Supplementary material for the article:

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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°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^{-1} 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 5×10^8 colony forming unit (CFU) mL^{-1}, 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^{-1} 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.

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

Formula/Name/Acronym	No. of CF_2 groups	Acronym	Analyte
$CF_3(CF_2)_mCO_2^-$ Perfluoroalkyl carboxylates (PFCAs)	m = 2	PFBA	Perfluorobutanoate
	m = 3	PFPeA	Perfluoropentanoate
	m = 4	PFHxA	Perfluorohexanoate
	m = 5	PFHpA	Perfluoroheptanoate
	m = 6	PFOA	Perfluorooctanoate
	m = 7	PFNA	Perfluorononanoate
	m = 8	PFDA	Perfluorodecanoate
	m = 9	PFUnDA	Perfluoroundecanoate
	m = 10	PFDoDA	Perfluorododecanoate
	m = 11	PFTrDA	Perfluorotridecanoate
	m = 12	PFTeDA	Perfluorotetradecanoate
$CF_3(CF_2)_nSO_3^-$ Perfluoroalkyl sulfonates (PFSAs)	n = 3	PFBS	Perfluorobutanesulfonate
	n = 5	PFHxS	Perfluorohexanesulfonate
	n = 7	PFOS	Perfluorooctanesulfonate
	n = 9	PFDS	Perfluorodecanesulfonate

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

Table S2. Model system setup used in this study.

No.	Model system	Microbial consortia	Microbiological media	PFAA tested	Sampling schedule (day)
1.	BT[1]	A[2]-CB[3]	Bushnel Haas with glucose	PFOS	0, 7, 14, 21, 28
2.	BT	B[4]-CB		PFOA	
3.	BT	A-YM[5]	Malt extract broth	PFOS	
4.	BT	B-YM		PFOA	
5.	BC[6]	A-CB	Bushnel Haas with glucose	-[7]	0, 7, 14, 21, 28
6.	BC	B-CB		-	
7.	BC	A-YM	Malt extract broth	-	
8.	BC	B-YM		-	
9.	AC[8]	-	Bushnel Haas with glucose	PFOS	0, 7, 14, 21, 28
10.	AC	-		PFOA	
11.	AC	-	Malt extract broth	PFOS	
12.	AC	-		PFOA	

[1]Biotic test, [2]sediment sample from Site A, [3]chemoorganoheterotrophic bacteria, [4]sediment sample from Site B, [5]yeast and molds, [6]biotic control, [7]0.05% dimethyl sulfoxide, [8]abiotic control

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

Instrument	Liquid chromatography-tandem mass spectrometry (LC/MS/MS) using Xevo TQ (Waters) coupled with ACQUITY UPLC (Waters)
Analytical column	ACQUITY UPLC BEH (C18, 2.1 × 50 mm, 1.7 µm, Waters)
Retention gap column	ACQUITY UPLC BEH (C18, 2.1 × 100 mm, 1.7 µm, Waters)
Column temperature	40 °C
Mobile phase	2 mM ammonium acetate and acetonitrile
Gradient profile	At a flow rate of 0.3 mL min^{-1}, the mobile phase gradient was 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-ion mode
Capillary voltage	0.5 kV
Desolvation gas flow	1000 L h^{-1}
Desolvation gas temperature	500 °C

Table S4. Instrumental parameters 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 *m/z* range	200–3000

Table S5. Method detection limit (MDL), method quantification limit (MQL) and recovery rates for sediments from Site A and Site B, and model system samples.

Site A (ng [g-dw]$^{-1}$)

	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]$^{-1}$)

	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 systems BT and AC (µg mL^{-1})

	PFOA	PFOS
MDL	0.008	0.016
MQL	0.024	0.048

Recovery rate of MPFAC-MXA (%)

	MPFBA	MPHxA	MPFOA	MPFNA	MPFDA	MPFUnDA	MPFDoDA	MPFHxS	MPFOS
Site A	113.4	108.5	113.9	103.2	92.5	97.8	92.3	126.7	118.6
Site B	104.7	106.9	107.3	79.9	92.4	97.3	89.0	104.2	132.8

Table S6. New peaks detected only in BT model systems after 28 days of incubation.

m/z	BT (Retention time)			
	A-CB	B-CB	A-YM	B-YM
218.103	15.6	-	15.5	-
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 17.0	-
325.179	-	16.2 16.5	16.2 16.5	-
339.158	-	-	16.6	16.1 16.5
341.173	15.5 16.2	15.5 16.1	15.4 16.1 16.6 17.0	15.4 16.1 16.7
343.189	-	15.7 16.0 16.2	15.7 16.2 16.7	15.7 16.0 16.5 16.7
345.205	-	15.8	15.6 16.4	15.5 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 15.6 16.4	15.4 15.7
361.200	-	15.0	14.9 15.5	15.4 15.6
363.215	-	14.7 15.5	14.4 15.3 15.9	14.5 15.4 16.1
419.278	17.4	17.4	-	-
453.262	-	16.6 16.8	16.7 16.9	16.4 16.8
455.277	-	-	16.4 16.8 17.6	16.4 16.8 17.5 17.9
457.293	-	-	16.9 17.2	16.8 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 (PFOA)	-	17.2	-	17.2
498.927 (PFOS)	17.5	-	17.5	-

