Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

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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SI 1. Sampling

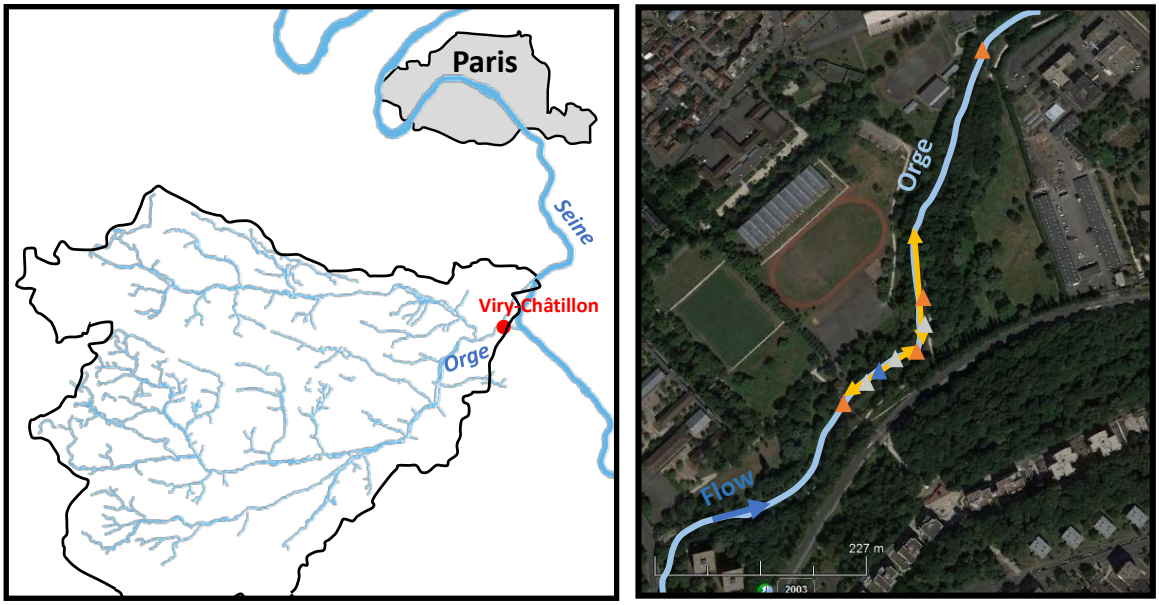

Figure S1: Location of the Orge River watershed (left) and location of sampling sites on the Orge River (right): water (blue triangle), sediments/leaf litter/biofilm/macrophytes (grey triangles), invertebrates (orange triangles) and fish (yellow arrows). The blue arrow indicates the water flow direction.

Table S1: Details on fish samples: size/weight of individuals (mean ± standard deviation and min–max in brackets) and humidity content (min–max).

Fish species	Number of samples	Number of individuals/sample	Size (cm)	Weight (g)	Humidity content (%)
B. barbus	1	1	13.6	25	71
C. gobio	4	1–2	9 ± 2 (6.9–10.8)	9 ± 5 (5.4–10.8)	73–78
R. rutilus	4	1–2	10 ± 2 (7.8–12.7)	10 ± 2 (7.0–19.5)	73–77
G. gobio	4	1–2	11 ± 1 (10.0–11.8)	11 ± 1 (7.5–16.0)	77–79
P. fluviatilis	4	2–3	7 ± 1 (4.8–8.0)	7 ± 1 (2.0–6.0)	77–79
L. gibbosus	4	1–3	7 ± 2 (5.5–10)	7 ± 2 (2.5–18.5)	74–75
I. melas	4	1	12 ± 2 (9.2–14.2)	12 ± 2 (10.5–31)	78–80
T. tinca	4	1	12 ± 1 (10–12.8)	12 ± 1 (15.4–32.0)	78–80

SI 2. Chemical analysis

SI 2.1. List of chemicals and reagents

Table S2: Acronyms, names, molecular formulas and associated internal standards of targeted PFASs

Acronym	Compound name	Molecular formula	Associated internal standard
PFBA	perfluoro-n-butanoic acid	C_3F_7COOH	[1,2,3,4-$^{13}C_4$] PFBA
PFPeA	perfluoro-n-pentanoic acid	C_4F_9COOH	[1,2-$^{13}C_2$] PFHxA
PFHxA	perfluoro-n-hexanoic acid	$C_5F_{11}COOH$	[1,2-$^{13}C_2$] PFHxA
PFHpA	perfluoro-n-heptanoic acid	$C_6F_{13}COOH$	[1,2,3,4-$^{13}C_4$] PFHpA
PFOA	perfluoro-n-octanoic acid	$C_8F_{15}COOH$	[1,2,3,4-$^{13}C_4$] PFOA
PFNA	perfluoro-n-nonanoic acid	$C_9F_{17}COOH$	[1,2,3,4-$^{13}C_4$] PFOA
PFDA	perfluoro-n-decanoic acid	$C_{10}F_{19}COOH$	[1,2-$^{13}C_2$] PFDA
PFUnDA	perfluoro-n-undecanoic acid	$C_{11}F_{21}COOH$	[1,2-$^{13}C_2$] PFUnDA
PFDoA	perfluoro-n-dodecanoic acid	$C_{12}F_{23}COOH$	[1,2-$^{13}C_2$] PFDoDA
PFTrDA	perfluoro-n-tridecanoic acid	$C_{13}F_{25}COOH$	[1,2-$^{13}C_2$] PFDoDA
PFTeDA	perfluoro-n-tetradecanoic acid	$C_{14}F_{27}COOH$	[1,2-$^{13}C_2$] PFTeDA
PFBS	perfluoro-1-butanesulfonic acid	$C_4F_9SO_3H$	[1,2,3,4-$^{13}C_4$] PFHxS
PFHxS	perfluoro-1-hexanesulfonic acid	$C_6F_{13}SO_3H$	[1,2,3,4-$^{13}C_4$] PFHxS
PFHpS	perfluoro-1-heptanesulfonic acid	$C_7F_{15}SO_3H$	[1,2,3,4-$^{13}C_4$] PFOS
PFOS	n-perfluoro-1-octanesulfonic acid	$C_8F_{17}SO_3H$	[1,2,3,4-$^{13}C_4$] PFOS
PFDS	perfluoro-1-decanesulfonic acid	$C_{10}F_{21}SO_3H$	[1,2,3,4-$^{13}C_4$] PFOS
4:2 FTSA	1H,1H,2H,2H-perfluorohexane sulfonate	$C_4F_9CH_2CH_2SO_3^-$	[1,2-$^{13}C_2$] 6:2 FTSA
6:2 FTSA	1H,1H,2H,2H-perfluorooctane sulfonate	$C_6F_{13}CH_2CH_2SO_3^-$	[1,2-$^{13}C_2$] 6:2 FTSA
8:2 FTSA	1H,1H,2H,2H-perfluorodecane sulfonate	$C_8F_{17}CH_2CH_2SO_3^-$	[1,2-$^{13}C_2$] 6:2 FTSA
10:2 FTSA	1H,1H,2H,2H-perfluorododecane sulfonate	$C_{10}F_{19}CH_2CH_2SO_3^-$	[1,2-$^{13}C_2$] 6:2 FTSA
FOSA	Perfluorooctanesulfonamide	$C_8F_{17}SO_2NH_2$	[$^{13}C_8$]FOSA
MeFOSA	N-methyl perfluorooctanesulfonamide	$C_8F_{17}SO_2N(CH_3)H$	D_3-MeFOSA
EtFOSA	N-ethyl perfluorooctanesulfonamide	$C_8F_{17}SO_2N(C_2H_5)H$	D_3-MeFOSA
FOSAA	perfluorooctanesulfonamido acetic acid	$C_8F_{17}SO_2NCH_2COOH$	D_3-MeFOSAA
MeFOSAA	2-(N-methylperfluorooctanesulfonamido) acetic acid	$C_8F_{17}SO_2N(CH_3)CH_2COOH$	D_3-MeFOSAA
EtFOSAA	2-(N-ethylperfluorooctanesulfonamido) acetic acid	$C_8F_{17}SO_2N(C_2H_5)CH_2COOH$	D_3-MeFOSAA
6:2 diPAP	bis(1H,1H,2H,2H-perfluorooctyl) phosphate	bis[$CF_3(CF_2)_5CH_2CH_2$]PO_4-	[1,2-$^{13}C_2$]$_2$ 6:2 diPAP
8:2 diPAP	bis(1H,1H,2H,2H-perfluorodécyl) phosphate	bis[$CF_3(CF_2)_7CH_2CH_2$]PO_4-	[1,2-$^{13}C_2$]$_2$ 6:2 diPAP
HFPO-DA	2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid (GenX)	$C_6HF_{11}O_3$	[1,2-$^{13}C_2$] PFHxA
ADONA	dodecafluoro-3H-4,8-dioxanonanoate	$C_6F_{12}O_2HCOO$-	[1,2,3,4-$^{13}C_2$] PFOA

Methanol (MeOH) and acetonitrile (ACN) were from J.T. Baker (Atlantic Labo, Bruges, France). Strata X-AW cartridges (200 mg/6 mL) were supplied by Phenomenex (Le Pecq, France). Supelclean ENVI-Carb cartridges (250 mg/6 mL), ammonium hydroxide (NH_4OH) (28.0–30.0 % NH_3 basis), potassium persulfate of analysis quality (≥ 99%) (Scharlau) and ammonium acetate (CH_3COONH_4) (Fluka) for HPLC (≥ 99.0 %) were obtained from Sigma-Aldrich (S^t Quentin Fallavier, France). Sodium hydroxide (ACS ISO, purity ≥ 99.0 %, Scharlau) was purchased from Atlantic Labo (Bruges, France).

