



# Does deforestation contribute to microplastic contamination in stream fish in the Southwestern Amazon?

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**Abstract** This study investigated the presence of microplastics (MPs) in fish from streams with different levels of forest cover in the southwestern Amazon. Fish were collected using seine and dip nets along an 80 m stretch of 20 streams. Among the 584 fish analyzed, 196 individuals (33%) contained microplastics in their digestive tracts, with MPs detected in all species examined. Blue and black fibers <0.1 mm were the predominant types of MPs found in the analyzed fish species. Polyester, polyamide and polypropylene

were identified as the main polymers. Vegetation cover in micro-watersheds influenced MPs ingestion by Amazonian stream fish, with more MPs found in the digestive tract of fishes from deforested areas compared to those from forested ones. Environmental characteristics, such as seasonal precipitation patterns, and ecological traits, including feeding strategies and habitat use, were also important drivers of MP incorporation by fish species. Our results indicate that fish from sparsely forested streams could become even more susceptible to microplastic contamination as the frequency and intensity of drought and flood events change.

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## Introduction

The generation of waste has increased proportionally to the growth of the global population (Das et al., 2023). According to Kaza et al. (2018), the volume of solid waste generated globally was 0.68 billion tons in 2002, reaching 2 billion tons in 2018. Furthermore, Perreard et al. (2024) reported that global plastic waste production surpassed 220,606 kilotons in 2024, with projections indicating that it could reach approximately 3.4 billion tons annually by 2050 (Voukkali et al., 2024). Plastic pollution becomes even more alarming when these materials degrade into microplastics (MPs). These MPs, which can be pellets, fibers, hard fragments, or films and range in size from 0.1 to 5 mm (Thompson et al., 2004; 2024), are highly dispersible and persist in the environment for extended periods (Stride et al., 2024), posing a severe global environmental threat (Jiang et al., 2019).

Direct sources of MPs into water bodies include discharges from sewage treatment plants (Browne, 2015), erosion and degradation of plastic waste in water bodies (Eerkes-Medrano et al., 2015) and terrestrial inputs from soil erosion or surface runoff (Horton et al., 2017). Rainfall events are also predominant, as they promote the leaching of soils near rivers and streams, introducing plastic waste into the aquatic environment (Su et al. 2020a). Deforestation is usually driven by regional policies, cattle ranching, mining, and illegal logging (Chen et al., 2024). The removal of riparian vegetation increases the input of human-sourced materials into the aquatic ecosystems (Casatti et al., 2012), including MPs. In this context, this vegetation serves as a natural barrier against pollution, acting as “forest filters” (Michael & Michael, 1998; Granados-Sánchez et al., 2024) and may temporarily retain MPs (Jiao et al., 2024; Gallitelli & Scalici, 2024).

In the context of the Amazon biome, research on MPs is still in its early stages. The first article on this topic was published in 2018 (Wen et al., 2018), analyzing MP ingestion by cichlid fish from Amazon rivers. However, the knowledge gap regarding MP pollution in the Amazon Basin remains significant

(Morais et al., 2024). Therefore, the impacts related to the presence of MPs in this region are: low performance of waste collection, recycling and disposal systems; low coverage of sewage and waste treatment networks, especially in urban centers; lack of sustainable practices and use of alternative materials (plastic free) in sectors such as fishing, agriculture (Morais et al., 2024) and mass tourism, which leads to high production of sewage and garbage, excessive use of water resources and unregulated urbanization (Davenport & Davenport, 2006) and habitat degradation, with an emphasis on deforestation (Morais et al., 2024). The Amazon River is already recognized as the seventh most polluted river by plastics globally (Lebreton et al., 2017; Giarrizzo et al., 2019). Although it is commonly perceived that Amazonian rivers traverse an isolated region covered by the world’s largest tropical rainforest, approximately 38 million people live in the Amazon Basin (Serrão & Thompson, 2005). Thus, this region has experienced accelerated economic growth, accompanied by the highest urbanization rate in Brazil in recent years (Becker et al., 2011).

Rivers play a crucial role as transportation routes for agricultural products, mining, and oil, but these activities also generate negative environmental impacts (dos Santos Silva et al., 2024), such as the introduction of macro- and microplastics into this environment (Liro et al., 2023; 2025). In their extensive review, Salerno et al. (2021) demonstrated that MPs negatively impact the feeding, behavior, and development of various fish species. The ingestion of MPs by fish promotes mechanical lesions, low growth rates, decreased fecundity (Ma et al. 2020a, b), reduction in feeding capacity, inflammatory responses, alterations in the intestinal microbiome (Parenti et al., 2019), and changes to behavior, metabolic processes, and swimming performance (Wen et al. 2018) and even death (Ma et al. 2020a). Moreover, MPs can act as vectors for pollutants (Fu et al., 2021), contributing to bioaccumulation and biomagnification (Mammo et al., 2020). These issues have generated growing public concern, leading to an exponential increase in studies on the ecological impacts of MPs (Zhang et al., 2021). In this regard, research analyzing MP ingestion by aquatic organisms, considering ecological factors such as trophic position and habitat characteristics, has gained attention, although results remain inconsistent (Dantas et al., 2020).

This study investigated the presence of MPs in fish from streams with different levels of forest cover in the southwestern Amazon. The objectives were: (i) to describe the composition, frequency, abundance, and diversity of MPs in the digestive tracts of fish; (ii) to evaluate which species, feeding zones, and trophic categories are more susceptible to MP ingestion; and (iii) to determine the influence of vegetation cover and periods (dry and rainy seasons) on MP ingestion across fish species. Based on the only previous study on Amazonian streams (Ribeiro-Brasil et al., 2020), the following hypotheses were proposed: (i) all fish species are contaminated with different types of MPs, (ii) the abundance and diversity of ingested MPs vary according to species, feeding zones and trophic categories and (iii) fishes from forested and deforested streams, sampled in different periods, are contaminated with MPs due to acute and chronic sources of contamination.

## Materials and methods

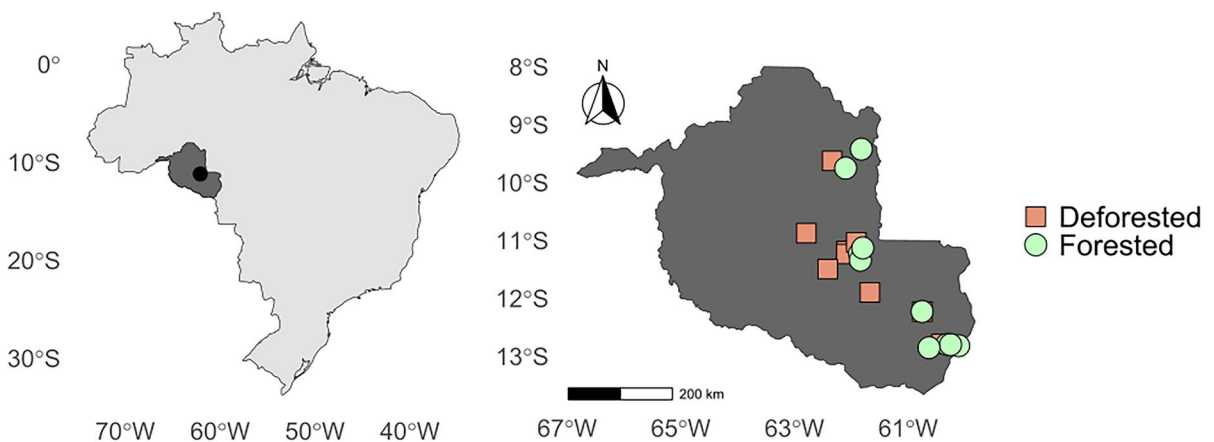
### Study area

The study was conducted in 20 s- and third-order streams, classified according to the Horton–Strahler system (Horton, 1932). These streams were identified using hydrographic maps at a 1:100,000 scale, covering the Rio Machado basin (also known as the Ji-Paraná River), which spans 75,400 km<sup>2</sup> in the state of Rondônia, Brazil (9–13° 30' S, 63–61° 30' W; Fig. 1).

