Supplementary material for the article:

Beškoski, V. P.; Yamamoto, A.; Nakano, T.; Yamamoto, K.; Matsumura, C.; Motegi, M.; Beškoski, L. S.; Inui, H. Defluorination of Perfluoroalkyl Acids Is Followed by Production of Monofluorinated Fatty Acids. *Science of the Total Environment* **2018**, *636*, 355–359. https://doi.org/10.1016/j.scitotenv.2018.04.243

# **Materials and Methods**

#### PFAA contents in the sediment samples

For extraction of PFAAs, 10 g from Site A and 1 g from Site B of sediments, previously dried to  $105^{\circ}$ C and homogenized, were used according to a previously described procedure (Beškoski et al., 2013). As mass-labeled surrogates, 10 µL of MPFAC-MXA (each 100 ng mL<sup>-1</sup> in methanol), were spiked into the sample. All results were calculated according to dry matter, while percentages were calculated according to mass. Fluorinated chemicals analyzed in the sediment samples are listed in Table S1.

#### Quality control, method limits of detection (LOD), and method limits of quantification (LOQ)

Mass axis calibration was conducted using a mixture of sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621 (Lancaster Synthesis, Ward Hill, MA). The instrumental LOD was defined empirically as the concentration producing a signal to noise ratio of 3, and the LOQ was defined as the concentration producing a signal to noise ratio of 10. The method detection limit (MDL) and the method quantification limit (MQL) were determined by dividing the LOD and LOQ by the concentration factor (Table S5). The sampling containers, glass jars, polypropylene bottles as well as all the glassware were rinsed with methanol and Milli-Q water prior to use. Teflon bottles and Teflon-lined caps were avoided throughout the analysis. HPLC grade water, SPE blank, solvents and sample bottle blank were all analyzed, and no analytes were detected.

### **Results and Discussion**

# Change of basic parameters during biotransformation study

After one week of incubation, the pH decreased in BT and BC for both A-CB and B-CB (Fig. S1a and S1b), suggesting microbiological production of organic acids. Increases in pH during second and third weeks suggested changes within the microbial community and consumption of the previously produced organic acids as a source of carbon or oxygen limitation in the later stages of the study. After an increase in the number of bacteria in the first seven days of incubation up to  $5 \times 10^8$  colony forming unit (CFU) mL<sup>-1</sup>, the number was stable until the end of the study. In A-YM and B-YM, the numbers of CB increased in BT and BC (Fig. S1c and S1d). In contrast, after an initial increase in the numbers of YM, their number decreased to  $10^6$  CFU mL<sup>-1</sup> in BT and BC, except for A-YM in BC. To confirm oxygen limitation, the number of anaerobic bacteria was analyzed in all BT model systems at the beginning ( $5x10^4$ ) and at the end of the experiments ( $5.2x10^5$ ,  $6.4x10^6$ ,  $7.7x10^6$  and  $8.1x10^6$  in A-CB, B-CB, A-YM and B-YM, respectively). Results are suggesting that in the later phases of the experiment, conditions were favorable for the growth of anaerobic bacteria. Changes within the composition of the microbial consortia were accompanied by changes in pH. The pH in all AC model systems did not change significantly.

Formula/Name/Acronym	No. of CF <sub>2</sub> groups	Acronym	Analyte
	m = 2	PFBA	Perfluorobutanoate
	m = 3	PFPeA	Perfluoropentanoate
	m = 4	PFHxA	Perfluorohexanoate
$CF_3(CF_2)_m CO_2^-$	m = 5	PFHpA	Perfluoroheptanoate
Deathrane allers	m = 6	PFOA	Perfluorooctanoate
Perfluoroalkyl	m = 7	PFNA	Perfluorononanoate
carboxylates	m = 8	PFDA	Perfluorodecanoate
(PFCAs)	m = 9	PFUnDA	Perfluoroundecanoate
(FICAS)	m = 10	PFDoDA	Perfluorododecanoate
	m = 11	PFTrDA	Perfluorotridecanoate
	m = 12	PFTeDA	Perfluorotetradecanoate
$CF_3(CF_2)_nSO_3^-$	<i>n</i> = 3	PFBS	Perfluorobutanesulfonate
	<i>n</i> = 5	PFHxS	Perfluorohexanesulfonate
Perfluoroalkyl sulfonates	<i>n</i> = 7	PFOS	Perfluorooctanesulfonate
(PFSAs)	<i>n</i> = 9	PFDS	Perfluorodecanesulfonate

 Table S1. Perfluoroalkyl acids (PFAAs) described in this study.

Two types of functional groups with variable  $CF_2$  chain length were included: perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs).