Glass fiber filters (Whatman GF/F) were supplied by VWR International (Fontenay Sous Bois, France). Ultrapure water was obtained using a Millipore Elix 10 system fitted with an EDS Pak polisher. Vittel mineral water was used for recovery tests.

SI 2.2. Analytical method for the determination of PFASs

Table S3: Mass spectrometry acquisition parameters

Acronym	Collision energy (V)	Fragmentor (V)	Analyte quantification (Q) transition (m/z)	Analyte confirmation (C) transition (m/z)	Retention time (min)
PFBA	4	380	213.0 → 168.9	-	1.2
PFPeA	2	380	263.0 → 218.9	-	2.7
PFHxA	2	380	313.0 → 268.9	313.0 → 118.9	3.8
PFHpA	2	380	363.0 → 318.9	363.0 → 168.9	4.7
PFOA	4	380	413.0 → 368.9	413.0 → 168.9	5.4
PFNA	4	380	463.0 → 418.9	463.0 → 218.9	6
PFDA	4	380	513.0 → 468.9	513.0 → 268.9	6.6
PFUnDA	4	380	563.0 → 518.9	563.0 → 268.9	7.2
PFDoDA	4	380	613.0 → 568.9	613.0 → 168.9	7.8
PFTrDA	4	380	663.0 → 618.9	663.0 → 369.0	8.9
PFTeDA	8	380	712.0 → 668.8	712.0 → 419.0	9.0
PFBS	28	380	298.9 → 79.9	298.9 → 98.9	3.9
PFHxS	52	380	398.9 → 79.9	398.9 → 98.9	5.7
PFHpS	56	380	448.9 → 79.9	448.9 → 98.9	6.3
PFOS	60	380	498.9 → 79.9	498.9 → 98.9	7
PFDS	76	380	598.9 → 79.9	598.9 → 98.9	8.2
4:2 FTSA	20	380	327.0 → 306.9	327.0 → 80.5	3.5
6:2 FTSA	20	380	427.0 → 406.8	427.0 → 79.9	5.1
8:2 FTSA	28	380	527.0 → 506.9	527.0 → 80.9	6.3
10:2 FTSA	36	380	627.0 → 606.8	427.0 → 79.9	7.5
FOSA	38	380	497.9 → 77.9	-	8.4
MeFOSA	24	380	512.0 → 218.9	512.0 → 168.9	10.1
EtFOSA	24	380	526.0 → 218.9	526.0 → 168.9	10.2
FOSAA	36	380	497.9 → 77.9	-	6.5
MeFOSAA	16	380	570.0 → 511.8	570.0 → 418.8	6.7
EtFOSAA	16	380	584.0 → 525.8	584.0 → 418.8	7
6:2 diPAP	20	380	789.0 → 443.0	789.0 → 96.9	8.2
8:2 diPAP	28	380	989.0 → 542.8	989.0 → 96.9	9.9
HFPO-DA	0	380	285.0 → 169.0	285.0 → 185.0	4.1
ADONA	10	380	377.0 → 251.0	377.0 → 85.0	5.0

The method was adapted from Munoz et al. (2015). PFAS quantification was performed by isotopic dilution with a five-point calibration curve (analyte concentration: 0.5–25 pg mg^{-1}; constant IS concentration = 10 pg mg^{-1}).

SI 2.3. QA/QC

Blanks

Contamination was minimized as much as possible using non-fluorinated materials: HDPE-bottles for water sampling and conservation, aluminium trays for sediments and biota sampling, glass and polyethylene vials for extraction and analysis. Procedural blanks were introduced in each sample series: ultra-pure water spiked with ISs for water samples, microwave cells filled with the extraction solvent and spiked with ISs for solid matrices, and bottle filled with ultra-pure water for TOP assay experiments. For each analysis series, instrument blanks were analysed and the absence of cross-contamination was verified by regularly injecting MeOH.

Determination of the limits of detection (LODs) and quantification (LOQs)

For analytes detected in blanks, LODs were set as the standard deviation of blanks corrected by the $t_{n-1,95}$ student coefficient, n being the number of blank replicates. Otherwise, LODs were derived from the signal-to-noise ratio (SNR) observed in low-contaminated samples or in spiked blank samples. LOQs were calculated by multiplying the LOD by 10/3. Levels in procedural blanks are indicated in table S4.

Recoveries

As regards targeted analysis, whole-method accuracy rates were determined using spiked matrices: Vittel mineral water (2 ng L^{-1}) and reference sand sample (10 ng g^{-1} dw) (Table S6). Method trueness was controlled through the analysis of NIST SRM 1947 reference samples (Lake Michigan Fish Tissue).

As regards the TOP assay, the recoveries of a wide range of PFAAs and pre-$PFAAs_{targeted}$ were determined using spiked sand samples (10 ng g^{-1} dw), ISs being added at the end of the analytical procedure to prevent their oxidation. The actual recoveries of individual pre-$PFAAAs_{unknown}$ could not be estimated since, by definition, the structure of these compounds was undetermined. It was however assumed that recoveries were i) within the range of that reported for targeted analytes (see table S6) and, more importantly, ii) that the extraction efficiencies of pre-$PFAAAs_{unknown}$ were similar in all the sample types investigated in the present work. Very recent work showed that conditions stronger than the one used in this work may provide higher yields for some cationic and zwitterionic PFAAs (Munoz et al., 2018). Nevertheless, the abovementioned assumptions were very reasonable since Munoz et al. (2017) previously demonstrated that the MeOH + 0.2 % NH_4OH extraction solvent provided similar recoveries for a wide range of targeted pre-PFAAs (including FTSAs and zwitterionic

compounds), in both sediment and fish. Thus, although the contribution of unattributed pre-PFAAs unknown might have been somewhat underestimated, the trends reported in the present work were not affected by recovery issues.

Table S4: Amounts of PFASs detected in procedural blanks (ng) and TOP assay procedural blanks (ng) (DF = detection frequency).

Procedure	**Procedural blanks**				**TOP assay procedural blanks**			
Matrix	Biota/sediments (n = 8)		Water (n = 4)		Ultra-pure water (n = 8)		Oxidized ultra-pure water (n = 8)	
Parameters	DF %	Mean ± SD	DF %	Mean ± SD	DF %	Mean ± SD	DF %	Mean ± SD
PFBA	100	0.10 ± 0.07	100	0.13 ± 0.07	37.5	0.05 ± 0.07	37.5	0.01 ± 0.02
PFPeA	100	0.12 ± 0.08	100	0.04 ± 0.01	50	0.02 ± 0.03	75	0.01 ± 0.01
PFHxA	37.5	0.01 ± 0.01	50	0.003 ± 0.004	75	0.01 ± 0.004	100	0.03 ± 0.03
PFHpA	37.5	0.001 ± 0.01	50	0.002 ± 0.002	87.5	0.004 ± 0.003	100	0.01 ± 0.01
PFOA	62.5	0.05 ± 0.04	50	0.003 ± 0.003	100	0.02 ± 0.01	100	0.02 ± 0.01
PFDA	37.5	0.09 ± 0.12	50	0.01 ± 0.02	50	0.01 ± 0.01	12.5	0.001 ± 0.001
PFUnDA	62.5	0.03 ± 0.02	100	0.03 ± 0.01	50	0.01 ± 0.01	75	0.005 ± 0.010
PFDoDA	37.5	0.01 ± 0.02	0	nd	0	nd	37.5	0.002 ± 0.004
PFHxS	25	0.001 ± 0.001	25	0.001 ± 0.001	62.5	0.001 ± 0.001	0	nd
PFOS	87.5	0.04 ± 0.04	75	0.005 ± 0.004	100	0.01 ± 0.003	100	0.01 ± 0.02
FOSA	87.5	0.001 ± 0.001	100	0.002 ± 0.001	87.5	0.001 ± 0.001	87.5	0.002 ± 0.002
6:2 diPAP	75	0.02 ± 0.01	25	0.02 ± 0.03	75	0.02 ± 0.02	75	0.02 ± 0.02
8:2 diPAP	25	0.00 ± 0.01	50	0.004 ± 0.006	62.5	0.02 ± 0.04	75	0.05 ± 0.05