The Rio Machado, a seventh-order river, is formed by the confluence of the Pimenta Bueno and Comemoração rivers, carrying an average annual discharge of 700 m<sup>3</sup>/s toward the Madeira River (Krusche et al., 2005). The region is characterized by ancient soils marked by high acidity, intense weathering, low fertility, and reduced cation exchange capacity (Krusche et al., 2005). The climate features average annual temperatures range of 19 and 33 °C and approximately 2500 mm of annual rainfall (Krusche et al., 2005). The region has two well-defined climatic seasons: a dry season from May to September and a rainy period from October to April (Fernandes & Guimarães, 2002). Sources of MPs introduction are observed in the streams of the Machado River basin, such as domestic effluent pipes (Fig. 2A, B), disposal of solid waste (Fig. 2C) besides severe erosion of river banks (Fig. 2D).

### Fish sampling

Sampling was conducted in 10 streams within the Rio Machado basin with less than 25% mature forest cover in their micro-watersheds (mean  $11.81 \pm 8.73\%$ ) and 10 streams with more than 70% mature forest cover (mean  $78.25 \pm 24.70\%$ ). Land use and forest cover analysis were performed using Landsat 5 TM satellite images (2011, 30×30 m resolution) provided by the Brazilian National Institute for Space Research (Instituto Nacional de Pesquisas Espaciais, [www.dgi.inpe.br/CDSR](http://www.dgi.inpe.br/CDSR)). These images were classified into three main categories: primary forest and pasture (absence



**Fig. 1** Sampling site location on 20 low-order Amazonian streams of the Machado River Basin studied during dry season

**Fig. 2** Source of Mps in the Machado River basin, Rondônia, Brazil. Domestic effluent pipes (A, B), disposal of solid waste on the banks (C) and erosion of river banks (D)



of forest), using the Supervised Maximum Likelihood method (Jensen, 2000) in Erdas 9.2 software. In the ArcGIS 9.3 software, a thematic map of soil use and the contours of the sampled micro basins were added, and the areas of each category of soil use within each microbasin were calculated using the Tabulate Areas tool. We used this information to get the percentage cover of each use category within the micro basins.

Sampling occurred during the dry and rainy seasons of 2017 and 2018. In each stream, three collectors conducted intensive fish sampling along 80 m stretches, which were previously blocked with 0.5 cm mesh nets to prevent fish escape. Sampling was performed during the day, for up to 1 h, using 2 mm mesh seines and dip nets. Captured specimens were euthanized with an overdose of eugenol, fixed in 10% formalin, and subsequently transferred to 70% ethanol. All specimens were deposited in the fish collection of the Department of Zoology and Botany at

São Paulo State University (Universidade Estadual Paulista, UNESP—São José do Rio Preto campus), Brazil (DZSJRP 14335 to DZSJRP 19916). The species were classified based on their diet into categories such as algivorous, omnivorous, piscivorous, insectivorous, invertivorous, herbivorous, and detritivorous,

**Table 1** Results of the analysis of variance testing the relationship between microplastic abundance and forest cover (FC)=deforested and forested streams, seasons (dry and rainy period), feeding zone (FZ) and trophic category (TC) in fish from the Machado River, Rondônia, Brazil

MPs abundance	Df	Sum Sq	Mean Sq	F value	P-value
FC	1	12.60	12.62	4.42	<b>0.04</b>
Periods	1	20.40	20.39	7.15	<b>0.01</b>
FZ	2	11.40	5.69	1.99	0.14
TC	7	63.90	9.12	3.20	<b>0.00</b>
Residuals	556	1557.52	2.80		

and according to their position in the water column (feeding zone) as pelagic, benthic, or benthopelagic, based on existing literature (Table 1). Fish sampling was conducted under authorization from the Chico Mendes Institute for Biodiversity Conservation (Instituto Chico Mendes de Conservação da Biodiversidade, ICMBio—authorization number 47345-1/2014).

### Digestive tract and filtration

For each fish analyzed, standard length (SL, in cm) and wet weight (in g) were recorded. Digestive tracts were removed, stored in individually cleaned test tubes, subjected to a wet oxidation process using 10% KOH, and maintained at 40 °C in an incubator for 48 h, adapted from Garcia et al. (2020). After oxidation, the samples were filtered using Whatman GF/C cellulose acetate filters (0.45 µm pore size) coupled to a vacuum pump, adapted from Garcia et al. (2020).

### Observation and identification of MPs

After the oxidation process, non-plastic residues were separated from synthetic plastics based on characteristics such as color, texture, gloss, and behavior under pressure, such as flaking and crumbling (Hidalgo-Ruz et al., 2012). Particles identified as MPs ranging from 0.1 to 5 mm were characterized under a Zeiss Stemi 305 compact stereo microscope with up to 80× magnification. MPs were morphologically classified as fibers (elongated shapes), fragments (angular or irregular shapes, hard or soft), films (irregular shapes, but all flat, flexible and thinner than fragments, and may have smooth or angular edges), or pellets (small plastic sphere) (Montagner et al., 2021). Additionally, they were categorized by color according to Galgani et al. (2013).

For polymer composition analysis, 87 MPs (30% of the total) were randomly selected. These particles were evenly distributed across samples and subjected to Fourier-transform infrared spectroscopy (FTIR) following the method described by Frias & Nash (2019). Selected particles were prepared using a Thermo diamond anvil compression cell, and infrared spectra were obtained with a Nicolet® Continuum Nexus spectrophotometer equipped with a coupled microscope (15× objective lens) and

liquid nitrogen-cooled MCT-A detector. Spectra were recorded in transmission mode, covering the range of 4000 to 700 cm<sup>-1</sup>, with a resolution of 4 cm<sup>-1</sup> and 128 scans. Only particles with a match greater than 85% compared to reference spectra available in the Open Specy—MicroplasticSpectra database (developed by Cowger et al., 2021) were considered as MPs.

### Quality assurance and quality control procedures (QA–QC)

Following rigorous quality assurance and quality control standards (Schymanski et al., 2021), several measures were implemented to prevent atmospheric contamination by microplastics (MPs) during collection and analysis. All equipment used was washed with filtered water and covered with aluminum foil, both in the field and in the laboratory. During collection, two previously washed Petri dishes with Milli-Q water were used as field controls and left open at the sampling site. In the laboratory, these dishes were filled with filtered water and subjected to the same analytical procedures as the main samples. In the laboratory, instruments were rinsed three times with filtered Milli-Q water and analytical-grade acetone before analysis and were kept protected with aluminum foil. Plastic equipment was avoided, prioritizing aluminum trays, glass containers, and the use of cotton clothing to minimize contamination sources. The analyses were conducted in a controlled environment without external ventilation or air conditioning, further reducing the risk of atmospheric MP deposition. The reagents used for sample digestion were pre-filtered with a vacuum pump and Whatman GF/C glass microfiber filters (0.45 µm). To monitor possible contamination in the laboratory environment (Bessa et al., 2018), three Petri dishes were placed near each sample during the analyses. A control dish was filled with Milli-Q filtered water and also subjected to filtration to check for the presence of MPs. No MP particles were detected in the control samples, confirming the absence of contamination during field and laboratory procedures (Bhat et al., 2024; Tauruzzi et al., 2024).