1 abic 52.	Widdel syste	III setup useu !	in this study.			
No.	Model	Microbial	Microbiological media	PFAA	Sampling	
INO.	system	consortia	Microbiological media	tested	schedule (day)	
1.	$\mathrm{BT}^{1}$	$A^2$ - $CB^3$	Pushnal Hass with glucosa	PFOS	_	
2.	BT	$B^4$ -CB	Bushnel Haas with glucose	PFOA	0 7 14 21 28	
3.	BT	$A-YM^5$	Malt extract broth	PFOS	0, 7, 14, 21, 28	
4.	BT	B-YM	Mait extract broth	PFOA		
5.	$BC^{6}$	A-CB	Duchnel Hoos with alugose	_7		
6.	BC	B-CB	Bushnel Haas with glucose	-	0 7 14 21 20	
7.	BC	A-YM	Malt averaget breath	-	0, 7, 14, 21, 28	
8.	BC	B-YM	Malt extract broth	-	-	
9.	$AC^8$	_	Duchnel Hoos with alugose	PFOS		
10.	AC	-	Bushnel Haas with glucose	PFOA	0 7 14 21 20	
11.	AC	-		PFOS	0, 7, 14, 21, 28	
12.	AC	-	Malt extract broth	PFOA		
1	2		2		4	

Table S2. Model system setup used in this study.

<sup>1</sup>Biotic test, <sup>2</sup>sediment sample from Site A, <sup>3</sup><u>c</u>hemoorganoheterotrophic <u>b</u>acteria, <sup>4</sup>sediment sample from Site B, <sup>5</sup>yeast and <u>m</u>olds, <sup>6</sup>biotic control, <sup>7</sup>0.05% dimethyl sulfoxide, <sup>8</sup>abiotic control

Table 55. Instrumental parame	ters for LC/MIS quantitative (targeted) analysis.
	Liquid chromatography-tandem mass spectrometry
Instrument	(LC/MS/MS) using Xevo TQ (Waters) coupled with
	ACQUITY UPLC (Waters)
Analytical column	ACQUITY UPLC BEH (C18, $2.1 \times 50$ mm, $1.7$ µm, Waters)
Detention con column	ACQUITY UPLC BEH (C18, 2.1 × 100 mm, 1.7 μm,
Retention gap column	Waters)
Column temperature	40 °C
Mobile phase	2 mM ammonium acetate and acetonitrile
	At a flow rate of 0.3 mL min <sup>-1</sup> , the mobile phase gradient was
Gradient profile	ramped from 1% to 95% acetonitrile in 8 min, kept at 95%
	for 1 min, and then ramped down again to 1%.
Injection volume	5μL
Ionization	Electrospray ionization (ESI) negative-ion mode SI negative-
Ionization	ion mode
Capillary voltage	0.5 kV
Desolvation gas flow	1000 L h <sup>-1</sup>
Desolvation gas temperature	500 °C

Table S3. Instrumental parameters for LC/MS quantitative (targeted) analysis.

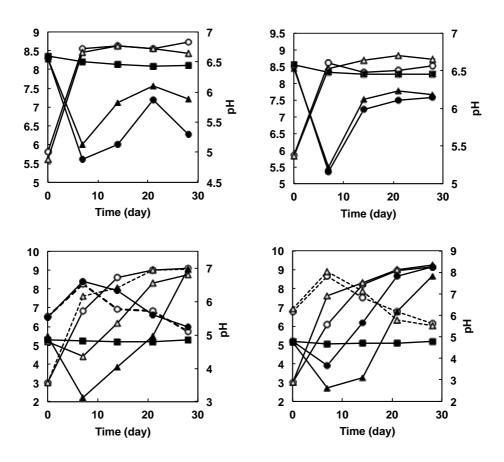
<b>Table 54.</b> Instrumental pa	arameters for LC/MS untargeted analysis.
Instrument	LC/MS using Ultimate 3000 and Exactive (Thermo Fisher)
Analytical column	TSK-GEL ODS-100S(C18, 2.0×150 mm, 5µm, Tosoh Corp.)
Retention gap column	TSK-GEL ODS-100S (C18, 2.0×150 mm, 5µm, Tosoh Corp.)
Column temperature	40 °C
Mobile phase	A: 2 mM ammonium bicarbonate water, B: 2 mM ammonium bicarbonate methanol
Gradient profile	10% B (0 min), 10% B (5 min), 80% B(10 min), 100% B (15 min), 100% B (23 min), 10% B (23.1 min) 10% B (28 min)
Injection volume	5μL
Ionization	ESI negative-ion mode
Capillary voltage	4.8 kV
Shealth gas flow	15
Capillary temperature	250 °C
Monitored <i>m/z</i> range	200–3000

Table S4. Instrumental parameters for LC/MS untargeted analysis.

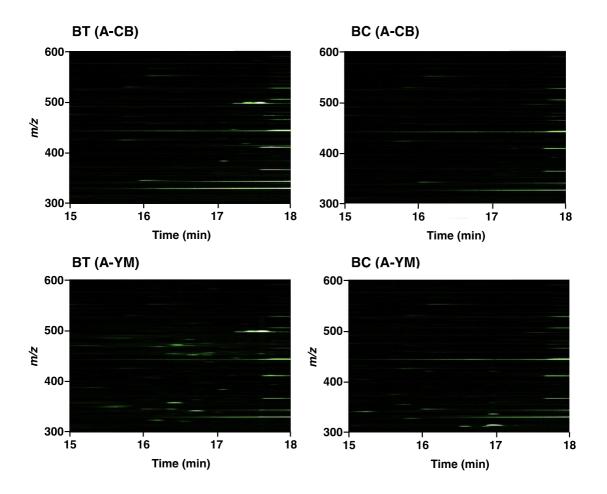
<b>Table S5.</b> Method detection limit (MDL), method quantification limit (MQL) and       Image: Comparison of the second secon	
recovery rates for sediments from Site A and Site B, and model system samples.	

