Supplementary Material

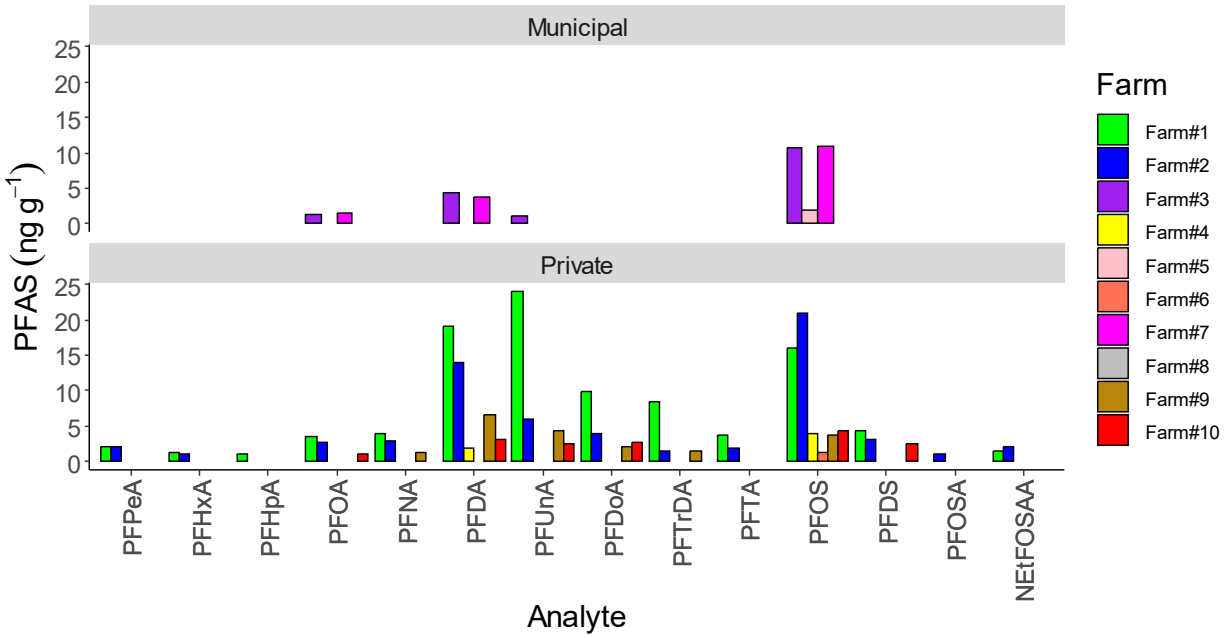
Biosolids as a Potential Source of PFAS to Farming Soils

Supplementary Table 1. Summary of number of observations (n), median, minimum and maximum PFAS concentrations, and results from Wilcoxon signed rank test comparing control versus treatment groups at surface and subsurface soil layers. Semi-quantitative value for samples <LOQ are shown in parenthesis.

