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Testing the Use of Biofilms to Measure PFAS in the South Fork Palouse River

Final Report

by

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Abstract

In August 2021, the Washington State Department of Ecology (Ecology) conducted a field study to test biofilms as a tool for measuring and identifying sources of per- and polyfluoroalkyl substances (PFAS) in the environment. Ecology chose the South Fork Palouse River watershed as the study area because high levels of PFAS have previously been documented in the river.

Ecology collected coinciding surface water and biofilm samples from 11 sites in the watershed during one sampling event. Ecology also collected sediment samples at a subset of four sites. Additionally, Ecology collected one influent and effluent sample from the Pullman Wastewater Treatment Plant (WWTP), which discharges effluent to the South Fork Palouse River.

PFAS were quantitatively detected in all 28 samples collected. Summed concentrations of 40 target analytes (total PFAS) were 13.4 – 118 ng/L in surface water, 0.11 – 3.6 ng/g in biofilm, and 0.27 – 5.4 ng/g in sediment samples. Ecology found the highest total PFAS concentrations downstream of the river's confluence with Paradise Creek and in Paradise Creek (a WWTP-influenced water body). Total PFAS concentrations in effluent from the Pullman WWTP were higher than in the influent, primarily due to increases in perfluoroalkyl carboxylic acids. Ecology also found relatively high PFAS concentrations in the surface water, biofilm, and sediment in Missouri Flat Creek, which is not affected by WWTP effluent.

Ecology found that biofilm samples were useful in identifying at least three major conveyances of PFAS to the South Fork Palouse River: Paradise Creek, the Pullman WWTP, and Missouri Flat Creek. Ecology also found that PFAS partition differently in the surface water, biofilm, and sediment. For this study, collecting and analyzing multiple matrices seemed most effective in characterizing PFAS contamination and identifying potential sources in the South Fork Palouse watershed.

Introduction

Background

Per- and polyfluoroalkyl substances (PFAS) are a large family of several thousand synthetic fluorinated chemicals that vary widely in their chemical and physical properties. Their thermal stability, stain-resistance, surfactant nature, and water and oil-repellent properties have made them useful in a wide array of industrial processes and consumer products. Because of their widespread use over the past few decades, PFAS are found globally in the environment, including surface water, groundwater, drinking water, rainwater, and animals (Brase et al. 2021; Xu et al. 2021; Cousins et al. 2022; Waterkeeper Alliance 2022; Andrews et al. 2023; Smalling et al. 2023).

Many PFAS are persistent, bioaccumulative, and toxic, causing concerns about their potential effects on human health and the environment. For this reason, many PFAS have been phased out of production, including those belonging to a group of PFAS known as perfluoroalkyl acids (PFAAs). PFAAs are further divided into perfluoroalkane sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) (Figure 1).

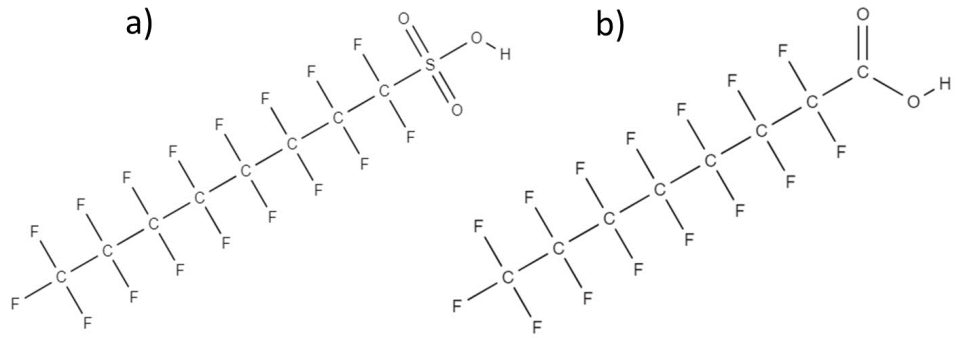

Figure 1. a) Example of a long-chain perfluoroalkane sulfonic acid (PFSA) and b) a long-chain perfluoroalkyl carboxylic acid (PFCA).

