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In situ effects of a microplastic mixture on the community structure of freshwater benthic macroinvertebrates

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Abstract:	Benthic communities represent some of the most threatened organisms in aquatic habitats due to the accumulation of plastic particles in sediments. The high abundance of microplastics (MPs) in sediments will continue to increase in the future, further increasing the probability of interactions between macroinvertebrates and MPs. In the present study, a benthic community in a pristine shallow pond was exposed either to an environmentally relevant high concentration of an MP mixture of 80 g m-2 in the sediment, or a control sediment, without the addition of MPs. The mixture of MPs contained irregular shaped polyethylene (PE), polyvinyl-chloride (PVC), and polyamide (PA) in a ratio of 50%: 25%: 25%, respectively. The in situ experiment lasted for 100 days. The total number of taxa that colonized the microcosms was 22 (17 in the control, 18 in the MP treatment). The most dominant group within the macroinvertebrate community was the dipteran family Chironomidae, both in the control and the MP treatment. No significant differences in the abundance and biomass at a community level were recorded between the groups by PERMANOVA ((F=0.993; p=0.456 and F=0.344; p=0.797, respectively). The mixture of MPs did not influence the abundance or biomass of the functional feeding groups (F=1.810; p=0.137 and F=0.377; p=0.736, respectively). The species richness (S), species abundance (N), species biomass (B), Shannon's diversity index (H) and Simpson's index of diversity (D) showed no statistically significant differences between the control and treatment groups. Czekanowski's quantitative similarity index indicated that 84% of the community remained unaffected after MPs exposure.

In situ effects of microplastic on benthic communities

In situ effects of a microplastic mixture on the community structure of benthic macroinvertebrates in a freshwater pond

Abstract

Benthic communities contain some of the most threatened organisms in aquatic habitats due to different anthropogenic pressures. The high abundance of microplastics (MPs) in sediments will continue to increase in the future, further increasing the probability of interactions between macroinvertebrates and MPs. In the present study, a benthic community in a relatively pristine shallow pond was exposed either to an environmentally relevant high concentration of an MP mixture of 80 g m^{-2} in the sediment, or a control sediment, without the addition of MPs. The mixture of MPs contained irregular shaped polyethylene (PE), polyvinyl-chloride (PVC), and polyamide (PA) in a ratio of 50%: 25%: 25%, respectively. The *in situ* experiment lasted for 100 days. The total number of taxa that colonized the microcosms was 22 (17 in the control, 18 in the MP treatment), and the colonization was not affected by the treatment. The most dominant group within the macroinvertebrate community was the dipteran family Chironomidae, both in the control and the MP treatment. No significant differences in the abundance and biomass at a community level were recorded between the groups by PERMANOVA ((F=0.993; p=0.456 and F=0.344; p=0.797, respectively). The mixture of MPs did not influence the abundance or biomass of the functional feeding groups (F=1.810; p=0.137 and F=0.377; p=0.736, respectively). The species richness (S), species abundance (N), species biomass (B), Shannon's diversity index (H) and Simpson's index of diversity (D) showed no statistically significant differences between the control and treatment groups. Czekanowski's quantitative similarity index indicated that 84% of the community remained unaffected after MPs exposure.

Keywords

Microplastics, Microcosms, Colonization, Benthos, Community structure, Sediment.

1. Introduction

Plastic particles within a size range from 1 µm to 5 mm are commonly known as microplastics (MPs), although the size range is often arbitrary, and the definition is subjective (Sorensen & Jovanović 2021). The world's plastic production still has an ascending trend with a staggering production of 359 million MT in 2018 (Plastics Europe 2019). In addition to this concern, approximately five billion metric tons of plastic remain in the environment, accumulated since the year 1950 (Geyer et al. 2017), which will eventually degrade into MPs.

