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Supplementary Material

BIOSURFACTANT-INDUCED PER- AND POLYFLUOROALKYL LEACHING FROM AQUEOUS FILM-FORMING FOAM IMPACTED SOIL

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1. MATERIAL AND METHODS

1.1. Contaminated Site and Sampling Event

In April 2023, a sampling campaign was conducted at the per- and polyfluoroalkyl substances (PFAS) Living Lab site at Utrecht University, a former firefighting training location. Soil samples were retrieved at three locations (A, B and C), with samples of 20 cm each up to a depth of 1 m. Three water samples (DW-B, DW-C and DW-D) were taken from a ditch at the location. The Site, including sample locations are displayed in **Figure S1**.

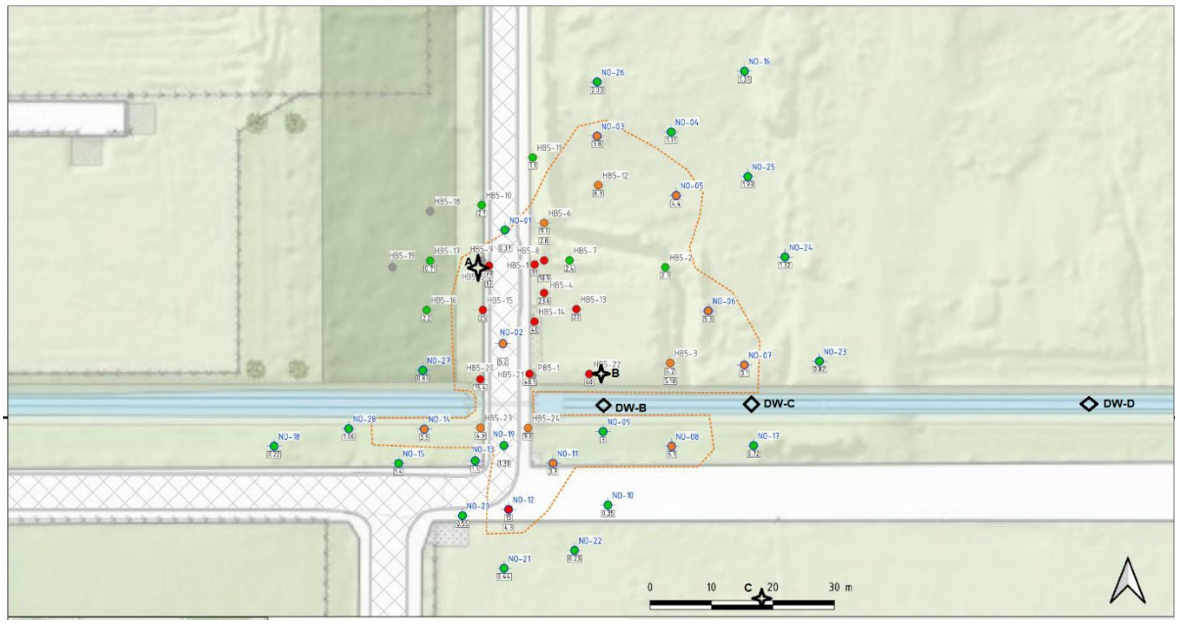

Figure S1: Soil sampling locations (A, B and C) and water sampling locations (DW-B, DW-C and DW-D) for the April 2023 sampling event overlaid on map from the BK Ingenieurs report (4).

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1.2. Laboratory Soil PFAS analysis methodology

Per- and polyfluoroalkyl (PFAS) analysis of soil samples from the April 2023 sampling event was completed in laboratories at the Vrije University, Amsterdam. Stock solutions of PFAS compounds were sourced from Wellington Laboratories (Ontario, Canada), Cambridge Isotope Laboratories (Andover, MA) and Chiron (Trondheim, Norway). Solvents were supplied by Biosolve (Valkenswaard, The Netherlands). Results of the analysis are summarized in **Table S1**.

Table S1: Per- and polyfluoroalkyl (PFAS) concentrations in soil samples of April 2023 sampling event, analysis completed at VU Amsterdam (*see next page*).

The soil samples were dried by lyophilization for 24 hours. A methanol-acetonitrile (1:1) solution with 0.01% NH_4OH was added to the samples and shaken for 15 minutes. Samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was transferred to a new vial, and the methanol-acetonitrile solution addition, shaking and centrifuging steps were repeated. Supernatant was added to the first extract, then it was evaporated down to 0.1 mL before adding 1 mL of methanol. For the clean-up, ENVI Carb (Supelco) was added to the extract and vortexed for 1 minute. Next, the extracts were centrifuged for 5 minutes at 3000 rpm. The supernatant was transferred to a new vial, and the process was repeated if the extract was still not clear. The extract was then entirely evaporated and reconstituted in 50 μL injection standard of 5 ng/mL each PFBA$^{13}C_3$, PFOA$^{13}C_2$, PFDA$^{13}C_2$, and PFOS$^{13}C_4$; and 50 μL millipore water.

The suite of PFAS were analyzed by liquid chromatography-mass spectrometry (LC-MS). An ExionLC (Sciex) system was used with an XBridge BEH XP Column (3.5 μL, 2.1 mm x 100 mm; Waters) and a 2.1 mm x 100 mm isolator colum (Waters). Volumes of 5 μL were injected at a rate of 0.6 ml/min. A gradient solvent program was run with 2 mM NH_4CHOO and methanol. The methanol eluate was increased to 99% over 10 minutes, held for 8 minutes, then decreased to 25% in 0.2 minutes and held for 2.8 minutes. A 6500+ (Sciex) system was used for MS with an ion spray voltage of -4000 V at 400°C. Quality control of results was confirmed with laboratory blanks and reproducibility analysis.