While long-chain PFSAs (containing six or more carbons) and PFCAs (containing eight or more carbons) have largely been phased out, alternative PFAS compounds have been and continue to be developed and used to replace them. These newer chemicals include, but are not limited to:

- Short-chain and ultra-short-chain PFAAs.
- PFAS precursor compounds (those that can transform into PFAAs).
    - Perfluorooctane sulfonamides and sulfonamidoacetic acids ("sulfonamides").
    - Fluorotelomer sulfonic and carboxylic acids ("fluorotelomers").
- Ether-based PFAS such as "GenX" and ADONA.

Some of these newer chemicals are as harmful as the chemicals they were meant to replace, and little is known about the environmental and health effects of the other thousands of PFAS that exist (Brase et al. 2021).

In Washington State, PFAS have been found in fresh and marine surface waters, sediments, stormwater, municipal wastewater discharges, fish tissue, and osprey eggs (Ecology and Health 2022). Washington's PFAS Chemical Action Plan (CAP) was developed to address PFAS contamination in the state and included recommendations for managing environmental contamination (Ecology and Health 2022). Specifically, the CAP recommended that the Washington State Department of Ecology (Ecology) conduct monitoring of PFAS in the environment to identify sources and assess exposure.

Ecology's PFAS CAP Implementation Monitoring Program addresses PFAS in the environment through research and monitoring to identify and better understand the sources and effects of PFAS. Because PFAS compounds have properties that can make them challenging to monitor, assessing different monitoring methods is important for better understanding their fate and transport and, thereby, their sources and effects on the environment. This CAP implementation study evaluates a novel approach — using biofilms to measure and trace sources of PFAS in the environment.

In aquatic environments, water sampling has traditionally relied on instantaneous grab samples. While the approach is relatively simple, source identification may be difficult if contaminant concentrations are at trace levels or are subject to variability over time. Furthermore, contaminants may partition differently in the water column than in other media, such as sediments or fish tissue. Using integrative passive samplers is one way to help address this issue, but these samplers can sometimes present logistical hurdles related to deployment, maintenance, and background contamination.

Natural passive samplers like biofilms are another way to sample contaminants. In aquatic systems, biofilms are complex matrices of microorganisms (e.g., algae, bacteria, protozoa), detritus, and other organic and inorganic materials attached to the surfaces of rocks. Contaminants in the water can bioaccumulate within biofilm over time, thus reflecting local conditions in the water over time. The biofilms also serve as a food source for aquatic invertebrates, which can serve as food for fish. Measuring the levels of contaminants in biofilms can, therefore, provide an understanding of effects at higher trophic levels.

Previous Ecology studies have measured organic contaminants, including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), and metals such as copper and zinc in biofilms (Hobbs 2018; Hobbs et al. 2019; Era-Miller and Wong 2022). However, Ecology has not conducted an environmental study to assess the use of biofilms as a PFAS monitoring and source identification tool. The purpose of this study was to fill this knowledge gap.

We chose the South Fork Palouse River watershed as our study area because elevated PFAS levels in the surface water have been previously documented in the river (Mathieu and McCall

2017). In addition, the river is influenced by effluents from wastewater treatment plants (WWTP) in the watershed, especially during summer low flows, and this serves as at least one known conveyance of PFAS to the river (Mathieu and McCall 2017). These factors provided a good opportunity to carry out our field test.

Goals and Objectives

The goal of this study was to assess the use of biofilms as a tool for measuring and identifying sources of PFAS in the environment. Based on previous studies documenting PFAS in WWTP influents and effluents (e.g., Mathieu and McCall 2017, Bothfeld and Mathieu 2022, Thompson et al. 2022), we expected that WWTPs within the South Fork Palouse River watershed would be identified as a conveyance of PFAS to the river.

Specific objectives were to:

- Collect and analyze PFAS in 11 biofilm and coinciding surface water samples from the South Fork Palouse River and tributaries to the river (Paradise Creek, Missouri Flat Creek, and Dry Fork Creek).
- Collect and analyze PFAS in 4 sediment samples coinciding with biofilm and surface water sample locations.
- Collect and analyze PFAS in one influent and effluent sample from the Pullman WWTP.
- Determine the presence and concentration ranges of total PFAS and 40 target analytes.
- Compare PFAS concentrations among sample locations and matrices to identify PFAS sources and pathways.
- Assess the effectiveness of using biofilms to measure and identify sources of PFAS in the environment.

Study Area

Our study was conducted in the South Fork Palouse River watershed in eastern Washington State (Figure 2). The river flows about 46 miles from its headwaters at Moscow Mountain in northern Idaho to its confluence with the Palouse River near Colfax, Washington. Tributaries to the South Fork Palouse River include Paradise Creek, Missouri Flat Creek, Four Mile Creek, Dry Fork Creek, and other smaller tributaries.