Table S5: Limits of detection and quantification

Matrix	**Water** ng L^{-1}		**Sediments** ng g^{-1} dw		**Biota** ng g^{-1} ww	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
PFBA	0.15	0.49	0.22	0.74	0.06	0.21
PFPeA	0.15	0.50	0.35	1.16	0.10	0.32
PFHxA	0.10	0.32	0.08	0.27	0.02	0.06
PFHpA	0.05	0.17	0.05	0.16	0.01	0.05
PFOA	0.19	0.62	0.08	0.28	0.02	0.08
PFNA	0.07	0.24	0.10	0.32	0.05	0.16
PFDA	0.16	0.52	0.14	0.45	0.03	0.09
PFUnDA	0.17	0.56	0.12	0.39	0.01	0.05
PFDoDA	0.27	0.90	0.09	0.30	0.01	0.03
PFTrDA	0.29	0.96	0.16	0.54	0.03	0.09
PFTeDA	0.23	0.77	0.06	0.20	0.03	0.10
PFBS	0.08	0.27	0.09	0.29	0.02	0.08
PFHxS	0.02	0.07	0.08	0.27	0.05	0.18
PFHpS	0.08	0.26	0.08	0.28	0.02	0.08
L-PFOS	0.06	0.19	0.08	0.25	0.02	0.07
Br-PFOS	0.08	0.26	0.08	0.25	0.02	0.07
PFDS	0.01	0.04	0.06	0.19	0.005	0.02
4:2 FTSA	0.06	0.19	0.02	0.05	0.005	0.02
6:2 FTSA	0.03	0.09	0.01	0.05	0.05	0.18
8:2 FTSA	0.003	0.01	0.05	0.15	0.03	0.08
10:2 FTSA	0.11	0.36	0.04	0.13	0.01	0.04
FOSA	0.02	0.08	0.08	0.27	0.01	0.03
MeFOSA	NA	NA	0.03	0.11	0.006	0.02
EtFOSA	NA	NA	0.02	0.08	0.009	0.03
FOSAA	0.10	0.32	0.03	0.11	0.01	0.03
MeFOSAA	0.05	0.16	0.05	0.15	0.01	0.04
EtFOSAA	0.18	0.59	0.08	0.27	0.01	0.04
6:2 diPAP	0.34	1.14	0.12	0.39	0.03	0.11
8:2 diPAP	0.09	0.29	0.23	0.76	0.06	0.21
HFPO-DA	0.05	0.17	0.15	0.51	0.04	0.14
ADONA	0.05	0.17	0.15	0.51	0.21	0.70

Table S6: accuracy (%) and recovery (%) of PFAS analysis in mineral water / Fontainebleau sand spiked at 10 ng g^{-1}. *: extracts were not oxidized since the aim of the test was to assess analyte recovery for the initial extraction/clean-up steps; ISs were added at the end of the analytical procedure to prevent their oxidation. NM: not measured

	Accuracy	**Accuracy**	**Recovery**
Procedure	Water extraction	Biota/sediment extraction for direct analysis	Biota/sediment extraction for the TOP assay*
Replicates	5	5	3
PFBA	94 ± 8	109 ± 3	66 ± 9
PFPeA	105 ± 7	117 ± 5	86 ± 7
PFHxA	97 ± 9	105 ± 1	76 ± 12
PFHpA	98 ± 9	107 ± 5	73 ± 4
PFOA	101 ± 8	110 ± 2	77 ± 10
PFNA	89 ± 6	101 ± 3	71 ± 7
PFDA	106 ± 10	113 ± 3	80 ± 9
PFUnDA	106 ± 9	110 ± 2	77 ± 6
PFDoDA	108 ± 2	114 ± 5	72 ± 9
PFTrDA	112 ± 4	113 ± 7	84 ± 31
PFTeDA	110 ± 9	109 ± 4	81 ± 26
PFBS	106 ± 6	118 ± 4	51 ± 5
PFHxS	108 ± 3	105 ± 3	51 ± 11
PFHpS	128 ± 3	138 ± 4	76 ± 5
L-PFOS	105 ± 10	109 ± 4	76 ± 1
PFDS	89 ± 3	102 ± 11	67 ± 7
4:2 FTSA	107 ± 16	107 ± 3	62 ± 8
6:2 FTSA	113 ± 9	119 ± 3	73 ± 9
8:2 FTSA	81 ± 7	99 ± 10	68 ± 3
10:2 FTSA	52 ± 8	79 ± 4	55 ± 5
FOSA	111 ± 9	109 ± 1	68 ± 6
MeFOSA	NA	108 ± 2	50 ± 13
EtFOSA	NA	101 ± 2	47 ± 16
FOSAA	102 ± 11	104 ± 8	84 ± 5
MeFOSAA	104 ± 4	100 ± 4	81 ± 6
EtFOSAA	123 ± 9	111 ± 5	66 ± 14
6:2 diPAP	109 ± 1	118 ± 13	55 ± 21
8:2 diPAP	139 ± 39	132 ± 9	55 ± 7
HFPO-DA	97 ± 1	112 ± 2	NM
ADONA	99± 2	114 ± 4	NM

Table S7: PFAS concentrations determined in NIST SRM 1947 reference material and in an in-house control matrix (common chub *S. Cephalus* from the Luynes River, France). For the latter, two procedures were compared: the usual extraction method applied for direct PFAS analysis and the extraction method used for the TOP assay. *: in the "usual" extraction procedure, ISs were added prior to the extraction step whereas in the procedure used for the TOP assay ISs were added after the oxidation step and prior to the Strata-XAW SPE cleanup step. †: oxidation was not performed on these extracts and amounts were determined for the calculation of recover only (see table S6). For the NIST SRM 1947 matrix, relative percent differences between concentrations measured in this work study and those found by Reiner et al. (2012) were calculated (only for compounds quantified in the two studies cases).

	NIST SRM 1947 Lake Michigan Trout			In-house control	
	This study	NIST Method 1 Reiner et al., 2012	Relative percent differences*	Common Chub from Luynes river (usual extraction procedure)	Common Chub from Luynes river (oxidation extraction procedure)
Replicates	4	24		4	3
PFBA	<LOD	< 0.861	NC	<LOD	<LOD
PFPeA	<LOD	< 0.441	NC	<LOD	<LOD
PFHxA	0.173 ± 0.070	< 0.917	NC	<LOD	<LOD
PFHpA	0.072 ± 0.007	< 0.069	NC	<LOD	<LOD
PFOA	0.105 ± 0.011	< 0.297	NC	0.42 ± 0.080	0.721 ± 0.176
PFNA	0.259 ± 0.064	0.179 ± 0.013	45	1.14 ± 0.27	0.969 ± 0.200
PFDA	0.279 ± 0.016	0.282 ± 0.062	-1.1	25.5 ± 4.8	27.1 ± 1.9
PFUnDA	0.271 ± 0.014	0.212 ± 0.024	22	15.7 ± 1.7	15.4 ± 1.7
PFDoDA	0.265 ± 0.017	< 0.137	NC	22.2 ± 3.4	26.5 ± 3.6
PFTrDA	0.187 ± 0.015	0.154 ± 0.020	18	8.4 ± 1.7	11.5 ± 0.8
PFTeDA	0.107 ± 0.015	0.198 ± 0.069	-85	8.4 ± 0.2	8.85 ± 1.3
PFBS	<LOD	< 0.194	NC	<LOD	<LOD
PFHxS	0.058 ± 0.006	< 0.049	NC	0.534 ± 0.193	0.46 ± 0.02
PFHpS	0.034 ± 0.013	NM	NC	<LOD	<LOD
L-PFOS	7.19 ± 0.556	6.17 ± 0.60	14	106.0 ± 17.3	102.0 ± 6.3
PFDS	0.202 ± 0.009	NM	NC	1.37 ± 0.18	1.75 ± 0.26
4:2 FTSA	<LOD	NM	NC	NM	NM
6:2 FTSA	<LOD	NM	NC	NM	NM
8:2 FTSA	<LOD	NM	NC	NM	NM
10:2 FTSA	<LOD	NM	NC	NM	NM
FOSA	0.120 ± 0.010	< 0.151	NC	NM	NM
N-MeFOSA	<LOD	NM	NC	NM	NM
N-EtFOSA	<LOD	NM	NC	NM	NM
FOSAA	<LOD	NM	NC	NM	NM
N-MeFOSAA	0.023 ± 0.007	NM	NC	NM	NM
N-EtFOSAA	0.063 ± 0.012	NM	NC	NM	NM
6:2 diPAP	<LOD	NM	NC	NM	NM
8:2 diPAP	<LOD	NM	NC	NM	NM
HFPO-DA	<LOD	NM	NC	NM	NM
ADONA	<LOD	NM	NC	NM	NM