1.3. Aqueous PFAS analysis methodology

Laboratory analysis of aqueous PFAS eluate samples (from column tests) was performed in facilities at the Utrecht University Veterinary School. The following stock solutions of PFAS were used: PFBA (52411-5ML-F; >99.5%), PFHxA (29226-5ML; >97%), PFOA (Aldrich; 171468-5G), PFBS (Merck; 294209-10G; 98%), PFHxS (TRC; technical grade; 50 mg) and PFOS (*Chemika*; 77282; 10 g; >98%)[a]. The methodology was based on the EPA *Method 1633* for PFAS analysis by LC-MS (3). Prior to analysis, the pH of each sample was confirmed to be between 6 and 7. *Oasis WAX* extraction cartridges with a weak ion exchange sorbent were used (150 mg, 30 μM, art. nr. 186002493, Waters). Each cartridge was conditioned with 10 mL 0.1% ammonium hydroxide (NH_4OH) in methanol (MeOH) followed with 5 mL of MeOH and 5 mL of millipore water. The entirety of each sample (approximately 400 mL) was slowly poured into the cartridge and allowed to gravity-filter. Note that for rhamnolipid flushing test samples at 1 pore volume (PV) (R1 T01) and 19 PVs (R1 T19), only 345 and 473.5 mL respectively were used because the SPE cartridge became clogged. The volume discrepancy was corrected by recalculating the PFAS concentrations accordingly. The cartridges were washed with 5 mL of millipore water followed by 5 mL of 1:1 MeOH:millipore water with 0.1% formic acid. The columns were then dried for 10 minutes under a vacuum. The PFAS was eluted off the column sorbent with 5mL NH_4OG in MeOH. The eluate was evaporated to 0.5 mL with nitrogen gas, then refilled to 1.0 mL with MeOH 0.1% formic acid. Each sample was centrifuged for 10 minutes at 1500 rpm and 150 μL was transferred to a new vial for analysis.

[a] *PFBA (Perfluorobutanoic acid); PFBS (Perfluorobutanesulfonic acid); PFHxA (Perfluorohexanoic acid); PFHxS (Perfluorohexanesulfonic acid); PFOA (Perfluorooctanoic acid); PFOS (Perfluorooctanesulfonic acid)*