When MPs enter the aquatic habitat, they potentially affect the aquatic biota in various ways (Scherer et al. 2018; Rezania et al. 2018; Villarrubia-Gómez et al. 2018). More precisely, MPs alter the parameters that are important for the life cycle of living organisms. Large concentrations of MPs and additives have been confirmed to be harmful for the ecophysiological functions performed by organisms (Browne et al. 2013). The presence of MPs on the water surface (Eriksen et al. 2013; Faure et al. 2015) interferes with sunlight by blocking it, while accelerating the aging of the MPs. MPs can affect the photosynthesis rate of phytoplankton, and the action of the biological pump, causing more greenhouse gas emissions (Shen et al. 2020). In the water column, MPs float and have the role of a vector for different chemicals or aquatic organisms (Mato et al, 2001; Rochman et al. 2013; Koelmans et al. 2013). MPs are bioavailable to a wide range of species, especially to filter-feeding groups (Scherer et al. 2017; Wagner & Lambert 2018). Finally, almost every particle over time reaches the sediments (Tibbetts et al. 2018), whether because of its original density, biofouling microbial activity (Arias-Andres et al. 2018), or by fecal pellets of organisms that have already ingested MPs (Cole et al. 2016).

The extent of exposure to MPs and its intake, beside its higher presence due to constant accumulation over time, is pronounced in benthic fauna due to their inability to discriminate between MPs and food particles (Bellasi et al. 2020). In comparison to marine ecosystems, studies on the potential adverse effects on aquatic biota caused by MP exposures are scarce for freshwater environments (Wagner & Lambert 2018). The available studies have documented various effects of MPs on living organisms, expressed as decreased filtration rate (Pedersen et al. 2020), MPs transfer from larvae to adult (Setyorini et al. 2021), reduced emergence and survival (Zlajahromi et al. 2018), reduction in growth (Ziajahromi et al. 2018; Redondo-Hasselerharm et al. 2018), lower assimilation efficiency in *Gammarus fossarum* (Straub et al. 2017; Blarer & Burkhardt-Holm 2016), as well as mouthpart deformities and postponed developmental time in *Chironomus riparius* (Silva et al. 2019; Stanković et al. 2020).

Various types of toxicity tests have been performed in recent decades to estimate the effect of MPs on benthic aquatic biota. The laboratory approach is mainly based on single-species toxicity tests and exposure to single polymer types of MPs (Scherer et al. 2017; Redondo-Hasselerharm et al. 2018; Silva et al. 2019; Ziajahromi et al. 2018, 2019). Field studies based on the passive monitoring of freshwater ecosystems have investigated the presence and quantities of MPs within freshwater sediments (Corcoran et al. 2015; Klein et al. 2015; Castañeda et al. 2014; González-Pleiter et al. 2020) and in aquatic organisms (Su et al. 2018; Scherer et al. 2017; Hurley et al. 2017). Only a few studies have been conducted in microcosms or mesocosms (Redondo-Hasselerharm et al. 2020; Fueser et al. 2020; Huang et al. 2021). So far, only Redondo-Hasselerharm et al. (2020) have investigated the effects of MPs on a benthic macroinvertebrate community in an outdoor experiment setup at an experimental field station.

Benthic macroinvertebrates are recognized as reliable indicators of the ecological condition of water bodies. They are good indicators because they spend all or most of their lives in the water, they are easy to collect, and they differ in their tolerance to pollution. Furthermore, macroinvertebrate assemblages reflect the state of local conditions since they are relatively sedentary and can only move short distances. The changes in structure and composition of benthic macroinvertebrate communities can reflect changes in the environmental quality (Rosenberg & Resh 1993). Environmental stressors affect community composition through the loss of sensitive taxa, while opportunistic (tolerant) species become dominant (Gray 1989). In addition, variation in the species diversity of aquatic ecosystems provides valuable information regarding the water quality status. Consequently, several diversity indices have been proposed, in order to obtain additional information about changes in community structure (Magurran 2004).

The aim of this study is to investigate the *in situ* effects of exposure to an MP mixture on the macroinvertebrate community structure within a natural freshwater ecosystem. It was hypothesized that the exposure of benthic macroinvertebrates to MPs will cause a change in (1) the community structure in terms of qualitative composition, abundance, and biomass, (2) the trophic structure of the community represented by functional feeding groups (FFG), (3) the species frequency and dominance, and (4) the α and β diversity indices in the form of a decline.

2. Methodology

2.1. Experimental setup of microcosms

The methodology as per Jovanović et al. (2016) was followed to the highest extent possible. To assure that the experiment would be performed in an environment with low MP pollution, a pristine pond in Barje Čiflik village (43.12911N, 22.543506E), Pirot, Serbia was selected (Figure 1).