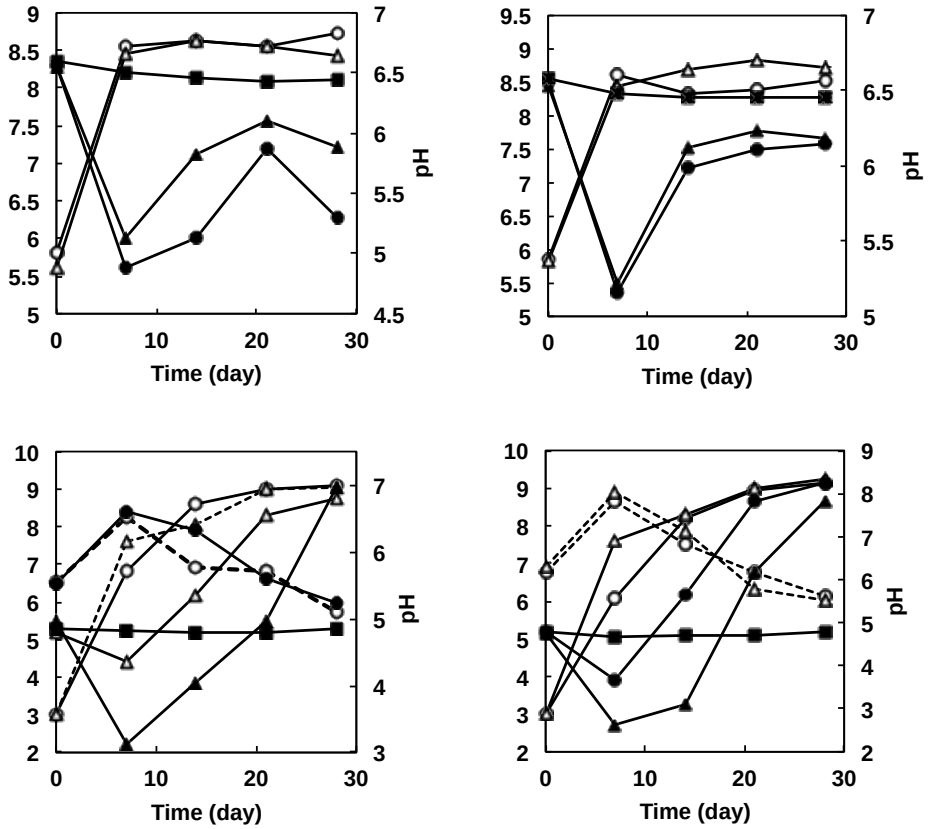

Fig. S1. Change of number of microorganisms and pH during biotransformation experiment with A-CB (a), B-CB (b), A-YM (c), and B-YM (d). Open and closed symbols on solid lines denote the number of bacteria and pH, respectively. Open symbols on dotted lines denote the number of yeast and