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	Sample code	A-2 0-20 cm	A-2 20-40 cm	A-2 40-60 cm	A-2 60-80 cm	A-2 80-100 cm	B-2 0-20 cm	B-2 20-40 cm	B-2 40-60 cm	B-2 60-80 cm	B-2 80-100 cm	C-2 0-20 cm	C-2 20-40 cm	C-2 40-60 cm	C-2 60-80 cm	C-2 80-100 cm	DW-C-4	DW-D-4	DW-B-4
	EH-code	23/0419	23/0420	23/0421	23/0422	23/0423	23/0424	23/0425	23/0426	23/0427	23/0428	23/0429	23/0430	23/0431	23/0432	23/0433	23/0434	23/0435	23/0436
Analysis	**W-PFAS100 and 104**	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	µg/kg DS	ng/liter	ng/liter	ng/liter
Perfluorobutyric acid	PFBA	0.4	0.3	0.2	0.1	0.09	0.3	0.4	0.5	0.2	0.2	0.4	0.08	<0.05	<0.05	<0.05	9.2	2.9	23
Perfluoropentanoic acid	PFPeA	0.9	1	0.3	0.1	0.2	1.2	2.2	2.4	1	1	0.1	<0.05	<0.05	<0.05	<0.05	36	1.1	100
Perfluorohexanoic acid	PFHxA	0.7	1	0.3	0.1	0.2	0.6	1.3	1.8	0.7	1.1	0.1	0.04	*0.02	<0.01	<0.01	54	1.2	120
Perfluoroheptanoic acid	PFHpA	0.3	0.3	0.2	0.05	0.09	0.6	0.6	0.7	0.3	0.4	0.2	*0.03	*0.02	<0.01	<0.01	11	0.6	28
Brominated Perfluorooctanoic acid	br-PFOA	*0.02	0.05	0.1	0.06	*0.02	*0.02	*0.02	*0.02	<0.01	0.06	0.06	0.05	*0.03	<0.01	<0.01	1.6	0.7	4.4
Linear Perfluorooctanoic acid	l-PFOA	1	0.8	2	0.8	0.3	0.8	0.8	1	0.6	1.1	1.9	0.9	0.4	0.2	0.05	17	8.5	59
Perfluorononanoic acid	PFNA	0.1	0.06	0.05	*0.01	<0.01	0.4	0.3	0.2	0.1	0.2	0.1	*0.02	<0.01	<0.01	<0.01	0.7	*0.06	3.6
Perfluorodecanoic acid	PFDA	0.07	0.04	0.3	<0.01	<0.01	0.2	0.1	0.07	0.08	0.05	0.07	<0.01	<0.01	<0.01	<0.01	*0.2	<0.05	1.8
Perfluoroundecanoic acid	PFUnDA	*0.02	*0.02	<0.01	<0.01	<0.01	0.07	0.03	*0.02	*0.02	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*0.07
Perfluorododecanoic acid	PFDoDA	*0.02	<0.01	<0.01	<0.01	<0.01	0.06	*0.02	<0.01	*0.02	<0.01	*0.02	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
Perfluorotridecanoic acid	PFTrDA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
Perfluorotetradecanoic acid	PFTeDA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
Hexafluoropropylene oxide dimer acid	HFPO-DA	*0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.2	<0.05	0.5
1H,1H,2H,2H-perfluorodecane-1-sulfonic acid	ADONA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
8:2 Fluorotelomer unsaturated carboxylic acid	8:2FTUCA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
Perfluorobutanesulfonic acid	PFBS	0.08	0.09	0.1	0.1	0.04	0.06	0.06	0.2	0.06	0.2	0.07	0.04	*0.02	<0.01	<0.01	12	3.3	22
Perfluoropentanesulfonic acid	PFPeS	0.03	0.04	0.06	0.04	0.03	0.1	0.2	0.4	0.2	0.4	<0.01	<0.01	<0.01	<0.01	<0.01	12	0.3	31
Brominated Perfluorohexanesulfonic acid	Br-PFHxS	*0.02	0.04	0.06	0.04	0.04	0.3	0.4	0.9	0.4	1.3	<0.01	<0.01	<0.01	<0.01	<0.01	25	0.4	64
Linear Perfluorohexanesulfonic acid	l-PFHxS	0.3	0.4	0.5	0.2	0.4	2.3	3.1	5.7	2.4	6.8	0.09	*0.03	<0.01	<0.01	<0.01	120	1	280
Perfluoroheptanesulfonic acid	PFHpS	*0.02	*0.02	*0.02	<0.01	<0.01	0.2	0.5	0.8	0.3	1.3	<0.01	<0.01	<0.01	<0.01	<0.01	2.9	<0.05	31
Brominated Perfluorooctanesulfonic acid	br-PFOS	0.6	0.6	0.5	0.2	0.1	6	20	34	26	38	0.3	0.1	0.04	*0.02	*0.02	47	0.9	670
Linear Perfluorooctanesulfonic acid	l-PFOS	1.7	1.3	0.7	0.2	0.2	17	48	61	43	36	0.6	0.1	0.05	*0.03	*0.02	73	0.2	800
Perfluorononanesulfonic acid	PFNS	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	0.06	0.04	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	0.2
Perfluorodecanesulfonic acid	PFDS	<0.01	<0.01	<0.01	<0.01	<0.01	*0.02	*0.02	*0.02	*0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
4:2 Fluorotelomer sulfonic acid	4:2FTS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
6:2 Fluorotelomer sulfonic acid	6:2FTS	<0.02	<0.02	<0.02	<0.02	<0.02	*0.04	*0.06	*0.06	0.09	0.4	<0.02	<0.02	<0.02	<0.02	<0.02	*1.1	<0.5	67
8:2 Fluorotelomer sulfonic acid	8:2FTS	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	*0.01	<0.01	*0.02	*0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	1
10:2 Fluorotelomer sulfonic acid	10:2FTS	*0.03	<0.02	<0.02	<0.02	<0.02	0.3	*0.06	*0.02	*0.05	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.1	<0.1	<0.1
N-Methyl perfluorooctane sulfonamido acetic acid	N-MeFOSAA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
N-Ethyl perfluorooctane sulfonamido acetic acid	N-EtFOSAA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
Perfluorooctane sulfonamide	FOSA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	*0.1	<0.1	1.9
9-Chloro Perfluoro-3-oxane sulfonate	9Cl-PF3ONS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
11-Chloro Perfluoro-3-octane sulfonate	11Cl-PF3ODS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.2	<0.2	<0.2
Total Perfluorooctanoic acid	PFOA-tot	1	0.9	2.1	0.8	0.3	0.8	0.8	1	0.6	1.2	2	0.9	0.4	0.2	0.05	18	9.2	63
Total Perfluorohexanesulfonic acid	PFHxS-tot	0.3	0.5	0.6	0.3	0.4	2.6	3.4	6.6	2.7	8.1	0.09	*0.03	<0.01	<0.01	<0.01	140	1.4	350
Total Perfluorooctanesulfonic acid	PFOS-tot	2.3	1.9	1.1	0.3	0.3	23	69	95	69	74	0.8	0.2	0.1	*0.05	*0.04	120	1.1	1500

* the value is between the limit of detection (LOD) and the limit of quantification (LOQ)

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Samples were analyzed for PFBA, PFBS, PFHxA, PFHxS, PFOA and PFOS using coupled high-performance liquid chromatography-mass spectrometry (HPLC/MS-MS) *Shimadzu Nexera XR* and *Shimadzu LCMS-8050*. GenX was also analyzed in all samples, but was not present at the site so has not been included in this report. A gradient solvent program was used with 5 mM NH_4ACE in millipore water and 5 mM NH_4ACE in LC-MS grade methanol. The solvents were injected at a rate of 0.2 mL/min. The methanol eluate was increased to 60% over 3 minutes, then 80% in the next 4 minutes, and decreased to 5% in 0.1 minute and held for 3 minutes. *GreatSmart RP18* 150 x 2.1 mm, 5μm columns with were used for chromatographic separation.