Figure 1. Experimental pond

The upper sediment layer (mud) was collected from the pond and processed through a sieve with a 5 mm mesh size, to remove all large debris. The sediment was further sterilized in order to remove all macroinvertebrates. The total organic carbon (TOC) in the sediment was determined following the standard procedure and methodology (Walkley & Black 1934). The percentage of TOC was 1.76 ± 0.15 (mean ± standard deviation) of dry weight, as determined using three replicates.

The experimental sediment consisted of two layers. The bottom layer was sterilized artificial sand and the upper layer was sterilized sediment collected from the experimental pond. Sterilization was performed separately for each layer at 180 ºC in the oven for 2 h. To spike the sediment (after cooling down) with MPs, a mixture of ultra-high molecular weight polyethylene (PE, surface modified, 40-48 µm particle size, Aldrich Chemistry cat. no. 434272), high molecular weight polyvinyl chloride (Aldrich Chemistry cat. no. 81387) and polyamide for column chromatography (Carl Roth cat. no. 9620.1) was added in powder form to the sterilized mud. The characterization of the MPs is presented in Table 1.

Table 1. Characterization of 100 particles per polymer used for the experiment: L- length [µm]; W- width [µm].

	PA		PVC		PE	
Specific gravitation	1.14 g/mL		1.4 g/mL		0.94 g/mL	
Particle dimensions	L [µm]	W [µm]	L [µm]	W [µm]	L [µm]	W[µm]
Average	43.66	27.98	90.43	66.15	41.10	30.68
SD	13.74	8.98	40.77	31.57	11.60	9.17

The MPs were examined using a Leica MZ16A stereomicroscope with 10 X/21 B ocular and 50 X objective magnification. All three polymers were irregularly shaped. The environmentally relevant concentration of MPs was considered to be 8 g m^{-2} of dry sediment, following previous calculations (Stanković et al. 2020). The MP treatment consisted of 80 g m^{-2}, 10 X higher than an environmentally relevant concentration, representing the worst-case scenario. The control group was not spiked with MPs, while the MP treatment consisted of PE, PVC, and PA in a ratio of 50%: 25%:25%, respectively. In order to prepare the MP treatment, 32 kg of sterilized sediment was mixed with 80 g of previously defined MP mixture. To ensure uniform distribution of the polymers throughout the sediment, the entire mixture was stirred for 30 minutes in 3 repetitions using a drilling machine (Bosch GSB 18-2 RE) and a special kneading attachment. In total, 40 trays were used for the experimental setup: 20 for the control and 20 for the MP treatment. The dimensions of the trays were 25 x 20 x 6 cm (L x W x H). All of the trays consisted of two layers: sterilized artificial sand (the lower layer) and sterilized sediment from the pond (the upper layer) with a ratio of 1:1. The trays were submerged into the pond in 4 transects of 5 replicas per control/treatment (Figure 2). The replicas in each transect were connected with a rope and metal rings. Each tray was fixed to a metal ring with 4 strings of equal length. In addition, each tray was attached to the adjacent tray in the same line to improve stability and pushed into the sediment, so that the top part of the plastic tray and the surrounding sediment in the lake were nearly in the same plane.

