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1 **Distinct Dynamics in Mountain Watersheds: Exploring Mercury and**  
2 **Microplastic Pollution—Unraveling the Influence of Atmospheric Deposition,**  
3 **Human Activities, and Hydrology**

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17

18 **ABSTRACT**

19 The intensification of human activities all around the globe has led to the spread of micropollutants  
20 in high-mountain freshwater environments. We therefore aimed to assess the geospatial  
21 distribution and determine the potential sources of (total-) mercury (THg) and microplastics (MPs)  
22 in mountain freshwater ecosystems. To do so, we analyzed THg and MP concentrations in brown  
23 trout, biofilm, and sediments from lotic and lentic ecosystems in the Pyrenees – all subjected to  
24 different types of human pressure. Additionally, we assessed the potential impacts of these  
25 pollutants on fish, and explored the bioindication capacity of brown trout (*Salmo trutta fario*) and  
26 biofilm regarding THg and MP pollution. For the first time, we measured concentrations of MPs  
27 trapped in the matrix of freshwater biofilm. Our results suggest that THg in the Pyrenees might be  
28 explained by both legacy (regional) and distant sources, in combination with environmental  
29 characteristics such as the presence of peatlands or streamwater physicochemistry, while MPs in  
30 fish are linked to recent local pollution sources such as single-use plastics. In contrast, MPs in  
31 biofilm matrix and sediments indicate a combination of distant (i.e., atmospheric deposition) and  
32 recent local pollution sources. Moreover, hydrodynamics and plastic density likely control MP  
33 distribution in rivers. Based on Fulton's condition factor, we also found that higher THg  
34 concentrations caused a negative impact on fish health ( $K < 1$ ), while no impact of MPs could be  
35 seen. Therefore, we suggest that brown trout and biofilm can serve as bioindicators of atmospheric  
36 deposition of THg in high-altitude lakes and that biofilm is a reliable bioindicator to assess MP  
37 pollution in remote environments. Brown trout may also act as a bioindicator of MP pollution, but  
38 only efficiently in more polluted areas.

39

40 **Keywords:** mercury, microplastics, brown trout, biofilm, long-range atmospheric transport,  
41 tourism

42

43 **1. INTRODUCTION**

44 Popular belief often associates remote areas, such as mountains, with pristine environments, where  
45 anthropogenic impacts are almost negligible. In reality, human activities heavily impact mountains  
46 due to the valuable resources they provide (e.g., freshwater sources, raw material extraction, and  
47 recreational activities; Price, 2015). Previous studies have shown high-altitude environments to be  
48 “contamination hotspots” due to their climatic and orographic characteristics that facilitate the  
49 deposition of distant airborne contaminants (Blais et al., 2006; Zhang et al., 2014; Le Roux et al.,  
50 2016). Moreover, the popularization of tourist activities has led to the presence of new pollutants  
51 such as plastic to mountain areas.

52

53 Past mining activities are often associated with the accumulation of legacy contaminant metals in  
54 reservoir compartments such as mountain soils or aquatic sediments (Kocman et al., 2017;  
55 Schuster et al., 2018; Chen et al., 2021a). These contaminants can later be released into other  
56 habitats as a result of environmental changes triggered by the intensification of human activities  
57 (e.g., soil erosion) or global change (Schmeller et al., 2018). Mercury (Hg) is a prioritized legacy  
58 contaminant due to its potent cardiovascular and neurotoxic effects that pose a significant risk to  
59 humans and wildlife alike, since it can bioaccumulate and biomagnify throughout the food-chain  
60 (Mergler et al., 2007; Lavoie et al., 2013; Dragan et al., 2023). Over 75-90% of mercury in our  
61 environments originate from anthropogenic emissions, with >60% emitted after 1850 (Mason et  
62 al., 1994; Streets et al., 2011). In freshwater and atmospheric compartments, ~50 and ~20-35%  
63 (respectively) of direct Hg anthropogenic emissions come from silver and gold mining activities  
64 (Kocman et al., 2017; Obrist et al., 2018). These emissions have led to the disruption of the natural  
65 cycle of Hg, posing a great risk to all living beings (Selin, 2009; Streets et al., 2011). Atmospheric  
66 deposition is the largest source of Hg in remote freshwater environments, and several studies

67 observed a more significant deposition of Hg as altitude increases (Fitzgerald et al., 1998;  
68 Rognerud et al., 2002; Lyman et al., 2020; Duval et al., 2023).

69

70 On a more recent timescale, plastic has been detected in all natural habitats, including remote  
71 mountain locations (Allen et al., 2019; Allen et al., 2021a, 2021b; Parolini et al., 2021). A recent  
72 report indicates that global annual plastic production is 450 million metric tons and is assumed to  
73 increase further by a factor of 2-3 by 2060 (OECD, 2022). The popularization of tourist activities  
74 in mountains and rural villages has led to the abandonment and mismanagement of plastic waste  
75 in these environments during peak seasons (Esteban Plazas, 2021). It is estimated that since 1950,  
76 ~60% of all plastic products have been discarded in landfills or natural environments (Geyer et al.,  
77 2017). Due to their persistence, plastics are slowly fragmented when exposed to sunlight,  
78 temperature variations, and natural oxidants. Microplastics (MPs) are small plastic particles (1  $\mu\text{m}$   
79 - 5 mm) that can be classified according to their shape (e.g., fibers, films, beads, fragments),  
80 colors, or origin (often described as primary MPs when they come from production, and secondary  
81 MPs as a result of the fragmentation of larger plastics) (Barnes et al., 2009; Rios Mendoza &  
82 Balcer, 2019). MPs can be distributed across different environments, including the atmosphere, be  
83 retained in temporal reservoirs, and potentially enter trophic food-webs (de Souza Machado et al.,  
84 2018; McIlwraith et al., 2021; D'Avignon et al., 2022; O'Brien et al., 2023; Prata & Dias-Pereira,  
85 2023). Although MP pollution is strongly associated with proximity to areas of intensive human  
86 activities and point sources ( $\sim 4 \text{ MPs L}^{-1}$  in urban water, and  $\sim 9 \text{ MPs g}^{-1}$  in urban sediment of MPs  
87  $> 50 \mu\text{m}$ ) (Chen et al., 2021b; Margenat et al., 2021a), diffuse sources also represent a significant  
88 contribution to the spread of these particles to remote environments (Allen et al., 2019; Evangelidou  
89 et al., 2020; González-Pleiter et al., 2020; Zhang et al., 2021). For instance, Allen et al. (2019)

90 showed significant amounts of airborne MPs deposited in the Pyrenees (365 MPs m<sup>-2</sup> day<sup>-1</sup>) had  
91 been transported over thousands of kilometers before final deposition (Allen et al., 2021b). The  
92 predicted increase in plastic production and its persistence in the environment may lead to higher  
93 small MPs (< 300 µm) atmospheric deposition in remote environments, from 1.1 Tg y<sup>-1</sup> to 3.5 Tg  
94 y<sup>-1</sup> (Sonke et al., 2022). The slow degradation and persistence of plastic compounds, along with  
95 their retention in temporal reservoirs, could indicate that MPs may become future legacy  
96 pollutants.

97  
98 Once in the mountains, the harsh conditions of these environments may affect the concentrations  
99 of THg and MPs. For example, the high solar radiation in high-altitude ecosystems increases the  
100 photodegradation of methylmercury (MeHg) (Duval et al., 2023). UV radiation also affects MP  
101 concentrations, accelerating the fragmentation of larger particles and potentially producing  
102 changes in their composition and distribution (Shi et al., 2023). Both Hg and MPs show persistence  
103 in the environment and can accumulate in common temporal reservoirs (e.g., aquatic sediments),  
104 where they can interact in adsorption-release processes leading to combined toxic effects on biota  
105 (Acosta-Coley et al., 2019; Naqash et al., 2020; Zhu et al., 2023). Bioindicator organisms are used  
106 to evaluate environmental changes resulting from anthropogenic or natural disturbances (Holt &  
107 Miller, 2010). The brown trout (*Salmo trutta fario*, Linnaeus 1785) is widely used as a bioindicator  
108 organism due to its broader distribution in Eurasian rivers and lakes, and its opportunistic feeding  
109 behavior (Fjeld & Rognerud, 1993; Elliot, 1994; Hansson et al., 2017, 2019; O'Connor et al.,  
110 2020). Biofilms are a complex community of prokaryotes, algae, fungi, and microfauna, embedded  
111 in a mucopolysaccharide matrix. These communities are ubiquitous in all freshwater systems and  
112 are considered reliable bioindicators of several environmental stressors due to their close

113 interaction with the water column, dissolved substances and quick response (Guasch et al., 2016;  
114 Argudo et al., 2019). Both brown trout and biofilm are used to monitor Hg contamination in  
115 freshwater systems (Dominique et al., 2007; Rhea et al., 2013; Hansson et al., 2017), and brown  
116 trout have been used to monitor MP pollution (O'Connor et al., 2020). Still, there is a lack of  
117 studies considering the biofilm matrix as a trap of MPs (Guasch et al., 2022).

118  
119 Our study therefore aimed to investigate the complex interplay between the environment,  
120 organisms, and micropollutants in mountain ecosystems (i.e., rivers, headwater streams, a peatland  
121 stream, and lakes), with a particular focus on evaluating their response to human activities such as  
122 long-distance atmospheric deposition, mining, and tourism. Specifically, we aimed to assess the  
123 accumulation of legacy pollutants (i.e., total mercury, THg) and emerging pollutants (MPs) in the  
124 biota of mountainous aquatic environments. We addressed several key questions: First, which  
125 environmental factors explain the distribution of THg and MPs in mountain freshwater habitats?  
126 Furthermore, what impact do these pollutants have on the health and growth of brown trout  
127 individuals? And finally, can brown trout and biofilm be reliable bioindicators for assessing THg  
128 and MP pollution in freshwater ecosystems found in mountain regions? To answer these questions,  
129 we quantified and compared the concentrations of THg and MPs in brown trout, biofilm, and  
130 sediments at ten sites in the Pyrenees. We also considered geographical factors, such as altitude  
131 and anthropogenic gradients, as well as river physicochemical variables, to explain their potential  
132 influence on the distribution of THg and MPs in the freshwater ecosystems of the Pyrenees.