Quality assurance and quality control (QA/QC) checks during PFAS analysis for data accuracy and precision were completed. Data accuracy was measured by percent recovery using laboratory control samples, and spiked surrogate samples. Acceptable average surrogate recoveries ranged from 95.3% to 108.7%. For each recovery analysis, one sample (the control) was analyzed directly by LC-MS, while a second sample spiked with the same amount of PFAS was processed using the same methodology as all other samples. This was repeated twice for each type of PFAS to confirm precision of the method. Additionally, the calibration standards of known PFAS concentration were injected in triplicate at equal time intervals throughout the LC-MS run to confirm there was no drift in results. Per each batch of six samples, one blank and three recovery samples were processed. No laboratory blanks returned results above the limit of quantification (LOQ). For the method blank, all PFAS concentrations were below method detection limits, except for PFBS, which was approximately equal to the LOQ (0.002 ng/L) and may therefore represent background contamination. Therefore, the method blank was deemed acceptable.

Quality assurance and quality control were successful in almost all cases, with a few exceptions. In the water-flushing PFAS test (W1), PFHxS for sample W1 T01 (2702.7 ng/L) was above the range of the calibration curve of which the highest concentration was 2177.8 ng/L. Blank PFOA samples had an average concentration of 4 ng/L, indicating a background concentration. The corresponding calibration curve was corrected to account for this background as well as PFOA results for this run. It is possible that the constant background is due to the Teflon tubing in the LC-MS machine, which needs to be confirmed at a later date. Additionally, a portion of the peak for the PFBA fell outside of the MS time window, such that the area method could not be used for concentration calculation. Instead, the height of the peak was used. In a follow-up sample run, the MS time window was adjusted and the area of the peak for PFBA was used, which resulted in comparable results to the height method. Therefore, the first analysis of PFBA with the height method was retained. The PFOS recovery for the first batch of samples (13 through 23) was low (68.4%, STD of 17.9%). The recovery for the second half of the analysis (samples 1 through 11) was acceptable (95.9%, STD 2.0%). Rather than using the average recovery for all samples, the recovery values for each group of samples were used separately for calculated concentration corrections. Following the poor recovery results from the first clean-up, the second batch of samples was poured directly into the SPE cartridges rather than vacuumed through the LDPE tubing as done for the first batch of samples. This seems to have resolved the issue with PFOS recovery; however, this methodology needs to be refined in future studies.

In the rhamnolipid-flushing test, the calibration lines were increased by 5% to adjust for concentrations that may be higher than in the water flushing test, and based on the PFHxS result above the calibration curve in that test. There was an average background concentration for PFHxA and PFOA of 1 ng/L and 3 ng/L, respectively. However, when this is corrected, the resulting calibration curve does not meet conventional requirements of 75% of the points falling within the accuracy range of 85% and 115%. Therefore, the raw results have been used in this report for the rhamnolipid test. For PFBS in the rhamnolipid test, there were many results falling just above the LOQ. While these results were retained for analysis, they should be used with caution as they may be a result of background noise from a low LOQ. The LOQ values for PFOA were higher for the rhamnolipid test than the water flushing test (15 ng/L versus 0.3 ng/L). This may result in an underestimation of the total mass of PFOA flushed, and the mass

balance result. There were no other QA/QC issues in the laboratory analysis for the rhamnolipid test samples.

1.4. Eluate Concentrations

Concentrations of all PFAS compounds and phosphate in the eluate of both flushing tests are provided in **Tables S2**, **S3**, and **S4**.

Table S2: Per- and polyfluoroalkyl (PFAS) concentrations in eluate from the water flushing test in ng/L. Sample number is approximately equal to pore volume (PV). Results below the corresponding limit of quantification (LOQ) values in gray. The LOQ for PFOA is 3.1 ng/L and PFOS is 0.4 ng/L.

Sample number	PFBA	PFHxA	PFOA	PFBS	PFHxS	PFOS
1	42.6	695.4	343.1	34.8	2702.7	1534.4
3	< 9.4	2.4	17.3	< 0.3	74.0	1733.7
5	< 9.4	1.4	3.2	< 0.3	22.1	1603.2
7	< 9.4	< 0.4	1.8	< 0.3	13.8	1183.8
9	< 9.4	< 0.4	1.4	< 0.3	8.9	1060.0
11	< 9.4	< 0.4	1.4	< 0.3	7.1	919.4
13	< 9.4	< 0.4	1.1	< 0.3	4.9	1090.3
15	< 9.4	< 0.4	1.3	< 0.3	5.0	894.5
17	< 9.4	< 0.4	0.8	< 0.3	4.6	841.5
19	< 9.4	< 0.4	0.8	< 0.3	3.8	699.4
21	< 9.4	< 0.4	0.9	< 0.3	3.0	575.4
23	< 9.4	< 0.4	0.8	< 0.3	2.8	507.0

PFBA (Perfluorobutanoic acid); PFBS (Perfluorobutanesulfonic acid); PFHxA (Perfluorohexanoic acid); PFHxS (Perfluorohexanesulfonic acid); PFOA (Perfluorooctanoic acid); PFOS (Perfluorooctanesulfonic acid)

Table S3: Per- and polyfluoroalkyl (PFAS) concentrations in eluate from the rhamnolipid flushing test in ng/L. Sample number is approximately equal to pore volume (PV). Results below the corresponding limit of quantification (LOQ) values in gray. Due to a different volume used in samples 1 and 19, the LOQ values are slightly different, as noted. The LOQ for PFHxS is 1.6 ng/L and PFOS is 1.9 ng/L (1.7 and 2.0 ng/L for sample 19, respectively). The LOQ for PFBS in sample 19 is 1.6 ng/L.