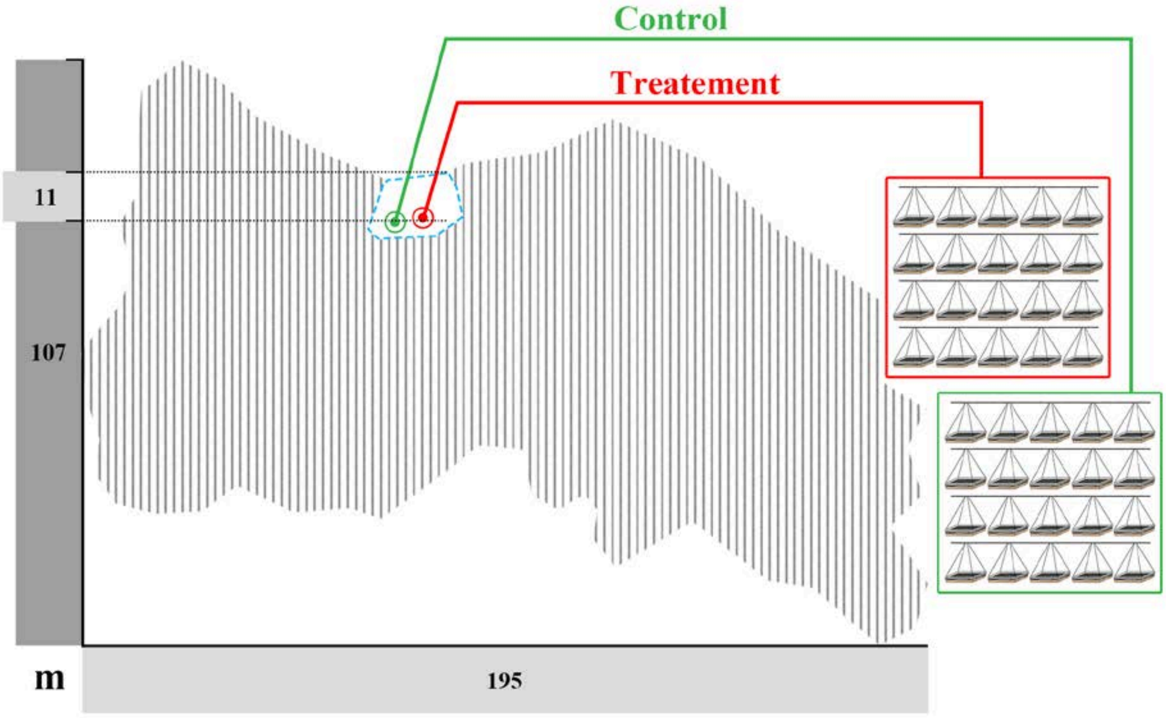

Figure 2. Position of the control and treatment experimental trays in the experimental pond. The hatched area represents overgrown part of the pond; blue circular line defines vegetation-free part of the pond.

The experiment lasted for 100 days (October 14-January 21). The time-frame and period of the year were selected to control the natural seasonal variability of the community structure caused by the phenology patterns of aquatic insects. After 100 days, the trays were carefully collected. All organisms from the samples were collected and preserved in 70% ethyl alcohol. The specimens were identified at the lowest possible taxonomic level with the use of relevant identification keys for the proper taxonomic group (Brinkhurst & Cook 1974; Hrabě 1981; Macan & Cooper 1994; Nilsson 1997; Pfleger 2000; Vallenduuk & Moller Pillot 2007). The concentration of miroplastic particles in the experimental trays was not measured at the end of the experiment, as there are methodological constraints of extracting microplastics from the sediments, separate the mixture of different polymers into specific polymer types and expressing the concentration in terms of mass for each polymer type.

To estimate the biomass, each species was dried in an oven at 105 ˚C (Leuven et al. 1985). All specimens of the same species from the same sample were placed in a glass petri dish and dried for 2 hours. After this period, the species were measured on an analytic scale and returned to the oven for another 1h. The weights remained the same after the second drying, which was considered as the final dry weight.

To apply the trait-based approach, all of the macroinvertebrate species were categorized into one of the five feeding categories: gathering collectors, filtering collectors, scrapers, shredders, or predators according to Schmidt-Kloiber & Hering (2015) (Table 2). The FFG abundance and FFG biomass were calculated for each plot, for both treatment and control.

Both frequency and dominance were determined according to Tischler's method (1949). Frequency (F) was expressed as a percentage of samples in which a given species was found, as follows: F= 75–100 % – euconstant; F= 50–75 % – constant; F= 25–50 % – accessory; F= 0–25 % – accident. Dominance (D) was expressed as the relative abundance of a species within a community and presented in the scale: D> 10 % – eudominant; D= 5–10 % – dominant; D= 2–5 % – subdominant; D= 1–2 % – recedent; D< 1 – subrecedent.

The α diversity was estimated as 1) Shannon's diversity index (H), calculated for each tray as

$$H = -\sum pi\ lnpi$$

where pi is the relative abundance of taxon i, calculated as the proportion of individuals in a given taxon in relation to the total number of individuals in the community, and n is the number of taxa in the community. The relative community abundance (N) was calculated for each tray as the total sum of the individual species present in each experimental unit, expressed as # individuals per cm, and statistically compared with the paired two-tail t-test, and 2) Simpson's index of diversity (D), sometimes dubbed as Simpson's evenness, that presents the probability that two individuals randomly selected from a sample will belong to different species according to the following formula:

$$D = 1 - \frac{\sum ni(ni - 1)}{N(N - 1)}$$

where n is the total number of specimens from a particular species and N is the total number of specimens from all species (Simpson 1949).