133

134

## 135 **2. MATERIAL & METHODS**

### 136 *2.1 Study sites*

137 Our study sites are located in the Pyrenean region between France, Andorra, and Spain, ranging  
138 from 1269 to 2076 m.a.s.l. (**Figure 1, Table S1**). Fieldwork was carried out in October 2015 and  
139 2016 for BER, ARG, BAS, and LAQ, and in October 2020 and 2021 for SET, BAR, CAT, BER,  
140 SOR, VAL, and ARC. According to the type of current human activities (i.e., hiking area, presence  
141 of ski resorts, presence of villages of > 200 inhabitants, agriculture and/or pastoralism area, science  
142 monitoring, fishing, and presence of roads at less than 300 m), we classify our sites in high (i.e.,  
143 SET, BAR, CAT), moderate (i.e., BER, SOR, ARC), and low (i.e., ARG, BAS, LAQ) human  
144 pressure areas. We sampled four types of freshwater bodies: five rivers (SET, BAR, SOR, VAL,  
145 ARC), two headwater streams (CAT, ARG), one peatland stream (BER), and two (shallow) lakes  
146 (BAS, LAQ). Rivers and headwater streams are expected to transport particles downstream (i.e.,  
147 transport systems). In contrast, the peatland stream and lakes are considered deposition zones  
148 because of their low velocity rates (i.e., deposition systems). The exposure to atmospheric  
149 deposition (i.e., % of canopy cover) was measured using CanopyApp (2018) by the University of  
150 New Hampshire. We measured water velocity, streamflow, and physicochemical variables (i.e.,  
151 conductivity, pH, temperature), characterized the substrate of the stream channel, quantified  
152 macro- and mesoplastics (MacP > 5 cm, and MesP 0.5 – 5 cm) in the riparian zone, and MPs (>333  
153  $\mu\text{m}$ ) from water column in SET, BAR, CAT, SOR, VAL, and ARC, following protocol described  
154 by Margenat et al., (2021b). Finally, we collected samples of brown trout, biofilm, and sediment  
155 at each site. Although our samples were collected in different campaigns (2015-2021), we did not  
156 expect a large temporal variation between sites since THg atmospheric deposition rates have been  
157 constant in the last 20 years (Enrico et al., 2016, 2017). We collected all samples during the same  
158 season (autumn) to reduce potential temporal variability in MP concentration due to the potential  
159 release of stocked pollutants from snow melt or high-flow events.



160

161 *2.2 Sampling and sample preparation*

162 *Fish.* Brown trout (*Salmo trutta fario*) were collected during the fall of 2015, 2016, 2020, and 2021  
163 using electric fishing. In total, 66 fish (3 to 15 per site) were collected following local regulations  
164 and guidelines. All captured fish were immediately exposed to an overdose of lethal anesthetic  
165 solution (Benzocaine for the French sites and MS/222 for the Andorran and Spanish sites), after  
166 which biological parameters (i.e., length, weight, and sex) were recorded. To ensure clean and cool  
167 conditions during transport from the field to the laboratory, all fish were individually wrapped in  
168 aluminum foil and placed in pre-labeled polyethylene bags, and all bags were placed in cool boxes  
169 on a mixture of ice and dry ice. At the laboratory, fish were dissected for the intestinal tract and  
170 muscle tissues from the caudal region following the ICP Waters Reports 105/2010 protocol.  
171 Collected intestinal tracts were then wrapped in aluminum foil, while muscle tissues were  
172 transferred to 50 mL Falcon tubes and placed in a freezer set at -20°C, where samples were kept  
173 until further analysis (intestinal tract was analyzed for MP identification, and muscle tissue for  
174 THg concentration and C- and N-isotopes). Before THg analysis, all muscle tissues were  
175 lyophilized using a Christ Alpha 1-2 freeze drier and manually ground into a powder using an acid-  
176 cleaned agate mortar. Fish health condition was assessed by calculating Fulton's condition factor  
177 ( $K$ ; Ricker, 1975) (**Table S2**):

178 
$$K = 100 \times \frac{W}{L^3}$$

179 *where  $W$  is the total wet weight (g) and  $L$  is the total length (cm)*

180

181 *Biofilm.* At each site, 2-4 rocks from three different points of the rivers and streams, and 5-10 rocks  
182 from the lake shore were collected, removed from the water, and gently brushed using clean

183 toothbrushes, after which the biofilms were transferred to acid-washed 50 mL Falcon tubes filled  
184 with 10 mL ultrapure water (PURELAB® Flex 1, 18.2 MΩ.cm) (BER, ARG, BAS, LAQ) or into  
185 previously decontaminated 25 mL glass vials (SET, BAR, CAT, SOR, VAL, ARC). All samples  
186 were kept in cool and dark conditions during transport back to the laboratory, where they were  
187 immediately frozen at -20°C. Prior to THg and MP analysis, biofilm samples were lyophilized  
188 using a Christ Alpha 1-2 freeze drier and manually homogenized using a clean stainless-steel  
189 spatula. A portion of the sample was used for THg analysis (one to three samples analyzed per  
190 site), and for C- and N-isotopes analysis (one to five samples analyzed per site), and the rest for  
191 MP analysis (one to three samples analyzed per site) (**Table S3a, S3b, S3c**).

192

193 *Sediments*. At each site, sediment samples were collected using either pre-cleaned stainless-steel  
194 scoop and grab sample technique (BER, ARG), a sediment corer with a 10 cm diameter PVC  
195 sample tube (BAS, LAQ) of which the top 2-5 cm were retrieved and mixed into one composite  
196 sample, or by placing a decontaminated 250 ml glass jar into the streambed and using a stainless  
197 steel spatula collecting the top layer of sediments (2-3 cm) (SET, BAR, CAT, BER, SOR, VAL,  
198 ARC). This procedure was repeated on three sites to ensure spatial representability and obtain one  
199 sediment sample per study site. Once in the lab, sediments were dried at 45-50°C, a portion of the  
200 sample was used for MP analysis (one to three per site), and the remaining sample was sieved  
201 through 50 µm mesh, and fine sediment (<50 µm) was used for THg analysis (two to four per site)  
202 (**Table S4a, S4b**). Due to the relatively high water content within the sediment samples, we opted  
203 to go for drying at slightly higher temperatures to ensure that the samples would be dry within a  
204 reasonable timeframe. We acknowledge that a small portion of the THg may have been lost, yet  
205 we consider this loss to be <5% of the THg content (Hojdová et al., 2015; Smeds et al., 2022).

206

207 *2.3 Analytical procedures*

208 *Mercury analysis.* Total Hg (THg) concentrations were measured in ~100 mg of muscle tissues  
209 (50-300 mg; n=66) and ~100 mg of sediment (10-260 mg; n=22) using a direct mercury analysis  
210 technique (DMA, i.e., thermal decomposition, amalgamation, and atomic absorption) with a  
211 Milestone DMA-80 or with an advanced mercury analyzer (AMA) for ~25 mg of biofilm (0.6-112  
212 mg; n=56 including replicates), and for sediment/muscle samples inferior to the detection limit of  
213 the DMA, at the Midi-Pyrenees Observatory/GET Laboratory, Toulouse, France. Analytical  
214 settings and calibrations followed the same protocol described in Hansson et al. (2017). Analytical  
215 quality was verified by including replicates and certified reference material (CRMs, i.e., NIST  
216 1547, NIST 1515, IAEA 336, BCR277r, DORM-3, DOLT-4) after every 10<sup>th</sup> sample and blanks  
217 after every 3<sup>rd</sup> sample throughout the analysis. The relative deviation for CRM replicates was  
218 <16% (10±4%), and THg concentrations in DMA blanks ranged from 0 to 0.69 ng THg (0.05±0.10  
219 ng THg) and AMA blanks <0 to 0.379 ng THg (-0.01±0.08 ng THg) (**Table S5**).

220

221 *Microplastic analysis.* MP identification of particles (i.e., MP composition) in the digestive tract  
222 of fish (n=66) from all sites and sediments (n=19) from recent campaigns (SET, BAR, CAT, BER,  
223 SOR, VAL, ARC) was carried out by ALS Scandinavia AB, Sweden. All biofilm (n=17) samples  
224 and sediments (n=6) from French sites (BER, ARG, BAS, LAQ) were analyzed in the  
225 Microplastics Lab of the LEFE-CNRS. To avoid contamination, we used cotton lab coats, and all  
226 glass equipment was rinsed three times with Milli-Q® ultrapure water (18.2 MΩcm). We also ran  
227 contamination controls to consider MP contamination in the lab environment while processing the  
228 samples (**Text S1**). Biofilm (0.27-2.58 g) and sediment (1.05-41.60 g) samples were digested with

229 30% H<sub>2</sub>O<sub>2</sub> (20-40 ml, 50°C, for 24-48 h). Density separation of ZnCl<sub>2</sub> (1.6 kg L<sup>-1</sup>, 1-3 days) was  
230 done to remove the mineral fraction. The supernatant was filtered through a 50 µm metal mesh,  
231 and the recovered material was transferred into a glass jar to apply a second H<sub>2</sub>O<sub>2</sub> (30%) digestion  
232 catalyzed with Fenton's reagent (stirring conditions, ambient temperature, 24 h). After digestion,  
233 the solution was filtered on 47 mm PVDF membranes, and MP quantification and identification  
234 were carried out with a Nicolet iN10 (Thermo Fisher Scientific) µ-FTIR microscope at the GET-  
235 CNRS, Toulouse, France (**Text S2**). Since our samples were analyzed by FTIR in two different  
236 laboratories, we could not obtain complete information about the shape and size of all samples;  
237 thus, we mainly focused on fragments >50 µm. Information about MP particle size in the biofilm  
238 can be found in **Table S6**.

239

240 *Carbon and Nitrogen isotopes.* Measurements of stable isotope ratios δ<sup>13</sup>C and δ<sup>15</sup>N of fish muscle  
241 (n=66) (**Table S2**) and biofilm (n=16) (0.3-0.6 mg) (**Table S3c**) were performed using an IRMS  
242 elemental analyzer (Isoprime 100) at the SHIVA analytical platform of ENSAT-INPT-LEFE  
243 Toulouse, France. Analytical quality was controlled using internal standards and replicates (i.e.,  
244 Urea, Acetanilide, Caffeine, Ammonium Sulfate), and detailed information on accuracy and  
245 reproducibility is presented in **Table S7** in the supplementary information file. δ<sup>13</sup>C indicates the  
246 carbon source, while δ<sup>15</sup>N indicates the trophic position, increasing at higher levels. It should be  
247 noted that data on THg, δ<sup>13</sup>C, and δ<sup>15</sup>N in trout muscle tissue from BAS have previously been  
248 reported by Hansson et al., (2017), all other data are novel and unreported.