SI 3. TOP assay

SI 3.1. Oxidation tests performed on spiked ultra-pure water samples

The TOP assay was initially validated with experiments performed using representative pre-PFAAs spiked into ultra-pure water at 50 ng L^{-1}: 8:2 FTSA, 6:2 diPAP, FOSA and MeFOSAA. Experiments were performed individually, i.e. only one precursor was added in each water sample. We observed the complete oxidation of these precursors under the selected conditions. Precursor to PFCA conversion rates were determined as the ratio between the molar concentrations of produced PFCAs and the initial molar concentration of precursors. The oxidation of 8:2 FTSA and 6:2 diPAP produced series of C_4–C_9 PFCA and C_4–C_7 PFCA homologues, respectively, while that of FOSA and MeFOSAA produced mainly PFOA. Overall, the conversion rates were similar to those estimated by Houtz and Sedlak (2012) (Table S8).

Then, the efficiency of the TOP assay was assessed for each sample batch. Ultra-pure water samples were spiked with 4 selected precursors: 8:2 FTSA, 6:2 diPAP, FOSA and MeFOSAA at about 50 ng L^{-1}. 100 mL of these spiked samples were transferred in separate 125mL-HDPE bottles. One replicate was stored at room temperature and one replicate was oxidized under after addition of persulfate (60 mM) and NaOH (150 mM) and incubation at 85°C for 6h. After IS addition, all replicates were subjected to the water extraction procedure. The complete oxidation of all precursors was systematically observed.

The same procedure was carried out on ultra-pure water samples spiked with the 16 analyzed PFAAs to determine their stability during the oxidation procedure. A significant decrease of C_{11}–C_{14} PFCAs and PFDS concentrations was observed upon oxidation (Figure S2). This loss of long-chain PFAAs was not attributed to their degradation because no concentration decrease of shorter-chain PFAAs was observed; rather, sorption onto the container and enrichment at the liquid/air interface may explain such a loss. A correction factor based on apparent recoveries was therefore applied to the concentration of these analytes in extracts subjected to oxidation.

Table S8: Molar conversion rates (%) of selected precursors into PFCAs in ultra-pure water upon oxidation.

		Δ[PFBA]/ $[precursors]_0$	Δ[PFPeA]/ $[precursors]_0$	Δ[PFHxA]/ $[precursors]_0$	Δ[PFHpA]/ $[precursors]_0$	Δ[PFOA]/ $[precursors]_0$	Δ[PFNA]/ $[precursors]_0$	Δ[PFOS]/ $[precursors]_0$
8:2 FTSA (n = 6)	this study	9 ± 4	13 ± 3	22 ± 4	32 ± 8	33 ± 6	3 ± 0.5	
	Houtz and Sedlak (2012)	11 ± 4	12 ± 4	19 ± 3	27 ± 3	21 ± 2	3 ± 0.1	
6:2 diPAP (n = 6)	this study	24 ± 6	61 ± 7	51 ± 7	21 ± 4			
	Houtz and Sedlak (2012)	27 ± 3	47 ± 3	47 ± 3	15 ± 3			
FOSA (n = 6)	this study			0.4 ± 0.5	1.1 ± 0.4	128 ± 16		1.5 ± 0.3
	Houtz and Sedlak (2012)					97 ± 3		
MeFOSAA (n = 6)	this study		0.6 ± 0.6	1.8 ± 0.2	4.1 ± 1.0	121 ± 24		1.0 ± 0.3
	Houtz and Sedlak (2012)					110 ± 8		

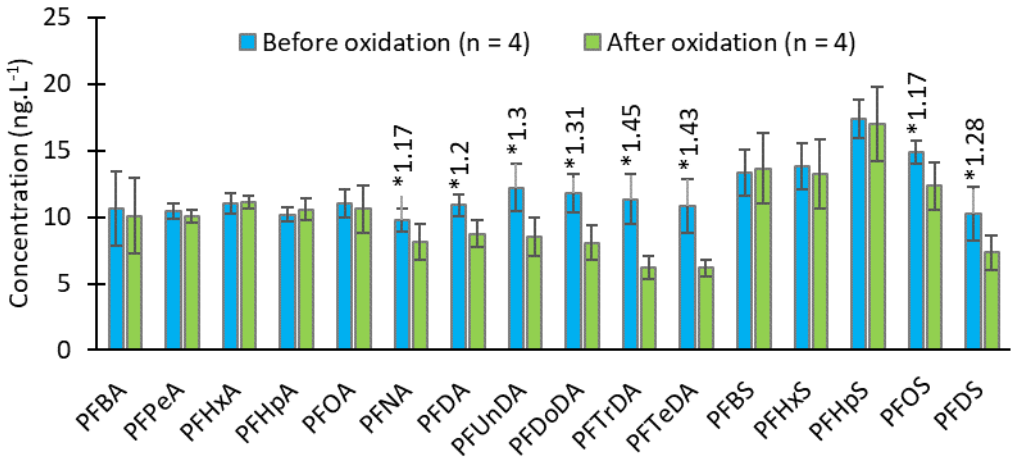

Figure S2: PFCA and PFSA concentrations in ultra-pure water before and after oxidation. The indicated numbers correspond to the correction factors applied to the concentrations after oxidation to compensate the losses observed during the TOP assay procedure.

SI 3.2. Oxidation tests performed on fish tissue extracts

A preliminary test was carried out on a sample of fish tissues (common chub *S. cephalius* from the Luynes River, SE France). The presence of PFAAs and some of their precursors (e.g. 8:2 FTSA) in this sample had been previously characterized. Samples consisting of 600 mg (dry weight) of ground tissues were extracted by microwave assisted extraction with MeOH + 0.2% NH_4OH, and the so-obtained extracts were cleaned-up with ENVI-carb cartridges and evaporated under a nitrogen stream to 300 µL. Then, 50 µL-aliquots were transferred into 6 HDPE 125mL-bottles and evaporated to dryness at room temperature under a nitrogen stream. Ultra-pure water (100 mL) was added to 3 bottles while 100 mL of ultra-pure water containing 60 mM of persulfate and 150 mM of NaOH were added to the other three bottles, which were placed in an oven at 85°C during 6h. After cooling and pH neutralization with HCl (1M), ISs were added to the six bottles (i.e. ISs were not added at the beginning of the sample preparation but after the TOP assay to avoid their own oxidation). Then, all samples were extracted on X-AW cartridges, evaporated to 300 µL and analyzed by LC-MS/MS. Note that persulfate and NaOH amounts were 16 times higher than in the method developed by Houtz et al. (2013) for soil samples, so as to maximize the conversion rates of pre-PFAAs in such complex biota extracts.

The results showed the complete conversion of targeted precursors (Figure S3) and a large increase of C_4–C_9 PFCA levels. Variation coefficients < 8 % highlighted the good repeatability of the assay.