The watershed encompasses about 295 square miles, about 9% of the greater Palouse River watershed (Sinclair and Kardouni 2009). Land use in the watershed is predominantly agricultural, with major crops being wheat, peas, and lentils (Pelletier 1993). The major urban centers are Pullman, Washington, and Moscow, Idaho, with populations of about 33,000 and 25,000, respectively, based on 2020 U.S. census data.

The average annual precipitation is 15 – 25 inches (Snouwaert 2011), occurring mostly as rain or snow from November to April. The driest months are typically July and August (Sinclair and Kardouni 2009).

Three WWTPs discharge to the South Fork Palouse River watershed. The Pullman WWTP is a secondary treatment plant that discharges directly to the South Fork Palouse River downstream of its confluence with Missouri Flat Creek. The Moscow Water Reclamation and Reuse Facility is a tertiary treatment plant discharging to Paradise Creek about half a mile east of the Washington-Idaho border. During summer low flows from July to November, discharges from the Moscow treatment plant make up most of the flow in Paradise Creek. During this time, the Moscow and Pullman treatment plants make up most of the downstream flow in the South Fork Palouse River (Pelletier 1993). The Albion treatment plant in Albion also discharges to the South Fork Palouse River. Its discharges typically occur from January through May and make up a relatively small portion of the total river flow.