Sample number	PFBA	PFHxA	PFOA	PFBS	PFHxS	PFOS
1	26.3	432.9	400.4	130.7	5067.3	959.9
3	< 4.6	< 5.8	45.4	2.0	305.3	1838.5
5	< 4.6	< 5.8	< 14.8	2.5	94.0	3764.1
7	< 4.6	< 5.8	< 14.8	1.6	62.5	2915.3
9	< 4.6	< 5.8	< 14.8	2.0	43.0	1269.3
11	< 4.6	< 5.8	< 14.8	1.6	37.7	650.3
13	< 4.6	< 5.8	< 14.8	< 1.5	27.1	374.4
15	< 4.6	< 5.8	< 14.8	1.7	23.9	239.2
17	< 4.6	< 5.8	< 15.8	2.1	19.1	182.4
19	< 4.9	< 6.2	< 14.8	2.0	17.8	138.6
21	< 4.6	< 5.8	< 14.8	1.8	15.4	111.1
23	< 4.6	< 5.8	< 14.8	< 1.5	13.4	89.8

PFBA (Perfluorobutanoic acid); PFBS (Perfluorobutanesulfonic acid); PFHxA (Perfluorohexanoic acid); PFHxS (Perfluorohexanesulfonic acid); PFOA (Perfluorooctanoic acid); PFOS (Perfluorooctanesulfonic acid)

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Table S4: Phosphate concentrations in eluate from the water flushing test and the rhamnolipid flushing test in μg/L. Sample number is approximately equal to pore volume (PV). The percent differences in sample results measured were all equal to or lower than 0.6%.

Sample number	Water PO_4	Rhamnolipid PO_4
2	249	100
4	258	133
6	262	182
8	160	148
10	241	135
12	233	130
14	211	115
16	165	112
18	237	117
20	177	117
22	180	109
24	173	108

1.5. Batch Testing

To confirm results of column flushing tests, an additional batch test was performed to observe efficacy of washing PFAS from the soil using water versus rhamnolipid. Soil from sample B3 0.40-0.60 m was dried in the same method as the other samples (Section 2.2.1: Column Preparation in paper). Approximately 13.2 g were placed in each of two 500 mL PP bottles. 400 mL of tap water was added to one bottle, and 400 mL of 0.005% rhamnolipid solution was added to the other. The ratio of liquid to soil in each was the same as the amount of solution passed through the columns over 24 PVs (30 mL per g). The bottles were placed on an orbital shaker at 150 rotations per minute for 48 hours, along with two blank samples containing 400 mL each of water and 0.005% rhamnolipid without soil. All samples were frozen at -20°C until analysis. Aqueous samples were delivered to the Vrije University, Amsterdam for analysis on March 12, 2024.

The batch testing resulted in similar percent masses of PFAS removed from the soil by water and by the rhamnolipid solution (see **Fig. S2**). This emphasizes the importance of groundwater flow in the desorption mechanisms activated by rhamnolipid surfactant.

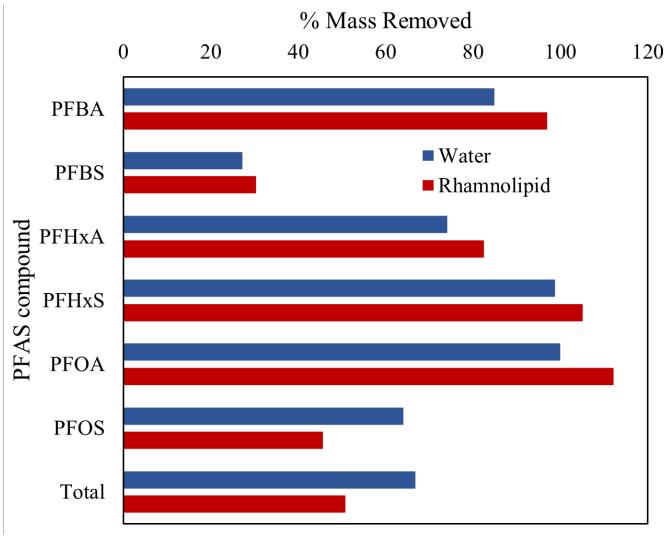

Figure S2: Percent mass of per- and polyfluoroalkyl removed from soil during batch testing by the rhamnolipid solution and by water. *PFBA (Perfluorobutanoic acid); PFBS (Perfluorobutanesulfonic acid); PFHxA (Perfluorohexanoic acid); PFHxS (Perfluorohexanesulfonic acid); PFOA (Perfluorooctanoic acid); PFOS (Perfluorooctanesulfonic acid).*

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2. BREAKTHROUGH CURVE ANALYSIS

2.1. Transport Model Formulation and CXTFIT Settings

We provide here a more detailed mathematical description of the model used for PFAS transport modeling. We apply the advection-dispersion equation with linear equilibrium adsorption accounting for fast-reacting adsorption sites and first-order kinetic adsorption. The governing transport equations in dimensionless form read as (1, 2) (**Eq. S1**):

$$\beta R\frac{\partial C}{\partial T} = -\frac{\partial C}{\partial X} + \frac{1}{P}\frac{\partial^2 C}{\partial X^2} - \omega(C - C_s) \tag{S1}$$
$$(1-\beta)R\frac{\partial C_s}{\partial T} = \omega(C - C_s)$$

The specification of all variables is given in **Table S5**.