### Data analysis

The frequency of occurrence of MPs in species was calculated using the formula  $FO \% = (Ni/N) \times 100$ ,

where FO%=frequency of occurrence of MPs; Ni=number of individuals of species *i* with MPs in the digestive tract; and *N*=total number of individuals sampled of species *i*. To test the relationships between the number of MPs and the length (SL) and trophic level (TL) of individuals, a bivariate linear regression was used (Zar, 1999). The trophic level of each species was determined based on the FishBase platform (Froese & Pauly, 2024). The tests were conducted using the *t* test and lm functions from the stats and ggplot2 packages in R software (R Core Team, 2021). Analysis of variance (ANOVA) was used to assess the effects of forest cover (forest and deforested), periods (dry and rainy seasons), feeding zone (bentopelagic, benthic, and pelagic) and trophic category (algivorous, carnivorous, omnivorous, piscivorous, insectivorous, invertivorous, herbivorous and detritivorous) on MP abundance, as well as interactions between these factors (Anderson, 2001). The assumptions of normality (Shapiro-Wilks test) and homoscedasticity (Levene's test) were confirmed (Zar, 1999).

A non-metric multidimensional scaling (nMDS) analysis was conducted to examine the dissimilarity of MP composition in fish from forested and deforested streams, based on the abundance of each type of MP, also considering the trophic category and feeding area. Abundance data were previously transformed using  $\log x + 1$ . The dissimilarity matrix was constructed using the Bray–Curtis index. The first axis scores of the nMDS were compared using a *t*-test with the *t.test* function (Wheeler & Torchiano, 2016). A Similarity Percentage (SIMPER) analysis was performed to indicate the individual contribution of each type of MP (> 1% contribution) found in the six species with abundance > 30 individuals to the dissimilarity between forested and deforested streams (Oksanen et al., 2017). ANOVA, *t*-tests, regressions, and SIMPER analyses were conducted using R software (R Core Team, 2021), while nMDS was calculated using PAST software (version 2.1.7, Hammer et al., 2001).

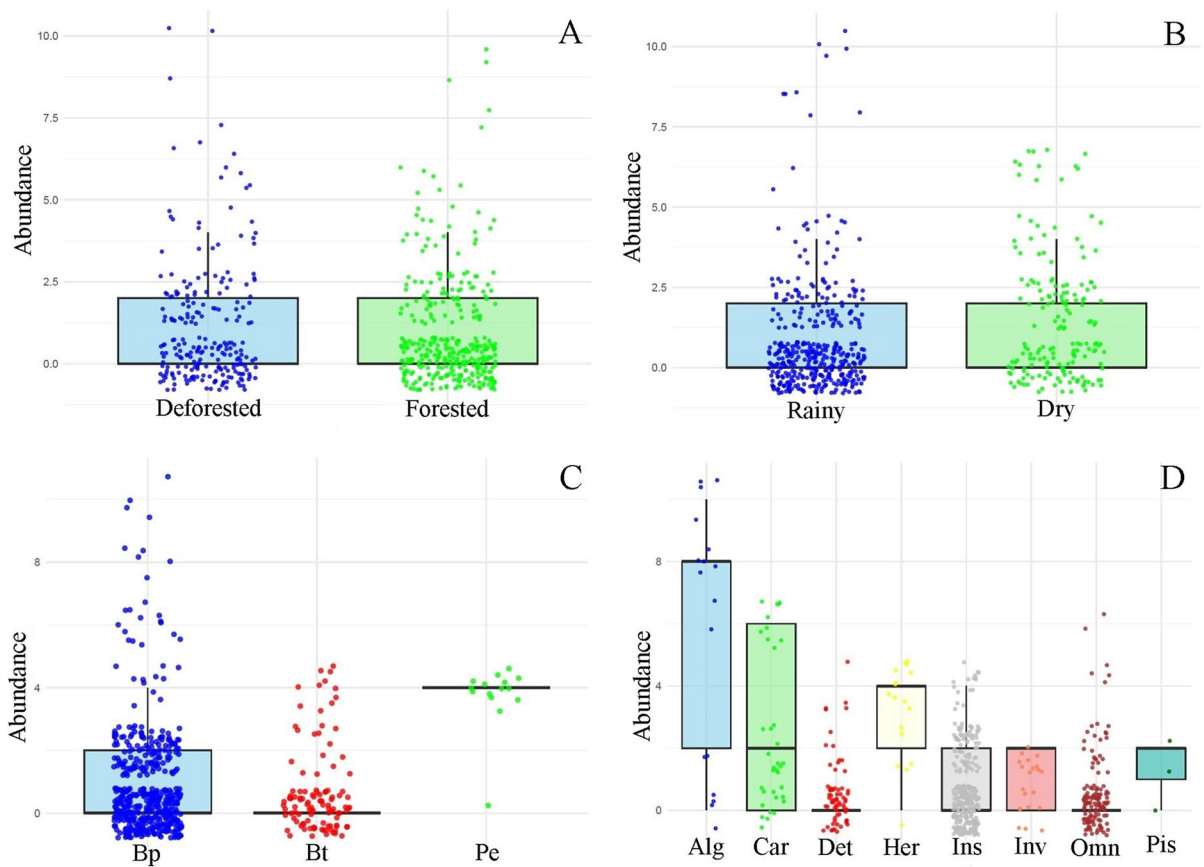
## Results

Out of 584 fish analyzed, 196 (33%) had MPs in their digestive tracts. All species (53 spp.) presented MPs, with an average of  $0.5 \pm 0.7$  MPs per individual

(Table 1). A total of 352 fish were sampled from forested streams and micro-watersheds, of which 103 (29%) had MPs. In deforested streams, a total of 232 individuals were analyzed, and 93 (40%) had ingested MPs. A total of 292 MPs ( $0.5 \pm 0.8$ ) were observed in the fish, with 137 ( $0.4 \pm 0.8$ ) from fish in forested streams and 155 ( $0.5 \pm 0.9$ ) from fish in deforested streams. The abundance of MPs showed no significant correlation with the fish length ( $F=2115.0$ ,  $R^2=0.04$ ,  $p=0.42$ ,  $n=584$ ) or with trophic level (TL) ( $F=2219.9$ ,  $R^2=0.03$ ,  $p=0.47$ ;  $n=56$ ).

The average abundance of MPs was significantly different between forested and deforested streams ( $F=4.42$ ;  $p=0.04$ ), periods ( $F=7.15$ ;  $p=0.01$ ), trophic category ( $F=3.20$ ;  $p=0.001$ ), and trophic level ( $F=2.24$ ;  $p=0.001$ ) (Table 1). Fish from deforested streams ( $1.1 \pm 1.8$ ) ingested on average 37% more MPs than those from forested streams ( $0.8 \pm 1.6$ ) (Fig. 3A). Individuals sampled during the rainy season ( $1.4 \pm 1.8$ ) had more MPs than those sampled during the dry season ( $0.8 \pm 1.3$ ) (Fig. 3B). The prevalence of MPs was higher in benthic fish ( $1.2 \pm 2.2$ ) compared to bentopelagic ( $0.9 \pm 1.5$ ) and pelagic fish ( $0.5 \pm 1.1$ ) (Fig. 3C). Piscivorous ingested more MPs ( $3.3 \pm 3.0$ ), followed by algivorous ( $2.0 \pm 1.8$ ), detritivorous ( $1.7 \pm 2.7$ ), herbivorous ( $1.2 \pm 1.7$ ), omnivorous ( $0.9 \pm 1.7$ ), carnivorous ( $0.8 \pm 1.2$ ), insectivorous ( $0.7 \pm 1.4$ ) and invertivorous ( $0.7 \pm 0.9$ ) (Fig. 3D).