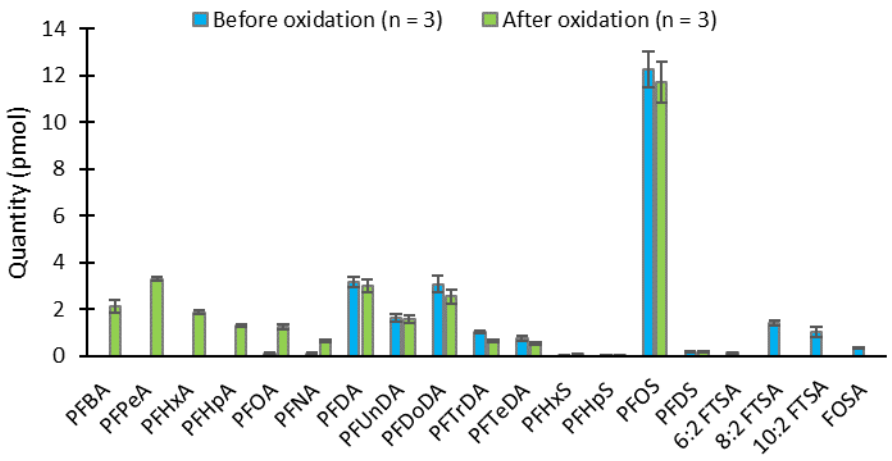

Figure S3: PFAS quantities in tissues of *S. cephalus* before and after oxidation (n = number of replicates, error bars indicate the standard deviation)

SI 3.3 Estimation of the contribution of ∑pre-PFAAs$_{unknown}$ to ∑PFASs

To estimate the contribution of ∑pre-PFAAs$_{unknown}$ to ∑PFASs, concentrations of PFCAs, PFSAs and pre-PFAAs$_{targeted}$ were first expressed on a molar basis (C_M). C_M(∑pre-PFAAs$_{targeted}$) were then converted to C_M(PFCAs) using previously determined conversion factors (Table S15) and this concentration was subtracted from ΔPFCAs to estimate the C_M(PFCAs) resulting from the oxidation of unknown precursors. Finally, to estimate C_M∑pre-PFAAs$_{unknown}$ on a molar basis, we assumed that one mole of each precursor produced one mole of PFCAs of similar chain length upon oxidation because i) the proportions of unidentified precursors with one, two (e.g. di-PAPs) or three perfluoroalkyl chains (such as tri-PAPs) were unknown, and ii) the molar yields of PFCAs from unidentified precursors were undetermined. In this way, C_M(∑pre-PFAAs$_{unknown}$) is given as a maximum estimate of the molar concentration of extracted and oxidized unidentified pre-PFAAs. Finally, it should be noted that since C_2–C_3 PFCAS were not analyzed, C_M(∑pre-PFAAs$_{unknown}$) may have been underestimated. However, the extent of such underestimation depends on the pre-PFAAs$_{unknown}$ pattern (Janda et al., 2019) that, by definition, remains undetermined at this stage.

SI 4. Supplementary results

Table S9: Concentration of PFASs detected in sediments (ng g^{-1} dry weight) and water (ng L^{-1}) samples.

	Sediments (n = 3) Mean ± SD	Water (n = 1)
PFBA	<0.22	6.88
PFPeA	<0.35	12.0
PFHxA	0.03 ± 0.05	13.9
PFHpA	<0.05	4.85
PFOA	0.04 ± 0.07	8.74
PFNA	<0.10	1.07
PFDA	0.13 ± 0.22	3.41
PFUnDA	0.05 ± 0.09	<0.17
PFDoDA	0.72 ± 0.61	<0.27
PFTrDA	0.10 ± 0.17	<0.29
PFTeDA	0.47 ± 0.35	<0.23
PFBS	<0.09	3.27
PFHxS	<0.08	9.51
PFHpS	<0.08	0.66
∑PFOS	0.53 ± 0.52	28.8
L-PFOS	0.47 ± 0.42	15.5
Br-PFOS	0.08 ± 0.07	13.3
6:2 FTSA	<0.01	8.03
8:2 FTSA	<0.05	<0.003
10:2 FTSA	0.13 ± 0.10	<0.11
FOSA	<0.08	0.21
FOSAA	<0.03	<0.10
MeFOSAA	<0.05	<0.05
EtFOSAA	0.02 ± 0.03	<0.18
6:2 diPAP	0.11 ± 0.08	<0.34
∑PFASs	2.28 ± 2.31	101.4

Table S10: Concentrations of PFASs detected in fish, expressed in ng g^{-1} ww (mean ± standard deviation, n = number of samples). Analytes never detected are not shown.

Species	**Detection frequency %**	***B. barbus***	***C. gobio***	***R. rutilus***	***G. gobio***	***P. fluviatilis***	***L. gibbosus***	***I. melas***	***T. tinca***
		Barbel	***European bullhead***	***Common roach***	***Gudgeon***	***Common perch***	***Sunfish***	***Catfish***	***Tench***
n		1	4	4	4	4	4	4	4
PFPeA	0	<0.10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PFHxA	21	<0.02	0.07 ± 0.03	0.02 ± 0.04	<0.02	0.01 ± 0.01	<0.02	<0.02	<0.02
PFHpA	17	<0.01	0.06 ± 0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PFOA	90	0.09	0.65 ± 0.17	0.05 ± 0.03	0.10 ± 0.01	0.07 ± 0.03	0.05 ± 0.03	0.04 ± 0.05	0.11 ± 0.02
PFNA	100	0.53	0.76 ± 0.15	0.21 ± 0.03	0.28 ± 0.06	0.23 ± 0.07	0.20 ± 0.05	0.22 ± 0.06	0.44 ± 0.10
PFDA	90	9.56	5.63 ± 1.96	5.19 ± 1.02	4.13 ± 2.85	7.32 ± 0.91	3.68 ± 2.56	2.60 ± 0.56	2.78 ± 1.94
PFUnDA	100	5.71	4.26 ± 1.32	3.88 ± 0.53	3.45 ± 0.52	4.42 ± 0.71	3.24 ± 1.01	2.28 ± 0.56	2.01 ± 0.39
PFDoDA	100	26.50	42.2 ± 13.1	28.7 ± 5.00	24.5 ± 4.89	32.5 ± 6.49	20.2 ± 8.03	19.8 ± 2.65	15.0 ± 4.44
PFTrDA	100	3.84	10.4 ± 2.92	6.48 ± 1.41	4.54 ± 1.29	6.47 ± 1.40	4.57 ± 2.08	4.22 ± 0.25	2.74 ± 1.57
PFTeDA	100	6.18	25.3 ± 4.81	16.9 ± 2.94	13.8 ± 4.13	16.7 ± 3.91	11.5 ± 4.71	12.7 ± 1.39	6.95 ± 3.87
PFHxS	93	0.13	1.32 ± 0.40	0.12 ± 0.05	0.49 ± 0.58	0.12 ± 0.03	0.08 ± 0.06	0.08 ± 0.06	0.59 ± 0.20
PFHpS	90	0.06	0.28 ± 0.17	0.03 ± 0.04	0.05 ± 0.01	0.12 ± 0.03	0.05 ± 0.03	0.03 ± 0.01	0.12 ± 0.05
ΣPFOS	100	45.8	52.7 ± 23.0	43.4 ± 6.21	24.1 ± 7.02	55.7 ± 4.41	41.5 ± 12.2	15.8 ± 2.59	25.3 ± 10.9
L-PFOS	100	40.3	44.5 ± 19.0	39.9 ± 5.51	21.6 ± 6.29	49.6 ± 4.05	37.6 ± 10.7	14.8 ± 2.42	22.0 ± 9.59
Br-PFOS	100	5.45	8.19 ± 4.00	3.42 ± 0.71	2.44 ± 0.84	6.04 ± 0.61	3.89 ± 1.78	0.95 ± 0.25	3.31 ± 1.36
PFDS	100	2.10	0.99 ± 0.43	1.17 ± 0.23	0.92 ± 0.43	1.20 ± 0.21	1.02 ± 0.23	0.67 ± 0.15	0.60 ± 0.22
6:2 FTSA	72	0.15	0.11 ± 0.04	0.03 ± 0.04	0.14 ± 0.04	0.14 ± 0.02	0.13 ± 0.01	0.01 ± 0.03	0.02 ± 0.04
8:2 FTSA	100	0.98	0.22 ± 0.09	0.26 ± 0.08	0.47 ± 0.33	0.58 ± 0.13	0.21 ± 0.06	0.09 ± 0.02	0.68 ± 0.28
10:2 FTSA	100	3.23	1.59 ± 0.23	2.27 ± 0.36	2.54 ± 0.40	2.68 ± 0.40	1.41 ± 0.48	1.62 ± 0.18	1.71 ± 0.47
FOSA	100	0.69	0.76 ± 0.23	0.60 ± 0.14	0.57 ± 0.20	0.44 ± 0.09	0.23 ± 0.03	0.34 ± 0.04	0.35 ± 0.15
FOSAA	31	0.01	<0.01	0.01 ± 0.01	0.01 ± 0.01	<0.01	<0.01	<0.01	<0.01
MeFOSAA	90	0.02	0.03 ± 0.01	0.01 ± 0.01	0.05 ± 0.04	0.09 ± 0.02	0.04 ± 0.004	0.01 ± 0.01	0.02 ± 0.01
EtFOSAA	93	0.04	0.06 ± 0.03	0.04 ± 0.02	0.07 ± 0.04	0.14 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.02
6:2 diPAP	59	0.08	0.03 ± 0.03	0.04 ± 0.03	0.08 ± 0.02	0.08 ± 0.01	0.02 ± 0.03	<0.03	0.02 ± 0.03
∑PFASs		106	147 ± 45.3	109 ± 15.8	80.3 ± 17.6	129 ± 17.0	88.3 ± 20.1	60.4 ± 1.63	59.6 ± 16.9

Table S11: Concentrations of PFASs detected in invertebrates, biofilm, macrophyte and leaf litter (ng g^{-1} ww): mean concentrations (± standard deviation) are given when n=3, and individual values are given when n <3 (nd: not detected).