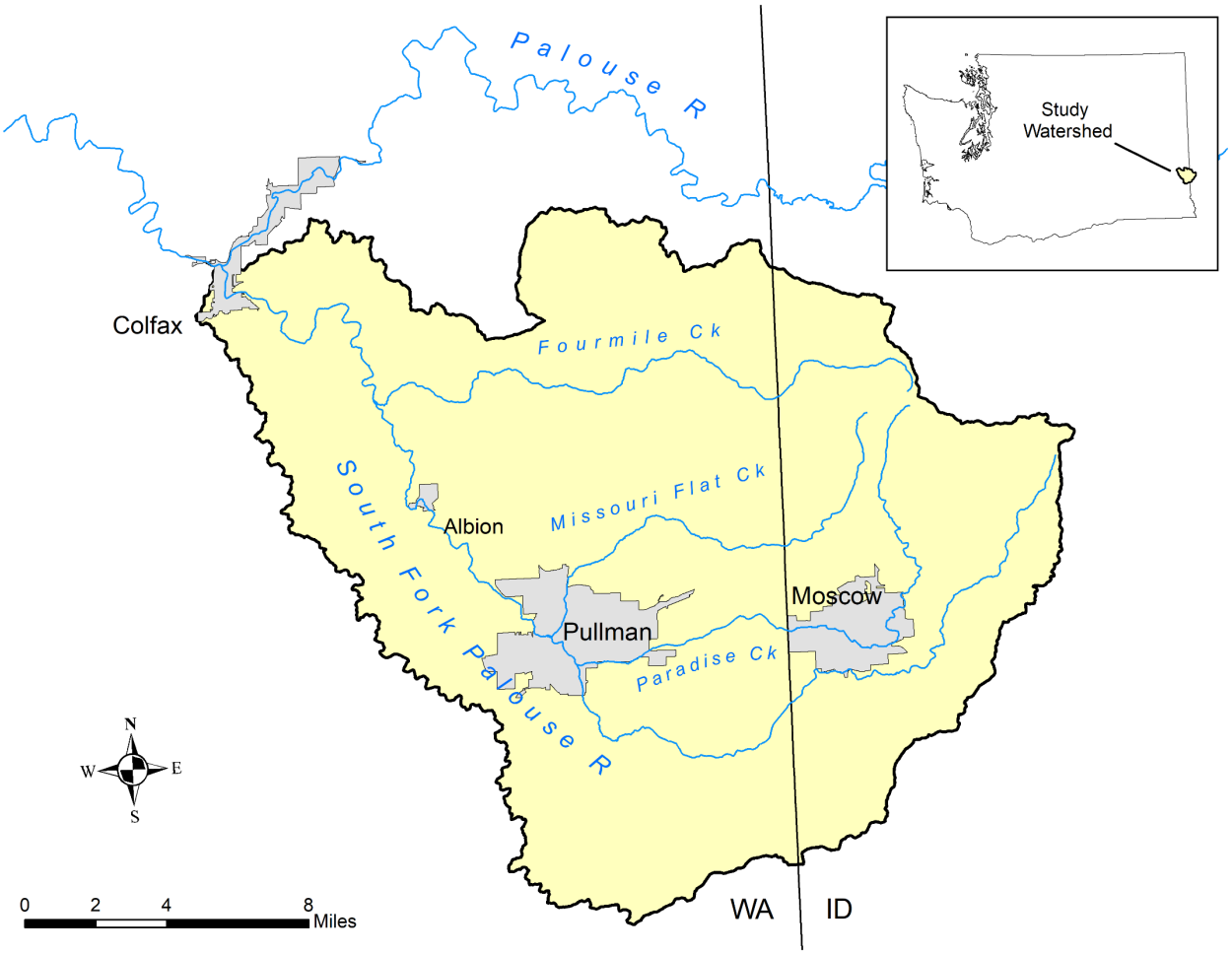

Figure 2. Overview map of study area.

Methods

All field methods used in this study followed Ecology's Environmental Assessment Program standard operating procedures and are detailed in the study's Quality Assurance Project Plan (QAPP) (Wong 2021). This section provides a summary of the methods.

Field Methods

Sampling Locations

Coinciding surface water and biofilm samples were collected during one event in August 2021 at 11 sites (Table 1). Seven sites were sampled on the South Fork Palouse River, ranging from upstream at the Washington-Idaho border to downstream in Colfax before the river empties into the Palouse River (Figure 3). Sites were selected based on river access and their positioning (upstream and downstream) relative to the Pullman WWTP and tributary confluences. South Fork Palouse River tributaries were also sampled (two sites in Paradise Creek, one in Missouri Flat Creek, and one in Dry Fork Creek).

Table 1. List of sampling sites and matrices collected at each site.

Location ID	General Sampling Location	Coordinates (WGS84)	Sample Matrix Collected
SFPR-Colfax	South Fork Palouse River, near confluence with the Palouse River in Colfax	46.88793, -117.36647	Water, Biofilm
SPFR-Shawnee	South Fork Palouse River, downstream of Albion	46.82696, -117.27481	Water, Biofilm
SFPR-Armstrong	South Fork Palouse River, between Albion and Pullman	46.76007, -117.22529	Water, Biofilm, Sediment
SFPR-Hayward	South Fork Palouse River, downstream of Pullman WWTP	46.739912, -117.191471	Water, Biofilm
SFPR-State Bridge	South Fork Palouse River, upstream of Pullman WWTP and downstream of Paradise Creek	46.732388, -117.181014	Water, Biofilm
SFPR-Bishop	South Fork Palouse River, upstream of Pullman WWTP and Paradise Creek	46.718876, -117.1654573	Water, Biofilm
SFPR-Stateline	South Fork Palouse River, upstream of Pullman WWTP and Paradise Creek	46.70060, -117.04147	Water, Biofilm, Sediment
PC-QI	Paradise Creek, near confluence with South Fork Palouse River	46.720558, -117.163572	Water, Biofilm, Sediment
PC-Stateline	Paradise Creek, near Washington-Idaho border	46.73239, -117.04694	Water, Biofilm
MF-State Bridge	Missouri Flat Creek, near confluence with South Fork Palouse River	46.73298, -117.18080	Water, Biofilm, Sediment
DF-Confluence	Dry Fork Creek, near confluence with South Fork Palouse River	46.731574, -117.179976	Water, Biofilm
Pullman WWTP-Influent	Pullman WWTP influent	46.73759, -117.18940	Water
Pullman WWTP-Effluent	Pullman WWTP effluent	46.73887, -117.19018	Water