Also, the concentration of MPs was different between forested and deforested streams ( $F=4.50$ ;  $p=0.03$ ), periods ( $F=7.27$ ;  $p=0.00$ ), trophic category ( $F=3.20$ ;  $p=0.00$ ), and trophic level ( $F=2.28$ ;  $p=0.00$ ). Fish from deforested streams ( $0.3 \pm 0.6$  MPs/g) presented higher concentrations of MPs than those from forested streams ( $0.2 \pm 0.5$  MPs/g) (Supplementary Fig. S1A). Individuals sampled during the rainy season ( $0.3 \pm 0.6$  MPs/g) had more MPs than those sampled during the dry one ( $0.2 \pm 0.4$  MPs/g) (Supplementary Fig. S1B). The prevalence of MPs was higher in benthic fish ( $0.4 \pm 0.6$  MPs/g) compared to bentopelagic ( $0.3 \pm 0.5$  MPs/g) and pelagic fish ( $0.1 \pm 0.3$  MPs/g) (Supplementary Fig. S1C). Piscivorous ingested more MPs ( $1.1 \pm 1.0$  MPs/g), followed by algivorous ( $0.6 \pm 0.8$  MPs/g), detritivorous ( $0.5 \pm 0.7$  MPs/g), herbivorous ( $0.4 \pm 0.5$  MPs/g), omnivorous ( $0.3 \pm 0.5$  MPs/g), carnivorous ( $0.2 \pm 0.3$  MPs/g), insectivorous ( $0.2 \pm 0.4$  MPs/g) and invertivorous



**Fig. 3** Absolute number of MPs (n) in fish from forested and deforested streams (A), rainy and dry seasons (B), feeding areas (C), and trophic categories (D), in the Machado River basin, northern Brazil. Bp benthopelagic, Bt benthic,

Pe pelagic. Trophic category: Alg algivorous, Car carnivorous, Omn omnivorous, Pis piscivorous, Ins insectivorous, Inv invertivorous, Her herbivorous, Det detritivorous

( $0.2 \pm 0.3$  MPs/g), respectively (Supplementary Fig. S1D).

The detritivore *Ancistrus* sp. ( $N=34$ ; 23 MPs;  $0.6 \pm 1.2$ ) showed the highest abundance of MPs in forested streams, followed by the insectivore *Brachyhalcinus copei* (Steindachner, 1882) ( $N=44$ ; 217 MPs;  $21.0 \pm 0.7$ ) and the insectivore *Bryconops giacopinii* (Fernández-Yépez, 1950) ( $N=32$ ; 15 MPs;  $15.0 \pm 0.8$ ). In deforested streams, the insectivore *Aequidens rondoni* (Miranda Ribeiro, 1918) ( $N=18$ ; 16 MPs;  $0.9 \pm 1.0$ ) was the species with the highest MP ingestion, followed by *Ancistrus* sp. ( $N=10$ ; 12 MPs;  $2.4 \pm 2.4$ ) and the insectivore *Characidium* sp. ( $N=8$ ; 9 MPs;  $1.1 \pm 1.2$ ) (Table 2).

The ordination of samples based on MP abundance explained 48% (axis 1) of the total abundance. Overall, forest cover (axis 1 = 20%;

stress = 0.38;  $F_{nMDS1} = 46.2$ ; d.f. = 1.0;  $p = 0.07$ ) (Fig. 4A), feeding zone (axis 1 = 19%; stress = 0.41;  $F_{nMDS1} = 44.1$ ; d.f. = 2.0;  $p = 0.06$ ) (Fig. 4B), and trophic categories (axis 1 = 13%; stress = 0.34;  $F_{nMDS1} = 48.2$ ; d.f. = 7.0;  $p = 0.06$ ) (Fig. 4C) did not affect MP composition.

Black fibers were the MP morphotypes with the greatest contribution for the dissimilarity between forested and deforested streams for the species *A. rondoni* (32%), *Ancistrus* sp. (25%), and *M. oligolepis* (45%). Blue fibers (50%), black fragments (41%), and blue fragments (17%) were the most representative MPs for *B. giacopinii*, *C. maxillares*, and *K. heteresthes*, respectively (Fig. 5).

Blue MPs were predominant (42%) among fish from the Machado River basin streams, followed by black (40%), transparent (10%), and red (2%)

**Table 2** List of the species collected in forested and deforested small streams showing the trophic level (TL), number of fish sampled (N), total number of individuals with MPs (MPs), occurrence of microplastics in the species (FO %), total number (T) of plastic particles recorded in the gastrointestinal tract of each specie and mean ( $M \pm$  standard deviation (SD)); feeding zone (FZ): benthopelagic (Bp), benthic (Bt) and pelagic (Pe); and trophic category (TC): Alg, algivorous, Car, carnivorous; Omn, omnivorous; Pis, piscivorous; Ins, insectivorous; Inv, invertivorous; Her, herbivores; Det, detritivorous and references for trophic category

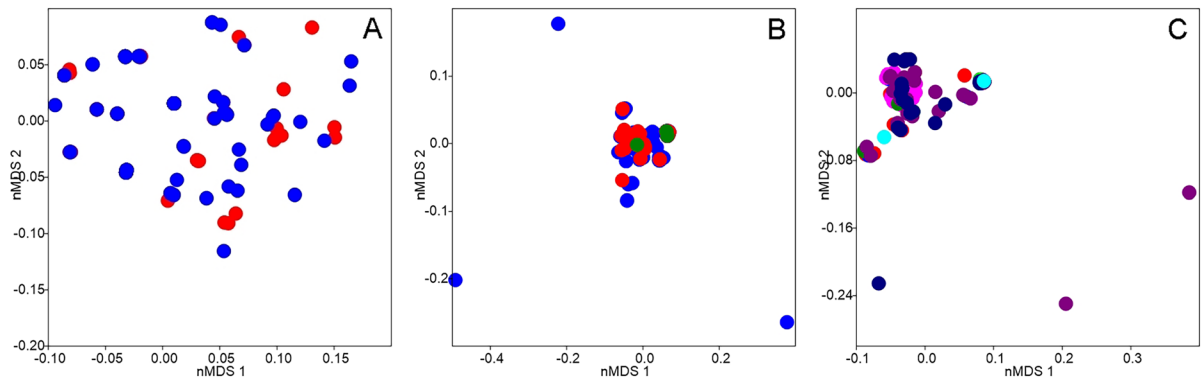
Order/family/species	TL Forested			Deforested			FZ	TC	Authors				
	N	MPs	FO (%)	T ( $M \pm$ SD)	N	MPs				FO (%)	T ( $M \pm$ SD)		
Characiformes													
Characidae													
<i>Asyanax bimaculatus</i> (Linnaeus, 1758)	2.4	15	3	20	3	(0.2±0.4)	1	0	0	(0.0±0.0)	Bp	Omn	Andrian et al. (2001)
<i>Asyanax</i> sp.	2.4	3	2	67	5	(1.7±2.1)	5	2	40	(0.8±1.3)	Bp	Omn	Andrian et al. (2001)
<i>Bryconops caudomaculatus</i> (Günther, 1864)	4.2	7	4	57	8	(1.1±1.5)					Bp	Ins	Carvalho (2008)
<i>Brachyhalcinus copei</i> (Steindachner, 1882)	3.1	44	16	36	21	(21.0±0.7)	7	3	43	(0.4±0.5)	Bp	Ins	Godoi (2008)
<i>Bryconops giacopinii</i> (Fernández-Yépez, 1950)	3.2	32	9	28	15	(15.0±0.8)	21	7	33	(0.4±0.7)	Bp	Ins	Carvalho (2008)
<i>Creagrutus beni</i> Eigenmann, 1911	2.3				3		0	0	0	(0.0±0.0)	Bp	Omn	Ortiz & Rocha (2001)
<i>Creagrutus maxillaris</i> (Myers, 1927)	2.3	43	8	19	8	(8.0±0.4)	18	5	28	(0.5±1.0)	Bp	Omn	Ortiz & Rocha (2001)
<i>Creagrutus petilus</i> Vari & Harold, 2001	2.5	5	3	60	8	(1.6±1.5)					Bp	Omn	Vari (2001)
<i>Hypessobrycon</i> sp.	2.9	1	1	100	2	(2.0±0.0)					Bp	Ins	Carvalho (2008)
<i>Knodus heterosthes</i> Eigenmann, 1908	3	21	4	19	6	(0.2±0.7)	10	2	20	(0.2±0.4)	Bp	Ins	Nogueira & Costa (2014)
<i>Moenkhausia colletii</i> (Steindachner, 1882)	3.4	10	2	20	2	(0.2±0.4)					Bp	Ins	Galuch (2007)
<i>Moenkhausia oligolepis</i> (Günther, 1864)	3.3	22	3	14	3	(0.1±0.4)	13	2	15	(0.3±0.8)	Bp	Ins	Carvalho (2008)
<i>Moenkhausia cotinho</i> Eigenmann, 1908	3.3				1		0	0	0	(0.0±0.0)	Bp	Ins	Carvalho (2008)
<i>Moenkhausia pankilopteryx</i> Bertaco & Lucinda, 2006	3.5				4		1	25	1	(1.0±0.0)	Bp	Ins	Bertaco & Lucinda 2006
<i>Poptella compressa</i> (Günther, 1864)	3.1				8		5	63	6	(0.7±0.5)	Bp	Ins	Pereira et al. (2007)
<i>Phenacogaster retropinnus</i> Lucena & Malabarba, 2010	3.4	1	0	0	0	(0.0±0.0)	14	3	21	(0.3±0.6)	Pe	Ins	Carvalho (2008)
<i>Serrapinnus microdon</i> (Eigenmann, 1915)	2.5	4	2	50	3	(0.8±1.0)	10	8	80	(1.3±0.9)	Bp	Alg	Teresa & Casatti (2010)
<i>Serrapinnus notomelas</i> (Eigenmann, 1915)	2.2				1		1	1	100	(1.0±0.0)	Bp	Alg	Costa & Rocha (2017)
Anostomidae													
<i>Leporinus friderici</i> (Block, 1794)	3.3				1		0	0	0	(0.0±0.0)	Bp	Omn	Röpke (2008)
Crenuchidae													
<i>Characidium zebra</i> Eigenmann, 1909	3.5	4	1	25	1	(0.3±0.5)					Bp	Ins	Casatti et al. (2012)
<i>Characidium</i> sp.	3.5				8		6	75	9	(1.1±1.2)	Bp	Ins	Casatti et al. (2012)
Curimatidae													
<i>Steindachnerina fasciata</i> (Vari & Géry, 1985)	3				5		4	80	9	(1.8±1.3)	Bp	Det	Godoi (2008)
Serrasalminae													
<i>Myelus setiger</i> Müller & Troschel, 1844	2.7				5		3	60	4	(0.8±0.8)	Bp	Her	Dary et al. (2017)