249

250 *2.4 Data analysis*

251 All statistical tests and analyses were performed using RStudio (v.2023.09.1) and Python (Python  
252 Software Foundation, <https://www.python.org/>). According to Shapiro-Wilk normality tests, the  
253 residuals of our fish variables (*THg and MP concentrations, K, and  $\delta^{13}C$  and  $\delta^{15}N$  content*) were  
254 not normally distributed (except for  $\delta^{15}N$ ), even after applying log (x+1) transformation. We  
255 standardized THg values at a mean fork length of 20 cm to explore the spatial distribution of THg  
256 concentration explained by environmental variables and remove the effect of bioaccumulation  
257 (Médiéu et al., 2022). Non-parametric Kruskal-Wallis test was used to test significant differences  
258 ( $p < 0.005$ ) across sites for each type of system (i.e., transport or deposition) and human pressure  
259 (i.e., low, moderate, high) for standardized THg and MP concentrations,  $K$ , and  $\delta^{13}C$  and  $\delta^{15}N$   
260 content in fish. Post-hoc Dunn's test was used to run pairwise multiple comparisons following  
261 Kruskal-Wallis test. We applied the non-parametric Spearman correlation test (statically  
262 significant differences at  $p < 0.05$ ) and linear regressions to explore how river variables (i.e.,  
263 streamflow, velocity, streambed characterization, physicochemical variables), MacP and MesP  
264 concentrations in the riparian zone, MP concentrations in the water column, correlate with THg  
265 and MP concentration in fish, biofilm, and sediments. We also applied Spearman correlations to  
266 explore the potential effects of THg and MP concentrations in fish variables (i.e., *fish length, fish*  
267 *weight, K,  $\delta^{13}C$  and  $\delta^{15}N$  content in fish*). Finally, we applied a Canonical Correlation Analysis  
268 (CCA) to explore the combined relationship between the explanatory variables to MP pollution  
269 (i.e., altitude, human pressure, canopy cover, streamflow, velocity, conductivity, and presence of  
270 mud and sand), and the presence of MPs according to the density (i.e., low, intermediate, and high-  
271 density MPs) (Thompson, 1984). CCA was applied to the overall MP pollution in each site,  
272 considering the MPs found in the three studied environmental compartments and at each  
273 compartment individually. Before running CCA, all variables were standardized using

274 *StandardScaler* function from *sklearn* package after which we applied CCA to explore the effect  
275 of those river environmental variables (i.e., explanatory variables) on the presence of THg and  
276 MPs in each site and compartment (fish, biofilm, and sediment).

277

278

### 279 **3. RESULTS**

280 *Mercury*. THg was detected in all analyzed fish samples of the Pyrenees. Observed THg values  
281 ranged from 0.02 to 2.69 mg kg<sup>-1</sup> dry weight (d.w.; mean 0.36±0.58), with the highest mean values  
282 observed in ARG (0.17±0.00), BAS (0.20±0.05), and LAQ (0.20±0.02). Significant differences in  
283 standardized THg concentrations in fish were identified for ARG-CAT, ARC-SET, ARG-SET,  
284 and SET-SOR transport systems, and BER-LAQ deposition system ( $p < 0.05$ ). We observed lower  
285 THg concentrations in biofilm samples than in fish, with values ranging from <0.01 to 0.13 mg  
286 kg<sup>-1</sup> d.w. (0.04±0.03 mg kg<sup>-1</sup> d.w.). In biofilm, higher THg concentrations were found in ARG  
287 (0.3±0.01 mg kg<sup>-1</sup> d.w.), BAS (0.07±0.03 mg kg<sup>-1</sup> d.w.) and LAQ (0.06±0.02 mg kg<sup>-1</sup> d.w.). In  
288 sediments, THg ranged from 0.01 to 0.21 mg kg<sup>-1</sup> d.w. (0.07±0.06 mg kg<sup>-1</sup> d.w.), being higher in  
289 ARG (0.15±0.06 mg kg<sup>-1</sup> d.w.) and BAS (0.14±0.07 mg kg<sup>-1</sup> d.w.) (**Figure 2**).

290

291 *Microplastics*. Almost 40% of the fish caught in the Pyrenees presented MPs in their digestive  
292 tract (26 out of 66 fish), ranging from 0 to 59 MPs g<sup>-1</sup> d.w. (4.0±9.9 MPs g<sup>-1</sup> d.w.). Higher average  
293 MP concentrations in fish were found in SET (10.8±16.2 MPs g<sup>-1</sup> d.w.) and CAT (8.0±17.9 MPs  
294 g<sup>-1</sup> d.w.) (**Figure 2**). Seven plastic compounds were identified in fish, of which polypropylene (PP)  
295 was the most abundant plastic (59%) present in all sites except in SOR, followed by ethylene  
296 propylene diene monomer (EPDM) rubber (15%), polyethylene (PE, 13%) and polystyrene (PS,  
297 11%), polyamide (PA, <1%), polyvinyl chloride (PVC, <1%), and polyoxymethylene (POM,

298 <1%). SET and CAT showed higher variability in MP composition, identifying four and six  
299 different compounds, respectively (**Figure 3**). MPs in the biofilm were detected in all studied sites,  
300 ranging from 0 to 421 MPs g<sup>-1</sup> d.w. (76.4±119.9 MPs g<sup>-1</sup> d.w.), observing higher concentrations  
301 (and variability) in CAT (93.9±132.8 MPs g<sup>-1</sup> d.w.), BER (127.5±91.6 MPs g<sup>-1</sup> d.w.), and BAS  
302 (227.7±211.4 MPs g<sup>-1</sup> d.w.) (**Figure 2**). Six plastic compounds were identified in biofilm, of which  
303 polytetrafluoroethylene (PTFE) was the most abundant polymer (36%), followed by polyurethane  
304 (PUR, 20%), PP (19%), PA (13%), PE (11%), and polyethylene terephthalate (PET, 1%). Higher  
305 polymer diversity (4 polymers) was observed in deposition systems (i.e., BER, BAS, and LAQ)  
306 (**Figure 3**). Finally, MPs present in sediment ranged from 0 to 60 MPs g<sup>-1</sup> d.w. (6.2±12.8 MPs g<sup>-1</sup>  
307 d.w.). Higher MP concentration in sediments was found in BER (13.8±8.7 MPs g<sup>-1</sup> d.w.) and LAQ  
308 (60 MPs g<sup>-1</sup> d.w.) (**Figure 2**). In total, nine polymer types were identified in sediments being  
309 EPDM (35%), ethylene-vinyl acetate (EVA, 29%), and PUR (27%) the most abundant, followed  
310 by PE (4%), PP (2%), PS (1%), and SBR, PA, PET (all <1%). SET, BAR, CAT, BER, SOR, and  
311 ARC showed greater diversity (6 to 8 polymers per site) (**Figure 3**).

312  
313 *THg and MP distribution in rivers.* Overall, THg concentrations in fish (standardized), biofilm,  
314 and sediment were higher in ARG, BAS, and LAQ (**Figure 2**). Standardized THg in fish was  
315 positively correlated with streamflow ( $r_s = 0.83$ ,  $p < 0.05$ ) measured at base flow conditions. THg  
316 in biofilm was positively correlated with THg in sediment ( $r_s = 0.94$ ,  $p < 0.01$ ), and negatively  
317 with conductivity in water ( $r_s = -0.94$ ,  $p < 0.001$ ). THg in sediments was negatively correlated with  
318 conductivity ( $r_s = -0.82$ ,  $p < 0.05$ ). Concerning plastic pollution, MP concentration in fish was  
319 positively correlated with water velocity ( $r_s = 0.83$ ,  $p < 0.05$ ). MPs in the biofilm were positively  
320 correlated with conductivity ( $r_s = 0.82$ ,  $p < 0.05$ ). In addition, MacP and MesP concentrations in

321 the riparian zone were positively correlated with altitude ( $r_s = 0.83$ ,  $p < 0.05$ ). According to the  
322 CCA, low-density MPs (i.e., PUR, PP, EVA, PE) are influenced by human activity and streamflow  
323 in the global river environment, biofilm, and sediment compartments. Low-density MPs in fish are  
324 moderately influenced by the presence of mud and sand in sediments and negatively by altitude,  
325 human activity, and streamflow. In all cases, the presence of mud and sand positively influences  
326 the presence of intermediate-density MPs (i.e., PS, PA). High-density MPs (i.e., PVC, PET, POM,  
327 EPDM, PTFE, SBR) are positively influenced by altitude in the river environment and by altitude,  
328 conductivity, and mud and sand presence in sediments, while in biofilms, high-dense MPs are  
329 influenced by increased water velocity (**Figure 4**).

330

331 *THg and MP effects on biota.* Fulton's condition factor ( $K$ ) values ranged from 0.53 to 1.64,  
332 showing an average value of  $1.18 \pm 0.18$ , indicating an overall good health status of trout in this  
333 region ( $K > 1$ ) (Ragheb, 2023) (**Table S2**). Significant differences were obtained for ARG-SET,  
334 BER-SET, and LAQ-SET. However, lower  $K$  was obtained in BER ( $0.95 \pm 0.27$ ), ARG  
335 ( $0.99 \pm 0.10$ ), and LAQ ( $1.08 \pm 0.14$ ).  $K$  was negatively correlated with THg in fish ( $r_s = -0.55$ ,  $p <$   
336  $0.001$ ).

337

338 *Trout trophic position.*  $\delta^{13}\text{C}$  values ranged from -29.52 to -19.75 ‰ in fish ( $-23.28 \pm 2.31$  ‰), and  
339 -26.46 to -16.40 ‰ in biofilm ( $-22.10 \pm 3.01$  ‰), while  $\delta^{15}\text{N}$  values ranged from 2.69 to 10.13 ‰  
340 in fish ( $5.68 \pm 1.41$  ‰), and -27.94 to 12.08 ‰ in biofilm ( $-4.89 \pm 9.90$  ‰). Trout from BER occupies  
341 different trophic niches, as indicated by their lower  $\delta^{13}\text{C}$  values ranging from -29.52 to -27.31.  
342 Trout from LAQ exhibit much more diverse food sources, with  $\delta^{13}\text{C}$  values ranging from -25.11  
343 to -19.94. The  $\delta^{15}\text{N}$  values show relatively low and similar trophic positions for fish from CAT



344 (5.61±0.56), BER (5.37±0.52), ARG (4.12±0.15), SOR (5.11±0.61), and VAL (4.57±0.55), being  
345 higher in SET (7.42±0.52) and BAR (7.90±0.16). Fish from LAQ display more varied trophic  
346 positions, with  $\delta^{15}\text{N}$  values ranging from 2.69 to 10.13 (**Figure S1**).

