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Biomagnification of perfluoroalkyl acids (PFAAs) in the food web of an urban river: assessment of the trophic transfer of targeted and unknown precursors and implications

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Abstract

The present work examined the trophic transfer of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in a typical urban river (Orge River, near Paris, France), and aimed to investigate the potential contribution of precursors to the biomagnification of perfluoroalkyl acids (PFAAs). Sixteen PFAAs, twelve of their precursors (pre-PFAAs_{targeted}) and two fluorinated alternatives to long-chain PFASs were analyzed in water, sediments and biota (including biofilm, invertebrates and fish). Twenty two compounds were detected in biological samples (2.0–147 ng g⁻¹ wet weight), perfluorooctane sulfonate (PFOS) and C₁₂–C₁₄ perfluoroalkyl carboxylates (PFCAs) being predominant while Σ pre-PFAAs_{targeted} contributed to 1–18% of Σ PFASs. Trophic magnification factors (TMFs) were > 1 (i.e. denoting biomagnification) for C₉–C₁₄ PFCAs, C₇–C₁₀ perfluoroalkyl sulfonates (PFSAs) and several pre-PFAAs (e.g. 8:2 and 10:2 fluorotelomer sulfonates). The significant decrease in Σ pre-PFCAs/ Σ PFCAs concentration ratio with trophic level suggested a likely contribution of selected precursors to the biomagnification of PFCAs through biotransformation, while this was less obvious for PFOS. The Total Oxidizable Precursor assay, applied for the first time to sediment and biota, revealed the presence of substantial proportions of extractable unknown pre-PFAAs in all samples (i.e. 15–80% of Σ PFASs upon oxidation). This proportion significantly decreased from sediments to invertebrates and fish, thereby pointing to the biotransformation of unattributed pre-PFAAs in the trophic web, which likely contributes to the biomagnification of some PFAAs (i.e. C₉–C₁₂ PFCAs and C₇–C₁₀ PFSAs).

1. Introduction

Perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs) are of major environmental concern because of their ubiquitous occurrence, persistence and toxicity¹. These chemicals, used as additives and surfactants in a large number of industrial applications and manufactured products, can be introduced into the environment following their production, their use and their disposal, either directly or indirectly via the degradation of precursors². Their worldwide presence in aquatic wildlife was reported since the early 2000s^{3,4}. and long-chain perfluoroalkyl acids (PFAAs) (i.e. PFCAs with carbon number $n_c > 8$ and PFSAs with $n_c > 6$) are recognized as bioaccumulative substances⁴.

Exposure to PFASs can occur via direct and dietary uptake. High PFAS levels reported in aquatic mammals from remote areas suggest the major role of the trophic pathway³. Biomagnification factors (BMFs) and trophic magnification factors (TMFs) are field-based metrics relevant to assess the biomagnification potential of a contaminant⁵. BMFs and TMFs > 1 have been consistently reported for PFOS and long-chain PFCAs in marine, lake or estuarine food webs, providing evidence for their biomagnification^{6–11}. However, data showed a considerable study-to-study variability that may be explained by factors such as sampling design, taxa and ecosystem properties, statistical data processing and, possibly, the biotransformation of precursors¹².

Numerous PFASs are less persistent than PFCAs or PFSAs and may be converted into PFAAs in the environment. Identified PFAA precursors (pre-PFAAs) include for instance (alkyl-)perfluoroalkyl sulfonamides ((alkyl-)FASAs)^{13–15}, (alkyl-) perfluoroalkyl sulfonamidoacetic acids ((alkyl-)FASAAs)² or polyfluoroalkyl phosphoric acid diesters (diPAPs)¹⁶. Gebbink et al.,¹⁷ proposed that precursors could play a significant role in the biomagnification of PFCAs in fish from the Baltic Sea and species-specific biotransformation of pre-PFAAs was suggested in fish from the Rhône River¹⁸. Additional pre-PFAAs including fluorotelomer sulfonates (FTSAs) previously detected in aquatic ecosystems^{19,20}, could also contribute to the biomagnification of PFAAs, since their biotransformation can lead to the production of PFCA homologues^{21–23}. However, like that of diPAPs, their trophic transfer in aquatic ecosystems

has been little investigated so far. Such pre-PFAAs may originate from numerous sources and urban rivers are considered as a major receptacle for these chemicals ²⁴.

The present study addressed the trophodynamics of PFASs in an urban river and it specifically focused on the potential contribution of a wide range of pre-PFAAs to the biomagnification of PFAAs. Water, sediment, and biota samples (including biofilm, invertebrates and fish) were collected in the Orge River (France), previously identified as a hotspot of PFAS contamination at French nationwide level ²⁵. Thirty PFASs were analyzed including PFCAs, PFSAAs and numerous pre-PFAAs such as FTSAAs, (alkyl-)FSAAs, (alkyl-)FSAAs and diPAPs. The levels of two per- and polyfluoroalkyl ethers (PFPEs), HFPO-DA (namely GenX) and ADONA, were also determined to provide additional knowledge on the presence and behavior of these emerging PFASs used as alternative to long-chain PFAAs ²⁶. In addition, the occurrence of unknown pre-PFAAs was indirectly estimated using the Total Oxidisable Precursor (TOP) assay, originally developed to convert pre-PFAAs into PFCAs in urban runoff samples ²⁷. This approach proved relevant to demonstrate the presence of unknown pre-PFAAs in wastewater, ground water and soils samples ^{27–31}. In the present work, the TOP assay was applied for the first time to biota and sediment samples to test the hypothesis that unknown pre-PFAAs contribute to the biomagnification of PFASs, which was estimated through the calculation of field-metrics such as BMFs and TMFs.

2. Materials and methods

2.1. Sampling

Sampling was performed in September 2016, along a 500 m-transect on the Orge River at Viry-Châtillon, a few kilometers upstream of the confluence with the Seine River (48°40'23"N; 2°21'30"E) (Figure S1 of the Electronic Supporting information (ESI)). This medium-sized river (mean flow rate $\approx 5 \text{ m}^3 \cdot \text{s}^{-1}$) receives both sewage discharge and urban runoff ³².

Samples (n=29, Table S1)) of eight fish species were collected by electrofishing: barbel *Barbus barbus*, European bullhead *Cottus gobio*, roach *Rutilus rutilus*, gudgeon *Gobio gobio*, common perch *Perca fluviatilis*, Pumpkinseed *Leopomis gibbosus*, bullhead catfish *Ameiurus melas* and tench *Tinca tinca*. Such sampling strategy allowed the collection of species with supposedly contrasted feeding behavior (i.e. benthic vs bentho-pelagic, omnivorous vs carnivorous...). The permit for the capture of fish for scientific purposes was delivered by local authorities (Departmental Direction of Territories). Fish were identified, anesthetized (tricaine methanesulfonate, 1 g L⁻¹ in river water), euthanized on the field and transferred into aluminium trays kept at 4°C. Once in the laboratory, they were measured, weighted and stored at -20 °C; the smallest individuals of each taxa were pooled (Table S1) while the others were processed individually.