Table 2 (continued)

Order/family/species	TL Forested			Deforested			FZ TC	Authors
	N	MPs	FO (%)	N	MPs	FO (%)		
<i>Erythrinidae</i>								
<i>Erythrinus erythrinus</i> (Bloch & Schneider, 1801)	3.7	1	100	1	1	100	Bt	Carvalho (2008)
<i>Hoplias malabaricus</i> (Bloch, 1794)	4.5	1	100	1	1	100	Bp	Villar & Costa (2014)
<i>Siluriformes</i>								
<i>Auchenipteridae</i>								
<i>Tatia autopygia</i> (Kner, 1858)	3.4	4	50	3	4	100	Bp	Ins Ramirez et al. (2015)
<i>Callichthyidae</i>	3	1	100	1	1	100	Bt	Inv Casatti (2002)
<i>Corydoras aeneus</i> (Gill, 1858)	3	1	0	0	0	0	Bt	Inv Casatti (2002)
<i>Cetopsidae</i>								
<i>Cetopsis</i> sp.	3.6	2	100	2	2	100	Bt	Omn Vari et al. (2005)
<i>Loricariidae</i>								
<i>Ancistrus dubius</i> Eigenmann & Eigenmann, 1889	2.4	3	67	5	3	67	Bt	Det Ferreira et al. (2021)
<i>Ancistrus</i> sp.	2.4	34	11	32	23	60	Bt	Det Ferreira et al. (2021)
<i>Farlowella oxyrryncha</i> (Kner, 1853)	2.4	2	0	0	0	0	Bt	Alg García-Alzate et al. (2012)
<i>Hypostomus pantherinus</i> Kner, 1854	2.4	7	29	7	2	29	Bt	Alg García-Alzate et al. (2012)
<i>Hypostomus pyrineusi</i> (Miranda-Ribeiro, 1920)	2.4	3	67	6	2	0	Bt	Alg García-Alzate et al. (2012)
<i>Hypostomus</i> sp.	2.4	7	0	0	0	0	Bt	Alg García-Alzate et al. (2012)
<i>Rineloricaria lanceolata</i> (Günther, 1868)	2.6	1	100	1	3	30	Bt	Her Ibáñez et al. (2009)
<i>Rineloricaria phoxocephala</i> (Eigenmann & Eigenmann, 1889)	2.5	1	100	1	1	100	Bt	Her Ibáñez et al. (2009)
<i>Heptapteridae</i>								
<i>Imparfinis stictonotus</i> (Fowler, 1940)	3.1	8	25	2	8	25	Bt	Ins Casatti et al. (2012)
<i>Phenacohandia</i> sp.	3.3	1	0	0	0	0	Bt	Ins Casatti et al. (2012)
<i>Phenacorhamdia boliviana</i> (Pearson, 1924)	3.3	2	0	0	0	0	Bt	Ins Casatti et al. (2012)
<i>Pimelodella howesi</i> Fowler, 1940	3.4	12	6	7	12	50	Bt	Omn Mazzoni et al. (2010)
<i>Pimelodella</i> sp.	3.4	1	100	1	1	100	Bt	Omn Mazzoni et al. (2010)
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	3.9	3	0	0	0	0	Bp	Ins Casatti et al. (2012)
<i>Pseudopimelodidae</i>								
<i>Batrachoglanis</i> sp.	3.7	1	0	0	0	0	Bt	Pis Carvalho (2008)
<i>Trichomycteridae</i>								
<i>Iuglans amazonicus</i> (Steindachner, 1882)	3.2	1	100	1	1	100	Bp	Ins Galuch (2007)
<i>Gymnotiformes</i>								

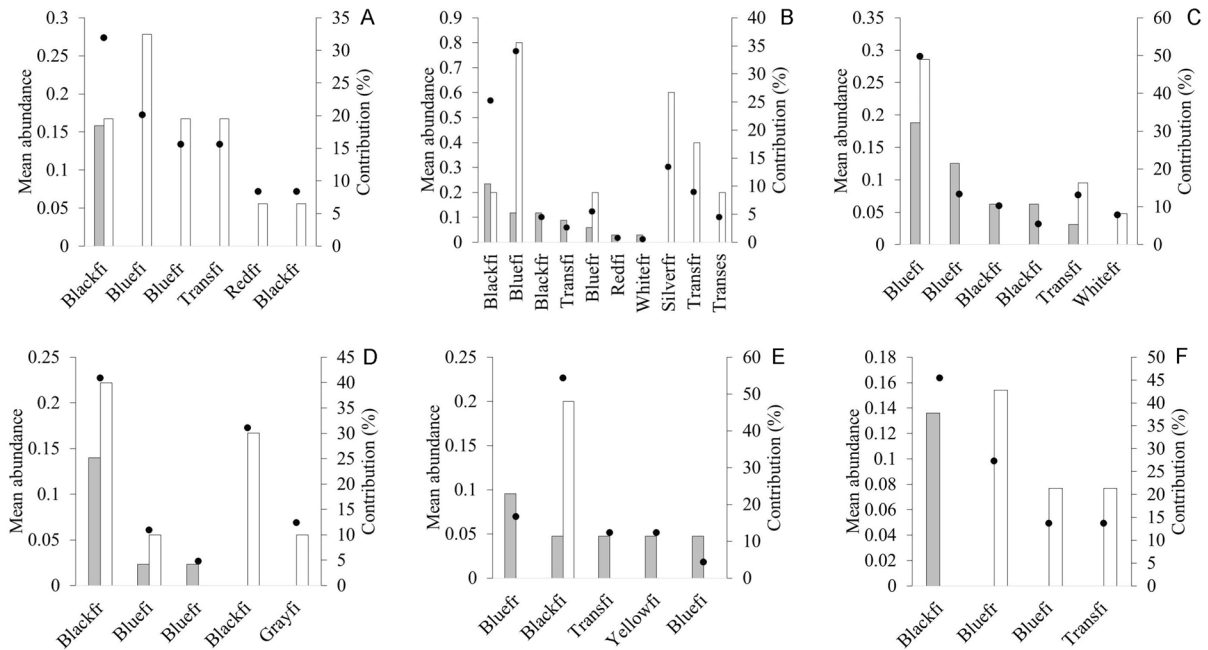
Table 2 (continued)