Depth	Analyte	Control				Treatment				Wilcoxon test *p*-value
		n	Median	Min	Max	n	Median	Min	Max	
0-15cm	NEtFOSAA	10	0.00	ND	<LOQ(0.1)	10	0.10	ND	2.2	0.014
0-15cm	PFDA	10	0.09	<LOQ(0.03)	<LOQ(0.9)	10	3.53	<LOQ(0.2)	19	0.001
0-15cm	PFDoA	10	0.04	<LOQ(0.01)	<LOQ(1)	10	1.50	<LOQ(0.08)	9.9	0.002
0-15cm	PFDS	10	0.00	ND	<LOQ(0.08)	10	0.45	ND	4.3	0.003
0-15cm	PFHpA	10	0.09	<LOQ(0.04)	<LOQ(0.3)	10	0.30	<LOQ(0.08)	1.1	0.011
0-15cm	PFHxA	10	0.07	<LOQ(0.03)	<LOQ(0.4)	10	0.35	<LOQ(0.06)	1.2	0.008
0-15cm	PFNA	10	0.20	<LOQ(0.08)	<LOQ(0.4)	10	0.80	<LOQ(0.1)	4.0	0.009
0-15cm	PFOA	10	0.25	<LOQ(0.1)	<LOQ(0.8)	10	0.98	<LOQ(0.2)	3.5	0.007
0-15cm	PFOS	10	0.45	<LOQ(0.1)	1.9	10	4.16	<LOQ(0.7)	21	0.001
0-15cm	PFOSA	10	0.00	ND	<LOQ(0.03)	10	0.09	ND	1.0	0.002
0-15cm	PFPeA	10	0.07	ND	<LOQ(0.7)	10	0.70	<LOQ(0.06)	2.1	0.006
0-15cm	PFTA	10	0.01	ND	<LOQ(0.6)	10	0.55	<LOQ(0.03)	3.7	0.002
0-15cm	PFTrDA	10	0.04	<LOQ(0.02)	<LOQ(0.8)	10	0.30	<LOQ(0.03)	8.5	0.035
0-15cm	PFUnA	10	0.10	<LOQ(0.05)	<LOQ(0.5)	10	0.98	<LOQ(0.08)	24	0.016
0-15cm	tPFAS	10	1.54			10	21.59			<0.001
15-30cm	NEtFOSAA	10	0.00	ND	ND	10	0.00	ND	<LOQ(0.2)	0.035
15-30cm	PFDA	10	0.03	ND	<LOQ(0.06)	10	0.50	<LOQ(0.04)	7	0.001
15-30cm	PFDoA	10	0.02	ND	<LOQ(0.03)	10	0.15	<LOQ(0.01)	<LOQ(0.9)	0.002
15-30cm	PFDS	10	0.00	ND	ND	10	0.04	ND	1.1	0.002
15-30cm	PFHpA	10	0.07	<LOQ(0.05)	<LOQ(0.1)	10	0.25	<LOQ(0.08)	<LOQ(0.5)	0.003
15-30cm	PFHxA	10	0.06	<LOQ(0.05)	<LOQ(0.2)	10	0.45	<LOQ(0.08)	<LOQ(0.7)	0.001
15-30cm	PFNA	10	0.10	<LOQ(0.07)	<LOQ(0.2)	10	0.50	<LOQ(0.07)	2.0	0.008
15-30cm	PFOA	10	0.30	<LOQ(0.1)	<LOQ(0.5)	10	0.75	<LOQ(0.2)	2.3	0.016
15-30cm	PFOS	10	0.30	<LOQ(0.08)	<LOQ(0.7)	10	2.54	<LOQ(0.2)	11	0.002
15-30cm	PFOSA	10	0.00	ND	ND	10	0.03	ND	<LOQ(0.2)	0.006
15-30cm	PFPeA	10	0.06	ND	<LOQ(0.3)	10	0.80	<LOQ(0.08)	<LOQ(1)	0.001
15-30cm	PFTA	10	0.00	ND	ND	10	0.06	ND	<LOQ(0.3)	0.002
15-30cm	PFTrDA	10	0.00	ND	<LOQ(0.03)	10	0.05	ND	<LOQ(0.8)	0.007
15-30cm	PFUnA	10	0.04	ND	<LOQ(0.06)	10	0.10	<LOQ(0.02)	3.9	0.036
15-30cm	tPFAS	10	1.16			10	7.75			<0.001

Supplementary Table 2. Summary of number of observations (n), median, minimum, and maximum PFAS concentrations in surface treatment soils, and results from Wilcoxon signed rank test comparing farms receiving biosolids from private and municipal applicators. Semi-quantitative value for samples <LOQ are shown in parenthesis.