Invertebrates were collected with a surber net and both poor diversity and population density were observed. Therefore, only four taxa could be collected and composite samples were obtained as follows: *lymnaeae* (n=3), *gammaridae* (n=3), *notonectidae* (n=1) and *corbiculidae* (n=1). Two samples of the macrophyte *Ranunculus pseudofluitans*, two periphytic biofilm samples and two leaf litter samples were also collected. Overall, 43 biological samples representative of the whole trophic web were collected, a number that is reasonable for TMF assessment ⁵.

In addition, three composite surface sediment samples (0–2cm) were taken along a 100 m-transect (Figure S1) with a stainless steel spoon. All samples were transported in aluminum trays kept at 4°C in the field, then stored at -20°C. Surface water was collected in a 1L-HDPE bottle (n=1); an aliquot was filtered on GF/F filters to determine the suspended matter content (15.7 mg L⁻¹).

2.2. Compounds and reagents

Thirty PFASs were analyzed: eleven PFCAs (C₄–C₁₄), five PFSAAs (C₄, C₆–C₈ and C₁₀), four FTSAAs (4:2, 6:2, 8:2 and 10:2 FTSA), three (alkyl-)FSAAs (FOSA, MeFOSA, EtFOSA), three (alkyl-)FSAAs (FOSAA, MeFOSAA, EtFOSAA), two diPAPs (6:2 and 8:2 diPAP) and two per- and polyfluoroalkyl ethers PFPEs

(ADONA and HFPO-DA) (see Table S2 for details, including internal standards (ISs)). Standard solutions (chemical purity > 98%) were acquired from Wellington Laboratories (via BCP Instruments, Irigny, France). A full list of chemicals and solvents is provided in the ESI.

2.3. Sample preparation, extraction and analysis

Biota samples were freeze-dried, ground and homogenized prior to analysis. Sediments were also freeze-dried, sieved at 2 mm and homogenized.

Biota samples were processed using a previously published method ¹¹: microwave-assisted extraction was performed on 0.2 g-samples (dry weight, dw) with 12 mL of MeOH + 0.2% NH₄OH and followed by Strata X-AW/graphitized carbon clean-up. Sediment samples were processed similarly but clean-up was performed using graphite only. Unfiltered water samples (100 mL) were concentrated using solid phase extraction on Strata X-AW cartridges ¹⁹. Extracts were evaporated to 300 µL under a nitrogen stream and stored at -20°C prior to analysis. ISs (1 ng each) were added at the beginning of the extraction procedure in samples, procedural blanks and spiked control samples.

PFAS analysis was carried out by liquid chromatography coupled with tandem mass spectrometry using a 1200 LC system and a 6490 triple quadrupole mass spectrometer from Agilent Technologies (Massy, France); the electrospray ionization source was operated in negative mode ¹¹. Further details are provided in the ESI. Note that sum-branched (Br-PFOS) and linear PFOS (L-PFOS) were quantified separately using the calibration curve of L-PFOS.

2.4. Total Oxidizable Precursor (TOP) assay

The principle of the TOP assay has previously been published ³¹. Briefly, extracts are exposed to hydroxyl radicals generated by the thermolysis of persulfate under basic pH conditions, to promote the conversion of pre-PFAAs into PFCAs of similar or shorter perfluoroalkyl chain length.

The TOP assay was applied to sediment and water samples; due to sample availability, a limited set of selected biota samples ($n = 15$) were also treated with this method. For water samples, the oxidation procedure was adapted from Houtz and Sedlak ²⁷: samples were amended with persulfate (60 mM) and NaOH (150 mM) and incubated at 85°C for 6 h. To prevent their oxidation, ISs (4 ng each) were added after sample cooling and pH neutralization with HCl (3 M); samples were subsequently extracted as described in the previous section. The biota and sediment extract oxidation procedure was adapted from Houtz et al. ²⁸. After microwave extraction (MeOH + 0.2 %NH₄OH) and graphite clean-up, extracts were concentrated to 100 μ L, transferred into 125 mL-HDPE bottles and evaporated to dryness. Then, 100 mL of ultra-pure water containing 60 mM of persulfate and 150 mM of NaOH were added to each bottle; sonication (20 min) was performed to promote the dissolution of PFASs. Samples were then processed as described above for water samples.

The sum of pre-PFAAs targeted in this study (i.e. FTSAs, (alkyl-)FASAs, (alkyl-)FASAs and diPAPs) was thereafter termed $\sum \text{pre-PFAAs}_{\text{targeted}}$. TOP assay data were used to calculate the increase of $\sum \text{PFCAs}$ after oxidation (noted thereafter ΔPFCAs , expressed on a molar basis), which allowed to estimate the total extractable amount of pre-PFAAs ($\sum \text{pre-PFAAs}_{\text{total}}$) in each sample (see ESI for calculation details). Based on these results, the fraction of unidentified precursors ($\sum \text{pre-PFAAs}_{\text{unknown}}$) could be determined for each sample treated with the TOP assay, i.e. $\sum \text{pre-PFAAs}_{\text{unknown}} = \sum \text{pre-PFAAs}_{\text{total}} - \sum \text{pre-PFAAs}_{\text{targeted}}$.

2.5. Stable isotope analysis

The isotopic composition of biota samples (C and N) was evaluated on defatted samples ³³ while carbonates were removed from sediments with diluted HCl. Analysis was performed on 0.2 (± 0.1) mg of matrix using a ThermoFinnigan Delta V EA-IRMS with a Conflo IV interface. Carbon and nitrogen isotope compositions were expressed as per mil (‰) in the δ notation relative to Vienna PeeDee Belemnite (VPDB) and atmospheric N₂, respectively. The reproducibility (i.e. the relative standard

deviation of triplicate analyses performed on selected samples), was less than 5%. Trueness was determined using reference materials: IAEA-N2 ($\delta^{15}\text{N} = 20.3 \text{ ‰} \pm 0.2 \text{ ‰}$) and USG-24 ($\delta^{13}\text{C} = -16.1 \pm 0.2 \text{ ‰}$). Experimental results were in good agreement with certified values and averaged $20.59 \pm 0.07 \text{ ‰}$ ($n = 15$) and $-15.82 \pm 0.26 \text{ ‰}$ ($n = 28$) for IAEA-N2 and USG-24, respectively.

2.6. QA/QC

C₄–C₁₂ PFCAs, PFOS and diPAPs were frequently detected at trace level in procedural blanks (Table S4). Blank correction was performed when applicable and the Limit of Detection (LOD) was determined as the standard deviation of the blanks multiplied by the $t_{n-1,95}$ Student coefficient¹⁹. For analytes not detected in the blanks, LODs were determined as the concentration yielding a signal to noise ratio of 3. Overall, LODs in water, sediments and biota were in the range 0.003–0.34 ng L⁻¹, 0.014–0.35 ng g⁻¹ dw and 0.005–0.21 ng g⁻¹ wet weight (ww), respectively (Table S5).