Species	Detection frequency %	*Lymnaeidae*	*Corbiculidae*	*Gammaridae*	*Notonectidae*	Biofilm	Macrophyte	Leaf litter
n		3	1	3	1	2	2	2
PFPeA	21	<0.10	<0.10	0.39 ± 0.34	<0.10	<0.10	<0.10	<0.10, 0.15
PFHxA	86	0.04 ± 0.04	<0.02	0.47 ± 0.09	0.09	0.04, 0.04	0.03, 0.03	0.06, 0.03
PFHpA	50	<0.01	<0.01	0.10 ± 0.18	0.28	0.02, 0.02	0.02, 0.02	0.01, <0.01
PFOA	100	0.09 ± 0.08	0.04	1.88 ± 0.04	3.19	0.14, 0.12	0.10, 0.11	0.06, 0.06
PFNA	86	0.12 ± 0.14	0.06	0.85 ± 0.09	1.62	0.09, 0.05	0.11, 0.12	<0.05, 0.05
PFDA	93	0.68 ± 0.68	<0.03	3.17 ± 0.24	5.79	0.20, 0.10	0.48, 0.59	0.17, 0.29
PFUnDA	100	0.82 ± 0.50	0.02	2.27 ± 0.14	2.91	0.08, 0.06	0.30, 0.32	0.09, 0.12
PFDoDA	100	8.53 ± 5.36	0.70	17.26 ± 0.98	19.56	0.75, 0.77	1.43, 1.37	0.55, 0.62
PFTrDA	100	0.87 ± 0.37	0.39	2.10 ± 0.25	2.89	0.16, 0.13	0.13, 0.13	0.07, 0.09
PFTeDA	100	4.66 ± 2.14	1.98	8.11 ± 1.15	3.62	0.46, 0.42	0.19, 0.23	0.15, 0.26
PFHxS	50	<0.05	<0.05	0.61 ± 0.05	4.47	<0.05, 0.06	<0.05	0.06, 0.10
PFHpS	29	<0.02	<0.02	0.07 ± 0.01	0.22	<0.02	<0.02	<0.02
ΣPFOS	100	1.69 ± 0.50	0.12	6.59 ± 0.24	32.29	0.79, 0.62	1.58, 1.75	0.61, 1.01
L-PFOS	100	1.53 ± 0.16	0.12	5.87 ± 0.24	27.6	0.71, 0.56	1.39, 1.55	0.55, 0.88
Br-PFOS	88	0.48 ± 0.04	<0.02	0.72 ± 0.01	4.70	0.07, 0.06	0.19, 0.21	0.06, 0.13
PFDS	93	0.15 ± 0.07	0.02	0.10 ± 0.03	0.19	0.02, 0.04	0.03, 0.04	<0.005, 0.03
6:2 FTSA	86	0.07 ± 0.07	<0.05	0.64 ± 0.06	5.24	0.12, 0.14	0.08, 0.08	0.06, 0.06
8:2 FTSA	86	0.08 ± 0.04	<0.03	0.50 ± 0.11	0.95	0.03, 0.03	0.05, 0.06	0.03, <0.03
10:2 FTSA	100	1.08 ± 0.60	0.50	1.35 ± 0.42	0.30	0.18, 0.20	0.22, 0.16	0.05, 0.10
FOSA	86	0.57 ± 0.16	0.21	0.38 ± 0.01	0.58	0.03, 0.01	<0.01, 0.01	<0.01, 0.01
FOSAA	64	0.03 ± 0.01	<0.01	0.06 ± 0.01	0.12	<0.01	0.01, 0.01	<0.01
MeFOSAA	50	0.03 ± 0.01	<0.01	0.06 ± 0.004	0.09	<0.01	<0.01	<0.01
EtFOSAA	50	0.08 ± 0.04	<0.01	0.04 ± 0.003	0.16	<0.01	<0.01	<0.01
6:2 diPAP	50	0.05 ± 0.05	<0.03	0.02 ± 0.04	<0.03	0.07, 0.10	0.03, 0.03	<0.03
∑PFASs		19.6 ± 8.97	4.03	47.0 ± 2.5	84.6	3.17, 2.92	4.81, 5.07	1.98, 2.98

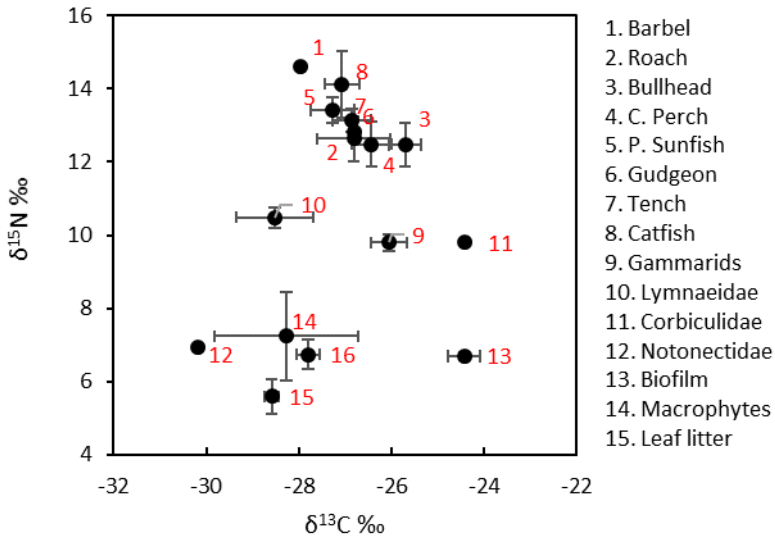

Figure S4: Mean stable isotope signature ($\delta^{15}N$ Vs $\delta^{13}C$) of taxa collected from the Orge River (error bars correspond to standard deviations).

TL ranges (or values when the number of samples was ≤ 2), calculated according to Post (2002), were as follows: barbel (3.4), roach (2.7-3.1), bullhead (2.6-3.0), C. perch (2.6-3.0), P. sunfish (3.0-3.2), gudgeon (2.8-3.0), tench (2.9-3.1), catfish (3.0-3.5), gammarids (1.9-2.1), lymnaeidae (2.1-2.3), corbiculidae (2.0), notonectidae (1.2), biofilm (1.1, 1.1), macrophytes (1.0, 1.5).

Table S12: Biomagnification factors corrected for trophic level (mean ± standard deviation). Concentrations were expressed in ng g^{-1} ww. BMF_{TL} was calculated only when the detection frequency of a given compound was 100 % in both the prey and the predator. NA: not available.