Site A (r	ng [g-dw]	<sup>-1</sup> )													
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	PFOS	PFDS
MDL	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
MQL	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.06	0.06	0.06
Site B (	ng [g-dw	] <sup>-1</sup> )													
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	PFOS	PFDS
MDL	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
MQL	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.6	0.6	0.6
Model s	ystems B'	T and AC	(µg mL <sup>-1</sup> )	)											
		PFOA							:	PFOS					
MDL		0.008								0.016					
MQL		0.024								0.048					
Recover	y rate of l	MPFAC-M	IXA (%)												
		MPI	BA	MPHxA	М	PFOA	MPF	NA	MPFDA	MPFUnDA	MPFDoDA	MPFHx	S MF	FOS	
Site A		11:	3.4	108.5	1	13.9	103	.2	92.5	97.8	92.3	126.7	11	18.6	
Site B		10	4.7	106.9	1	107.3	79.	9	92.4	97.3	89.0	104.2	13	32.8	

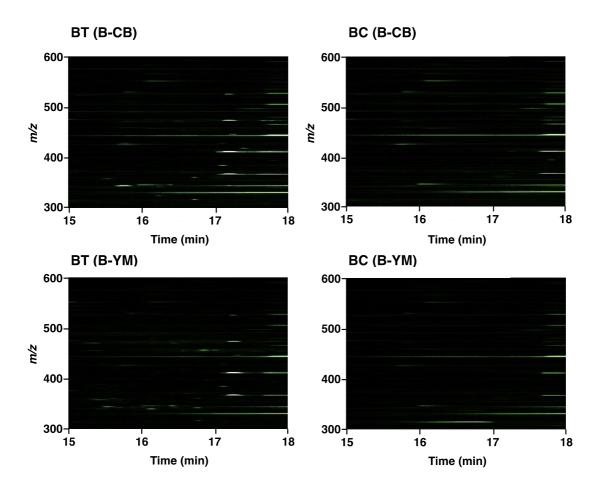
m/z	A-CB	B-CB	ntion time) A-YM	B-YM	
218.103	15.6	<u>-</u>	15.5	D-1 M	
244.154	-	-	16.1	16.1	
263.077	15.7		-	-	
264.061	15.5	15.5	-	-	
295.132	15.9	15.8	-	-	
323.163	16.6	16.6	16.5	-	
525.105	10.0	10.0	17.0	-	
325.179	-	16.2	16.2	-	
		16.5	16.5		
339.158	-	-	16.6	16.1 16.5	
341.173	15.5	15.5	15.4	15.4	
541.175	16.2	16.1	16.1	16.1	
	10.2	10.1	16.6	16.7	
			17.0	10.7	
343.189	-	15.7	15.7	15.7	
		16.0	16.2	16.0	
		16.2	16.7	16.5	
		10.2		16.7	
345.205	_	15.8	15.6	15.5	
0.01200		1010	16.4	15.8	
				16.5	
347.220	16.4	16.4	16.4	16.4	
				16.6	
357.226	-	-	-	15.6	
359.184	-	15.5	15.3	15.4	
0091101			15.6	15.7	
			16.4		
361.200	-	15.0	14.9	15.4	
			15.5	15.6	
363.215	-	14.7	14.4	14.5	
		15.5	15.3	15.4	
			15.9	16.1	
419.278	17.4	17.4	-	-	
453.262	-	16.6	16.7	16.4	
		16.8	16.9	16.8	
455.277	-	-	16.4	16.4	
			16.8	16.8	
			17.6	17.5	
				17.9	
457.293	-	-	16.9	16.8	
1 400 0 0 -		4	17.2	17.0	
469.256	-	16.2	16.2	16.1	
471.272	-	16.6	16.5	16.4	
473.288	-	16.5	16.5	16.4	
475.303	16.7	16.9	16.8	16.6	
477.319	-	-	16.9	16.9	
483.272	-	16.6	16.6	16.6	
501,057	15.3	-	-	-	
519.068	14.7	-	-	-	
412.964		17.2		17.2	
(PFOA)	-	11.2	-	17.2	
498.927	17.5		17.5		
(PFOS)					



**Fig. S1.** Change of number of microorganisms and pH during biotransformation experiment with A-CB (a), B-CB (b), A-YM (c), and B-YM (d). Open and closed symbols on solid lines denote the number of bacteria and pH. respectively. Open symbols on dotted lines denote the number of veast and



**Fig. S2.** LC/MS spectra of biotic test (BT) and biotic control (BC) model systems incubated with PFOS after 28 days of incubation.



**Fig. S3.** LC/MS spectra of biotic test (BT) and biotic control (BC) model systems incubated with PFOA after 28 days of incubation.