347

348

#### 349 **4. DISCUSSION**

350 THg and MPs are found at all the studied sites across the altitudinal (i.e., altitude ranging from  
351 1269 m.a.s.l. to 2076 m.a.s.l.) and anthropogenic gradient (i.e., low, moderate, and high), and both  
352 are present in all freshwater compartments (fish, biofilm, and sediment). The average THg  
353 concentrations in the studied freshwater habitats (i.e., 0.36±0.58 mg kg<sup>-1</sup> d.w. for fish, 0.04±0.03  
354 mg kg<sup>-1</sup> d.w. for biofilm, 0.07±0.06 mg kg<sup>-1</sup> d.w. for sediment) are relatively low and do not exceed  
355 the recommended limits established by the European Food Safety Authority (EFSA) for fish  
356 consumption (2 mg kg<sup>-1</sup> d.w., for a wet:dry weight ratio of 4). The THg concentrations we obtained  
357 in biofilm and sediment are relatively low compared to previous studies. For instance, THg  
358 concentrations in biofilm from Canadian Boreal lakes were 0.14±0.04 THg mg kg<sup>-1</sup> d.w.  
359 (Desrosiers et al., 2006), and THg concentrations found in an ancient mining site were 0.16 THg  
360 mg kg<sup>-1</sup> d.w. in both river biofilm and sediments (Rhea et al., 2013). THg concentrations  
361 significantly differed across study sites depending on the type of system (i.e., transport or  
362 deposition). Overall, THg concentrations were higher in ARG, BAS, and LAQ in all the studied  
363 compartments. In ARG, a historical Ag-Pb mine (Dubois, 1999) could explain the higher levels of  
364 THg found in fish, biofilm, and sediments. Although the activity in the mine stopped in the 19<sup>th</sup>  
365 century, the high levels of THg in the sediments suggest that accumulated THg is being  
366 remobilized to other compartments, such as fish and biofilm. THg concentrations in BAS are  
367 higher than LAQ, potentially because of the proximity of BAS valley to ARG valley, as previously

368 reported concerning lead concentrations, and driven by atmospheric deposition (Hansson et al.,  
369 2019). Still, other Hg sources have been identified in the studied region. For instance, fish  
370 introductions via stocking programs are a source of marine Hg found in freshwater ecosystems  
371 (Hansson et al., 2017). In addition, ARG and BAS valleys could be more prone to accumulate Hg  
372 due to interactions with organic matter from the nearby peatlands, especially as peatlands are  
373 known hotspots for Hg methylation (Simola & Lodenius, 1982; Kolka et al., 1999; Mitchell et al.,  
374 2008; He et al., 2019). In LAQ, atmospheric deposition could explain high levels of THg in fish  
375 and biofilm since THg concentration in sediments ( $0.05 \text{ mg kg}^{-1} \text{ d.w.}$ ) was close to the average  
376 ( $0.07 \pm 0.06 \text{ mg kg}^{-1} \text{ d.w.}$ ). Other studies have observed an increase in THg concentration when  
377 increasing altitude, suggesting long-range atmospheric transport as the main source of THg in  
378 high-altitude environments (Blais et al., 2006; Blackwell & Driscoll, 2015). In our study, we did  
379 not find a significant correlation between increasing altitude and the concentrations of THg in the  
380 studied compartments. However, atmospheric deposition is the most likely source of THg in high-  
381 altitude aquatic environments (Fitzgerald et al., 1998; Duval et al., 2023). The strong connectivity  
382 between rivers and their watershed makes rivers sensitive to atmospheric deposition. Moreover,  
383 the characteristics of the watershed influence the cycle of Hg in rivers. For example, the presence  
384 of wetlands may increase the presence of MeHg in rivers (Brigham et al., 2009). We found a  
385 positive correlation between THg in fish and streamflow, which could be related to runoff inputs  
386 from the watershed (Bishop et al., 1995). Freshwater biofilms and sediments are known to be  
387 hotspots for the production of MeHg (Clarkson, 1993; Hammerschmidt et al., 2006). Allen-Gil et  
388 al. (1995) suggested that optimal conditions for methylation of Hg and its bioaccumulation in fish  
389 are slow streamflow and low conductivity. We found that those sites with higher conductivity  
390 presented lower THg in biofilm and sediments.

391  
392 Concerning MPs, distribution patterns across habitats differ from those obtained for THg  
393 concentrations: MP concentrations in biofilm ( $76.37 \pm 119.90$  MPs  $g^{-1}$  d.w.) were one order of  
394 magnitude higher than those found in fish ( $3.96 \pm 9.92$  MPs  $g^{-1}$  d.w.) and sediments ( $6.24 \pm 12.81$   
395 MPs  $g^{-1}$  d.w.). We identified MPs in 39% of fish ( $6.06 \pm 12.53$  MPs fish $^{-1}$ ). Although the  
396 prevalence of MPs is lower in the Pyrenees, the average number of MPs is higher than those from  
397 trout captured in an Irish river (72% of fish,  $1.88 \pm 1.53$  MPs fish $^{-1}$ ; O'Connor et al., 2020). More  
398 than 80% of the MPs identified in our fish were associated with the packaging industry (PP, PS,  
399 and PE). Low-density ( $\sim 0.8$  to  $1$  g  $cm^{-3}$ ) plastics are buoyant and can easily be found in the water  
400 column. Fish in SET and CAT present higher MP concentrations and a more diverse MP  
401 composition than other sites, which may be linked to the different type of human activities in the  
402 surrounding area. Moreover, CAT stream discharges into the SET river, connecting fish  
403 populations of both rivers. We found a positive correlation between MPs in fish and water velocity  
404 and a positive influence of mud and sand on MPs in fish. This could be explained by the generalist  
405 and opportunistic behavior of brown trout and the fact that individuals residing in rapid waters  
406 with higher velocity (e.g., juveniles) often feed on benthos macroinvertebrates and are thereby at  
407 risk of also ingesting sediment grains, as well as inherent exposure to microplastics (Jonsson, 1989;  
408 Heggenes et al., 1999; Montori et al., 2006; Krause et al., 2021). However, these relationships  
409 found in our study might not be causal since more MPs are found in fish from SET and CAT,  
410 where we measured a higher proportion of fine sediments and the highest velocity (SET  $0.61$  m  $s^{-1}$   
411 while the average was  $0.39 \pm 0.13$  m  $s^{-1}$ ). Biofilms are described as transient storage compartments  
412 of MPs (Guasch et al., 2022), and our results showed the highest MP concentration in biofilm  
413 compared to fish and sediment. To our knowledge, this is the first study to quantify MPs in the

414 biofilm matrix, so more studies are needed to understand the role of biofilm in trapping MPs.  
415 Deposition systems (i.e., BER and BAS) present greater MP concentration and variability in MP  
416 composition. The presence of low- and high-density MPs is almost equal and related to the  
417 automotive and insulating industry (PTFE, PUR), and the packaging industry (PP, PE). MP  
418 concentration in biofilms is positively correlated with conductivity and associated with higher  
419 streamflow and human pressure (low-density MPs), presence of mud and sand (intermediate-  
420 density MPs), and velocity (high-density MPs). These associations are consistent with those found  
421 in the overall river environment. Temporal changes in conductivity are associated with discharge,  
422 and greater values are usually found in polluted areas ( $>1000 \mu\text{S cm}^{-1}$ ). In our rivers, differences  
423 in conductivity ( $102 \pm 53 \mu\text{S cm}^{-1}$ ) may be related to different transport capacity of each body rather  
424 than pollution. Thus, our results indicate that the transport capacity of each studied waterbody  
425 explains MP presence in biofilm. In sediments, higher concentrations were found in LAQ, with  
426 PUR as the single plastic detected. The proportion of low- and high-density MPs is almost equal,  
427 however, the most abundant MPs are linked to the automotive industry and mountain technical  
428 gear (EPDM, EVA, PUR). Greater diversity of MPs was observed in those areas with high- and  
429 moderate- human pressure.

430

431 The Fulton factor obtained within this study indicates a good health status of the trout analyzed  
432 from the Pyrenees, given the contaminant concentrations observed (i.e.,  $K = 1.182 \pm 0.186$ ,  
433  $0.361 \pm 0.582 \text{ THg mg kg}^{-1} \text{ d.w.}$ ,  $3.96 \pm 9.92 \text{ MPs g}^{-1} \text{ d.w.}$ ); yet, lower Fulton's factor was obtained  
434 in BER ( $0.95 \pm 0.27$ ), ARG ( $0.99 \pm 0.10$ ), and LAQ ( $1.08 \pm 0.14$ ). Since THg concentrations were  
435 higher in ARG and LAQ, the lower Fulton's factor values obtained there suggest that THg  
436 potentially has a negative impact on fish health. However, the Fulton factor is a basic indicator,

437 and other variables, such as the harsh and oligotrophic conditions in high-altitude lakes, might  
438 affect fish growth. The lower Fulton's factor values observed in BER could indicate that trout from  
439 BER occupy a different trophic niche, since these fish have a less carbon-rich food source than  
440 those from other sites. We found that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in trout differed from sites and waterbody  
441 type, suggesting different resources and feeding behaviors. For example, the higher  $\delta^{15}\text{N}$  content  
442 in trout from SET and BAR suggests a more complex trophic structure in these environments than  
443 in SOR and ARC. Our results did not indicate a negative effect of MPs in fish health in the studied  
444 Pyrenees region. However, other studies have detected a negative impact of MPs on fish health at  
445 different scales, like physiological harm due to MP ingestion, toxicity, oxidative stress, changes in  
446 feeding behavior, growth retardation, reproductive abilities, and other metabolic disruption,  
447 leading to an increase in mortality (Amelia et al., 2021; Bhuyan, 2022). Further, severe  
448 neurotoxicity has been associated with aged MPs compared to virgin MPs in larval zebrafish  
449 (Xiang et al., 2023). Therefore, the Fulton factor used here is a basic indicator, and that perhaps  
450 more sensitive indicators should be used to detect potential effects at these MP concentrations  
451 (Alexandre, 2017). It has been shown that MPs bioaccumulate with increasing trophic levels  
452 (Farrell & Nelson, 2013; Parolini et al., 2023), and though our study focuses on the presence of  
453 MPs in digestive systems (which usually is not consumed by humans), MPs can be transferred to  
454 other tissues, like the muscle (Barboza et al., 2020; McIlwraith et al., 2021) in which case it would  
455 pose a potential risk to humans via dietary exposure.

456

457 The use of fish as bioindicators is widely extended across all aquatic systems for trace metals  
458 (Authman, 2015), persistent organic pollutants (Ueno et al., 2003), antibiotics (Ballash et al.,  
459 2022), and more recently, MPs (Saad et al., 2022). The dynamic nature of fish allows for assessing

460 the overall ecological status of aquatic systems. Because of its wider distribution and generalist  
461 feeding behavior, brown trout is considered a key species in the Pyrenees and can be a good  
462 bioindicator for assessing environmental pollution. In addition, biofilm has been largely used as a  
463 passive bioindicator of several pollutants (Guasch et al., 2016; Leguay et al., 2016; Hansson et al.,  
464 2019). Our results suggest that brown trout and biofilm are suitable bioindicators of THg distant  
465 and local pollution. Concerning MPs, our results suggest that MPs in fish are linked to local sources  
466 of pollution, specifically from single-use plastics, while MPs in biofilm (and sediments) suggest a  
467 combination of distant (i.e., atmospheric deposition) and local pollution sources. Moreover, MP  
468 presence in biofilm corresponds with MP distribution in the river environment. Thus, we conclude  
469 that biofilm is a more reliable bioindicator than fish when assessing global MP pollution in  
470 Pyrenees freshwater ecosystems.