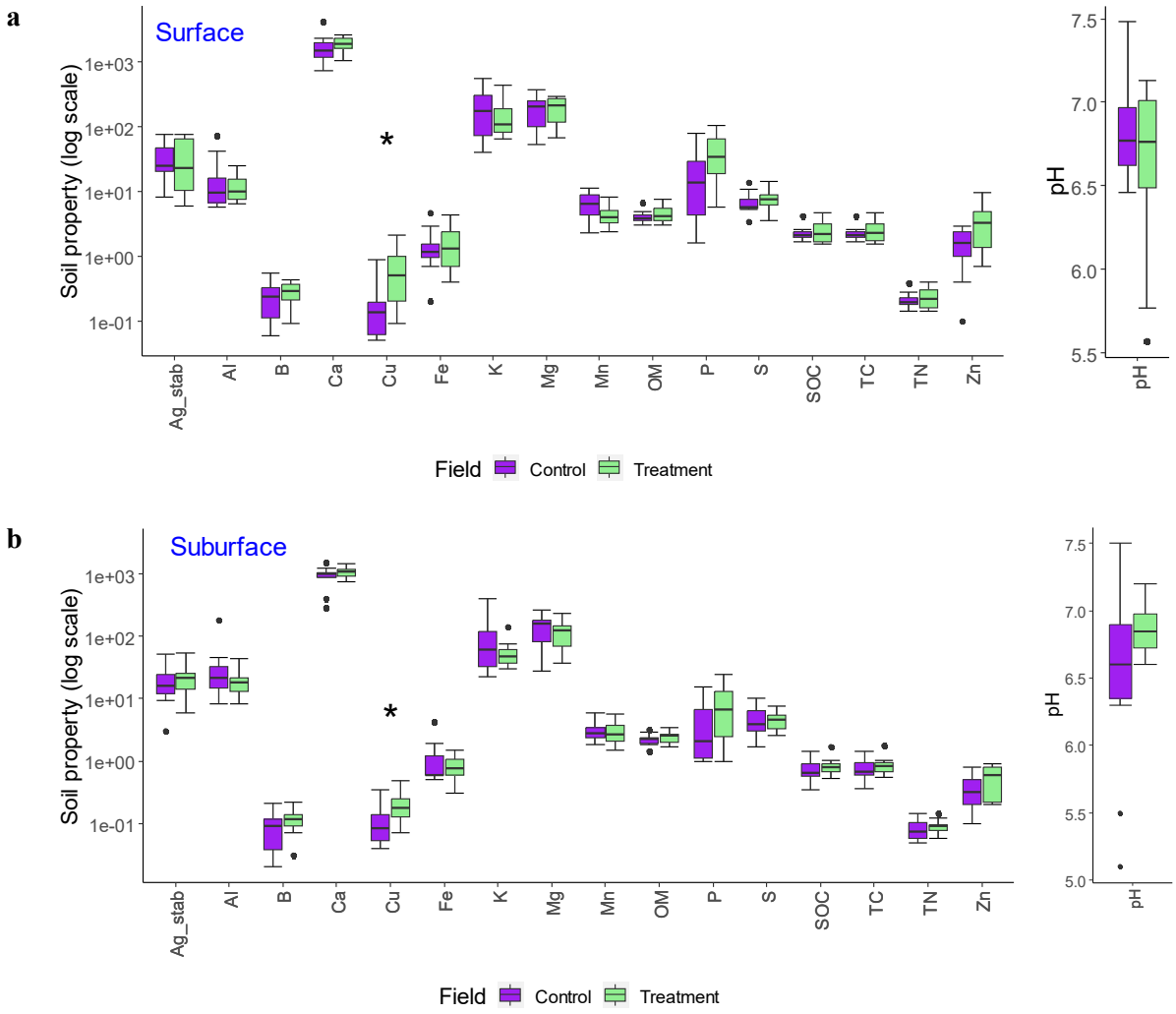
	Municipal				Private				Wilcoxon
Analyte	**n**	**Median**	**Min**	**Max**	**n**	**Median**	**Min**	**Max**	**test *p*-value**
NEtFOSAA	8	0.00	ND	<LOQ(0.2)	12	0.08	ND	2.2	0.168
PFDA	8	0.35	<LOQ(0.04)	4.5	12	2.59	<LOQ(0.05)	19	0.076
PFDoA	8	0.10	<LOQ(0.01)	2	12	0.70	<LOQ(0.02)	9.9	0.097
PFDS	8	0.04	ND	<LOQ(0.7)	12	0.40	ND	4.3	0.173
PFHpA	8	0.20	<LOQ(0.08)	<LOQ(0.5)	12	0.30	<LOQ(0.1)	1.1	0.199
PFHxA	8	0.25	<LOQ(0.06)	<LOQ(0.7)	12	0.45	<LOQ(0.2)	1.2	0.174
PFNA	8	0.30	<LOQ(0.07)	<LOQ(0.8)	12	1.24	<LOQ(0.1)	4.0	0.018
PFOA	8	0.60	<LOQ(0.3)	2.3	12	0.98	<LOQ(0.2)	3.5	0.587
PFOS	8	2.82	<LOQ(0.2)	11	12	4.16	<LOQ(0.7)	21	0.315
PFOSA	8	0.01	ND	<LOQ(0.2)	12	0.07	ND	1.0	0.116
PFPeA	8	0.40	<LOQ(0.06)	<LOQ(1)	12	0.90	<LOQ(0.2)	2.1	0.198
PFTA	8	0.04	ND	<LOQ(0.6)	12	0.25	ND	3.7	0.088
PFTrDA	8	0.04	ND	<LOQ(0.3)	12	0.20	<LOQ(0.01)	8.5	0.044
PFUnA	8	0.09	<LOQ(0.02)	1.1	12	0.87	<LOQ(0.04)	24	0.020
tPFAS	8	5.85			12	16.54			0.115



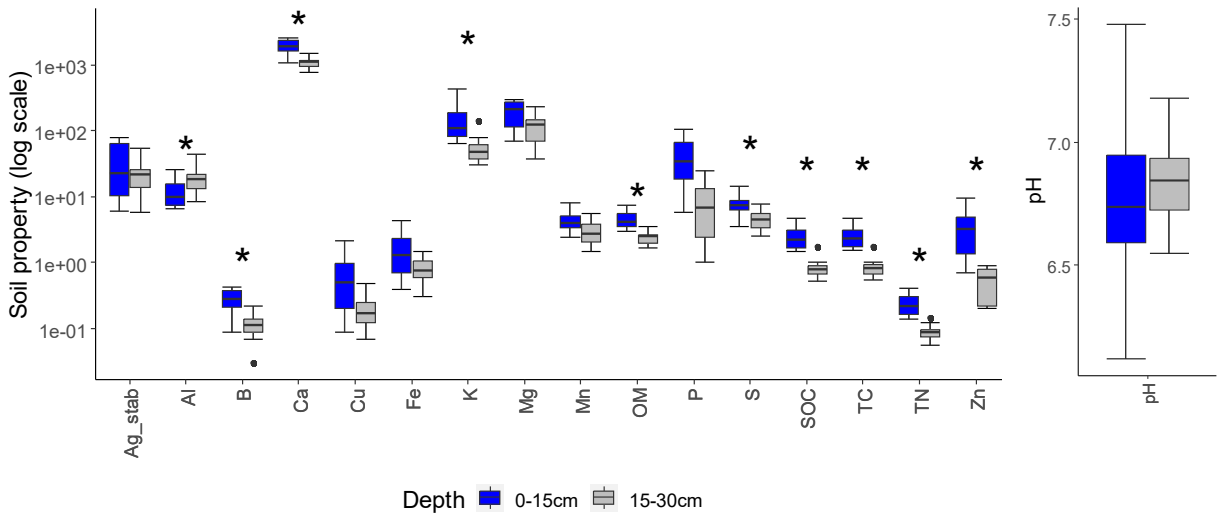
Supplementary Figure 1. Concentrations of PFAS ≥LOQ in surface (0-15cm) soils receiving biosolids from municipal (top) vs private (bottom) applicators in 10 working farms in noreastern US. Empty bars indicate PFAS concentrations were <LOQ.