Regarding the targeted analysis, reproducibility was lower than 10% except for 8:2 diPAP in water samples (28%). For this compound, qualitative water concentration data are given, for information only. Accuracy ranged between 80 and 120% except for PFHpS (128–138%), 10:2 FTSA (52–79%), EtFOSAA (111–123%) and 8:2 diPAP (132–139%); the concentration of these four analytes in samples were corrected according to their mean accuracy value (Table S6). Method trueness was controlled through the analysis of NIST SRM 1947 reference samples (Lake Michigan Trout Tissue, $n = 4$) (Table S7) and PFAS concentrations were in good agreement with previous reports³⁴.

Full details on the TOP assay validation are given in the “QA/QC” and “TOP assay” sections of the ESI (e.g. extraction efficiencies, conversion rates, oxidation product patterns...). Overall, acceptable analyte recovery rates were achieved (Table S6) and the complete conversion of pre-PFAAs_{targeted} was observed for all sample extracts.

2.7. Statistical analysis and TMF/BMF calculation

Since data were not normally distributed, statistical differences between two or several groups were conducted using either the Mann-Whitney or the Kruskal-Wallis tests, using the “R commander” package for R statistical software (R version 3.3.3, R core team 2017). The Spearman’s rank correlation coefficient was used to investigate correlation between variables. The difference in PFCA concentrations before and after oxidation were conducted using the paired samples Wilcoxon Test. For all tests, significance was set at $p \leq 0.05$. Hierarchical clustering based on Ward’s minimum variance classification and Euclidian distance methods was performed using RcmdrPlugin.FactoMiner (function hclust) to identify pattern similarity of PFCAs formed upon oxidation among samples.

Trophic levels (TLs) were determined according to equation 1 where 2 corresponds to the TL of the organism selected as baseline (corbiculidae = primary consumer), $\delta^{15}\text{N}_{\text{consumer}}$ and $\delta^{15}\text{N}_{\text{BL}}$ are the $\delta^{15}\text{N}$ of the consumer and the baseline, respectively, and 3.4 (‰) is the mean trophic enrichment ³⁵.

$$TL_{\text{consumer}} = 2 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{BL}}) / 3.4 \quad (1)$$

The biomagnification of PFASs was assessed using two metrics: BMF_{TL} (i.e. TL-normalized BMF) and TMF. BMF_{TL} was calculated using equation 2 where C_{predator} and C_{prey} are the PFAS concentrations (ng g^{-1} ww whole body) in the predator and in its prey, and TL_{predator} and TL_{prey} their trophic level, respectively ⁵.

$$\text{BMF}_{\text{TL}} = 10^{\left[\frac{\log_{10}(C_{\text{predator}}/C_{\text{prey}})}{TL_{\text{predator}} - TL_{\text{prey}}} \right]} \quad (2)$$

Predator-prey relations were determined using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, as well as the existing knowledge about fish trophic ecology ³⁶.

TMFs were obtained using the slope of the linear regression between the concentration (log-transformed, ng g^{-1} ww) and TL (equation 3 and 4), using the function *lme4* (linear mixed-effects models

with censored data) from the LMEC R package ¹¹. The TMF calculation was performed only for compounds with a detection frequency > 40% ³⁷.

$$\text{Log}C = \sim TL + 1|\text{intercept} \quad (3)$$

$$TMF = 10^{\text{slope}} \quad (4)$$

3. Results and discussion

3.1. PFAS concentrations and composition profiles

Among the 30 analyzed PFASs, 4:2 FTSA, MeFOSA, EtFOSA, 8:2 diPAP, HFPO-DA and ADONA were never detected. The detection frequency and the mean concentrations of the other PFASs are provided in the ESI (Tables S9–S11), while Σ PFASs and molecular patterns are illustrated by Figure 1.

A single water sample was analyzed to indicate an order of magnitude of PFAS concentrations in this compartment. 13 PFASs were detected and Σ PFASs was 101.4 ng L⁻¹. The predominant compound was PFOS followed by C₄–C₈ PFCAs and PFHxS, which individual concentrations ranged between 4.9 and 28.8 ng L⁻¹. Long-chain PFCAs (C₁₁–C₁₄), PFDS, 8:2 FTSA, 10:2 FTSA and alkyl-FASAs were not detected. Among pre-PFAAs_{targeted}, only 6:2 FTSA and FOSA were found at respective concentrations of 8.0 and 0.21 ng L⁻¹.

In sediments (n = 3), 12 PFASs were detected and Σ PFASs was variable (2.3 ± 2.3 ng g⁻¹). Short-chain PFAAs (e.g., PFBA and PFBS) were not found, likely because of their low sediment-water partitioning coefficient (K_D) ²⁵. PFDoDA, PFOS and PFTeDA dominated the molecular pattern with mean concentrations of 0.72, 0.53 and 0.47 ng g⁻¹, respectively. Among pre-PFAAs_{targeted}, 10:2 FTSA, 6:2 diPAP and EtFOSAA in sediments were either not detected or found at levels close to the LOD. Overall, Σ pre-PFAAs_{targeted} represented less than 8% of Σ PFASs in both abiotic compartments. Note that the

reported PFAA concentrations and patterns are consistent with those previously observed at this site ²⁵ and exceed the average value determined at French nationwide scale for both water and sediments ¹⁹.

In biota samples, the detection frequency of C₁₁–C₁₄ PFCAs, PFOS and 10:2 FTSA was 100%, while C₈–C₁₀ PFCAs, PFDS, 8:2 FTSA, FOSA, MeFOSAA and EtFOSAA were detected in more than 80% of samples. Short-chain PFAAs were not found since they are quickly excreted and, consequently, poorly bioaccumulated ³⁸. The average Σ PFASs ranged between 59.5 and 147 ng g⁻¹ ww in fish with an inter-individual variability lower than 30% within the same species (Figure 1A). These levels are, for instance, of the same order of magnitude than previously published values for whole fish (including juveniles and adults) from other sites in France (Rhône River and Gironde estuary) ^{11,18} or in the United States (Ohio, Missouri and Upper Mississippi Rivers) ³⁹.