471

472

## 473 **CONCLUSION**

474 THg and MPs were found in all the studied sites, and both were present in all freshwater  
475 compartments (i.e., biota, biofilm, and sediments). This study, therefore, provides, for the first  
476 time, a quantification of MPs embedded in the biofilm matrix of mountain ecosystems. Long-range  
477 transport appears to be the main source of Hg to these high-altitude environments, however, our  
478 results also showed that local (legacy) sources, combined with environmental characteristics such  
479 as peatlands or streamwater physicochemistry, also modulate the presence and distribution of THg  
480 in the studied areas. The MP distribution and composition indicate that MPs were driven by both  
481 distant and local sources, even though the dominant source differed between the studied media.  
482 We therefore highlight that studying different habitats (e.g., biota, water column, benthos)

483 simultaneously is essential to fully understanding the distribution patterns of MPs in remote  
484 aquatic ecosystems. Overall, the obtained Fulton's condition factor ( $K$ ) indicated a good health  
485 status ( $K > 1$ ) of the studied trout. However, values  $K < 1$  were obtained in fish with greater THg  
486 concentration, indicating a negative impact of THg on fish health, whereas MP – albeit in low  
487 concentrations - showed no such impact. Finally, we showed that concerning THg pollution, brown  
488 trout and biofilm may act as bioindicators of atmospheric deposition of Hg in high-altitude lakes,  
489 whereas, for plastic pollution, biofilm can be a reliable bioindicator to assess the global MP  
490 pollution in pristine freshwater mountain environments, while brown trout can be a suitable  
491 dynamic biosensor of local MP pollution at sites of higher human pressure.

492

493

## 494 **ENDNOTES**

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522

### 523 ***Conflict of interest/Competing interest***

524 The authors declare no competing financial interest, nor any conflict of interest.

525

### 526 ***Availability of data and material***

527 Information on measures to avoid MP contamination during sampling and processing and  
528 parameters used for  $\mu$ FT-IT for MP identification is described in detail in the SI file (Text



529 **S1** and **Text S2**, respectively). Information on each sampling site is presented in **Table S1**. All  
530 fish data is reported in **Table S2**, including THg and MP concentrations, weight, length, and sex  
531 of the collected fish, calculated Fulton's condition factor, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  content.  
532 Concentrations of THg and MPs, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  content in biofilm are detailed in **Table**  
533 **S3a**, **S3b**, and **S3c**, respectively. Concentrations of THg and MPs in sediments are presented  
534 in **Table S4a**, **S4b**, and **S3c**, respectively. **Table S5** gives detailed information about CRM,  
535 including recommended and measured values. **Table S6** presents information concerning MPs  
536 found in biofilm, including MP length, width, and type of polymer. Stable isotope ratios of  
537 reference standards measured for fish and biofilm samples and the reference value are presented  
538 in **Table S7**. **Table S8** gathers the abbreviations used in the present study. Finally, **Figure S1**  
539 shows linear regression for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in fish.

540

#### 541 *Author contributions*

542 HM and SVH designed the study with input from all authors; all authors participated in various  
543 aspects of the fieldwork, sample collection, and sample preparation; HM performed all lab work  
544 and analysis together with LC, with input from SVH, JES, LL, REZ and GLR; HM performed all  
545 statistical analysis in close discussion with SVH and GLR; all authors participated in the  
546 interpretation of the results, HM wrote the manuscript in close discussion with SVH and with input  
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548

#### 549 *Ethics*

550 The authors declare that collection and sacrificing of all fish included in this study was performed  
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885



886 **FIGURES and TABLES**

887

888 **Figure 1.** Location of the Pyrenees region sampled for the present study. Sites located in (1) and  
889 (2) are part of France, while (3) are in Andorra and (4) in Spain. We selected different sites  
890 according to their altitude (m.a.s.l.) and the level of human pressure (i.e., low, moderate, high).  
891 The level of human pressure was calculated according to the number of current human activities  
892 (i.e., hiking, skiing, village (>200 inhabitants), agriculture and/or pastoralism, science monitoring,  
893 fishing, and the presence of road (< 300 m). Source of images: Google Earth.

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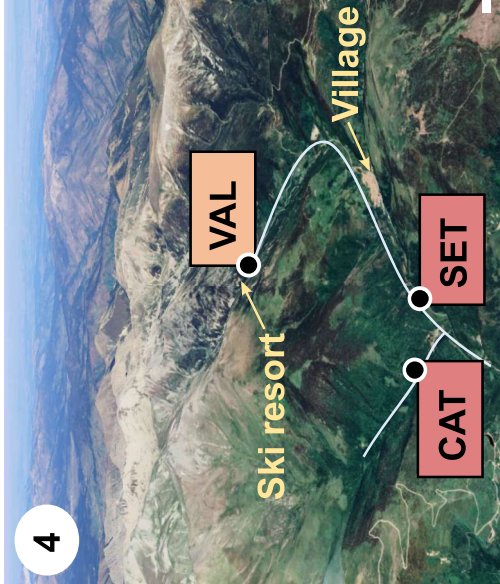
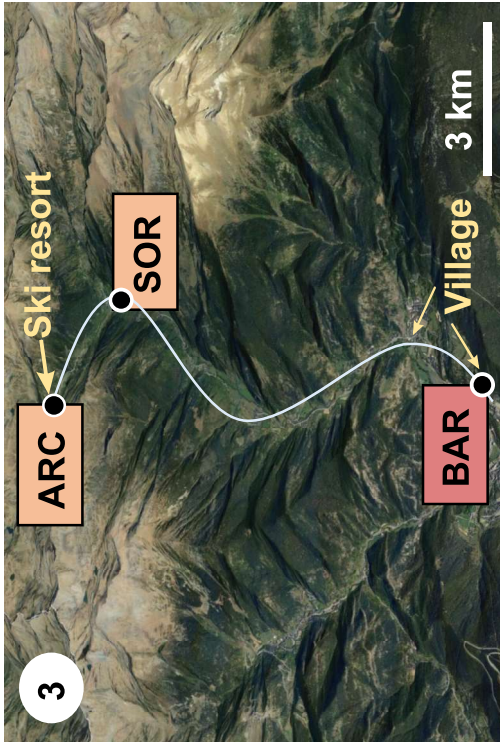
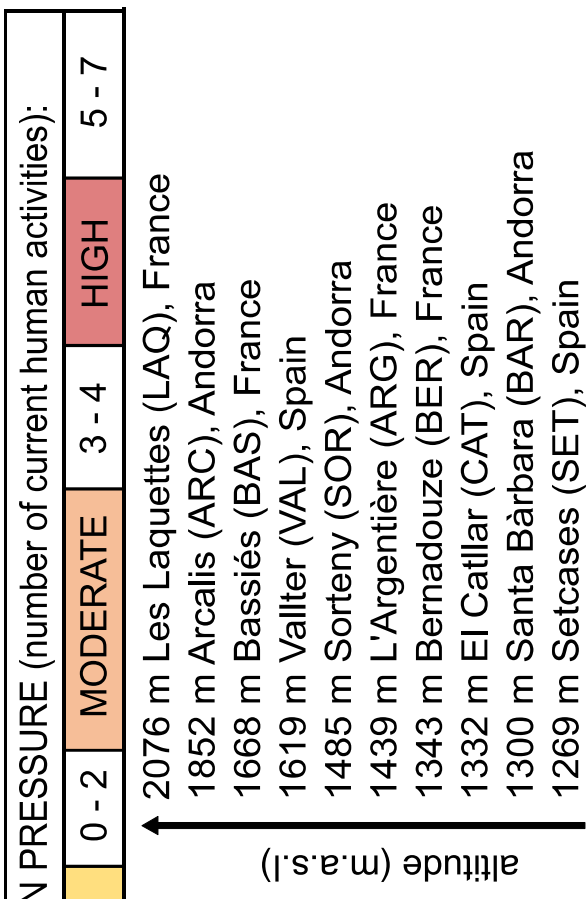
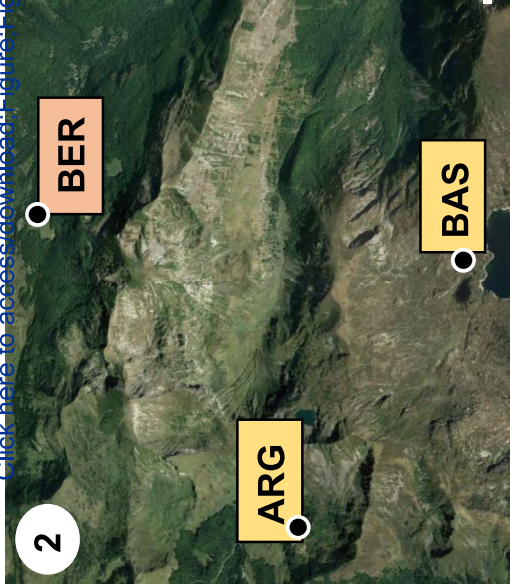
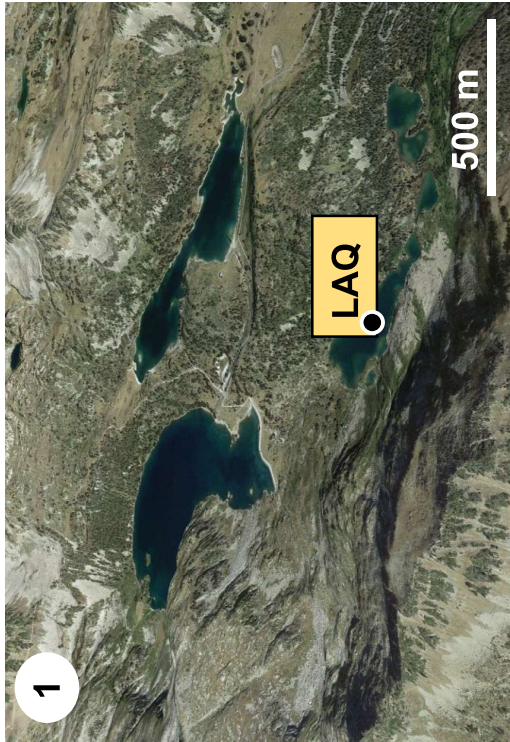
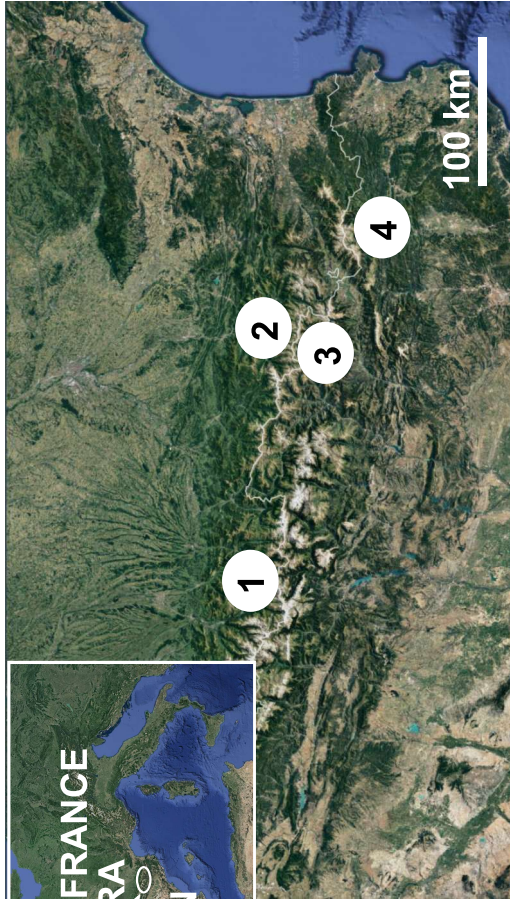
895 **Figure 2.** Average THg (mg kg<sup>-1</sup> per dry weight (d.w.)) and MPs (MPs g<sup>-1</sup> d.w.) concentrations in  
896 fish, biofilm, and sediments. THg concentrations in fish are standardized according to the average  
897 length (20 cm). Note that the range of the y-axis shifts between graphs. Transport systems (i.e.,  
898 SET, BAR, CAT, ARG, SOR, VAL, ARC; white background) and deposition systems (i.e., BER,  
899 BAS, LAQ; grey background) are ranked according to altitudinal gradient for THg, and according  
900 to local human pressure (H-high: red, M-moderate: orange, L-low: yellow) for MP concentrations.  
901 (\*) Indicates those sites that showed significant differences according to Dunn's test (p < 0.05).