Table S5: Quantities and parameters used in the transport model. Dimensional parameters first, second column provides unit; dimensionless parameters after line, second column provides relation to dimensional parameters.

Variable	Unit/Relation	Quantity
x	[cm]	Distance from column inlet
t	[min]	Time
L	[cm]	Column length
c	[g/ml]	Measured mass concentration
c_i	[g/ml]	Initial concentration
c_0	[g/ml]	Background concentration
c_s	[g/ml]	Adsorbed mass concentration
v	[cm/min]	Average flow velocity
D	[cm^2/min]	Dispersion coefficient
α_L	[cm]	Longitudinal dispersivity
ρ_b	[g/mL]	Bulk density
K_d	[mL/g]	Linear adsorption coefficient
θ	[-]	Porosity
f	[-]	Fraction of the exchange sites that are at equilibrium
λ	[1/min]	First-order kinetic adsorption rate coefficient
C	c/c_i	Dimensionless concentration
C_s	c_s/c_i	Dimensionless adsorbed concentration
X	x/L	Dimensionless distance
T	tv/L	Dimensionless time
P	vL/D	Peclet number
R	$1 + \rho_b \cdot K_d/\theta$	Retardation factor
β	$\frac{\theta + f\rho_b K_d}{\theta + \rho_b K_d}$	Dimensionless partitioning coefficient
ω	$\frac{\lambda(1-\beta)RL}{v}$	Dimensionless mass transfer coefficient

Initial conditions for the setting of our column experiments are: $c(x,0) = c_i$ in dimensional form and $C(x,0) = 1$ in dimensionless form corresponding to **Equation S1** for the aqueous PFAS concentration. The adsorbed concentrations have the initial condition of $c_s(x,0) = c = c_i$ in dimensional and $C_s = 1$ in dimensionless form. c_i represents here the initial concentration of the relevant dissolved PFAS compound based on eluted concentration in the first PV, as this model does not allow for setting an initial adsorbed concentration. The concentration eluted in the first PV functions as the initial concentration of dissolved PFAS following the 24 hour equilibration period.

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Boundary conditions are represented by **Equation S2**:

$$\left(-\frac{1}{P}\frac{\partial C}{\partial X}+C\right)|_{X=0}=C_0 \qquad \frac{\partial C}{\partial X}(\infty,T)=0 \tag{S2}$$

where C_0 is the concentration at the inflow, for our experimental setting $C_0 = 0$ representing solute-free input water with no production. The first represents a third type flux boundary condition at the inlet. The latter implies a semi-infinite column for the lower column boundary. The analytical solutions we used in CXTFIT correspond to these two boundary conditions. See Genuchten (1) for a discussion of the correctness of the lower column boundary condition.

Note that a different definition of the dimensionless concentration in the form of $C = (c - c_i)/(c_0 - c_i)$ would lead to other values of the dimensionless initial and boundary conditions, namely $C(x, 0) = 0$ and $C_0 = 1$ which is a common formulation for inflow experiments. However, the analytical solution used in CXTFIT is not impacted by the choice of dimensionless concentration formulation.

Input prompts for the CXTFIT module of STANMOD are provided in **Table S6** to display the workflow we used within the software. Along with retardation factor R, β and ω were fitted to the data. Fitting three parameters at once can make the model converge on different results depending on initial estimates for each parameter. The model was re-run tens of times for each PFAS compound to ensure that the best fit was chosen, i.e., the one that did not allow any parameter to converge on its bounding value; and with the lowest correlation between parameters (2).

Table S6: Table of model input settings for fitting PFAS data to Advection-dispersion equation (S1) with 2-Site Adsorption in CXTFIT.

Input Prompt	**Model setting choice**
Type of Problem	Inverse Problem (parameter estimation)
Type of Model	Deterministic nonequilibrium CDE
Input and Output Data Code	Dimensionless time and dimensional position
Units: Length , Time , Concentration	cm, dimensionless, mg/L
Concentration Mode	Flux-averaged concentration, C_f
Characteristic length for dimensional parameters	37.5 (water), 37 (rhamnolipid)
Parameter Constraint Code	No constraints for parameter estimation
Static	No estimation for total mass
Maximum number of iterations	20
Nonequilibrium model code	Two-site chemical nonequilibrium model
Degradation estimation code	Solution and adsorbed phase degradation rates are independent (both set to zero)
Boundary Value problem	Solute free input water
Initial Value Problem	Constant initial concentration
Production Value Problem	Zero production
Data Structure Input Code	T(I), C(I) for a fixed depth (BTC), position of breakthrough curve = characteristic length
Space discretization: Number of output positions, spatial increment, initial value of output position	1, 0, 37.5 (water) or 37 (rhamnolipid)
Time discretization: Number of output times, time increment, initial value of output time	48, 0.5 (water) or 0.52 (rhamnolipid), 0
Output print code	Concentration vs. time

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2.2. Fitted Breakthrough Curves of Tracer Tests

(a)