	Roach/ Lymnaeidae	Roach/ Macrophyte	Gudgeon/ Gammarids	Gudgeon/ Lymnaeidae	Gudgeon/ Macrophyte	Tench/ Lymnaeida	Tench/ Macrophyte	Catfish/ Lymnaeidae	Catfish/ Gammarids
PFOA	NA	NA	0.04 ± 0.003	1.2 ± 0.1	1.0 ± 0.04	1.4 ± 0.3	1.0 ± 0.1	NA	NA
PFNA	2.3 ± 0.6	1.5 ± 0.1	0.3 ± 0.1	NA	1.7 ± 0.2	NA	2.2 ± 0.3	NA	0.3 ± 0.1
PFDA	25.2 ± 7.8	4.2 ± 0.5	NA	NA	NA	NA	NA	3.5 ± 0.7	0.9 ± 0.1
PFUnDA	11.6 ± 2.5	4.9 ± 0.4	1.6 ± 0.3	8.1 ± 1.8	4.3 ± 0.4	3.2 ± 4.8	2.9 ± 0.3	2.6 ± 0.6	1.0 ± 0.2
PFDoDA	6.9 ± 1.8	6.7 ± 0.7	1.5 ± 0.3	4.7 ± 1.3	5.7 ± 0.7	2.1 ± 6.8	3.9 ± 0.7	2.2 ± 0.3	1.1 ± 0.1
PFTrDA	24.1 ± 8.1	11.7 ± 1.6	2.4 ± 0.8	11.3 ± 4.5	8.7 ± 1.5	4.5 ± 11.4	5.7 ± 1.8	4.3 ± 0.2	1.7 ± 0.1
PFTeDA	7.7 ± 2.1	15.8 ± 1.7	1.8 ± 0.6	5.0 ± 2.1	12.7 ± 2.3	1.7 ± 15.3	7.3 ± 2.3	2.5 ± 0.3	1.4 ± 0.1
PFHxS	NA	NA	0.8 ± 1.1	NA	NA	NA	NA	NA	NA
PFHpS	NA	NA	0.8 ± 0.2	NA	NA	NA	NA	NA	NA
∑PFOS	169.0 ± 36.5	7.7 ± 0.7	4.3 ± 1.4	48.9 ± 19.8	5.0 ± 0.9	33.2 ± 7.0	4.7 ± 1.2	8.0 ± 1.2	2.0 ± 0.3
L-PFOS	172.8 ± 36.0	8.0 ± 0.7	4.4 ± 1.4	48.2 ± 19.4	5.1 ± 4.6	31.4 ± 17.2	4.7 ± 1.2	8.3 ± 1.3	2.1 ± 0.3
Br-PFOS	133.9 ± 41.4	6.0 ± 0.8	4.0 ± 1.5	56.7 ± 28.7	0.9 ± 0.9	52.0 ± 26.6	5.0 ± 1.2	5.4 ± 1.3	1.2 ± 0.3
PFDS	25.7 ± 7.6	8.9 ± 1.1	12.2 ± 6.2	14.8 ± 9.0	7.1 ± 2.1	6.0 ± 8.8	5.0 ± 1.1	4.0 ± 0.8	4.4 ± 0.8
6:2 FTSA	NA	NA	0.2 ± 0.1	2.5 ± 0.9	NA	NA	NA	NA	NA
8:2 FTSA	7.3 ± 3.5	2.6 ± 0.5	0.9 ± 0.7	15.7 ± 16.3	3.5 ± 1.4	17.2 ± 2.1	4.2 ± 0.9	1.2 ± 0.3	0.3 ± 0.1
10:2 FTSA	3.3 ± 0.8	4.7 ± 0.5	2.0 ± 0.4	3.5 ± 0.8	4.8 ± 0.5	1.8 ± 4.7	3.5 ± 0.5	1.5 ± 0.2	1.1 ± 0.1
FOSA	1.1 ± 0.4	NA	1.6 ± 0.6	1.0 ± 0.5	NA	NA	NA	0.6 ± 0.1	0.9 ± 0.1
FOSAA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MeFOSAA	NA	NA	0.8 ± 0.8	2.5 ± 3.1	NA	NA	NA	NA	NA
EtFOSAA	0.4 ± 0.3	NA	2.2 ± 1.3	0.9 ± 0.7	NA	NA	NA	NA	NA
6:2 diPAP	NA	NA	NA	NA	1.7 ± 0.3	NA	NA	NA	NA

Table S13: TMF calculations: details on regressions of Log C (concentrations expressed in ng g^{-1} ww, whole body) vs TL obtained with the *lmec* function of the NADA R-package.

	Slope (95% confidence interval)	Intercept (95% confidence interval)
PFHxA	-0.64 (-0.74 – -0.53)	-0.54 (-1.11 – 0.02)
PFOA	-0.25 (-0.30 – -0.20)	-0.34 (-0.63 – -0.05)
PFNA	0.20 (0.17 – 0.22)	-1.12 (-1.28 – -0.97)
PFDA	0.41 (0.34 – 0.47)	-0.87 (-1.30 – -0.43)
PFUnDA	0.34 (0.32 – 0.36)	-0.74 (-0.88 – -0.59)
PFDoDA	0.38 (0.36 – 0.40)	0.06 (-0.07 – 0.19)
PFTrDA	0.46 (0.44 – 0.48)	-0.86 (-0.99 – -0.74)
PFTeDA	0.46 (0.44 – 0.48)	-0.43 (-0.56 – -0.30)
PFHxS	0.07 (-0.01 – 0.15)	-1.11 (-1.64 – -0.58)
PFHpS	0.19 (0.14 – 0.24)	-1.97 (-2.31 – -1.62)
∑PFOS	0.19 (0.16 – 0.21)	0.53 (0.31 – 0.75)
L-PFOS	0.20 (0.17 – 0.23)	0.41 (0.19 – 0.63)
Br-PFOS	0.27 (0.23 – 0.31)	-0.70 (-1.00 – -0.39)
PFDS	0.74 (0.72 – 0.76)	-2.33 (-2.43 – -2.23)
6:2 FTSA	-0.24 (-0.28 – -0.20)	-0.45 (-0.72 – -0.17)
8:2 FTSA	0.10 (0.07 – 0.14)	-1.04 (-1.27 – -0.80)
10:2 FTSA	0.48 (0.47 – 0.48)	-1.15 (-1.19 – -1.11)
FOSA	0.40 (0.37 – 0.42)	-1.60 (-1.78 – -1.42)
FOSAA	-0.19 (-0.29 – -0.09)	-1.90 (-2.53 – -1.28)
MeFOSAA	0.17 (0.13 – 0.21)	-2.17 (-2.44 – -1.90)
EtFOSAA	0.16 (0.11 – 0.22)	-2.02 (-2.37 – -1.68)
6:2 diPAP	0.07 (0.04 – 0.11)	-1.80 (-2.05 – -1.56)

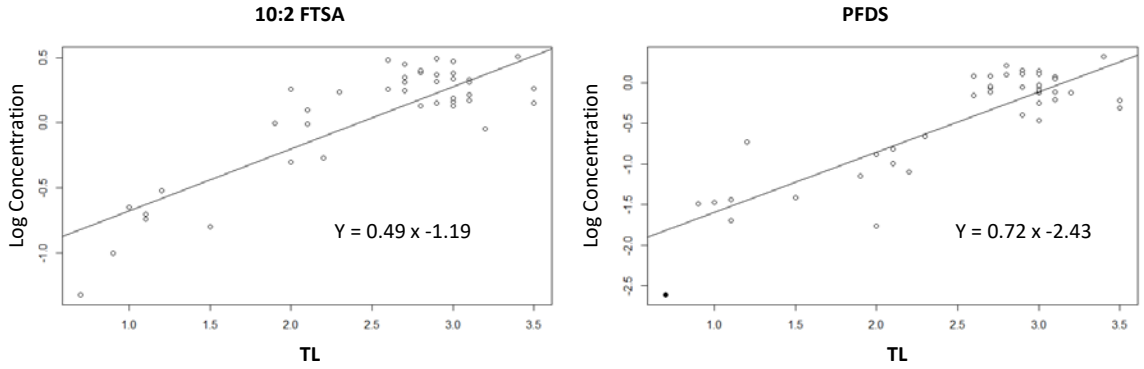

Figure S5: Linear regression of Log Concentration vs TL for 10:2 FTSA and PFDS, obtained using the lmec function.

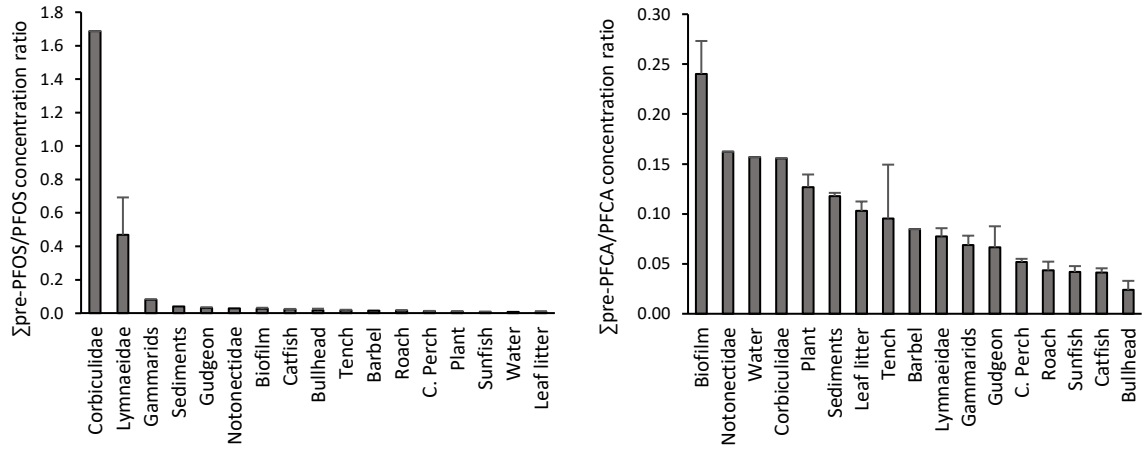

Figure S6: ∑pre-PFOS/PFOS and ∑pre-PFCAs/PFCA concentration ratios in the trophic web of the Orge river. Error bars indicate the standard deviation.