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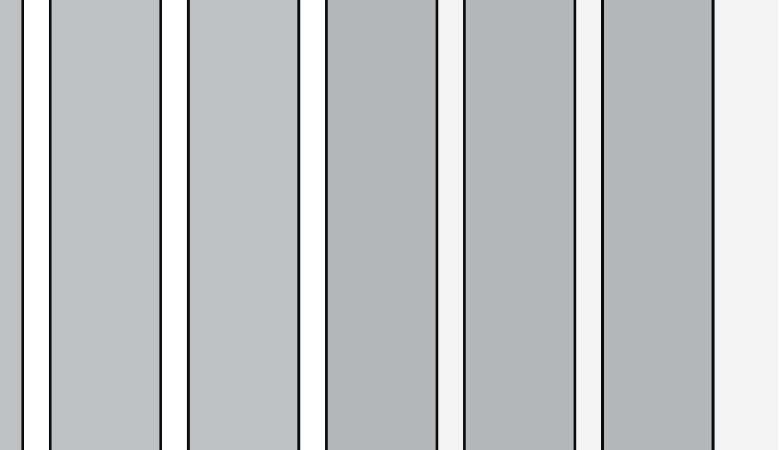
903 **Figure 3.** MP composition in the three freshwater compartments analyzed: fish, biofilm, and  
904 sediments. The presented data indicate the percentage of each polymer found. Transport systems  
905 (i.e., SET, BAR, CAT, ARG, SOR, VAL, ARC; white background) and deposition systems (i.e.,  
906 BER, BAS, LAQ; grey background) are ranked according to local human pressure from the highest  
907 (i.e., SET in transport systems, and BER in deposition systems) to the lowest (i.e., ARG in  
908 transport systems, and BAS and LAQ in deposition systems).

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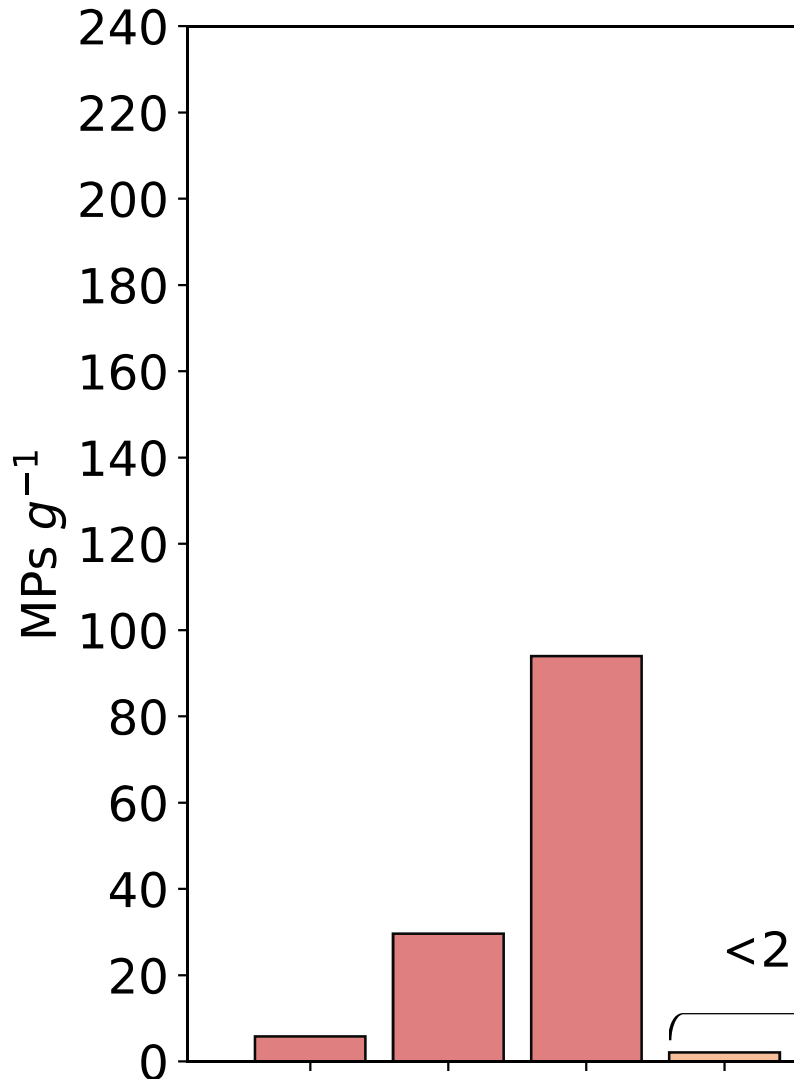
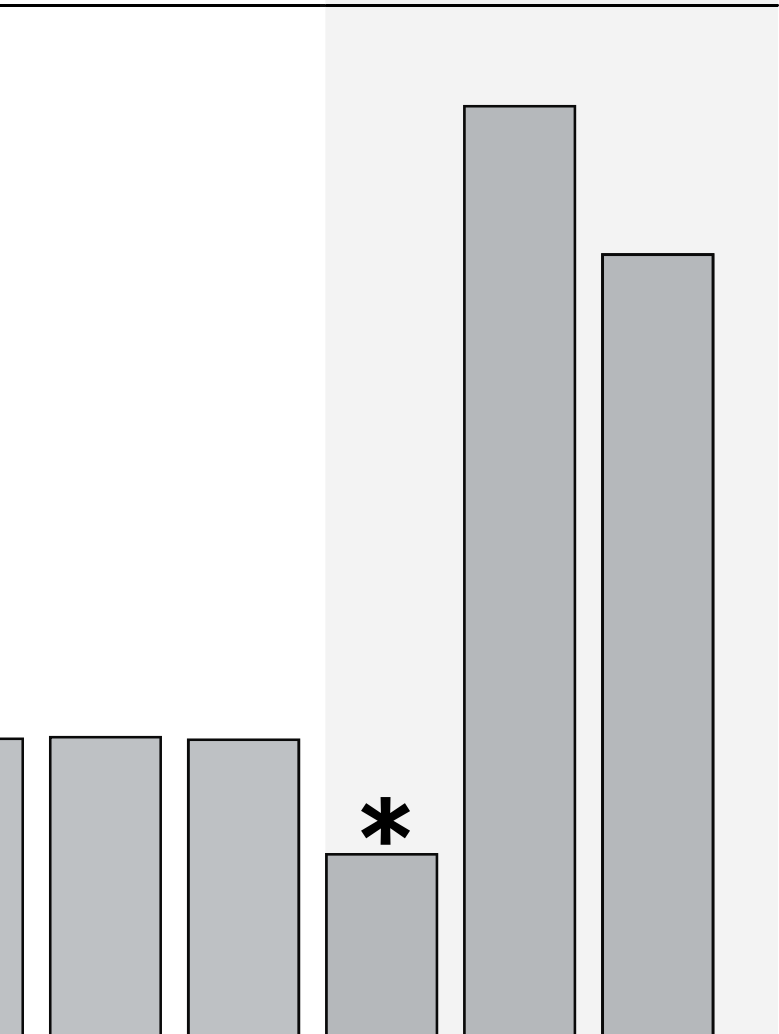
910 **Figure 4.** CCA biplots represent the distribution of MPs (*canonical variates of Y*) according to  
911 plastic density: low (*low*), intermediate (*int*), and high (*high*) in the river environment, fish,  
912 biofilm, and sediment. The river environment comprises all MPs found in fish, biofilm, and  
913 sediment. Explanatory variables (*canonical variables of X*) are altitude (*asl*), human pressure  
914 (*hum\_act*), exposition to atmospheric deposition (*canopy\_cover*), streamflow (*Q*), water velocity  
915 (*vel*), water conductivity (*cond*), and proportion of mud and sand in the streambed (*mud\_sand*).  
916 Canonical X and Y variates were calculated from the average values of each variable in each  
917 studied site.