The β diversity was measured by 1) the Jaccard similarity index, calculated for all trays combined as

$$Sj = c/(A + B - c),$$

2) the Sørensen-Dice qualitative similarity index as

$$Ss = 2c/(A + B),$$

where A is the total number of species in the control, B is the total number of species in the treatment, and c is the number of shared species, and finally, 3) Czekanowski's quantitative similarity index (S_C), calculated for all trays combined as

$$Sc = 2\sum c\ (A + B)$$

where A and B are the community absolute abundance for the treatment and control, respectively, while c is the lowest abundance of all species common to both groups. Czekanowski's similarity index is recommended as the most convenient index for measuring the level of impact of a toxicant on a community, since it can describe the most important changes in the community after the introduction of a toxicant into an ecosystem (Sanchez-Bayo and Goka 2012; Jovanović et al. 2016).

Species richness (S), species abundance (N), species biomass (B), Shannon's diversity index (H), and Simpson's index of diversity (D) for the control and treatment groups were statistically compared with the Mann–Whitney test using SPSS software version 15.0 (SPSS Inc, Chicago, IL, USA).

2.2. Data analysis

Non-parametric multidimensional scaling (NMDS) was used to visualize any differences in the qualitative and quantitative structure of the macroinvertebrate community. To test the effect of the MP mixture on the macroinvertebrate community, a distance-based permutational multivariate analysis of variance (PERMANOVA) was applied. The fixed factor for the analysis was the mixture of MPs. All analyses were based on Bray-Curtis dissimilarities of previously transformed data (fourth root).

To investigate whether the MP treatment affected FFG composition within the macroinvertebrate community, PERMANOVA analysis based on Bray-Curtis dissimilarities was used.

3. Results

All 40 trays were successfully collected and analyzed. In total, 22 taxa were identified in samples - 17 taxa in the control trays and 18 taxa in the treatment trays, with a total abundance of 415 and 504 specimens, respectively. The distributional pattern of sampling sites (trays) on an NMDS plot did not reveal any differences in terms of the community structure presented by the taxa abundance and biomass (Figure 3). In addition, the PERMANOVA results showed no differences in community structure in terms of both abundance and biomass between the control and treatment trays (F=0.993; p=0.456 and F=0.344; p=0.797, respectively). Similarly, the MP treatment did not influence the FFG abundance or FFG biomass within the macroinvertebrate community (F=1.810; p=0.137 and F=0.377; p=0.736, respectively). Finally, no statistically significant differences were detected between the control and treatment groups for species richness (S), species abundance (N), biomass (B), Shannon's diversity index (H), or Simpson's index of diversity (D) (Table 3). Czekanowski's quantitative similarity index value was 0.84, indicating that 84% of the community remained unaffected by the MP treatment, while only 16% was affected, either positively or negatively. The Jaccard and Sørensen-Dice qualitative similarity indices had values of 0.66 and 0.80, respectively, indicating that the Jaccard similarity index measured less similarity in the qualitative species composition between the control and treatment groups (Table 3).