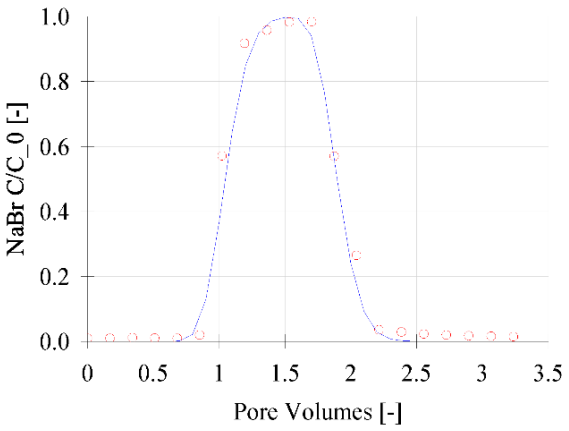
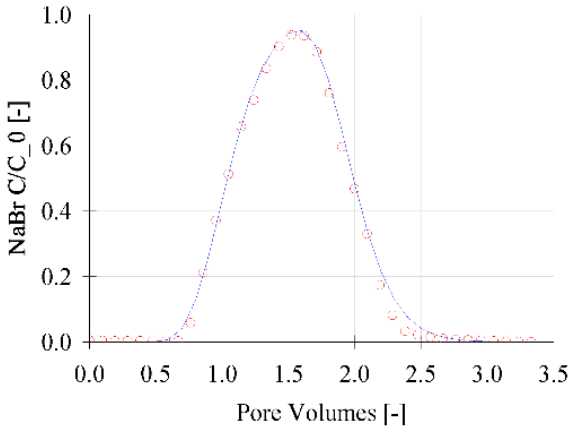


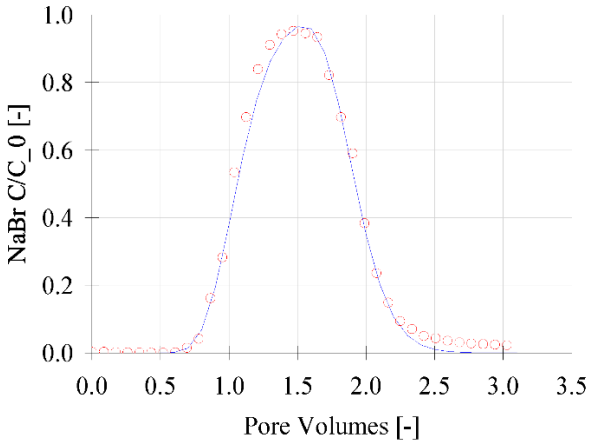
Figure S3: Breakthrough curves (BTC) of preliminary tracer tests. Laboratory data in red circles with fitted curve as a blue line. **(a)** BTC of 100% sand tracer test. Fitted parameters $\boldsymbol{D = 0.507}$ [cm^2/min]. Coefficient of determination $\boldsymbol{R^2 = 0.99}$.

(b)



(b) BTC of 17% soil tracer test. Fitted parameters $\boldsymbol{D = 1.82}$ [cm^2/min], $\boldsymbol{R^2 = 0.997}$.

(c)



(c) BTC of 22% soil tracer test. Fitted parameters $\boldsymbol{D = 1.15}$ [cm^2/min], $\boldsymbol{R^2 = 0.99}$.

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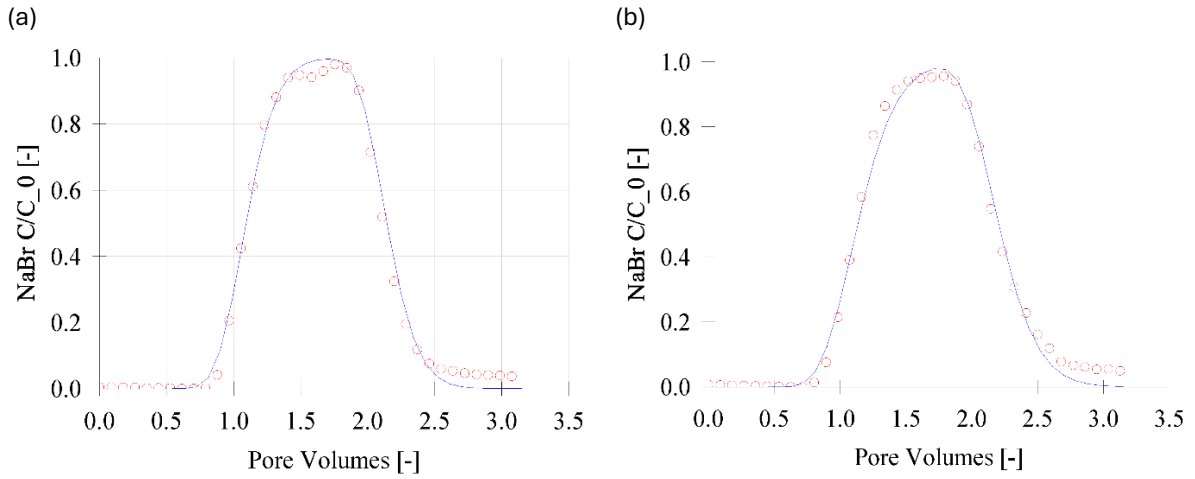

Figure S4: Breakthrough curves (BTC) of tracer tests performed after flushing experiments. Laboratory data in red circles with fitted curve as a blue line. **(a)** BTC of tracer test for water PFAS flushing experiment. Fitted parameters D = 0.91 [cm^2/min], R^2 = 0.995. **(b)** BTC of tracer test for rhamnolipid PFAS flushing experiment. Fitted parameters D = 1.28 [cm^2/min], R^2 = 0.995.