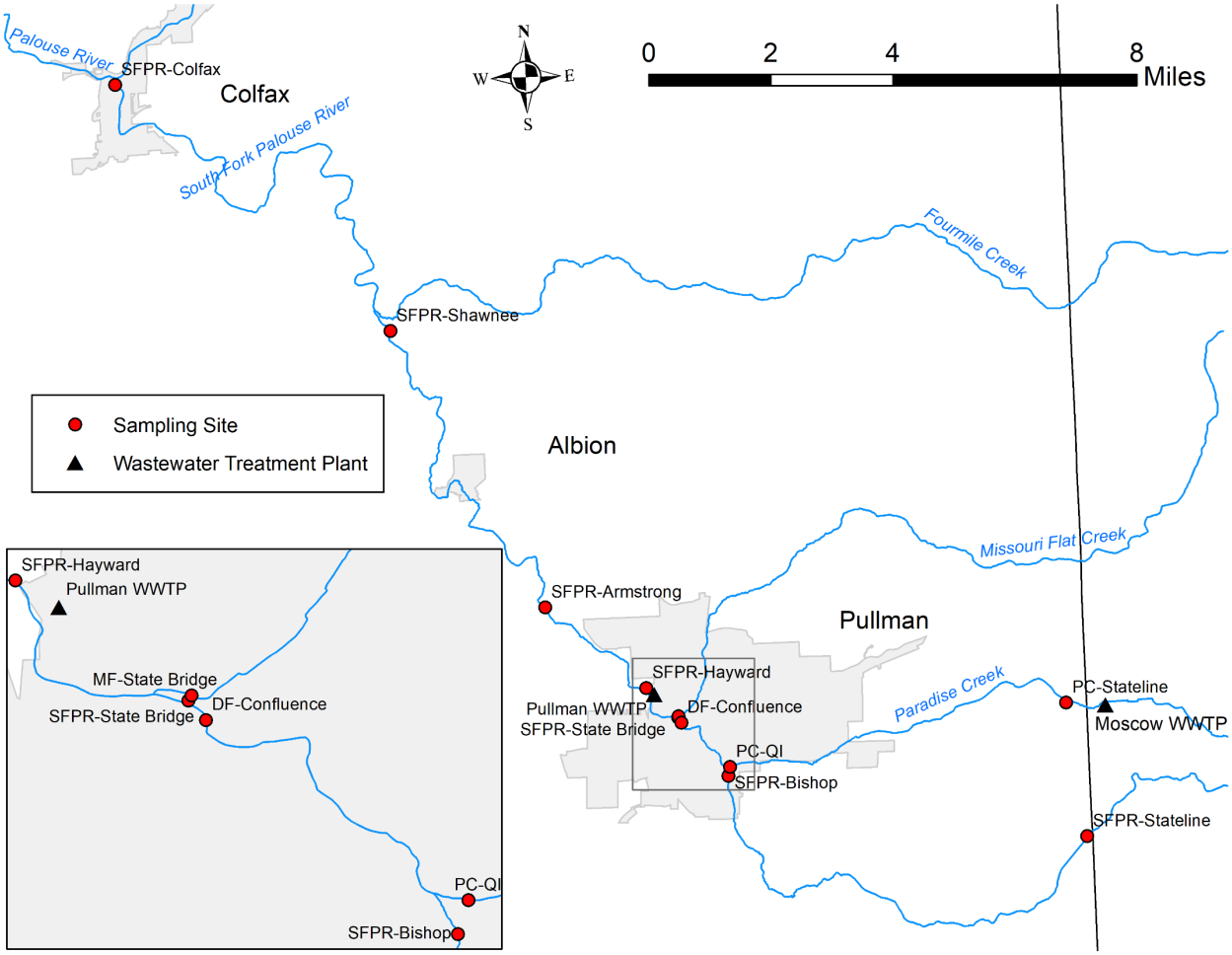

Figure 3. Map of sampling sites in the South Fork Palouse River watershed study area.

To characterize PFAS in sediment, samples were collected at a subset of 4 of the 11 sampling sites (two in the South Fork Palouse River, one in Paradise Creek, and one in Missouri Flat Creek).

One influent and effluent sample was also collected from the Pullman WWTP to characterize PFAS from the treatment plant and assess its potential influence on downstream surface water concentrations.

Sampling Procedures

Water, biofilm, and sediment samples were collected for analyses of PFAS (Table 1). Samples were also collected to analyze ancillary parameters, including total organic carbon (TOC) and dissolved organic carbon (DOC) in water; grain size and TOC in sediment; and ash-free dry weight and carbon and nitrogen isotopes in biofilm. Before field sampling, equipment used to collect biofilm and sediment samples was decontaminated using a methanol rinse.

Surface water grab samples were collected at each site about 15 – 30 cm below the water surface. Separate containers were filled for PFAS, TOC, and DOC analyses. Field duplicates for PFAS,

TOC, and DOC were collected by repeating the water sampling procedure. A calibrated YSI sonde was used to measure water temperature, dissolved oxygen, pH, and conductivity.