Supplementary Table 3. Summary of number of observations (n), median concentrations of soil physicochemical properties, and results from Wilcoxon signed rank test comparing soils from control vs treatment fields.

Depth	Analyte	Control		Treatment		Wilcoxon test *p*-value
		n	median	n	median	
0-15cm	Ag_stab	10	24.6	10	23.6	0.796
0-15cm	Al	10	9.7	10	10.0	0.912
0-15cm	B	10	0.2	10	0.3	0.472
0-15cm	Ca	10	1501.2	10	1939.3	0.218
0-15cm	Cu	10	0.1	10	0.5	0.011
0-15cm	Fe	10	1.2	10	1.3	0.970
0-15cm	K	10	175.5	10	110.9	0.853
0-15cm	Mg	10	206.0	10	211.9	0.739
0-15cm	Mn	10	6.6	10	4.0	0.150
0-15cm	OM	10	3.8	10	4.1	0.631
0-15cm	P	10	14.7	10	34.8	0.123
0-15cm	pH	10	6.8	10	6.7	0.850
0-15cm	S	10	5.7	10	7.4	0.315
0-15cm	SOC	10	2.1	10	2.2	0.912
0-15cm	TC	10	2.1	10	2.3	0.796
0-15cm	TN	10	0.2	10	0.2	0.684
0-15cm	Zn	10	1.6	10	3.5	0.140
15-30cm	Ag_stab	10	16.5	10	21.8	0.631
15-30cm	Al	10	21.5	10	18.7	0.436
15-30cm	B	10	0.1	10	0.1	0.305
15-30cm	Ca	10	1010.0	10	1096.3	0.353
15-30cm	Cu	10	0.1	10	0.2	0.034
15-30cm	Fe	10	0.6	10	0.8	1.000
15-30cm	K	10	60.7	10	48.4	0.650
15-30cm	Mg	10	162.2	10	125.4	0.393
15-30cm	Mn	10	2.8	10	2.7	0.733
15-30cm	OM	10	2.3	10	2.5	0.529
15-30cm	P	10	2.1	10	7.1	0.160
15-30cm	pH	10	6.6	10	6.8	0.241
15-30cm	S	10	3.9	10	4.6	0.853
15-30cm	SOC	10	0.7	10	0.8	0.579
15-30cm	TC	10	0.7	10	0.8	0.481
15-30cm	TN	10	0.1	10	0.1	0.449
15-30cm	Zn	10	0.4	10	0.6	0.133