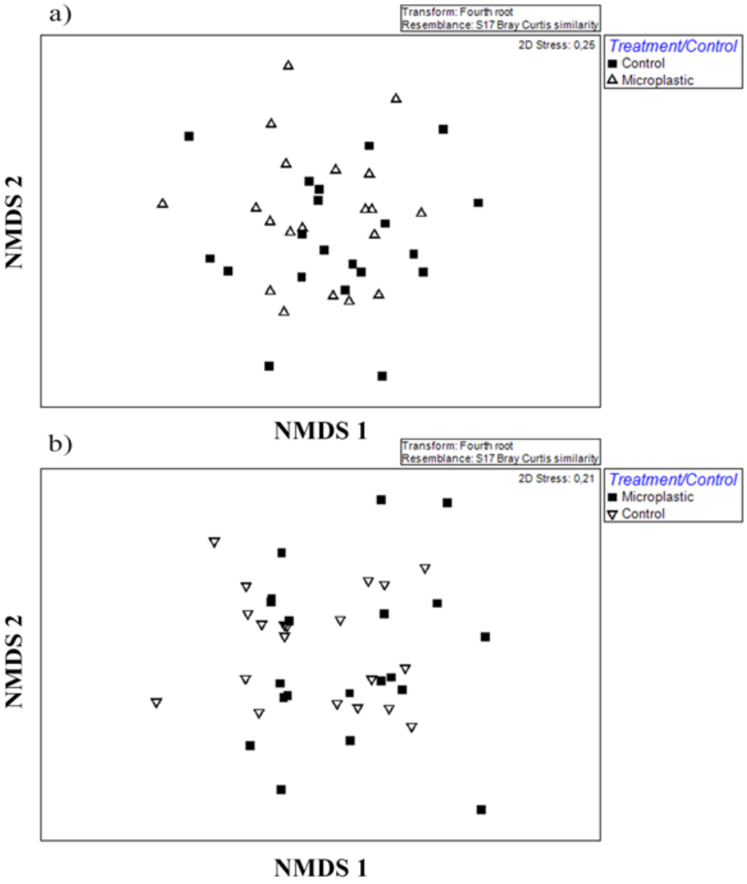

Figure 3. The nMDS plots showing the distributional pattern of sampling sites in terms of the community structure, presented by (a) taxa abundance and (b) biomass. NMDS 1 - ; NMDS 2- .

The most dominant group in the experimental trays was ordo Diptera, Insecta, with an extremely high abundance of the family Chironomidae, followed by Gastropoda. The dominant species both in the control and the MP treatment was *Chironomus plumosus*.

Table 2. Functional feeding group composition (FFG), dominance (D), and frequency (C) within the macroinvertebrate community in the MP treatment and control.

	Treatment	**Control**	**Treatment**	**Control**

	FFG	Dominance		Frequency	
Acroloxus lacustris **(Linnaeus, 1758)**	*gra/scr*	E	E	EC	C
Bithynia tentaculata **(Linnaeus, 1758)**	*gra/scr*	D	D	C	C
Lithoglyphus naticoides **(C. Pfeiffer, 1828)**	*c-g*	R	R	AD	AD
Physella acuta **(Draparnaud, 1805)**	*gra/scr*	E	E	C	EC
Peregriana peregra **(Müller, 1774)**	*gra/scr*	R	S	AC	AC
Planorbis carinatus **Müller, 1774**	*gra/scr*	E	E	EC	EC
Pisidiusm **sp.**	*c-f*	S	S	AC	AC
Haplotaxis gordioides **(Hartmann, 1821)**	*c-g*	-	SR	-	AD
Helobdella stagnalis **(Linnaeus, 1758)**	*prd*	S	S	AC	AD
Culicoides simulator **Edwards, 1939**	*prd*	S	S	AC	AD

Ceratopogon **sp.**	*prd*	S	-	AD	-
Chironomus plumosus agg	*c-g*	E	E	EC	EC
Chironomus riparius **Meigen, 1804**	*c-g*	-	SR	-	AD
Chironomus tentans **Fabricius, 1805**	*c-g*	SR	SR	AD	AD
Chironomus vallenduuki **Ashe & O'Connor 2015**	*c-g*	SR	SR	AD	AD
Chironomus sp.	*c-g*	SR	-	AD	-
Dicrotendipes lobiger **(Kieffer, 1921)**	*c-g*	-	SR	-	AD
Endochironomus gr.dispar	*c-g*	SR	SR	AD	AD
Procladius sp.	*prd*	SR	-	AD	-
Psectrocladius sp.	*c-g*	SR	-	AD	-

Note: EC – euconstant; C – constant; AC – accessory; AD – accident.

E– eudominant; D– dominant; S – subdominant; R – recedent; SR – subrecedent.

scr- scrapers (grazers); c-g – collector gathers, c-f collector filters, prd- predators.

Table 3. Differences in the community structure of benthic macroinvertebrates exposed to MPs: S- species richness; N- species abundance; B- biomass; H- Shannon's diversity index; D- Simpson's index of diversity; Sj- Jaccard similarity index; Ss- Sørensen-Dice qualitative similarity indices; Sc- Czekanowski's quantitative similarity index.