At each surface water site, cobbles containing an attached layer of brownish, flocculent biofilm were collected for biofilm sampling (Figure 4). Biofilm was scraped off each cobble and composited into a stainless steel bowl using a stainless steel blade. Excess water was carefully siphoned using a syringe. Composited biofilm was mixed using a stainless steel spoon and then scooped into separate containers for PFAS and carbon and nitrogen isotope analysis. A field duplicate was collected by splitting the composited biofilm into a second container for PFAS analysis.

To get a rough estimate of areal biomass, a separate sample of cobbles was collected and scraped for biofilm. For each cobble, the scraped surface area was estimated using aluminum foil cutouts, which were then digitized and processed using Image J software (Dudley et al. 2001). Biofilm from these cobbles was composited into a separate container and analyzed for ash-free dry weight. Areal biomass was calculated as Ash-Free Dry Weight (mg) / Surface Area (cm^2).

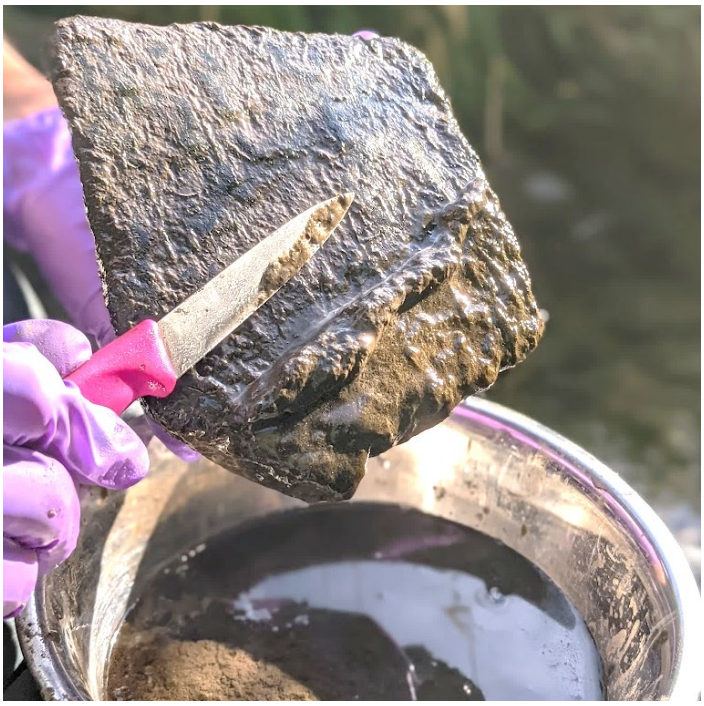

Figure 4. Biofilm scraped from a cobble during field sampling.

Sediment samples were collected using stainless steel scoops. The top 0 – 2 cm sediment was scooped from the river bed and composited into a stainless steel bowl. Excess water was carefully siphoned using a syringe. The composited sediment was then mixed and scooped into separate sample containers for PFAS, TOC, and grain size analyses. Field duplicates were collected by splitting the composited sediment into additional separate containers for PFAS, TOC, and grain size analyses.

Water samples from the Pullman WWTP were collected as a composite of subsamples in the morning and afternoon of the same day. The influent sample was collected on-site at the facility's post-solids screening sampling point. The effluent sample was collected at the facility's effluent discharge sampling point. Samples were collected for PFAS, TOC, and DOC analyses.

All water, biofilm, and sediment samples were stored in a cooler on ice until further processing at Ecology Headquarters. Biofilm and sediment samples were carefully decanted at Ecology Headquarters to remove excess water. PFAS samples were stored frozen at Ecology Headquarters before shipping to the laboratory.

Laboratory Methods

PFAS samples were shipped overnight to and analyzed by SGS AXYS Laboratory in Sydney, B.C., Canada. Samples were analyzed for 40 target PFAS analytes by liquid chromatography-tandem mass spectrometry with isotopic dilution following the laboratory's procedure at the time, *MLA-110 Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues, AFFF Products, Blood/Serums and Solvent Extracts by LC-MS/MS*. The MLA-110 procedure is essentially equivalent to draft Method EPA 1633, which was first published in August 2021 (EPA 2021) and has since undergone multiple drafts.

The following samples were sent to Manchester Environmental Laboratory in Port Orchard, WA, for analysis: TOC and DOC (water), TOC (sediment), and ash-free dry weight (biofilm). TOC and DOC samples were analyzed using SM5310B. TOC-sediment samples were analyzed using EPA 440.0. Ash-free dry-weight samples were analyzed using SM10300C.