2.3. Fitted PFAS Elution Curves

Per- and polyfluoroalkyl (PFAS) compounds with non-zero concentrations in the eluent throughout the tests were fitted to the two-site adsorption model. Fits are displayed in **Figures S3** and **S4**. For PFHxA and PFBA in both tests as well as PFBS in the water and PFOA in the rhamnolipid test, concentrations went below detection limit within a few pore volumes (**Tables S1** and **S2**).

All PFAS except PFOS exhibited rapid decreases in concentration during flushing, resulting in a good model fit. In contrast, PFOS data were more difficult to fit because, in both experiments, the concentration measured at the first pore volume (PV) was not the highest. To address this, the fitting procedure was adapted by adjusting the initial concentration or by using data from later stages of each experiment.

Because the PFOS concentration in the first pore volume (PV) was not the peak in either experiment, the model showed a poor fit to the PFOS elution data. Resulting fits using the maximum PFOS concentration as the initial dissolved concentration and using the initial PFOS concentration as the initial concentration were considered. The model was also run considering only the concentration data after the highest concentration of PFAS eluate to confirm fitted R-values were in a reasonable range (not shown).

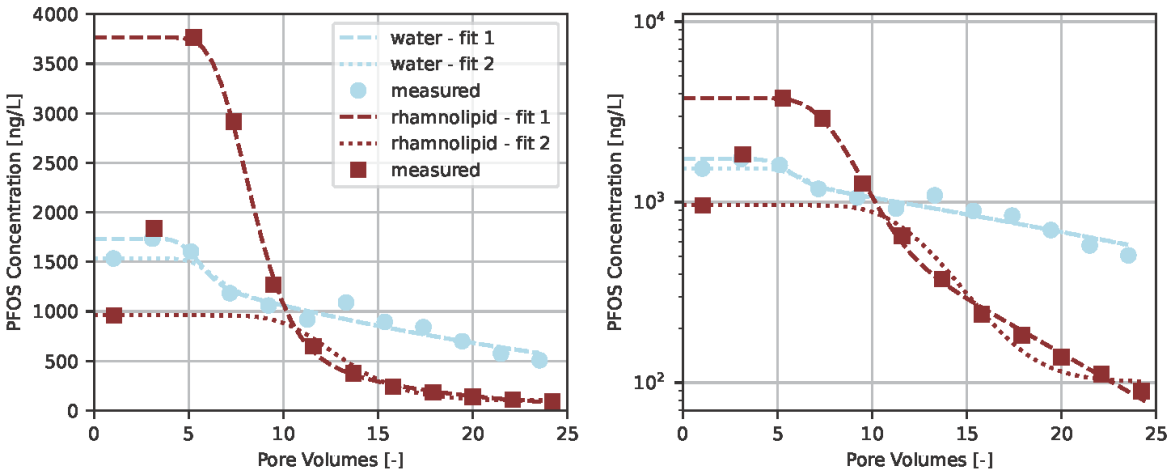

Figure S5: Measured and fitted perfluorooctanesulfonic acid (PFOS) concentration in linear (left) and semi-log scale (right).

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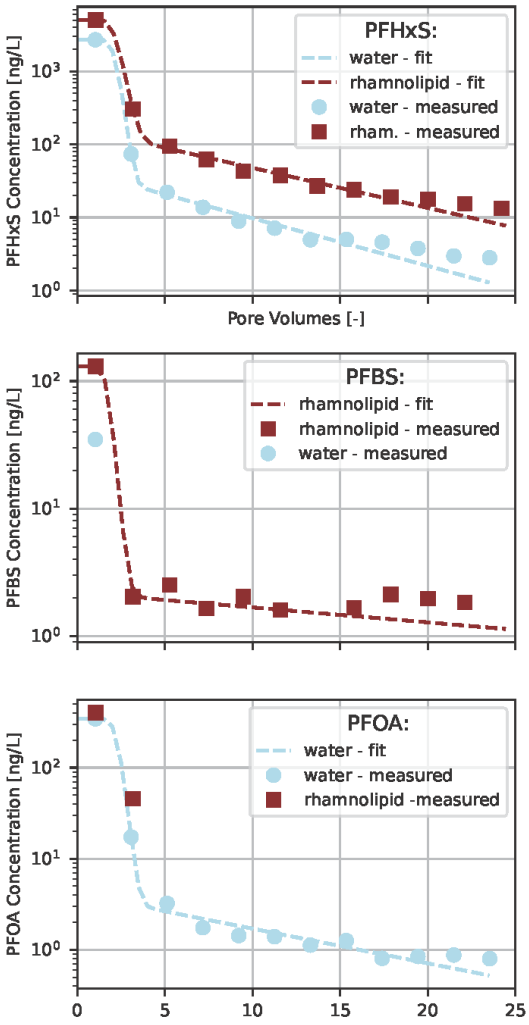

Figure S6: Measured and fitted concentrations of perfluorohexanesulfonic acid (PFHxS), perfluorobutanesulfonic acid (PFBS) and perfluorooctanoic acid (PFOA) for those flushing tests showing non-zero concentrations throughout the entire test in semi-log scale.

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