Total PFAS levels were significantly different between fish species: bullhead and common perch had higher Σ PFAS levels than catfish, tench and gudgeon while roach was more contaminated than catfish and tench. Except for PFDS, significant differences between taxa were also found for the most frequently detected PFASs when considered individually. Such differences might be partly explained by the dietary behavior of these taxa. Overall, piscivorous fish generally present higher Σ PFASs than omnivorous and herbivorous species ⁴⁰. Bullhead and common perch may feed on fish juveniles ³⁶. The isotopic carbon signature is usually used to identify the feeding behavior since $\delta^{13}\text{C}$ values are not impacted by trophic enrichment ³⁵. Bullhead and common perch exhibited the narrowest range of $\delta^{13}\text{C}$ values (-26.2 – -25.5 ‰ and -26.7 – -26.0 ‰, respectively) (Figure S4). Tench and catfish seemed to have a different and more varied diet, showing slightly more negative and higher amplitude of $\delta^{13}\text{C}$ values (-27.1 – -26.1 ‰ and -27.8 – -26.8 ‰, respectively). The widest range of $\delta^{13}\text{C}$ values was observed for roach (-27.9 – -26.1 ‰), a known omnivorous species. The mean $\delta^{13}\text{C}$ of fish ranged from -27.9 to -25.5 ‰ and was framed by those of invertebrates that varied between -30.2 ‰

(Notonectidae) and -24.4 ‰ (Corbiculidae), suggesting that carbon sources exploited by fishes were appropriately taken into account.

Notonectidae were the most contaminated invertebrate taxon with Σ PFASs (84.6 ng g⁻¹ ww) comparable to that of gudgeons and pumpkinseed, whereas corbiculidae presented the lowest levels (4.0 ng g⁻¹ ww) similar to those observed in biofilm (2.9–3.2 ng g⁻¹ ww), macrophyte (4.8–5.1 ng g⁻¹ ww) and leaf litter (2.0–3.0 ng g⁻¹ ww). Notonectidae are mainly insectivorous and they are expected to feed essentially on insect larvae whereas corbiculidae are filter feeders; in addition, notonectidae are air breather insects, which may favor PFAS bioaccumulation⁸. These results are still to be confirmed with a larger sample set. The mean Σ PFASs in gammarids was 47.0 ± 2.5 ng g⁻¹ ww, about 7 times lower than in the Rhône River¹⁸ and about 5 times higher than in the Gironde Estuary¹¹.

The dominant compounds in biota were PFOS, PFDoDA and PFTeDA which, on average, contributed to 31%, 28% and 16% of Σ PFASs, respectively (Figure 1b). Labadie and Chevreuil (2011) reported a larger predominance of PFOS (76% in average) in tissues of European chub *Squalius cephalus* collected at the same site in 2010²⁵. In this study, the highest PFOS relative abundance was found in pumpkinseed (47%) common perch (43%), barbel (43%) and tench (42%). PFDoDA was dominant in lymnaeidae (42%), gammarids (37%), catfish (33%) and gudgeon (31%) whereas PFTeDA dominated the PFAS composition in corbiculidae (49%). The largest proportion of Σ C₄–C₈ PFCA (3–7%) were found in gammarids, corbiculidae, biofilm and macrophyte, in agreement with previous results¹⁰. Physiological characteristics, biotransformation capacities, life traits and feeding behavior might result in different exposure routes to PFAAs and their precursors, thereby possibly explaining these differences.

As for the abiotic compartment, Σ pre-PFAAs_{targeted} represented on average less than 5% of Σ PFASs in biota. The highest relative abundances were found in corbiculidae (18%), biofilm (13–17%) and lymnaeidae (9–12%). 10:2 FTSA was the dominant precursor in all biota samples (except notonectidae) with concentrations ranging from 0.16 ng g⁻¹ ww (macrophyte) to 3.2 ng g⁻¹ ww (barbel). To our knowledge, its presence in aquatic organisms has never been reported before. Other recurring

precursors were FOSA, 6:2 FTSA and 8:2 FTSA (Tables S10 and S11). The presence of 6:2 diPAP was also reported in 59% of biota samples but this compound was systematically detected at low levels (< LOQ).

3.2. Biomagnification of PFAAs: BMF_{TL} and TMF

The combined use of C and N stable isotopes allowed for the determination of the trophic web structure (Figure S4). Biofilm (TL = 1.1) and macrophyte (TL= 1.2) were found at the lowest trophic positions, followed by corbiculidae (TL = 2.0), gammarids (TL = 2.0) and lymnaeidae (TL = 2.2). Fish TLs ranged between 2.8 and 3.4 with barbel and catfish at the top of the food web.

BMF_{TL} were calculated for a total of 9 well-established predator-prey pairs³⁶; data ranges are shown in Table 1 while detailed values for each predator-prey pair are provided in Table S12. BMF_{TL} systematically > 1 were estimated for C₁₁–C₁₄ PFCAs, PFOS, PFDS and 10:2 FTSA. As regards PFAAs, these results are consistent with previous reports^{7,11,18} while these are the first BMF_{TL} values reported for 10:2 FTSA. High variability of BMF_{TL} was found for several compounds, including PFOS, PFTTrDA or PFTeDA, depending on prey-predator pairs. For PFOS (BMF_{TL}=2–169), the highest value was observed for the roach–lymnaeidae pair while the lowest one was calculated for the catfish–gammarid pair. This is partly due to the fact that such invertebrates with close TLs may exhibit contrasted contamination levels, as a result of different feeding behavior or differences in the ability to biotransform and eliminate chemicals⁴¹.

Considering the variability of BMF_{TL} observed for several PFAAs, the use of TMF appeared more relevant, since it is a more integrative and holistic metrics that reflects the mean behavior of a chemical in a food web comprising complex trophic relations⁵. TMFs ranged from 0.2 to 5.5 (Table 1; slope and intercept values of the regression lines given in Table S13). As indicated by TMF >1, C₉–C₁₄ PFCAs, PFHpS, PFOS, PFDS, 8:2 FTSA, 10:2 FTSA, FOSA, MeFOSAA, EtFOSAA appeared to biomagnify in this trophic web, contrary to shorter chain-PFCAs, e.g. 6:2 FTSA or PFOA. The highest TMFs were

determined for PFDS and 10:2 FTSA and a significant increase of TMF with chain length was observed for PFCAs (C₉–C₁₄) (Spearman test: p-value = 0.004, rho = 0.90). For the latter compounds, such a trend has already been reported in lake and marsh ecosystems ^{6–9}, whereas decreasing TMF with increasing chain length was reported in an estuarine benthic food web ¹¹. These differences further suggest that the chemical structure and, hence, the chemical properties of PFASs are not the only determinant of biomagnification and that ecosystem characteristics, contamination pattern and exposure routes likely contribute to the TMF variability.

TMF_{PFOS} (1.5 ± 0.1) was comparable to that reported for a subtropical marsh food web in Hong-Kong ⁹, the estuarine trophic web from the Gironde in France ¹¹ or the marine bottlenose dolphin food web from Charleston Harbor (based on estimated whole body burden) ⁷. The TMFs of long-chain PFCAs were also in the range of values previously reported for marine and freshwater ecosystems ^{7–10}.

