or to false satiation and to a decreased activity of the decomposers (Ockeden et al., 2022). Opposite results were obtained by Silva et al. (2021b, 2022) which observed changes in macrobenthic communities but did not detect differences in leaf litter decomposition mediated by macrobenthic invertebrates in relation to MP exposure. However, this latter work was related to a short exposure period (eight days). Microplastic can also have direct effects on fungal activities and indirect consequences on shredders (Batista et al., 2022). Seena et al. (2019) showed that average litter decomposition decreased in relation to nanosized plastic exposition, due to interference with the fungal activities and similar results were reported by Du et al. (2022), which highlight that nanoplastics can inhibit stream leaf decomposition by affecting the microbial activity and the structure of the fungal community. In fact, plastic can slow down decomposition of leaves because it physically hinders microbes to access the leaves (Stangl, 2022).

However, results found in literature could be very difficult to compare, due to different study designs, and to the complex interplay among organisms, ecosystem processes and anthropogenic impacts, that could lead to divergent results (Lòpez-Rojo et al., 2020, Ma et al., 2020; Koelmans et al., 2022; Stangl, 2022). Moreover, many studies were carried on in laboratory under controlled conditions, while the present work was entirely performed on field.

Generally, the discharge is one of the main factors affecting decomposition in rivers, due to strong effect in the leaching phase, in relation to sediment and nutrient transportation (Sangiorgio et al., 2008). A positive contribution to the decomposition rate values was expected, especially in a system such as the Vipacco River, which is heavily influenced by discharge variation (Mosetti, 1983). In fact, during the study period the discharge varied between 1.6 and 54.4 $m^3 s^{-1}$, while the range reported by Mosetti (1983) was wider (1–300 $m^3 s^{-1}$). In the same area of the present study, a significant decrease of the rainfall was observed during the latter 30 years, associated to a significant increase of air temperature (Bertoli et al., 2022). This could be related to the global warming effects, that could affect river discharges, especially for a watercourse like the Vipacco River, which hydrometric level is strictly depending on precipitation regimes (Mosetti, 1983).

5. Conclusions

This study represents an attempt to analyze the organic matter decomposition rates in riverine ecosystems, in relation to biotic and abiotic features, including the MP items retained by the leaf bags.

The results presented herein provide new insights about the effects due to MP pollution on freshwater system dynamics: as it is well known that water temperature represents the main driver in decomposition processes and other drivers play a secondary role in decay dynamics, a significant negative contribution was found in relation to MPs items observed in the leaf bags. This contribution was similar to those recorded for other parameters, such as discharge, and the abundance of shredders which were among the most contaminated invertebrates observed in the present study.

As MPs are present globally in all freshwater environments, it would be of pivotal interest to extend the analyses also to watercourses with different hydrological characteristics, where water temperature could have a less marked effect than the watercourse analyzed herein (i.e., alluvial spring ecosystems or karstic systems) and other freshwater environments (lakes, wetlands). The leaf bag technique was used worldwide to study decomposition dynamics in many environments (such as rivers, lakes, wetlands, karstic systems) but it could be also a useful tool to analyze the effects of the MPs presence freshwater ecosystems, as leaf bags simulate the natural litter accumulation. Moreover, they could be used to analyze the MP retention in different conditions. A further step in riverine systems should regard the relationship between discharge and MPs accumulation in leaf litter. In fact, the reduction of the discharges observed in the last years in European rivers could represent and enhancing factor for pollutant effects, as the autodepuration riverine processes can be negatively affected by the climate changes. Finally, under a scenario of water scarcity, the effects of MPs (and other chemicals as pharmaceuticals attached to them) on macrobenthic invertebrates could be more important and need to be investigated in the future.

CRediT authorship contribution statement

Marco Bertoli: Writing – original draft, Investigation, Conceptualization, Methodology, Data curation, Supervision, Writing – review & editing. **Monia Renzi:** Investigation, Conceptualization, Methodology, Data curation, Writing – review & editing. **Paolo Pastorino:** Investigation, Conceptualization, Methodology, Data curation, Writing – review & editing. **Davide Lesa:** Investigation, Conceptualization, Methodology, Writing – review & editing. **Antonella Mele:** Investigation, Methodology, Writing – review & editing. **Serena Anselmi:** Investigation, Methodology, Writing – review & editing. **Damià Barceló:** Investigation, Conceptualization, Methodology, Writing – review & editing. **Marino Prearo:** Investigation, Conceptualization, Methodology, Writing – review & editing. **Elisabetta Pizzul:** Investigation, Conceptualization, Methodology, Data curation, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.109995.

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