Order/family/species	TL		Forested		Deforested		FZ	TC	Authors		
	N	MPs	FO (%)	T (M±SD)	N	MPs				FO (%)	T (M±SD)
Apteronotidae											
<i>Apteronotus albifrons</i> (Linnaeus, 1766)	3.6	1	0	0 (0.0±0.0)	2	0	0	0 (0.0±0.0)	Bp	Ins	Röpke (2008)
Gymnotidae											
<i>Gymnotus carapo</i> Linnaeus, 1758	3	4	1	1 (0.2±0.5)	11	4	36	4 (0.3±0.5)	Bp	Inv	Planquette et al. (1996)
Rhamphichthyidae											
<i>Gymnorhamphichthys rondoni</i> (Miranda Ribeiro, 1920)	3.1	4	2	1 (0.3±0.6)					Bp	Ins	Virgilio et al. (2019)
Sternopygidae											
<i>Sternopygus macrurus</i> (Bloch & Schneider, 1801)	3.2	4	0	0 (0.0±0.0)	10	5	50	6 (0.6±0.7)	Bp	Car	Carvalho (2008)
<i>Eigenmannia trilineata</i> López & Castello, 1966	3.1	1	0	0 (0.0±0.0)	5	0	0	0 (0.0±0.0)	Bp	Ins	Carvalho (2008)
Perciformes											
Cichlidae											
<i>Aequidens rondoni</i> (Miranda Ribeiro, 1918)	3.2	19	3	3 (0.2±0.4)	18	10	56	16 (0.9±1.0)	Bt	Ins	Kullander (2003)
<i>Aequidens tetramerus</i> (Heckel, 1840)	3.2	9	3	3 (0.3±0.5)					Bt	Ins	Costa and Soares (2015)
<i>Crenicichla santosi</i> Ploeg, 1991	3.2	9	1	1 (0.1±0.4)	12	6	50	6 (0.5±0.5)	Bp	Car	Anjos (2005)
<i>Satanoperca jurupari</i> (Heckel, 1840)	3.1	5	2	2 (0.8±0.0)	6	2	33	2 (0.3±0.5)	Bp	Omn	Santos et al. (2004)



**Fig. 4** Ordination of samples along the first two axes of the non-metric multidimensional scaling (nMDS) analysis (Bray–Curtis index) based on MP abundance in fish from forested streams (blue circles) and deforested streams (red circles) (A);

(green circle) (B); algivorous (green circle), carnivorous (blue circle), omnivorous (dark blue circle), piscivorous (light blue circle), insectivorous (purple circle), invertivorous (light green circle), herbivores (dark green circle), and detritivorous (pink circle) (C)

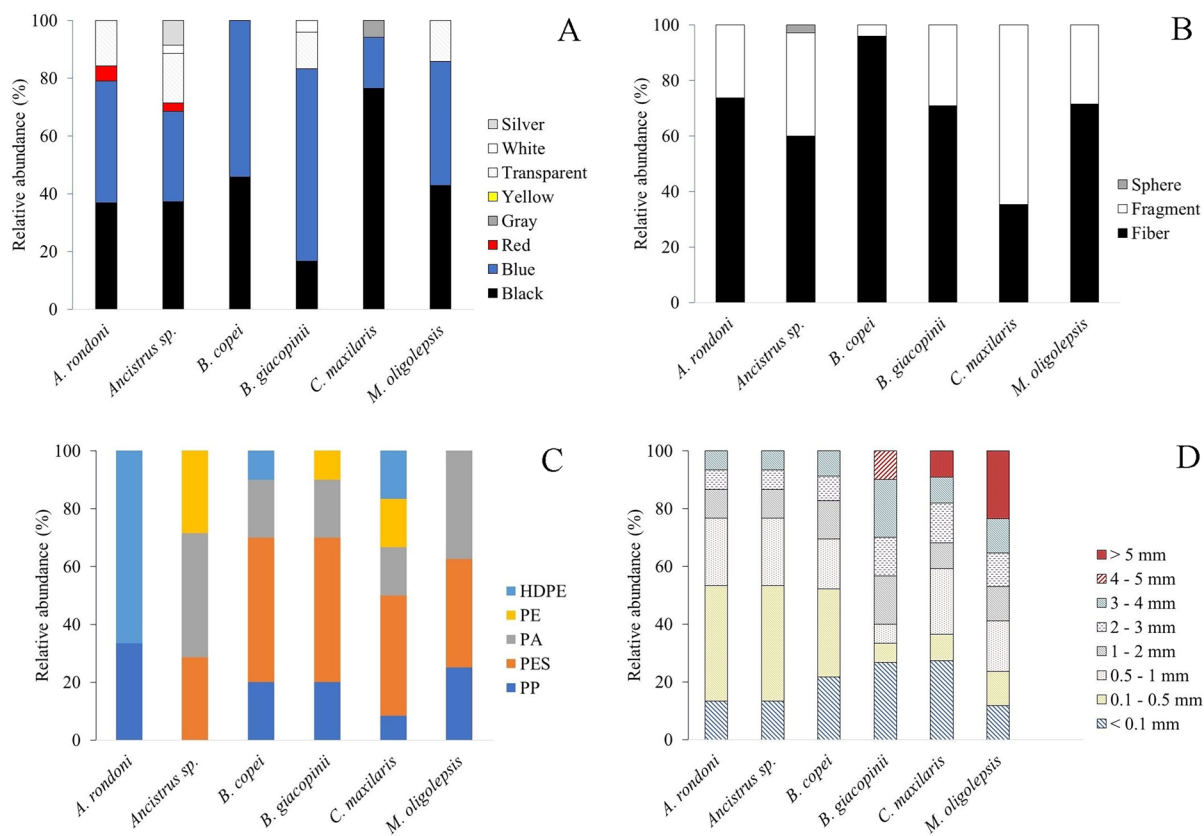


**Fig. 5** Average abundance (bars) and relative contribution of MPs (circles) to the dissimilarity in MP composition in the digestive tracts of fish species from forested (gray bars) and deforested (white bars) streams through a SIMPER analysis. **A** *A. rondoni*, **B** *Ancistrus sp.*, **C** *B. giacopinii*, **D** *C. maxillaris*, **E** *K. heteresthes*, and **F** *M. oligolepsis*. Blackfr black

fiber, Bluefr blue fiber, Redfr red fiber, Greenfr green fiber, Grayfr gray fiber, Yellowfr yellow fiber, Transfr transparent fiber, Blackfr black fragment, Greenfr green fragment, Bluefr blue fragment, Redfr red fragment, Whitefr white fragment, Transfr transparent fragment, Silverfr silver fragment, Blues blue sphere, Transes transparent sphere

(Fig. 6A). Three forms of MPs—fibers, fragments, and microbeads—were observed. Fibers represented 70% of the total MPs (Fig. 6B).

Five types of plastic polymers were detected in the fish: polypropylene (PP), polyamide (PA), polyester (PES), polyethylene (PE), and high-density polyethylene (HDPE).