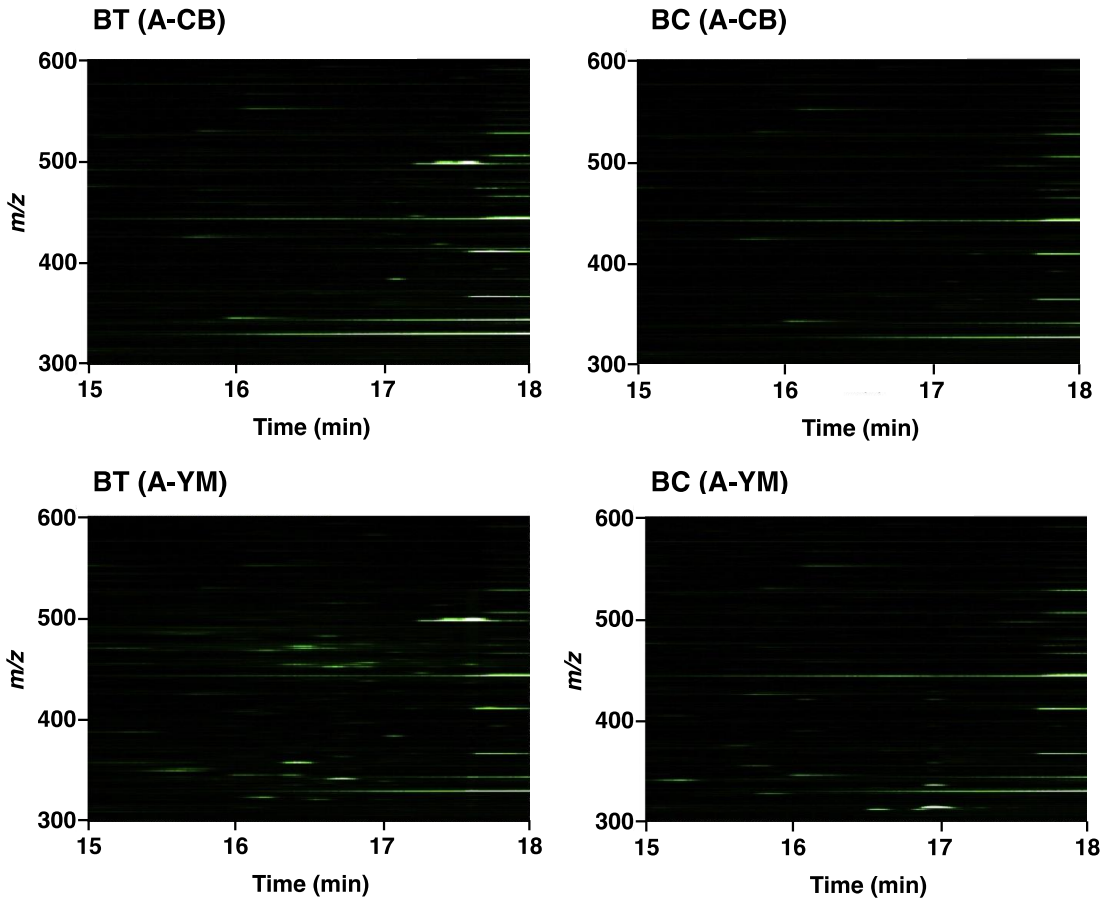

Fig. S2. LC/MS spectra of biotic test (BT) and biotic control (BC) model systems incubated with PFOS after 28 days of incubation.

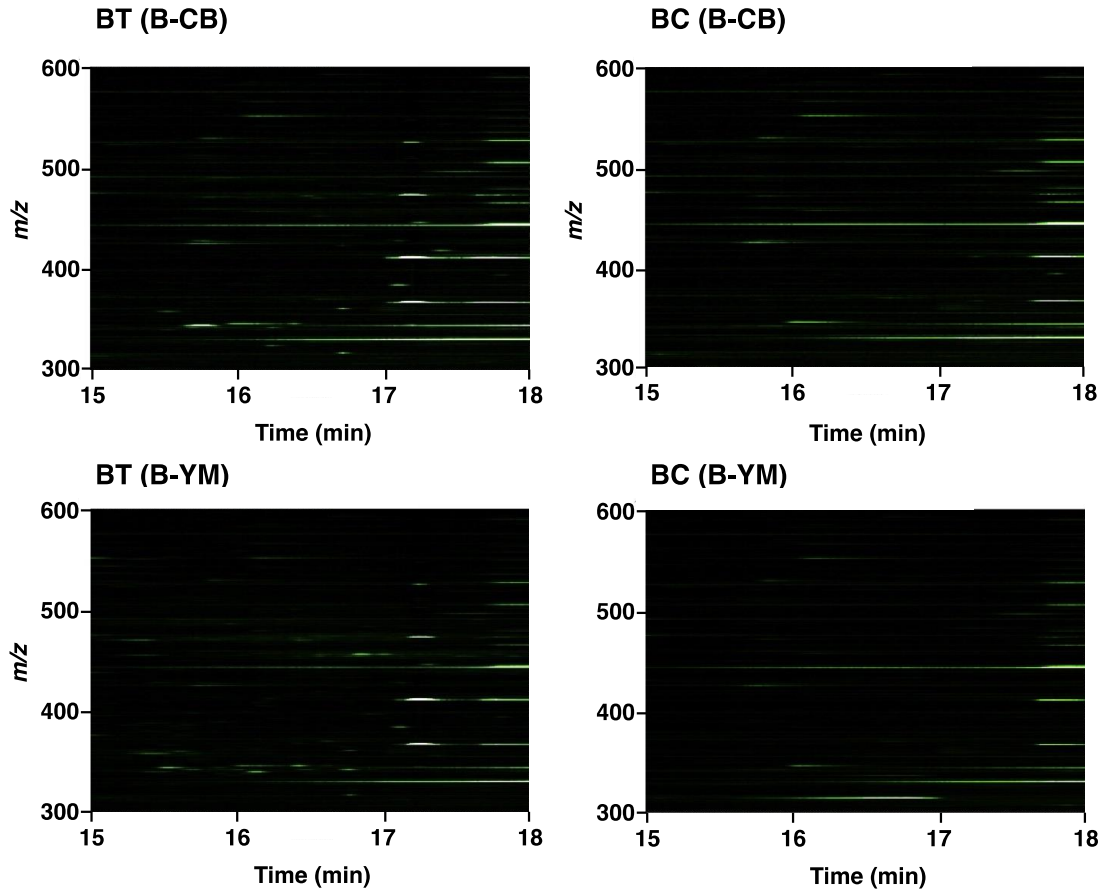

Fig. S3. LC/MS spectra of biotic test (BT) and biotic control (BC) model systems incubated with PFOA after 28 days of incubation.