Pre-PFAAs were seldom investigated in previous works, with the notable exception of FOSA. In agreement with our results, several studies reported TMF_{FOSA} > 1 ^{7,8,11,42} whereas TMF_{FOSA} < 1 was reported for a lake Ontario foodweb ⁶. MeFOSAA was not biomagnified in the Gironde estuary (TMF = 0.18) ¹¹, unlike in the present work; this suggests that the oxidation of MeFOSAA precursors may occur in the trophic web of the Orge river (see section 3.3). To our knowledge, the trophic magnification of FTSA and diPAPs has never been studied before. In the Orge river, 6:2 FTSA was not biomagnified contrary to its homologues with longer perfluoroalkyl chains, probably because the elimination rates of PFASs increase with decreasing chain length ³⁸. In addition, the TMF of 6:2 diPAP appeared to be

close to 1 but not significantly > 1 and, thus, further data are needed to better estimate its biomagnification potential.

3.3. Trophic transfer of pre-PFAAs_{targeted}

The biotransformation of (alkyl-)FASAs and (alkyl-)FASAAAs can lead to the formation of PFOS while FTSAAs and diPAPs can be converted into PFCA homologues with similar or lower number of perfluorinated carbon atoms⁴³. The ratio between the concentrations of pre-PFAAs_{targeted} and those of PFAAs was investigated at different TLs, as a proxy of precursor biotransformation in the food web¹⁷. Leaf litter was also included in the analysis, with an estimated TL of 0.8 (based on its $\delta^{15}\text{N}$ signature).

Log Σ pre-PFOS_{targeted} (i.e. FOSA and (alkyl-)FASAAAs) to PFOS concentration ratios were plotted against TL and no significant trend was observed (Figure 2a). However, the mean ratio was significantly higher in invertebrates (0.42 ± 0.57) than in fish (0.02 ± 0.01); this suggests that the biotransformation of pre-PFOS might occur at the highest TLs, likely because fish have higher metabolic capacities than invertebrates. Previous studies reported on the metabolization of EtFOSA to FOSA and of FOSA to PFOS by the rainbow trout *Onchorhynchus mykiss*^{15,16}. Babut et al. (2017) showed that FOSA/ MeFOSAA ratios varied between fish species and that the mean value was lower in invertebrates than in fish, thereby suggesting the increase of biotransformation rates of MeFOSAA at the highest TLs¹⁸. However, in the present study, similar TMFs were observed for MeFOSAA, EtFOSAA and PFOS, indicating that the biotransformation of MeFOSAA and EtFOSAA precursors occurred in this trophic web (e.g. *N*-ethylperfluorooctane sulfonamidoethanol, EtFOSE).^{18,44} The latter hypothesis might also explain why the Σ pre-PFOS_{targeted} to PFOS concentration ratio was significantly higher in invertebrates than in biofilm and leaf litter, suggesting that the biotransformation of unidentified (alkyl-)FASAA precursors present in water, sediments, biofilm or leaf litter also occurs in invertebrates.

Unlike in fish, $\Sigma \text{pre-PFOS}_{\text{targeted}}/\text{PFOS}$ concentration ratios exhibited large variations between invertebrate taxa (variation coefficient of 134%). Corbiculidae (1.69) and lymnaeidae (0.47) displayed higher ratios than gammarids (0.08) or notonectidae (0.03), possibly because of higher pre-PFOS metabolization capacities in the latter taxon (Figure S6). In addition to taxon-specific metabolic capacities or exposure routes, PFAS toxicokinetics might also differ between taxa ⁴⁵.

$\text{Log } \Sigma \text{pre-PFCAs}_{\text{targeted}}$ (i.e. all pre-PFAAs_{targeted} except FOSA and (alkyl-)FASAAs) to ΣPFCAs concentration ratios were negatively correlated with TLs (Figure 2b), clearly indicating an increase of the biotransformation rates of pre-PFCAs into PFCAs along the trophic web. To date, the biotransformation of FTSA and diPAPs has been little studied in aquatic organisms. Yeung and Mabury (2013) demonstrated that the biotransformation of 6:2 and 8:2 FTSA by juvenile rainbow trout (*Oncorhynchus mykiss*) could induce the formation of 5:3 and 7:3 FTCA, respectively, and subsequently C₅–C₈ PFCAs. In the same line, the metabolization of 10:2 FTSA (i.e. the most abundant pre-PFAAs_{targeted} in the biota from the Orge River), could significantly contribute to the formation of longer-chain PFCAs (e.g. C₉–C₁₀) along the trophic web. Further investigation of 10:2 FTSA biotransformation kinetics would therefore be helpful to estimate its contribution to PFNA and PFDA bioaccumulation. The fate of 8:2 diPAP was recently assessed for the first time in a marine fish species (*Sparus aurata*) ¹⁶. The biotransformation of 8:2 diPAP yielded saturated and unsaturated fluorotelomer carboxylic acids (FTCAs and FTUCAS, respectively), as well as PFOA. These findings suggest that 6:2 diPAP could be metabolized similarly, yielding shorter-chain fluorotelomer carboxylic acids and PFCAs. As observed for (alkyl-)FASAAs, increasing concentrations of these pre-PFAAs along the Orge River trophic web suggest that the metabolization of their unidentified precursors occurs in this food web.

3.4. Trophic transfer of pre-PFAAs_{unknown}

To get further insight into the bioaccumulation of organic fluoride in fish exposed to AFFF formulations, Yeung and Mabury⁴⁷ implemented a mass balance analysis based on the determination of both total fluorine (TF) and extractable organic fluorine (EOF) in fish tissues. They demonstrated that known PFASs explained only 0.9–7% of EOF in unexposed fish and < 7%–60% in exposed fish. They concluded that both known PFASs and unknown organofluorines could be bioconcentrated in fish tissues.

To investigate whether unknown PFASs could play a role in the trophic magnification of PFAAs in the Orge River, the TOP assay was applied to selected samples: water (n = 1), sediment (n = 1) and biota (n = 15). Upon oxidation, positive Δ PFCAs were systematically observed, i.e. + 15–424%, depending on the sample (Table S14). The largest relative increase was found at the base of the trophic web, e.g. in biofilm (424%), sediment (319%), leaf litter (298%), and macrophytes (196%). On the contrary, the lowest Δ PFCAs was found in fish, especially in catfish (+ 15–22%). Note that, the limited number of considered samples does not allow for inter-individual nor spatial variability. While a significant increase of C₄–C₁₂ PFCA concentrations was observed in all samples upon oxidation, this was not the case for PFTTrDA and PFTTeDA. Thus, pre-PFAAs_{unknown} bearing perfluoroalkyl chains with more than 12 carbons were either absent or present at extremely low levels in these samples, suggesting that the biomagnification of PFTTrDA and PFTTeDA was not affected by the biotransformation of precursors. These results are consistent with previous reports on the lack of very long-chain PFCA precursors in urban runoff and waste water effluent, indicating that they were little emitted into the aquatic environment^{28,29,31}.