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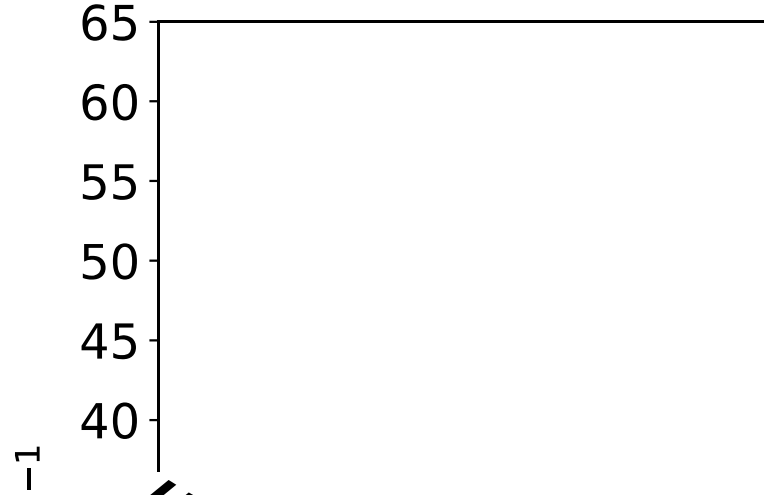
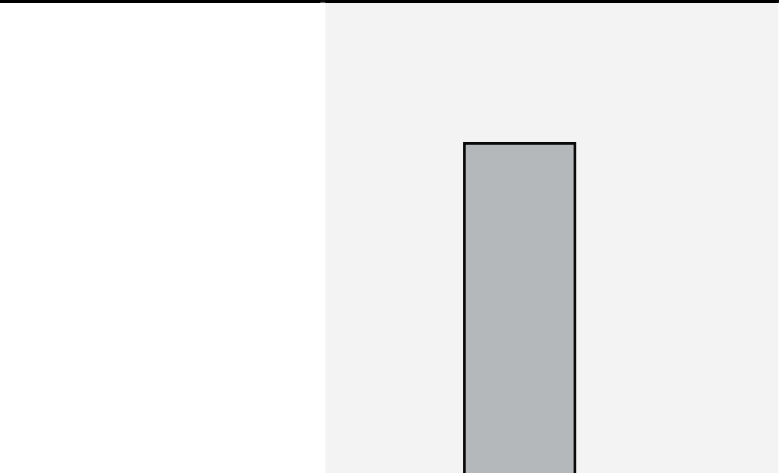
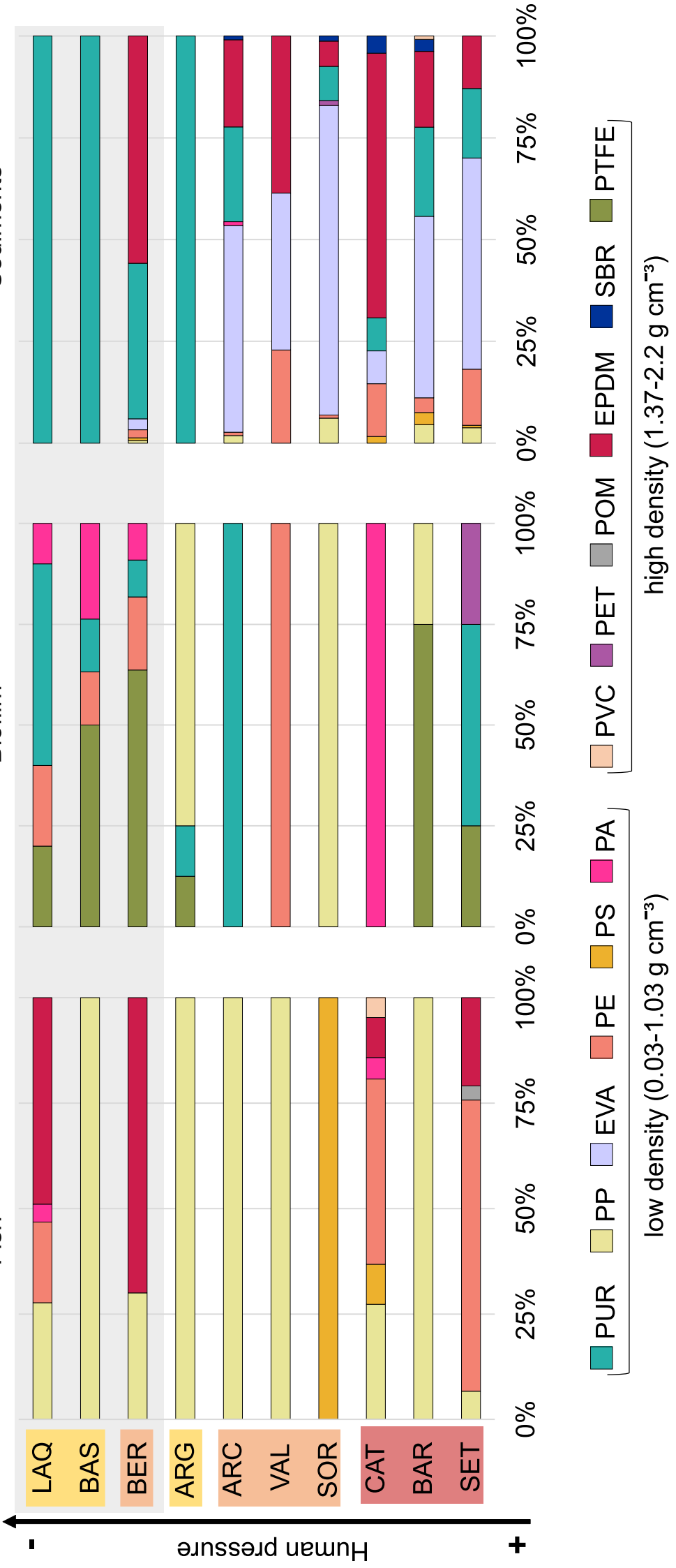
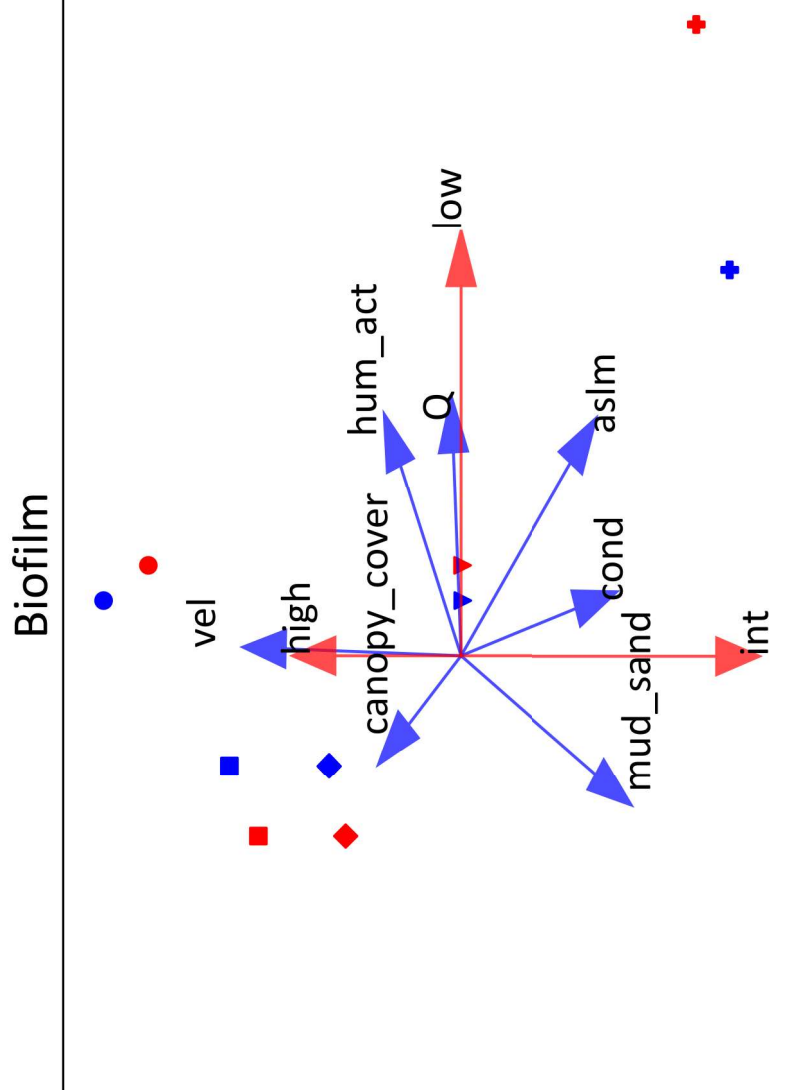
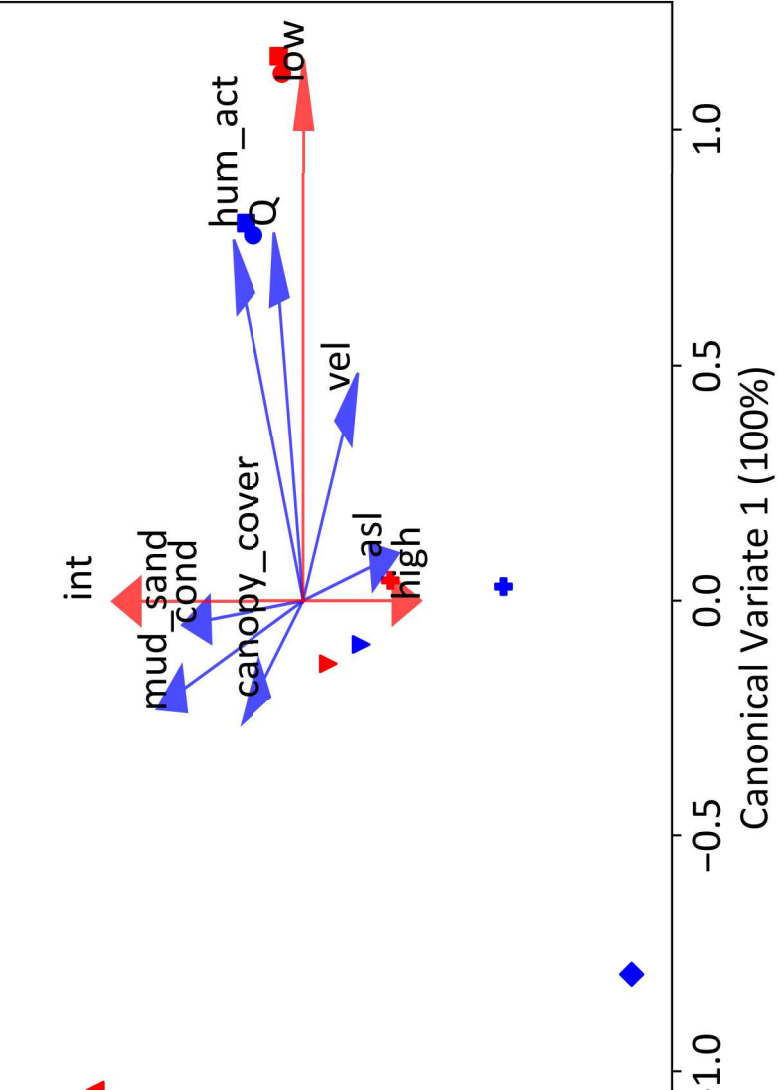
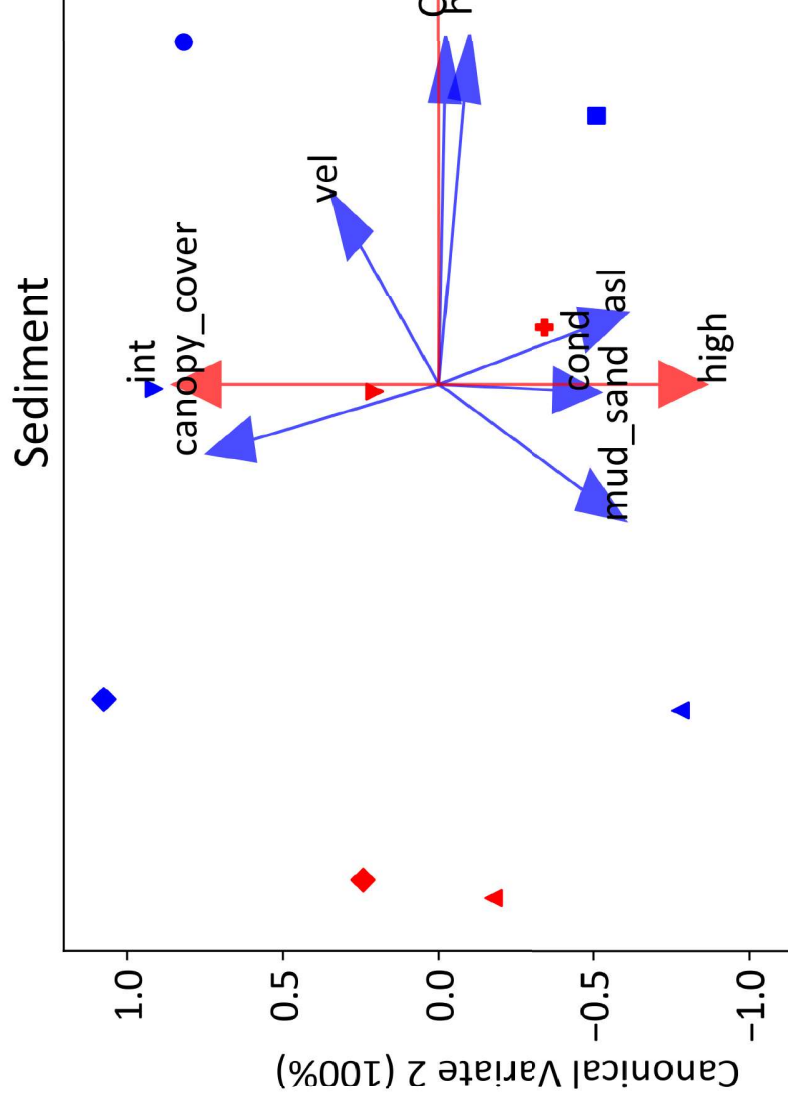
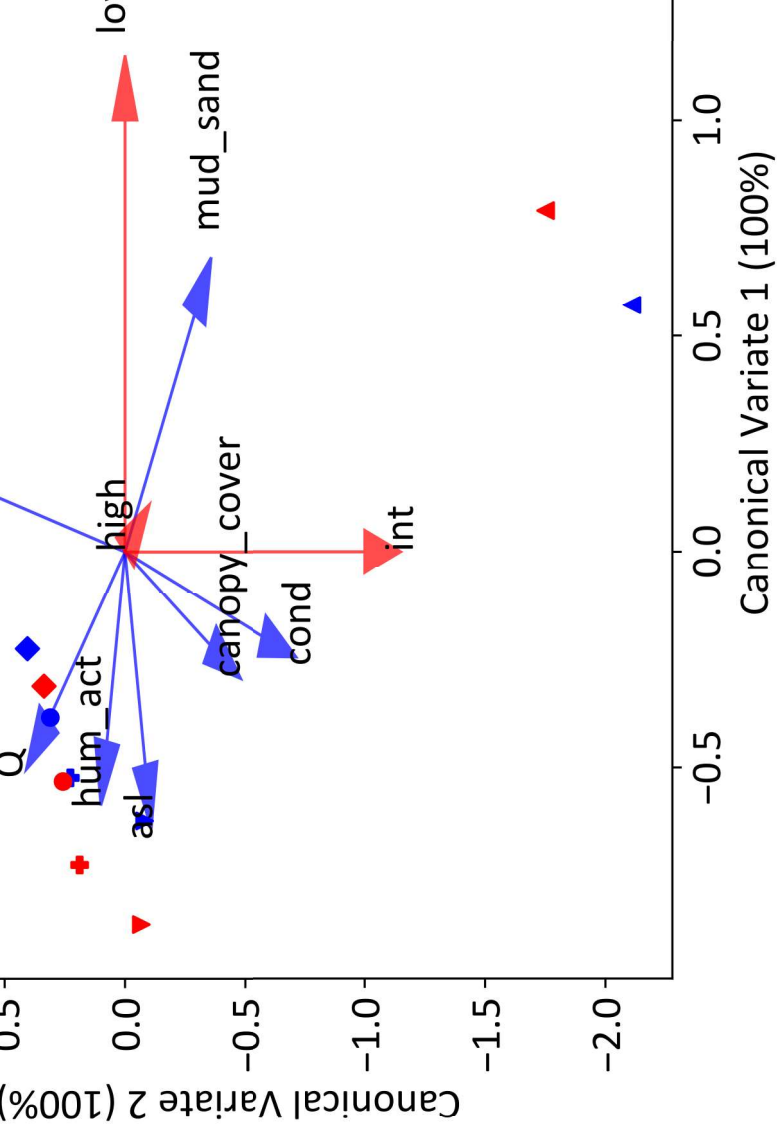
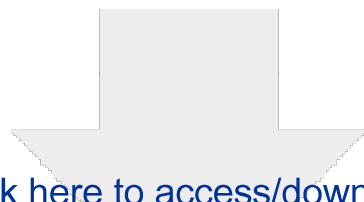