Table S14: Increase of PFCA concentrations (ΔPCFAs) after oxidation in ng L^{-1} for water, ng g^{-1} dw for sediments and ng g^{-1} ww for biota samples and in % according to the initial concentration for all matrices.

	ΔPFBA		ΔPFPA		ΔPFHxA		ΔPFHpA		ΔPFOA		ΔPFNA		ΔPFDA		ΔPFUnA		ΔPFDoA		Δ(PFCAs)	
Sample	C	%	C	%	C	%	C	%	C	%	C.	%	C	%	C	%	C	%	C	%
water	2.0	29	24.4	202	8.4	60	3.0	62	0.1	1	0.9	86	0.0	0	0.0	0.0	0.0	0.0	39.7	78
sediment	1.7	NA	1.5	NA	1.1	1346	1.0	NA	1.6	1323	1.1	NA	0.9	246	0.7	439	0.6	44	10.7	319
biofilm	1.1	NA	1.4	NA	0.9	2310	0.9	4643	1.0	690	0.7	821	0.8	400	0.5	658	0.6	86	8.2	424
macrophyte	0.8	NA	1.4	NA	0.5	2071	0.5	2975	0.5	523	0.4	378	0.2	46	0.3	93	0.7	47	5.5	196
leaf litter	0.6	NA	0.6	NA	0.3	461	0.4	2524	0.4	702	0.4	NA	0.3	154	0.3	306	0.2	32	3.5	298
gammarids 1	1.4	NA	1.6	NA	0.9	155	1.1	368	2.2	117	1.7	197	1.3	41	1.5	63	5.0	28	18.4	49
gammarids 2	1.4	NA	1.2	239	0.9	216	1.3	NA	1.3	69	1.1	149	1.4	46	1.4	58	3.1	17	14.8	39
limnaeidae 1	1.3	NA	1.6	NA	1.3	1862	1.5	NA	1.9	1024	2.3	810	0.8	52	0.9	67	0.0	0	5.5	23
limnaeidae 2	1.6	NA	1.6	NA	1.3	NA	1.7	NA	2.2	3837	2.5	3423	1.3	383	1.5	279	0.6	11	11.6	82
bullhead 1	1.4	NA	2.1	NA	1.6	1648	1.6	2048	2.9	330	2.7	281	2.1	25	3.5	59	19.6	33	35.1	30
bullhead 2	2.0	NA	1.6	NA	1.4	3612	1.5	1671	2.8	497	2.9	443	2.5	59	1.6	57	8.4	31	26.2	43
roach 1	1.7	NA	1.7	NA	1.4	1833	1.6	NA	3.1	4522	2.9	1291	3.0	56	2.7	70	9.7	32	33.5	52
roach 2	1.5	NA	1.7	NA	1.5	NA	1.7	NA	2.7	3615	2.8	1419	1.7	34	2.4	62	6.3	22	21.5	36
c. perch 1	1.2	NA	1.9	NA	1.5	NA	1.3	NA	2.4	2934	3.1	1246	1.6	20	2.4	50	4.8	16	18.6	29
c. perch 2	2.0	NA	2.3	NA	2.2	NA	1.7	NA	2.8	4474	3.1	1514	0.6	11	2.6	77	1.9	8	17.6	34
catfish 1	1.3	NA	1.3	NA	1.6	NA	2.1	NA	3.0	5048	2.4	1055	0.8	40	0.6	38	0.0	0	8.7	22
catfish 2	0.9	NA	0.7	NA	1.0	NA	1.0	NA	2.1	NA	2.1	1372	1.2	57	1.8	86	0.1	1	6.0	15

Table S15: Conversion factors used to estimate the molar concentration of PFCAs produced by the oxidation of known precursors.

PFCAs / Precursors	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	Reference
6:2 FTSA	0.22	0.27	0.22	0.02					Houtz and Sedlak (2012)
8:2 FTSA	0.09	0.13	0.22	0.32	0.33	0.03			This study
10:2 FTSA			0.09	0.13	0.22	0.32	0.33	0.03	Estimated based on results obtained for 8:2 FTSA
FOSA					1.28				This study
FOSAA					1.2				Estimated based on results obtained for N-MeFOSAA
MeFOSAA					1.2				This study
EtFOSAA					0.92				Houtz and Sedlak (2012)
6:2 diPAP	0.24	0.62	0.51	0.21					This study

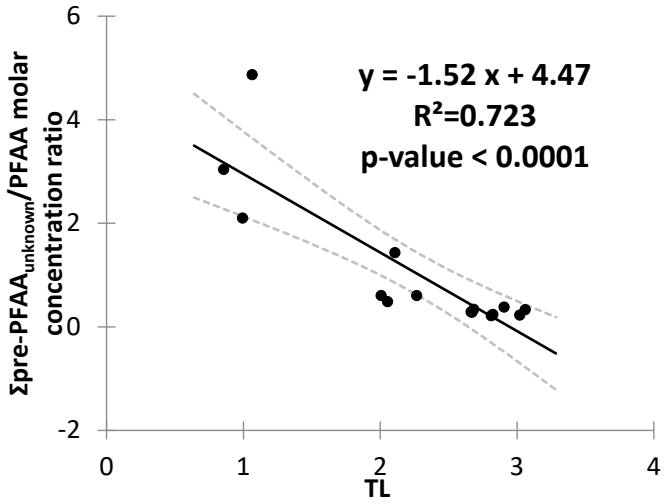

Figure S7: Relation between ∑pre-PFAA$_{unknown}$/PFAA molar concentration ratios and the trophic level (TL).

References

Janda, J.; Nödler, K.; Scheurer, M.; Happel, O.; Nürenberg, G.; Zwiener, C.; Lange, F. T. Closing the Gap – Inclusion of Ultrashort-Chain Perfluoroalkyl Carboxylic Acids in the Total Oxidizable Precursor (TOP) Assay Protocol. Environ. Sci.: Processes Impacts (2019) https://doi.org/10.1039/C9EM00169G

Houtz, E.F., Sedlak, D.L., Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff, Environ. Sci. Technol. 46 (2012) 9342–9349.

Houtz, E.F. , Higgins, C.P., Field, J.A., Sedlak, D.L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil, Environ. Sci. Technol. 47 (2013) 8187–8195.

Martin, D. Munoz, G., Mejia-Avendaño, S. , Duy, S.V. , Yao, Y. , Volchek, K. , Brown, C.E., Liu, J., Sauvé, S. Zwitterionic, cationic, and anionic perfluoroalkyl and polyfluoroalkyl substances integrated into Total Oxidizable Precursor Assay of contaminated groundwater, Talanta, 2019, 195, 533–542

Munoz, G., Giraudel, J.-L., Botta, F., Lestremau, F., Dévier, M.-H., Budzinski, H. , Labadie, P. Spatial distribution and partitioning behavior of selected poly- and perfluoroalkyl substances in freshwater ecosystems: A French nationwide survey, Sci. Total Environ. 517 (2015) 48–56

Munoz, G., Desrosiers, M. , Duy, S.V., Labadie, P., Budzinski, H., Liu, J., Sauvé, S. Environmental Occurrence of Perfluoroalkyl Acids and Novel Fluorotelomer Surfactants in the Freshwater Fish *Catostomus Commersonii* and Sediments Following Firefighting Foam Deployment at the Lac-Mégantic Railway Accident. Environ. Sci. Technol. (2017) 51 (3), 1231–1240

Munoz, G., Ray, P., Mejia-Avendaño, S., Sung, V.D., Dat, T.D. Liu, J., Sauvé, S. Optimization of Extraction Methods for Comprehensive Profiling of Perfluoroalkyl and Polyfluoroalkyl Substances in Firefighting Foam Impacted Soils. Analytica Chimica Acta 1034 (2018) 74–84

Munoz, G., Ray P., Mejia-Avendaño., Sung, V.D., D. Do, D.T., Liu, J., Sauvé, S. Optimization of Extraction Methods for Comprehensive Profiling of Perfluoroalkyl and Polyfluoroalkyl Substances in Firefighting Foam Impacted Soils. Analytica Chimica Acta (2018) 1034: 74–84

Post, D. M. Using stable isotopes to estimate trophic position : models, methods, and assumptions, Ecology 83 (2002) 703–718

Reiner, J.L. , O'Connell, S.G., Butt, C.M., Mabury, S.A., Small, J.M., De Silva, A.O., Muir, D.C.G., Delinsky, A.D., Strynar, M.J., Lindstrom, A.B., Reagen, W.K., Malinsky, M., Schäfer, S., Kwadijk, C.J. F., Schantz, M.M., Keller, J.M. Determination of perfluorinated alkyl acid concentrations in biological standard reference materials, Anal. Bioanal. Chem. 404 (2012) 2683–2692