**Fig. 6** Different proportions of colors (A), shape (B), sizes (C), and plastic polymers (D) found in the six most abundant species in the streams of the Machado River basin, northern Brazil

PES was the predominant polymer (40%), followed by PA (28%) and PP (14%) (Fig. 6C). The majority of MPs were < 0.1 mm (23%), followed by those between 0.1 and 0.5 mm (21%) and between 0.5 and 1 mm (15%) (Fig. 6D).

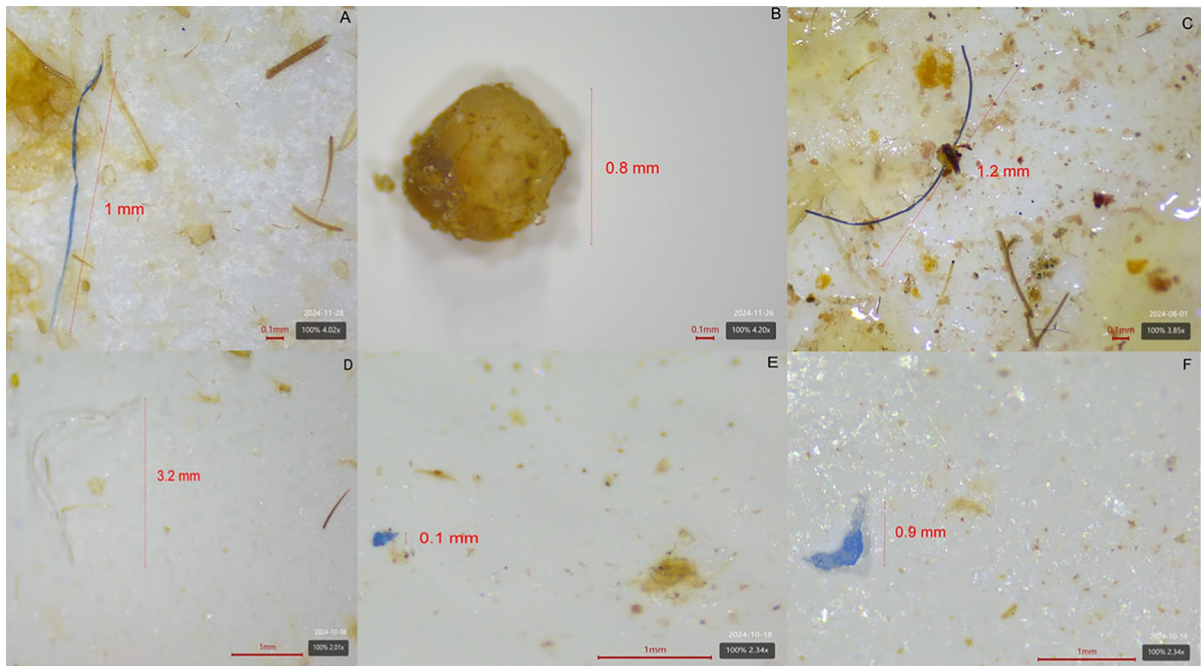
Based on the shape of the MPs recovered from the gastrointestinal tract of the species, they were classified as primary (Fig. 7B) and secondary (Fig. 7A, C–F), meaning they were a result of the fragmentation of larger plastic items.

## Discussion

The results indicate significant MP contamination of fish fauna from Southwestern Amazonia. Approximately 30% of the fish and all species analyzed were contaminated with MPs. Some species, such as *M. setiger*, *E. erythrinus*, and *H. malabaricus*, are

consumed by the local population, while others play important roles in the food webs of tropical streams (Reis et al., 2020). These results support the initial hypothesis that fish from both forested and deforested streams in the Machado River basin ingest plastic. The presence of MPs in fish from large rivers (Andrade et al., 2019; Rojas et al., 2023), riverine beaches (da Costa et al., 2023), and estuaries (Schmid et al., 2018; Pegado et al., 2021) has been more widely studied than in small streams of the Amazon (e.g., Ribeiro-Brasil et al., 2020). According to Rico et al. (2023), small rivers and streams located near urban areas in the Amazon have sufficiently high concentrations of MPs to cause ecotoxicological effects in aquatic organisms due to the ingestion of food resources or translocation across biological membranes.

Confirming our third hypothesis, fish from deforested streams exhibited a higher abundance of MPs



**Fig. 7** Photomicrographs of MPs ingested by the fish fauna of streams in the Machado River basin. **A** Blue fiber in *B. giacopinii*, **B** transparent sphere in *Ancistrus sp.*, **C** blue fiber in

*Astyanax sp.* gray, **D** transparent fiber in *B. giacopinii*, and **E**, **F** blue fragments in *C. petilus*

than those from forested streams. Data on MPs in soils under dominant land uses such as forests (39 million km<sup>2</sup>) or shrublands (12 million km<sup>2</sup>), as well as cultivated pastures (40 million km<sup>2</sup>) are incipient (Ren et al., 2021). However, land use analyses are important to allow an assessment of total soil pollution and to obtain basic knowledge on MPs in less anthropogenic areas (e.g., forests), where only atmospheric deposition of MPs acts as a main source (Weber & Bigalke, 2022). Corroborating our results, McNeish et al. (2018), in analyses of MPs in fish and water from three major tributaries of Lake Michigan, USA, observed higher concentrations of MPs in anthropized watersheds compared to forested ones. According to Nizzetto et al. (2016), soils covered by vegetation have a greater ability to retain MPs than deforested areas due to higher permeability and lower surface runoff. The dense vegetation cover in forests protects the soil from airborne pollutants, such as MPs or heavy metals, considering the retention capacity of vegetation (Diener & Mudu, 2021). The ingestion of MPs by fish can be more pronounced when aquatic fauna relies on resources from riparian vegetation

(riparian forest), as is the case in streams (Vannote et al., 1980), especially in regions where the forest has been removed due to urbanization, facilitating the transport of solid waste into water bodies (Ribeiro-Brasil et al., 2020).

Surface runoff can be one of the main pathways for transporting MPs from terrestrial to aquatic environments (Su et al., 2020b), with precipitation being a crucial factor in this process (Dikareva & Simon, 2019). Our results showed a higher presence of MPs in fish during the rainy period compared to the dry period, confirming our third hypothesis. The occurrence of rainfall promotes leaching of soils near rivers and streams, resulting in the introduction of plastic waste into aquatic environments (Su et al., 2020b). Considering that the degradation rate of MPs in terrestrial environments is faster than in aquatic environments due to greater exposure to temperature variation and UV radiation (Andrady et al., 2022), freshwater bodies receive a higher amount of MPs than terrestrial environments. Considering that 20 species (~40% of the total species) are common to both types of streams (deforested and forested), sampled in the periods, and that the number of trophic

guilds and feeding areas are reduced, we highlight that such differences in the abundance of ingested MPs are linked to land use and local climate events. According to Quadroni et al., 2024, in a review of MPs pollution in rivers under climate change, they describe that the interaction between river hydrology and MPs pollution is primarily governed by factors such as land use in the river basin, position within the basin, channel morphology, and MPs characteristics. In addition to these factors, ecological characteristics such as vegetation cover and the structure along river banks can significantly influence this relationship, highlighting the complexity of studying and understanding MPs pollution in river environments (Quadroni et al., 2024).

Benthic fish exhibited a higher abundance of MPs than benthopelagic and pelagic fish, corroborating our second hypothesis. In line with our study, Seetapan & Prommi (2023), in studies conducted in the Ban Tam River in northern Thailand, found that benthic fish had higher concentrations of MPs than benthopelagic and pelagic fish, suggesting that plastic ingestion by fish may be related to their feeding habitat. As micro-particles tend to accumulate in the substrate (Bellas et al., 2016), secondary ingestion can occur among species that consume bottom resources, such as algae and insects, which explains the high number of MPs found in the benthic species analyzed. Furthermore, benthic organisms may unintentionally ingest MPs after feeding on sediment or consuming MPs resuspended due to bioturbation processes in the substrate (Tien et al., 2020). Sediment is the main reservoir of MPs, capturing 1–2 orders of magnitude more MPs than found on the surface (de Smit et al., 2021). Studies on MPs in potential prey (such as gastropods, insects, crustaceans, and fish) are essential for a broader understanding of secondary ingestion of MPs by bentophagous species (da Costa et al., 2023).