The profiles of PFCAs formed upon oxidation (Δ PFCA) are presented in Figure 3. While these patterns cannot be directly associated to precursors chain length profiles since oxidation of some precursors leads to the formation of a series of variable chain-length PFCAs, such information are used to provide general insights on the range of chain length of unknown PFAA-precursors homologues present in the different matrixes and a large approximation of their relative abundance. Different patterns of PFCAs

formed upon oxidation were observed between samples as highlighted by hierarchical clustering analysis based on Euclidian distance. In water, PFPeA presented the largest increase (+24.4 ng L⁻¹, i.e. + 202%) followed by PFHxA (+8.3 ng L⁻¹, i.e. + 60%) and PFHpA (+ 3 ng L⁻¹, i.e. + 62%) explaining 88% of Δ PFCAs. Although these compounds can result from the oxidation of precursors with higher number of perfluorinated carbon atoms (Table S8), the predominance of C₄–C₆ PFAA precursors in water would not be surprising for two reasons: i) the PFAS solubility decreases with increasing chain length and ii) regulatory bodies encourage the use of PFASs with less than 8 perfluorinated carbons. Among pre-PFAAs_{targeted}, only 6:2 FTSA was detected in water and its concentration could explain 44%, 5%, 15% and 5% of the increase upon oxidation of PFBA, PFPeA, PFHxA and PFHpA levels, respectively. This strongly suggests the presence of unidentified short-chain pre-PFAAs in the water column, although chemicals behaving similarly to FTSAs or diPAPs upon oxidation could also yield such short-chain PFCAs. Some PFAA-precursors, including 6:2 FTSA, may also generate C₂–C₃ PFCAs upon TOP assay⁴⁷. Ultrashort-chain PFASs, as named, were not addressed in the present study. However, a recent review pointed out that such PFASs pass through conventional wastewater treatment processes and were found in aqueous environment including rivers⁴⁸.

In sediment and biota samples, the formation of C₈–C₁₂ PFCAs upon oxidation was more predominant than in water, explaining 40–70% of Δ PFCAs (Figure 3). These results are consistent with previous reports indicating that both the K_o and the bioaccumulation factor (BAF) of PFASs increase with increasing chain length^{25,41}. It should be noted that no significant increase of PFDoDA concentrations was observed in lymnaeidae and catfish upon oxidation (blue cluster on Figure 3). Conversely, the largest Δ PFDoDA was observed in bullhead and its main prey, gammarids (black cluster on Figure 3). The reason for this observation remains unclear, considering that no such trend was observed in the gammarid main food sources (i.e. leaf litter and biofilm, grouped in red cluster on Figure 3).

Σ pre-PFAAs_{targeted} accounted for a higher contribution to Δ PFCAs in fish and invertebrates than at the base of the trophic web. For instance, based on the conversion factors obtained in ultra-pure water

(Table S15), the oxidation of 10:2 FTSA would explain on average 38% of Δ PFDA in fish and invertebrates against less than 11% in sediments and biofilm. Altogether, 8:2 FTSA, 10:2 FTSA and sulfonamides would contribute to 23–63% of Δ PFOA in fish and invertebrates against 3–11% in biofilm. The relative proportion of Σ PFCAs, Σ PFASs, Σ pre-PFAAs_{targeted} and Σ pre-PFAAs_{unknown} in the trophic web of the Orge River is shown on Figure 4. In water and sediment, Σ pre-PFAAs_{unknown} accounted respectively for 33% and 78% of Σ PFASs. The contribution of pre-PFAAs_{unknown} to Σ PFASs in biofilm, leaf litter and macrophytes was in the same range that in sediment (64–80%). Pre-PFAAs_{unknown} accounted for 30–42% of Σ PFASs in gammarids and lymnaeidae. In fish, the proportion of extractable Σ pre-PFAAs_{unknown} was similar between taxa (18–23%) and was significantly lower than in invertebrates or abiotic samples. Thus, a significant sharp decrease of the Σ pre-PFAAs_{unknown}/ Σ PFASs molar ratio was observed according to TL (Figure S7). Considering the large relative abundance of extractable pre-PFAAs_{unknown} at the base of the trophic web, it is therefore likely that the biotransformation of these chemicals contributes to the observed increase of the levels of some PFCAs and PFASs at the higher TLs (i.e. C₉–C₁₂ PFCAs and C₇–C₁₀ PFASs, based on the profiles of PFAAs formed upon oxidation, see Figure S7 and discussion above). The actual contribution of pre-PFAAs unknown to the biomagnification of these PFAAs cannot currently be estimated because i) the structure of pre-PFAAs_{unknown} occurring in the Orge River remains by definition undetermined at this stage, ii) the individual conversion rates and oxidation product patterns of these compounds using the TOP assay are unknown and iii) toxicokinetics may differ among these compounds. Additionally, the TOP assay process does not mime biotransformation meaning that results from such approach cannot be directly used in the state to quantify the precursors contribution to the biomagnification of individual PFAAs. Indeed, Indeed, PFCAs formed upon oxidation do not systematically correspond to biotransformation products. For instance, PFOA is the main and single product of FOSA and MeFOSAA oxidation upon TOP assay (refer to Table S15) whereas PFOS has been identified as their major products through biotransformation^{15,49}. However, our results strongly point to the biotransformation of unattributed precursors along this trophic web that warrants further investigation.

Additionally, these results might also indicate that extractable sediment-bound unattributed precursors are either mainly metabolized by invertebrates or are poorly bioavailable. It should also be noted that no correlation was observed between $\Sigma \text{pre-PFAAs}_{\text{unknown}}/\Sigma \text{PFAAs}$ and $\delta^{13}\text{C}$, indicating the absence of an obvious relationship between carbon sources and exposure to pre-PFAAs.

4. Conclusion

The bioaccumulation and biomagnification of PFAAs in aquatic biota have raised a growing concern over the last 15 years. Numerous studies have addressed this issue but very few investigated the role of pre-PFAAs in such processes. Here, an original approach was implemented based on i) the quantitative analysis of selected pre-PFAAs and ii) on the estimation of unknown extractable pre-PFAAs via the TOP assay. Such a strategy was applied to the food web of the Orge River that may be considered as a typical urban river, i.e. with point and diffuse inputs of PFASs mainly linked to domestic sources and atmospheric deposition.

While selected precursors overall accounted for a relatively minor proportion of ΣPFASs , the TOP assay revealed the occurrence of substantial proportions of extractable unknown pre-PFAAs in all compartments of the Orge River trophic web. Sediments appear to be a sink for a complex mixture of unidentified pre-PFAAs and a source of long-chain fluoroalkyl chemicals that, upon biotransformation, could significantly contribute to the biomagnification of PFAAs (i.e. $\text{C}_7\text{--C}_{10}$ PFASs and $\text{C}_9\text{--C}_{12}$ PFCAs in the present study). Thus, these results highlight the need of future research to get further insight into the exposure of aquatic biota to PFASs and into the trophodynamics of these chemicals. There is a clear need to identify the structure of the unattributed PFAA precursors occurring in the environment using a complementary approach (e.g. TOP and extractable organic fluorine assays vs suspect or non-target screening based on high-resolution mass spectrometry). Further investigation of the toxicokinetics of newly identified pre-PFAAs is essential. The determination of biotransformation rates in biofilm, invertebrates and selected fish species would allow to accurately quantifying their

contribution to PFAA biomagnification, thereby contributing to better explain the spatial variability of TMFs. Additionally, modelling and experimental studies performed under controlled conditions would help getting further insight into the actual contribution of pre-PFAAs to PFAA biomagnification.