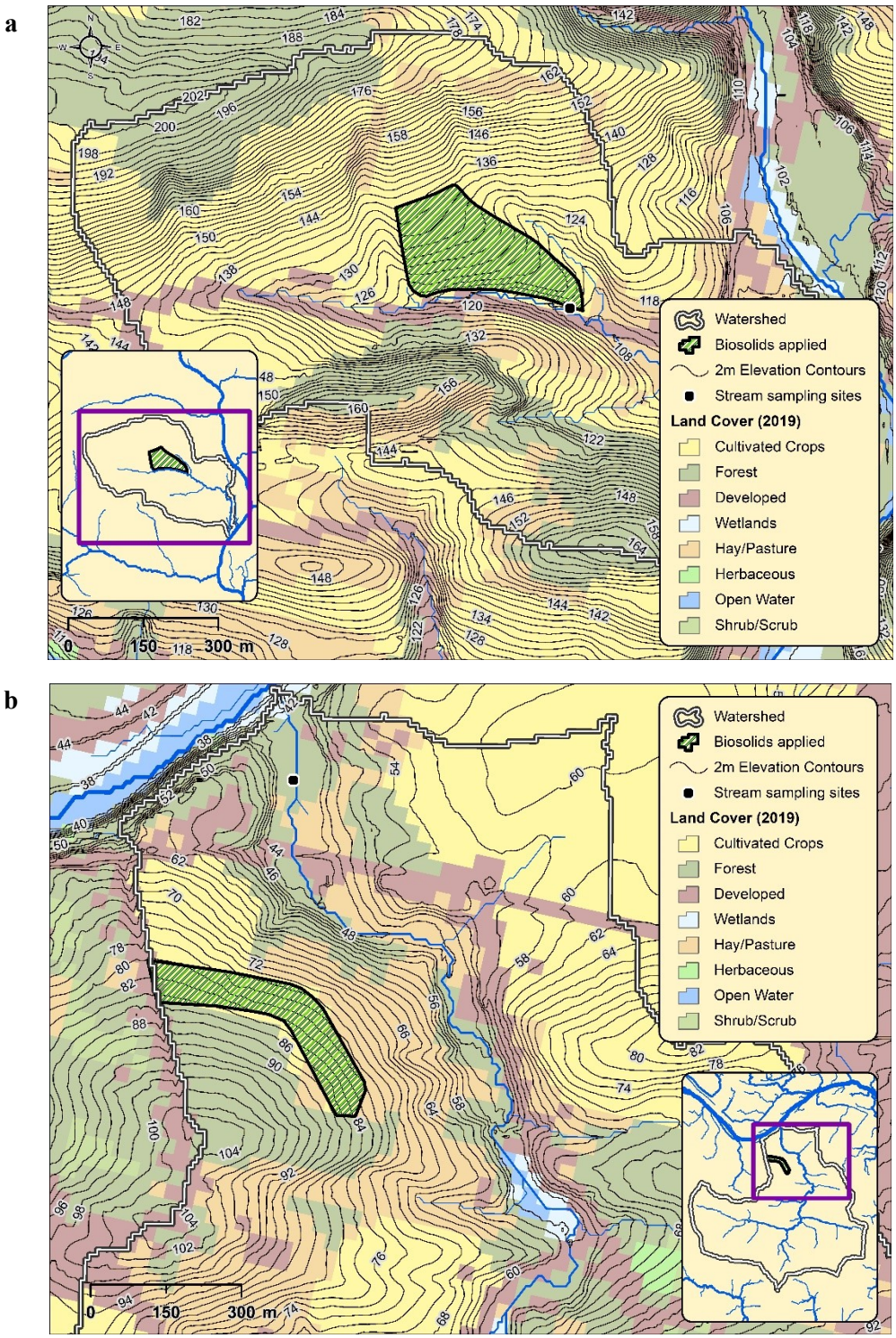
Supplementary Figure 2. Box plots showing the differences in soil physicochemical properties between treatment and control fields at 0-15cm (**a)** and 15-30 cm **(b)**. The box represents the interquartile range, the line inside the box represents the median, the whiskers represent the minimum and maximum and the dots represent outliers. *indicates significant differences between treatment and control fields (Supplementary Table 4). Ag_stab, OM, SOC, TC, and TN are in units of %; P, K, Mg, Fe, Mn, Zn, Al, Ca, Cu, S, and B are in units of ppm. pH is plotted on a linear scale.



Supplementary Figure 3. Box plots showing the differences in soil physicochemical properties between surface (0-15cm) and subsurface (15-30cm) of treatment soils. The box represents the interquartile range, the line inside the box represents the median, the whiskers represent the minimum and maximum and the dots represent outliers. *indicates significant differences between depths (Supplementary Table 3). Ag_stab, OM, SOC, TC, and TN are in units of %; P, K, Mg, Fe, Mn, Zn, Al, Ca, Cu, S, and B are in units of ppm. pH is plotted on a linear scale.

Supplementary Table 4. Summary of number of observations (n), median concentrations of soil physicochemical properties, and results from Wilcoxon signed rank test comparing concentrations in surface (0-15cm) vs subsurface (15-30cm) layers of treatment soils.

Analyte	0-15cm		15-30cm		Wilcoxon test *p*-value
	n	median	n	median	
Ag_stab	10	23.6	10	21.8	0.579
Al	10	10.0	10	18.7	0.036
B	10	0.3	10	0.1	0.002
Ca	10	1939.3	10	1096.3	0.001
Cu	10	0.5	10	0.2	0.031
Fe	10	1.3	10	0.8	0.129
K	10	110.9	10	48.4	<0.001
Mg	10	211.9	10	125.4	0.063
Mn	10	4.0	10	2.7	0.063
OM	10	4.1	10	2.5	<0.001
P	10	34.8	10	7.1	0.001
pH	10	6.7	10	6.8	0.677
S	10	7.4	10	4.6	0.016
SOC	10	2.2	10	0.8	<0.001
TC	10	2.3	10	0.8	<0.001
TN	10	0.2	10	0.1	<0.001
Zn	10	3.5	10	0.6	0.002



Supplementary Figure 4. Maps of (**a**) Farm #2 and (**b**) Farm #10 showing the topography, land use, and location of the sampled treatment field and the sampled headwater stream.

PFAS Analysis Detailed Methodology

1. Scope And Applicability

This method uses solid phase extraction (SPE) and dispersive solid phase extraction (DSPE) to concentrate and purify water samples and extracts of solid samples, followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) for determination of selected per- and polyfluoroalkyl substances (PFAS) using isotopically-labelled internal standards. The following analytes are determined using this method:

Supplementary Table 5. PFAS compounds determined in this study via targeted analysis. LOQ = limit of quantification.