	Treatment (mean ± SE)	**Control (mean ± SE)**	**Overall value**
S	7±1.86	6.65±1.22	
N	25.25±7.92	20.75±6.86	
B	0.05±0.02	0.05±0.02	
H	1.64±0.28	1.66±0.17	
D	0.78±0.10	0.81±0.04	
Sj			0.66
Ss			0.80
Sc			0.84

4. Discussion

MPs are currently recognized as a major environmental problem for freshwater ecosystems, while at the same time freshwater ecosystems serve as the main receptors of MPs from various inland activities (Silva et al. 2020). In addition, the aquatic biota is affected by a wide variety of types, sizes, shapes, and physicochemical properties of MPs. The most commonly studied polymers are polyethylene (PE) and polystyrene (PS), followed by polypropylene (PP) and polyester (PES) (de Sá et al. 2018). In this study, we used a mixture composed of three polymer types (PE, PVC, PA), which are among the top 10 most widely used in industry and daily life (Plastics Europe 2018). Moreover, the mixture mainly consisted of high-density polymers to ensure that they remained in the sediment.

So far, previous studies have demonstrated the ingestion rate of MPs for numerous taxa (Scherer et al. 2017; Fueser et al. 2019, 2020). However, the implications of MP ingestion are still unclear. De Sá et al. (2018) stressed that there is a lack of knowledge on the effects of various types of MPs on all groups of organisms except fish and small crustaceans. For instance, gastropods are widely known as organisms that have a high capacity to ingest MPs (Wagner & Lambert 2018). However, grazing freshwater gastropods have a digestive system that enables the separation of digestible and non-digestible particles (Weber et al. 2021). In the present study, Gastropoda (Mollusca) was the second most dominant group of aquatic macroinvertebrates in both the treatment and control trays. Based on the results, there was no observed difference in the gastropod community composition between the treatment and control trays. It is, therefore, assumed that their evolutionary adaption to a particle-rich environment may be a key mechanism limiting MP toxicity (Weber et al. 2021). For instance, Weber et al. (2021) clearly emphasized that despite the high level of ingestion, MPs particles neither affected the growth nor the reproduction of the freshwater gastropod species *Lymnaea stagnalis* (Linnaeus, 1758). In addition, chironomids, a biomass-rich group (Milosević et al. 2020), and the most dominant group in the present study, did not show any structural differences between the treatment and control groups. This result is similar to a previous study in which indoor experiments (Silva et al. 2019; Stanković et al. 2020) showed the absence of any lethal effects (mortality rate) of MPs. However, the previously discovered sublethal effects of MPs, such as morphological changes in the mouthparts and wings of chironomids (Stanković et al. 2020), may not necessarily be reflected in the community structure, at least not within one season. Silva et al. (2021) confirmed that the retention of MPs larger than 10 µm in the gut of *C. riparius* may cause mechanical/proteolytic damage in the epithelial cells of the gut lumen and induce an immune response through the activation of the phenoloxidase system. The previous outdoor study with a similar experimental setup showed that from chironomids, only the representants of the subfamily Orthocladiinae reacted to nanoplastics, significantly changing their abundances (Redondo-Hasselerharm et al. 2020). In the present study, the chironomid community was mainly composed of Chironominae larvae (Table 2) which in comparison to orthoclads were in general more resilient to anthropogenic degradation (Milošević et al. 2013).

Furthermore, knowledge about the uptake and biological effects of MPs mostly comes from laboratory studies, often with limited environmental relevance due to the simplified ecotoxicological protocols (e.g., one single plastic polymer and size and/or extremely high concentrations, with short exposure time) (Scherer et al. 2020). Conclusions obtained from laboratory experiments should be considered cautiously since they may fail to provide the ecological realism required to detect such ecological implications. Additionally, they lack the ecological processes that drive community change in the long term, such as various abiotic and biotic interactions. Scherer et al. (2017) showed that the presence of natural matter (food, sediment) significantly reduces the uptake of MPs by freshwater invertebrates. More precisely, the internal exposure seems to be lower in the chronic toxicity tests and experiments in natural habitats, where the availability of particulate matter is much higher. Therefore, the studies investigating the effects of MPs should be more frequently conducted under field conditions and for much longer periods, in order to take all these processes into account (Redondo-Hasselerharm et al. 2020).