Conflicts of interest

There are no conflicts of interest to declare

Acknowledgements

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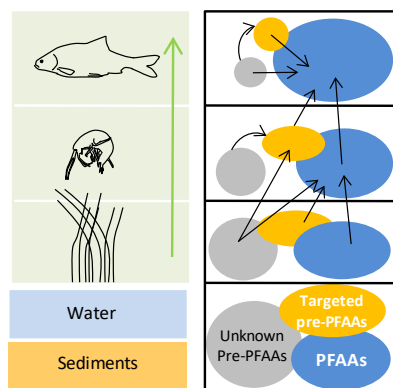
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Graphical abstract



In the Orge River, the contribution of perfluoroalkyl acid precursors decreases between the base and the top of the trophic web.

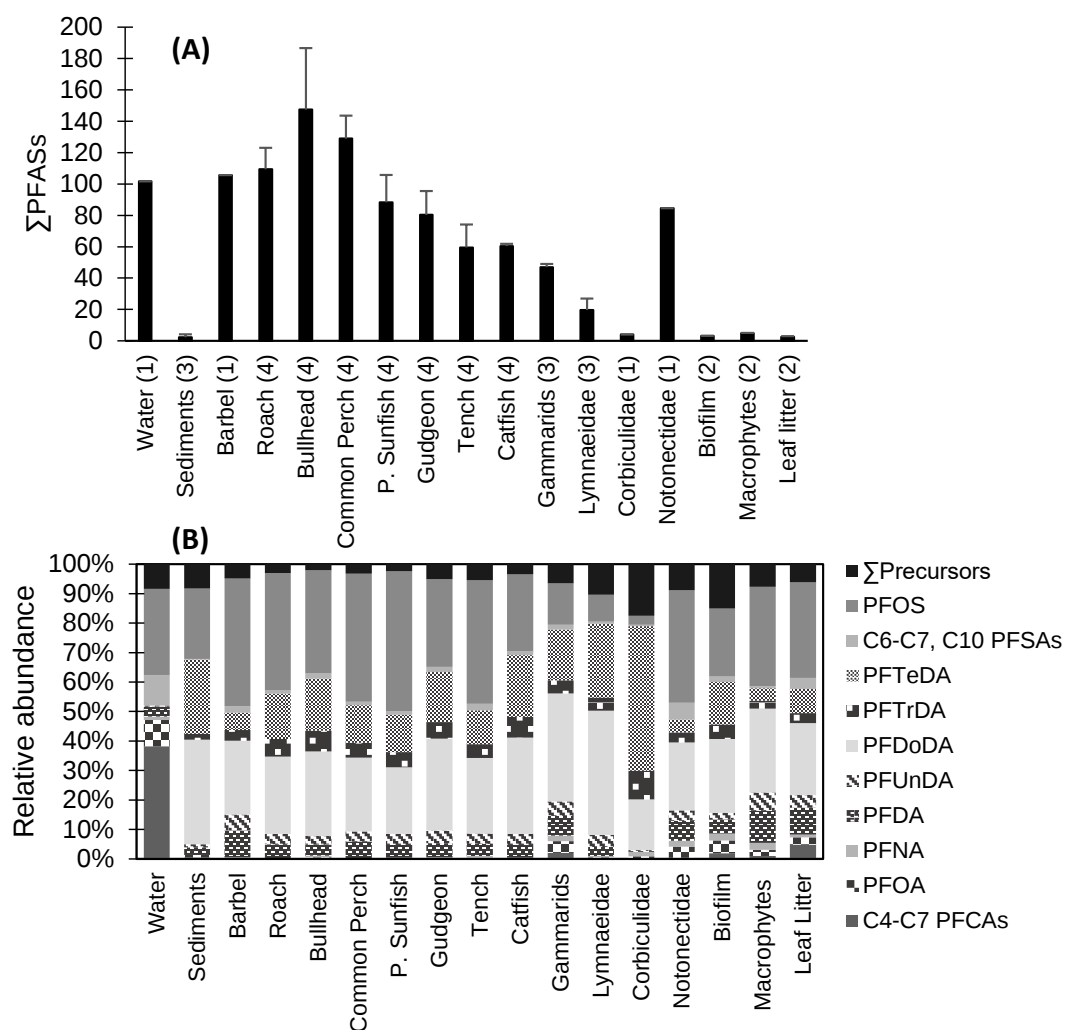


Figure 1: Mean total PFAS concentrations and molecular pattern in water, sediment and biota samples (the number of replicates is indicated between brackets). Concentrations are expressed in ng L⁻¹ for water, ng g⁻¹ dw for sediments and ng g⁻¹ ww for biota samples.

Table 1: BMF_{TL} and TMF determined in the Orge River trophic web; values between brackets indicate the 95% confidence interval while bold characters denotes TMF values significantly higher than 1. L-PFOS: linear isomer of PFOS; Br-PFOS: sum of PFOS branched isomers.

	Detection frequency (%)	Concentration range (ng g ⁻¹ ww)	BMF _{TL} (min–max)	TMF
PFHxA	39	<LD–0.57	<i>not calculated</i>	0.2 (0.2;0.3)
PFOA	93	<LD–3.19	0.04–1.4	0.6 (0.5;0.6)
PFNA	98	<LD–1.62	0.3–2.3	1.6 (1.5;1.7)
PFDA	90	<LD–9.56	0.9–25.2	2.6 (2.2;3.0)
PFUnDA	100	0.02–5.98	1.0–11.6	2.2 (2.1;2.3)
PFDoDA	100	0.70–58.6	1.1–6.9	2.4 (2.3;2.5)
PFTTrDA	100	0.13–14.1	1.7–24.1	2.9 (2.8;3.0)
PFTeDA	100	0.19–29.5	1.4–15.8	2.9 (2.7;3.0)
PFHxS	78	<LD–4.47	0.8	<i>not calculated</i>
PFHpS	73	<LD–0.50	0.8	1.6 (1.4;1.8)
ΣPFOS	100	0.12–84.0	2.0–169	1.5 (1.4;1.6)
L-PFOS	100	0.12–70.54	2.1–173	1.6 (1.5;1.7)
Br-PFOS	98	<LD–13.50	0.9–134	1.8 (1.7–2.0)
PFDS	100	0.02–2.10	4.0–25.7	5.5 (5.3;5.7)
6:2 FTSA	76	<LD–5.24	0.2–2.5	0.6 (0.5;0.6)
8:2 FTSA	98	<LD–1.09	0.3–17.2	1.3 (1.2;1.4)
10:2 FTSA	100	0.16–3.23	1.1–4.8	3.0 (3.0;3.0)
FOSA	98	<LD–0.97	0.6–1.6	2.5 (2.3;2.6)
FOSAA	44	<LD–0.12	<i>not calculated</i>	0.6 (0.5;0.8)
MeFOSAA	80	<LD–0.11	0.8–2.5	1.5 (1.3;1.6)
EtFOSAA	83	<LD–0.16	0.4–2.2	1.5 (1.3;1.6)
6:2 diPAP	59	<LD–0.11	1.7	1.2 (0.9;1.4)