Analyte	Acronym	CAS Number	LOQ (1633) ppb
Perfluorobutanoic acid	PFBA	375-22-4	4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	2
Perfluoropentanoic acid	PFPeA	2706-90-3	2
Perfluorobutanesulfonic acid	PFBS	375-73-5	1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	2
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	2
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	2
1H, 1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	4
Perfluorohexanoic acid	PFHxA	307-24-4	1
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	1
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	2
Perfluoroheptanoic acid	PFHpA	375-85-9	1
Perfluorohexanesulfonic acid	PFHxS	355-46-4	1
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	2
1H, 1H, 2H, 2H-Perfluoroctane sulfonic acid	6:2FTS	27619-97-2	4
Perfluorooctanoic acid	PFOA	335-67-1	1
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	1
Perfluorononanoic acid	PFNA	375-95-1	1
Perfluorooctanesulfonic acid	PFOS	1763-23-1	1
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	2
1H, 1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	4
Perfluorodecanoic acid	PFDA	335-76-2	1
Perfluoroundecanoic acid	PFUnA	2058-94-8	1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	2
Perfluorododecanoic acid	PFDoA	307-55-1	2
Perfluorotridecanoic acid	PFTrDA	72629-94-8	1
Perfluorotetradecanoic acid	PFTA	376-06-7	1
Perfluorononane sulfonic acid	PFNS	68259-12-1	1
Perfluorodecane sulfonic acid	PFDS	335-77-3	1
Perfluorododecane sulfonic acid	PFDoS	79780-39-5	1
Perfluorooctanesulfonamide	PFOSA	754-91-6	1

N-methyl perfluorooctanesulfonamide	NMeFOSA	31506-32-8	1
N-ethyl perfluorooctanesulfonamide	NEtFOSA	4151-50-2	1
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9	1
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6	1
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7	10
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2	10
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5	4
2H, 2H, 3H, 3H-Perfluorooctanoic acid	5:3FTCA	914637-49-3	20
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4	20

2. Analytical standards and reagents

2.1 Native PFAS Standard Mix F (Wellington Laboratories catalog no. PFAC-MXF), hereafter referred to as MXF

2.2 Native PFAS Standard Mix G (Wellington Laboratories catalog no. PFAC-MXG), hereafter referred to as MXG

2.3 Native PFAS Standard Mix H (Wellington Laboratories catalog no. PFAC-MXH), hereafter referred to as MXH

2.4 Native PFAS Standard Mix I (Wellington Laboratories catalog no. PFAC-MXI), hereafter referred to as MXI

2.5 Native PFAS Standard Mix J (Wellington Laboratories catalog no. PFAC-MXJ), hereafter referred to as MXJ

2.6 Mass-Labelled Perfluorinated Compound Isotope Internal Standard (Wellington Laboratories catalog no. MPFAC-HIF-IS), hereafter 1633IS

2.7 Mass-Labelled PFAS Isotope Extraction Standard (Wellington Laboratories catalog no. MPFAC-HIF-ES) hereafter 1633ES

3. Equipment

3.1 Automated solid-phase extraction apparatus – PromoChrom Model SPE-03.

3.2 SPE cartridges packed with 150 mg weak anion exchange (WAX) sorbent. Example: Chromabond HR-XAW (150 mg/6 mL), Macherey Nagel catalog no. 730749.

3.3 Liquid chromatograph equipped with a pump capable of binary gradient elution, an automated sample injector capable of injecting 1 to 20-µL aliquots, controlled temperature column compartment, triple quadrupole mass spectrometer, and a data processing system. This method was implemented on an Agilent Technologies Model 1290 Infinity II Liquid Chromatograph interfaced to an Agilent Model 6495C triple quadrupole mass spectrometer.

3.4 Analytical Column: Halo® 90 Å PFAS

Length = 10 cm
I.D. = 2.1 mm
Particle Diameter = 2.7 µm
AMT Catalog No. 92812-613

3.5 Delay Column (inserted between pump and injector): Halo® 160 Å PFAS Delay
Length = 5 cm
I.D. = 3.0 mm
Particle Diameter = 2.7 µm
AMT Catalog No. 92113-415

4. Instrumentation parameters

Mobile Phase A: 20 mM ammonium acetate in water (1.5 g in 1 L water)

Mobile Phase B: methanol

Operating Conditions

4.1 Binary Pump

Time (min)	% Mobile Phase A	% Mobile Phase B
0.00	80.0	20.0
5.00	40.0	60.0
12.00	10.0	90.0
12.10	80.0	20.0

4.2 Autosampler Conditions
Injection Volume: 2.0 µL
Needle Wash: On

4.3 Column Conditions
Column: Halo® 90 Å PFAS
Flow Rate: 0.5 mL/min
Temperature: 40°C
Maximum Pressure Limit: 1000 bar

4.4 Detector Conditions (MS/MS)
Scan Type: Dynamic Multiple Reaction Monitoring (DMRM)
Ionization Mode: Negative Ion Electrospray
Q1 Resolution: Unit
Q3 Resolution: Unit
Gas Temperature: 130°C
Gas Flow: 11 L/min
Nebulizer: 25 psi
Sheath Gas Heater: 250°C
Sheath Gas Flow: 12 L/min
Capillary: 2000 V
VCharging: 300 V
High Pressure RF: 90
Low Pressure RF: 60

Cell Accelerator: 3 V

Supplementary Table 6. Mass Spectrometer settings for targeted analytes and labeled standards.