Feeding behavior is essential for assessing plastic waste ingestion by fish (Jabeen et al., 2017). Due to their small size and floating properties, MPs are easily mistaken for food and ingested by aquatic organisms (Anderson et al., 2017). However, the relationship between feeding habits and MPs ingestion is still not well understood (Justino et al., 2021), especially in freshwater systems. In the present study, piscivorous fish exhibited a higher abundance of MPs compared to other trophic guilds in the streams of the Machado River basin, confirming our second hypothesis. In a

review on MPs ingestion by freshwater fish, Collard et al. (2019) found that piscivorous fish were more contaminated than omnivorous fish, suggesting that predator fish are at a higher risk of MP ingestion than those with different feeding strategies. Predator fish, both in freshwater and marine environments, may be more exposed to MP contamination compared to organisms at lower trophic levels, due to greater energy demand, consumption rate, secondary ingestion, and trophic transfer (Ferreira et al., 2019).

The colors of the MPs observed in this study were similar to those found in other studies, both in the Amazon region (Andrade et al., 2019; Pegado et al., 2021; da Costa et al., 2023) and globally (Nkosi et al., 2023), with blue and black being the most predominant. Some evidence suggests a preference for blue and black colors in clothing (Bakker et al., 2013), which may contribute to the presence of these synthetic and semi-synthetic colored textile fibers in rivers and streams. In fact, due to poor sanitation coverage, washing machine effluents are released into these environments. The similarity between the colors of MPs and available food resources in the environment may even increase the likelihood of ingestion by fish species (Parvin et al., 2021). Regardless of the color or shape of the microparticles, it is important to highlight that when ingested, these particles can cause mechanical damage, reduce growth rates, decrease fertility, and even lead to the death of organisms (Ma et al., 2020b).

The shape of MPs is a crucial factor, due to the wide diversity of forms found in aquatic environments (Wagner & Lambert, 2018). Similar to Ribeiro-Brasil et al. (2020), in studies on MPs ingestion by fish in streams in northern Brazil the fibers were predominant (70%) in the digestive tract of fishes collected in the present study. In general, secondary MPs in the form of fibers are the most abundant in Amazonian environments (water, sediment, and biota), representing up to 96% of the MPs recorded (Morais et al., 2024). These results follow the global pattern, as fibers are the most frequently identified MP shapes according to the scientific literature (Orona-Návar et al., 2022). The accumulation of synthetic fibers in stream fish is particularly concerning, as this type of particle is considered the most dangerous, due to its high bioavailability and high potential of being accumulated by numerous organisms (Qiao et al., 2019).

The main polymers ingested by the analyzed fish were PES and PA, representing over 50% of the analyzed MPs. These polymers are widely used and produced globally (PlasticsEurope, 2019), also being the most commonly found in the Amazon region (Morais et al., 2024). PES is used in various products, such as clothing fabrics and general consumer items, with significant global annual production (Wang et al., 2019). PA, like PES, is primarily used in the manufacturing of synthetic textile products, being responsible for most of the MP fibers released by domestic effluents, especially wastewater from washing machines (Acharya et al., 2021). Its prevalence confirms that inadequate sanitation conditions in the Amazon are a primary source of MPs to the aquatic ecosystems. Moreover, PA is also used in the production of fishing nets and ropes, indicating that fishing activities play a significant role as another source of MPs in the region (Morais et al., 2024).

The size of the ingested MPs can affect the retention time of these particles in organisms (Grigorakis et al., 2017). MPs smaller than 0.5 mm were predominant in the analyzed fish. These particles are produced by processes of biological degradation, photodegradation, chemical deposition, and physical decomposition of larger plastic fragments (Andrady et al., 2022). Rehm et al. (2021) observed that MPs smaller than 0.1 mm in deforested areas are more prone to horizontal transport, being more susceptible to erosion and, consequently, leaching into water bodies compared to larger particles. However, leaching mechanisms of MPs, influenced by precipitation, are still not fully understood, as studies in this area are limited mainly because processing soil samples to quantify MPs smaller than 1 mm is challenging (Möller et al., 2022).

According to Morais et al. (2024), in MPs analyses in 313 municipalities in the Brazilian Amazon, only 2.6% were classified as adequate in terms of basic sanitation services, while 34.8 and 15% were classified as having low and precarious sanitation services, respectively. In addition, the inadequate management and disposal of fishing equipment represent a source of introduction of plastics into the environment (Morais et al., 2024). In the short-term it is important to make immediate improvements to waste collection, recycling, and disposal systems; promote educational campaigns and actions aiming the communities, industries, and stakeholders to raise awareness

about the sources, impacts, and mitigation of MPs pollution. Also, in the medium-term it is necessary to increase funding for research on plastic and MP pollution and mitigation, reinforcing the application of environmental regulations on waste management and water treatment, and to improve sewage and waste treatment facilities, especially in urban centers. In the longer-term it is indicated to develop and implement regulations aimed at preventing and mitigating plastic and MPs pollution and to initiate restoration projects for affected habitats with new methods to safely reduce and recycle MPs from the environment (Morais et al., 2024).

The main limitations of the present study were related to the difficult access and/or lack of authorization by the owners to the sampling points and the difficulty in comparing our results with data from the literature, considering the lack of standardization regarding the collection methods and units of measurement used to quantify the MPs. Our study helps to fill a gap in research highlighting the role of riparian vegetation in enhancing the resilience of aquatic ecosystems to microplastic contamination. Studies on the extent of MP contamination in forested watersheds remain limited, with most studies confined to mangroves (Ding et al. 2022). More research is needed to assess the effects of MPs in the biodiversity of Amazonian streams and other terrestrial environments. Furthermore, little is known about the transport, degree of remobilization, and long-term fate of MPs in the soil (Van den Berg et al., 2020). Given the potential for these particles to migrate through the soil profile, they may leach into surface water systems. As MPs degrade over very long time scales, they may persist and be available in the soil for centuries, possibly millennia (MacLeod et al., 2021). This suggests that soils with forest cover may represent a significant sink for MPs, delaying their entry into streams. However, it may also expose aquatic ecosystems to acute contamination during intense flood events, which are becoming more intense in the context of climate change.

## Conclusion

The presence of vegetation in micro basins is an important factor in the ingestion of MPs by the

ichthyofauna of Amazonian streams. Environmental characteristics, such as seasonal precipitation regime, and ecological factors, such as feeding behavior and habitat, were determinants for MPs incorporation by fish species. The blue and black fibers <0.1 mm, with polyester, polyamide, and polypropylene were the main polymers in our study. Clothing fibers were the most common type of MPs found, indicating that urbanization and poor sanitation observed at the Machado river basin, are important factors in the introduction of MPs into freshwater environments. The results of this study suggest that piscivorous fish could be used in bio-monitoring of Amazonian streams, as they appear to accumulate more MPs. Considering that the northern region of Brazil has one of the highest fish consumption rates in the world, with some of the fish species analyzed being commonly consumed by the local population and the other species supporting a complex food web, it is crucial to investigate the potential impact of MPs ingestion on human health. Future studies that aim to analyze the impacts of MPs, as well as plastic leachates, on freshwater ichthyofauna are necessary to elucidate the impacts of these synthetic materials on riverine aquatic biota. Additionally, considering that climate change affects the frequency and intensity of drought and flooding events, our results have significant implications for the management of MPs in organisms from Amazonian river systems with varying levels of forest cover, linking this landscape aspect to the availability of this contaminant for aquatic biota.

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**Data availability** Data will be made available on request.

**Declarations**

**Conflict of interest** The authors have no relevant conflicting or competing interests to declare.

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