Figure 3

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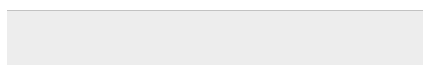
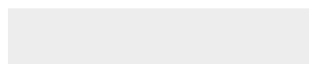




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**Supplementary Material**

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Atmospheric deposition, human pressure, and hydrology as drivers of mercury and microplastic pollution in mountain freshwater environments

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### *Author contributions*

HM and SVH designed the study with input from all authors; all authors participated in various aspects of the fieldwork, sample collection and sample preparation; HM performed all lab work and analysis together with LC, with input from SVH, JES, LL, REZ and GLR; HM performed all statistical analysis in close discussion with SVH and GLR; all authors participated in the interpretation of the results, HM wrote the manuscript in close discussion with SVH and with input from all other authors.



### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

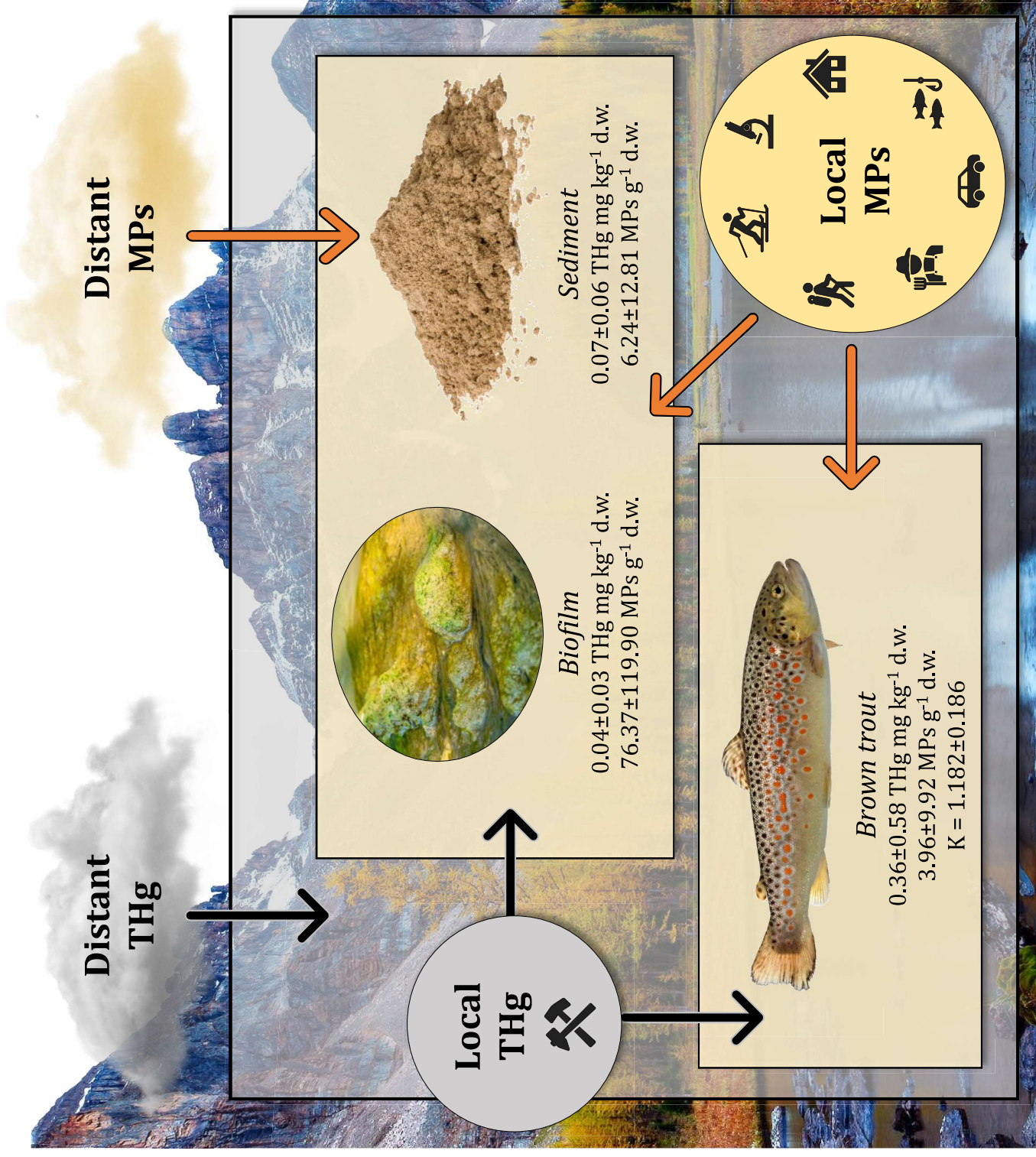
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What source of THg and MPs dominate on the Pyrenees?

How do THg and/or MPs impact the health (K) of the fish?

Can brown trout and biofilms be reliable bioindicators of THg and pollution in mountains?



Abbreviations: total mercury (THg), microplastics (MPs), Fulton's condition factor (K)