Acronym	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)	1633 ES Internal Standard	1633 IS Internal Standard
PFBA	213	169	10	13C4-PFBA	
PFMPA	229	85	10	13C5-PFPeA	
PFPeA	263	219	8	13C5-PFPeA	
PFBS	299	80	38	13C3-PFBS	
PFMBA	279	85	10	13C5-PFPeA	
PFEESA	315	135	20	13C5-PFHxA	
NFDHA	295	201	8	13C5-PFHxA	
4:2FTS	327	307	18	13C2-4:2FTS	
PFHxA	313	269	6	13C5-PFHxA	
PFPeS	349	80	35	13C3-PFHxS	
HFPO-DA	285	169	4	13C3-HFPO-DA	
PFHpA	313	269	6	13C4-PFHpA	
PFHxS	399	80	40	13C3-PFHxS	
ADONA	377	251	8	13C3-HFPO-DA	
6:2FTS	427	407	22	13C2-6:2FTS	
PFOA	413	369	6	13C8-PFOA	
PFHpS	449	80	48	13C8-PFOS	
PFNA	463	419	8	13C9-PFNA	
PFOS	499	80	50	13C8-PFOS	
9Cl-PF3ONS	531	351	28	13C3-HFPO-DA	
8:2FTS	527	507	28	13C2-8:2FTS	
PFDA	513	469	10	13C6-PFDA	
PFUnA	563	519	10	13C7-PFUnA	
11Cl-PF3OUdS	631	451	24	13C3-HFPO-DA	
PFDoA	613	569	12	13C2-PFDoA	
PFTrDA	663	619	9	13C2-PFTA	
PFTA	713	669	12	13C2-PFTA	
PFNS	549	80	55	13C8-PFOS	
PFDS	599	80	55	13C8-PFOS	
PFDoS	699	80	60	13C8-PFOS	
PFOSA	498	78	34	13C8-PFOSA	
NMeFOSA	512	219	22	D3-NMeFOSA	
NEtFOSA	526	219	20	D5-NEtFOSA	
NMeFOSAA	570	419	20	D3-NMeFOSAA	
NEtFOSAA	584	419	20	D5-NEtFOSAA	
NMeFOSE	616	59	10	D7-NMeFOSE	
NEtFOSE	630	59	11	D9-NEtFOSE	
3:3FTCA	241	177	0	13C5-PFPeA	
5:3FTCA	341	237	18	13C5-PFHxA	
7:3FTCA	441	317	18	13C5-PFHxA	
13C2-4:2FTS	329	309	18		18O2-PFHxS
13C2-6:2FTS	429	409	22		18O2-PFHxS
13C2-8:2FTS	529	509	28		18O2-PFHxS

13C2-PFDA	515	470	6		
13C2-PFDoA	615	570	12		13C2-PFDA
13C2-PFHxA	315	270	3		
13C2-PFTA	715	670	12		13C2-PFDA
13C3-HFPO-DA	287	169	4		13C2-PFHxA
13C3-PFBA	216	172	10		
13C3-PFBS	302	80	38		18O2-PFHxS
13C3-PFHxS	402	80	40		18O2-PFHxS
13C4-PFBA	217	172	10		13C3-PFBA
13C4-PFHpA	367	322	6		13C2-PFHxA
13C4-PFOA	417	172	6		
13C4-PFOS	503	80	50		
13C5-PFHxA	318	273	6		13C2-PFHxA
13C5-PFNA	468	423	8		
13C5-PFPeA	268	223	8		13C2-PFHxA
13C6-PFDA	519	474	10		13C2-PFDA
13C7-PFUnA	570	525	10		13C2-PFDA
13C8-PFOA	421	376	6		13C4-PFOA
13C8-PFOS	507	80	50		13C4-PFOS
13C8-PFOSA	506	78	34		13C4-PFOS
13C9-PFNA	472	427	8		13C5-PFNA
18O2-PFHxS	403	84	40		
D3-NMeFOSA	515	219	22		13C4-PFOS
D3-NMeFOSAA	573	419	166		13C4-PFOS
D5-NEtFOSA	531	219	20		13C4-PFOS
D5-NEtFOSAA	589	419	20		13C4-PFOS
D7-NMeFOSE	623	59	11		13C4-PFOS
D9-NEtFOSE	639	59	11		13C4-PFOS

5. Water sample preparation

The Promochrom SPE-03 was prepared with the solutions needed for draft EPA method 1633 which includes: methanol, water, 0.3 M formic acid in water, 1:1 0.1 M formic acid:methanol, and 2% ammonium hydroxide in methanol. The pH of each sample was checked to be between 6 – 7. The pH was adjusted using dilute acid and base if the sample was outside the desired pH range. Sample bottles were weighed, and the mass was recorded. 100.0 µL 1633 ES was added to each ~250 mL sample and QCs that were in 250 mL PP bottles. Bottles were shaken using a wrist action shaker for 10 minutes and then attached to the automated SPE system. SPE cartridges were pre-conditioned with 15 mL of 2% ammonium hydroxide in methanol followed by 5 mL of 0.3 M formic acid. Samples were then passed through the cartridges at 5 mL/min. Sample bottles were rinsed with two, 5 mL of water and the rinsate was passed through the cartridges. Sample bottles were then rinsed with 5 mL of 1:1 0.1 M formic acid/methanol and the rinsate was passed through the cartridges. Cartridges were then dried with 10 mL of air at a flow rate of 10 mL/min. Each sample bottle was rinsed with 8 mL of 2% ammonium hydroxide in methanol and the rinsate was passed through the cartridges and collected into 15 mL centrifuge tubes. The samples were then evaporated under nitrogen and water bath (30°C) to just under 2 mL sample volumes. Internal standard was added to each sample along with 75 uL glacial acetic acid and sufficient methanol to bring each sample up to approximately 2 mL. Tubes were vortexed and then 0.5 mL of samples