Our investigation is the first *in situ* study that evaluates the influence of an MP mixture on the aquatic macroinvertebrate community in terms of not only abundance but also the biomass and composition of functional feeding groups. So far, only Redondo-Hasselerharm et al. (2020) have conducted an outdoor experiment to evaluate the long-term effects of nano- and microplastics on the natural recolonization of sediments by a macroinvertebrate community under ecologically realistic conditions. More precisely, their study reported the effects of different concentrations of irregular polystyrene fragments on the abundance and diversity of the freshwater macroinvertebrate community. The results showed a lower total abundance of macroinvertebrates, which was correlated with a lower number of Naididae worms, while the total number of macroinvertebrate taxa and Shannon diversity index remained unaffected.

Based on the present experiment, the MP mixture of irregularly shaped PE, PVC, and PA in a concentration 10 X higher than an environmentally relevant had no effect on the macroinvertebrate community abundance and biomass. The qualitative similarity indices used in this study, especially the Jaccard similarity index, showed a minor difference in terms of community structure in treatment and control. However, qualitative similarity indices are based on the number of species in common and the number of species present for the assemblages investigated. Their main disadvantages are the equal treatment of rare and abundant species (Jovanović et al. 2016). Thus, quantitative indices that reflect abundance have been increasingly used. Czekanowski's quantitative similarity index, as a convenient indicator of toxicity effects of various pollutants, revealed that only 16 % of the community reacted to MP pollution. Thus, based on this result, it can be confirmed that this concentration of MPs had a minor effect on the macroinvertebrate community studied here.

The results showed no difference in the abundance and biomass of FFG within the macroinvertebrate community when exposed to MPs. Even though many authors have confirmed that the feeding type greatly affects ingestion of MPs in aquatic biota (Scherer et al. 2017), we believe that the high uptake rate of individuals does not necessarily cause significant or negative effects at the community level. This is particularly true for freshwater gastropods that have become tolerant to MPs due to specific adaptations, despite high uptake rates (Weber et al. 2021), or for freshwater Oligochaeta whose MP exposure did not express any lethal or sublethal effects (Silva et al. 2020; Castro et al. 2020) as well as for amphipod and isopod crustaceans (Redondo-Hasselerharm et al. 2020).

No statistically significant differences in species richness (S), species abundance (N), species biomass (B), Shannon's diversity index (H), or Simpson's index (D) between the control and treatment groups were observed. These diversity metrics describe the community structure, which is shaped by local environmental factors, dispersal, and biotic interactions (Medeiros et al. 2020).

As a result, the number of publications investigating interactions with the environment is rapidly growing. The number of publications per year investigating this topic has increased by 2,300%, compared to only 10 years ago (Sorensen & Jovanović, 2021). In conclusion, to fill all the gaps in MP pollution knowledge, both laboratory and *in situ* field studies are needed. However, microcosm studies seem to be the most convenient approach to understanding the effects of MP pollution on the environment. The impact of many aquatic toxicants has been investigated from the cellular to population levels of organization, but data on the community level are still lacking for many of them (Jovanović et al. 2016). In this regard, de Sá et al. (2018) clearly emphasized that it is highly recommended for researchers to publish even negative results. Considering the societal concern about the increase of MPs in the environment, the present investigation greatly contributes to the knowledge about the potential of their negative effects. In the present study colonization was not affected by the MPs and the immediate impact of MPs on the community structure is negligible, likely due to the lack of mortality and slow, but long-term, interaction. However, since the MPs are present in the sediment over multiple generations of benthic organisms, than the change in the community structure may become significant after several seasons due to the sublethal effects previously mentioned. Thus, further investigation should be focused on carrying out more long-term outdoor experiments, which will encompass various freshwater habitats inhabited by different hydrobiocenoses, whose vulnerability to MPs is still unknown.

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Figure 1. Experimental pond

Figure 2. Illustration of transects with experimental trays.

Figure 3. The nMDS plots showing the distributional pattern of sampling sites in terms of the community structure, presented by (a) taxa abundance and (b) biomass

Graphical abstract caption

Exposure of freshwater benthic macroinvertebrate community to high concentration of microplastic mixture of irregular shaped polyethylene (PE), polyvinyl-chloride (PVC), and polyamide (PA), followed with control.