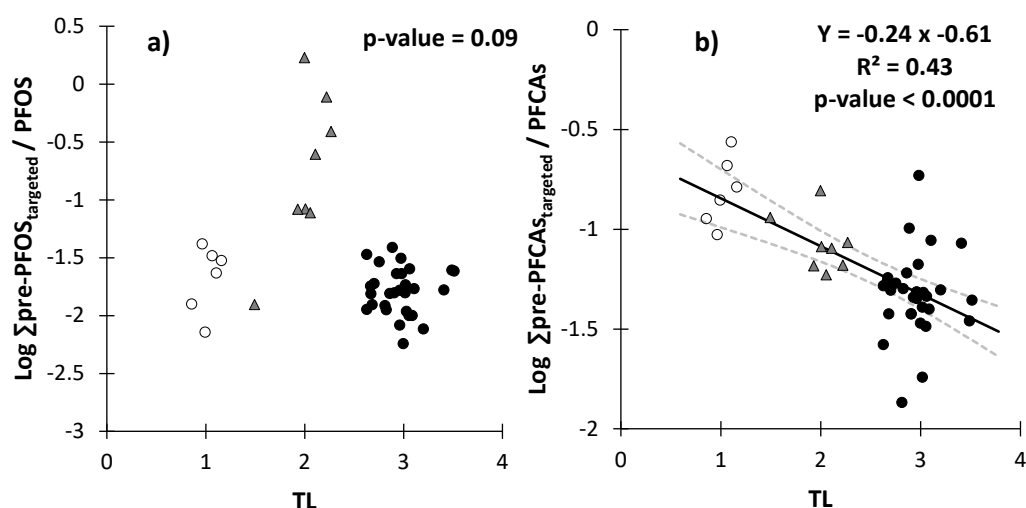


Figure 2: Log Σ pre-PFOS_{targeted}/ PFOS molar concentration ratios (a) and Log Σ pre-PFCAs_{targeted}/PFCAs molar concentration ratios (b) according to the trophic level. White circles correspond to the base of the trophic web (e.g. biofilm, leaf litter and macrophytes), grey triangle and black circles refer to invertebrates and fish, respectively.

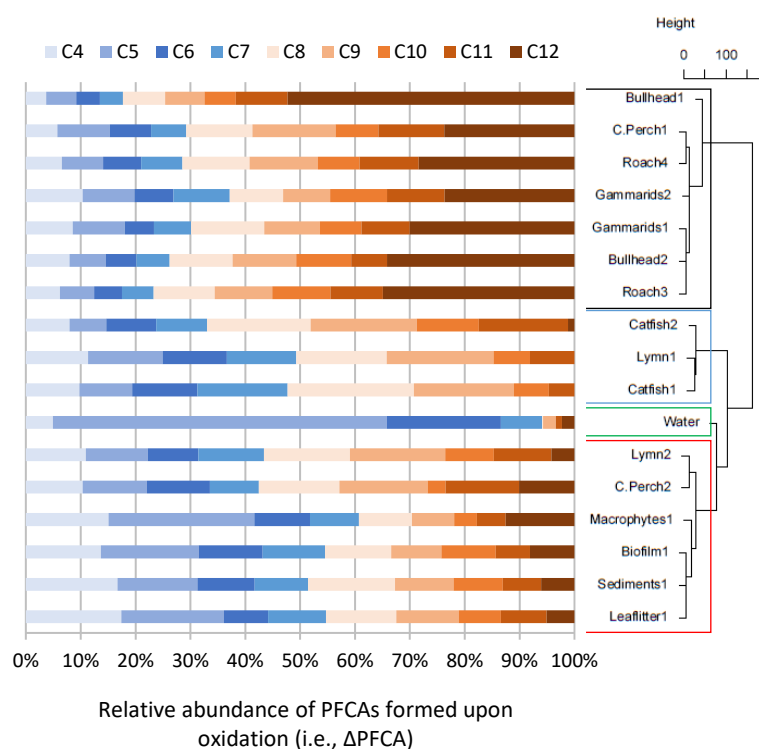


Figure 3: Hierarchical clustering analysis (method=ward, distance=Euclidian) of the pattern of PFCAs formed upon oxidation (i.e., Δ PFCA) in each sample.

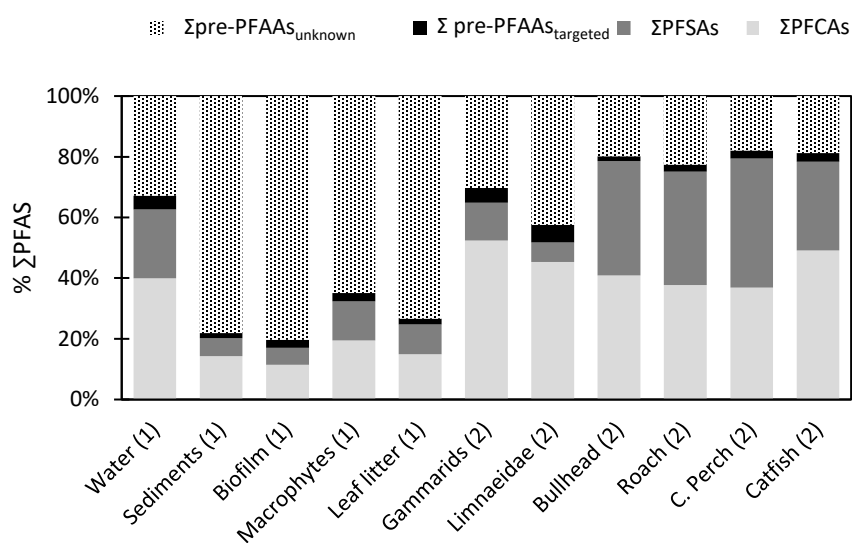


Figure 4: Relative contribution of pre-PFAAs_{unknown}, pre-PFAAs_{targeted}, PFASs and PFCAs to Σ PFASs (expressed on a molar basis) in the trophic web of the Orge River. The number of replicates is indicated between brackets.