was transferred to auto sampler vials for LC-MS/MS analysis. Empty sample bottles were weighed and their masses recorded. The processed sample volumes were calculated using:

$$\text{Volume (mL)} = \frac{\text{Initial weight (g)} - \text{Empty bottle weight (g)}}{1.0\ \text{g/mL}}$$

6. Solid sample preparation

Solid samples (soils and biosolids) were dried to a constant weight in a fume hood and then homogenized in a glass mortar and pestle. One gram of each homogenized solid sample was weighed into a 15 mL PP centrifuge tube. Weights were recorded to the nearest 10 mg. 100 µL of extraction standards were added to each sample. 10 mL of 0.3% ammonium hydroxide in methanol was added to each sample tube. Samples were vortexed and then shaken via wrist action shaker for 30 min. Samples were then centrifuged for 10 min at 4,000 rpm. Supernatant was decanted into new 15 mL centrifuge tubes and 75 µL glacial acetic acid was added to each tube. Samples were evaporated to just under 2 mL under nitrogen and a water bath set to 30°C.

Microcentrifuge tubes (1.5 mL) were prepared with approximately 25 mg Envicarb. 1 mL of each sample was pipetted into each microcentrifuge tubes, vortexed for 20 seconds, and then centrifuged for 2 min @ 10,000 rpm. 0.5 mL sample was transferred to auto sampler vials where 25 µL internal standard was added. Samples were then ready for analysis via LC-MS/MS.

7. Total oxidizable precursor (TOP) sample preparation - water

The same instructions outlined in section 5 is completed for TOP water samples, although stopping before evaporating, plus extraction standards were not added. The eluate was divided into two equal portions (portion 1: "TOP Assay", and portion 2: "oxidation efficiency indicator") in 5 mL PP tubes. 50 µL of M8FOSA & M2-4:2 FTS mixture (20 & 18.76 ng/mL) was added to tube 2 as an oxidation indicator. Samples were evaporated to dryness under nitrogen and a water bath set to 30°C.

0.2 grams of potassium persulfate, 0.5 mL of 10 N NaOH, and 4.5 mL of LCMS grade water was added to each portion. Samples were vortexed until the salt dissolved and then they were heated (85 °C) in a heating bath for 10 hours. Each portion was decanted into separate 250 mL PP bottles containing 250 mL LCMS grade water. SPE was repeated as above, except, 100 µL of 1633 ES internal standard mix was added to portion 1 and 100 µL M4PFOA and $^{18}O_2$-PFHxS mixture (10 & 9.5 ng/mL) was added to portion 2 to quantify the M8FOSA and M2-4:2 FTS. Sample was then ready for LC-MS/MS analysis.

8. TOP sample preparation – soil and biosolids

Solid samples (1.0 ± 0.1 gram) were weighed into 15 mL PP centrifuge tubes, with weights recorded to the nearest 0.001 g. 10 mL of 0.3% (v/v) ammonium hydroxide in methanol was added into each tube and place on wrist-action shaker set at maximum deflection for 30 min. Samples were then centrifuged at 4000 rpm for 10 min. Supernatant was decanted into 250 mL PP bottles containing 250 mL LCMS grade water. The pH of the samples solutions were adjusted with dilute acid and base to be between 6 to 7. Sample preparation was continued, following the instructions followed for section 7.

PFAS data quality assurance and control

Calibration standards (native and isotopically-labeled) were obtained from Wellington Laboratories (Guelph, Ontario). Each laboratory analytical set (20 samples or less) included at least one blank (HPLC-grade water or washed sand, as appropriate) plus three blanks fortified with known levels of the PFAS analytes. The lowest fortification level was taken as the Method Reporting Limit (MRL) or Limit of Quantification (LOQ). In addition, the absolute responses of the isotopic standards were monitored to ensure that no unacceptable recovery losses or matrix effects occurred. All data was reviewed and found to meet the laboratory's acceptability criteria. Any detections in blanks for all analytes (excluding fortified isotopically labeled standards) were ≤ 1/3 of the MRL. Fortifications at the MRL were within 50 to 150% of the true value. Fortifications at higher levels were within 70 to 130% of the true value. The isotopically labeled standard peak areas were within 50-150% of the average areas of the calibration standards and the recoveries were within 50-150% of the fortified values. Back-calculated agreement values for individual calibration standards were within 70 to 130%.