ALS Environmental, Kelso, WA, analyzed the sediment grain size using a sieve-pipette method (PSEP 1986).

UC Santa Cruz Stable Isotope Laboratory analyzed carbon and nitrogen isotopes in the biofilm samples. Samples were analyzed using nanoEA-iRMS.

Data Reporting and Analysis

Concentrations of the 40 target PFAS analytes in an individual sample were summed to calculate the sample's total PFAS concentration. All detected analytes, including those qualified as J and NJ, were included in total PFAS calculations.

Scatterplots were used to explore the relationships between PFAS and ancillary parameters. Correlations between PFAS and ancillary parameters were tested using Pearson's correlation coefficient, with significance defined at $p<0.05$. Correlations were not tested for PFAS analytes with fewer than three detections in the given sample matrix.

Pearson correlation was used to test the relationship between PFAS concentration in water versus corresponding biofilm samples, with significance defined at $p<0.05$. Analytes with fewer than

three detections in both corresponding matrices were not tested. Seven analytes had three or more detections in both corresponding water and biofilm samples and were therefore tested: MeFOSAA, PFDA, PFHxA, PFHxS, PFOA, PFOS, and PFPeA.

Quality Assurance/Quality Control

An independent data validator at Manchester Environmental Laboratory validated the PFAS data for this study using a stage 4 data validation following Table B-15 in DoD and DoE (2019).. The data validator provided a detailed description of data quality in the form of a written case narrative. This included information on laboratory quality control checks and an assessment of results compared to the study's measurement quality objectives.

Appendix A summarizes the data quality results based on this study's measurement quality objectives, including data qualifications. Overall, data from this study were deemed acceptable for use, with some qualifications. A description of some of the qualified results is provided below.

Several results were qualified as non-detect (U-qualified) due to method blank contamination (Detected Analyte Result < 5 x Detected Method Blank Result). These included:

- 6:2 FTS in 2 sediment samples
- PFHxA in 4 sediment samples
- PFHpA in 3 biofilm samples
- PFNA in 10 biofilm samples
- PFOSA in 3 biofilm samples
- PFTrDA in 1 biofilm sample
- PFUnA in 7 biofilm samples

Although PFAS analytes were detected in the method blank (with lingcod as the laboratory reference matrix), all method blank results met the QAPP criterion (< ½ limit of quantitation). Additionally, the blank-qualified sample result values were relatively low, between the sample detection and reporting limits, and therefore qualified J as estimated values. Qualifications due to method blank contamination are likely due to a combination of background levels in the method blank and low levels in the sample results.

Seven of 13 water samples had low surrogate recovery (<50%) for PFBA. However, PFBA surrogate recoveries were within the quality control acceptance criteria range for aqueous matrices (5% – 130%) in the most recent draft of EPA 1633 (EPA 2023). The draft method notes that surrogate recovery for PFBA can be problematic in some field samples. Results in samples for this study were qualified as estimates (J-qualified) with potential low bias.

Several sediment samples had low surrogate recoveries (<50%) for the following analytes: MeFOSA, EtFOSA, MeFOSE, and EtFOSE. However, these results were mostly within the range of analyte recoveries derived from the method's single-laboratory validation study (EPA

2021). Nonetheless, the results of these samples were qualified as estimates (J-qualified) with a potential low bias.

The Pullman WWTP-Influent sample (Sample ID 2108067-23) had low surrogate recoveries for several analytes: PFBA, PFDoA, PFTeDA, 8:2 FTS, MeFOSA, EtFOSA, MeFOSAA, EtFOSAA, MeFOSE, and EtFOSE. Dilution and reanalysis yielded similar results, indicating matrix interferences.

Field and laboratory duplicates were collected and analyzed for the biofilm sample from SFPR-State Bridge (Sample ID 2108067-05). The relative percent differences for PFPeA and PFHxA were over 100%, above quality control limits for this study. Reanalysis of the sample and laboratory duplicate yielded similar results. The high RPD in both the field and lab dups could be due to relatively low concentration in the samples (non-detect – 1.51 ng/g for PFHxA and non-detect – 0.368 ng/g for PFPeA) or natural variability in the biofilm.

A matrix spike/matrix spike duplicate was also analyzed for the SFPR-State Bridge biofilm sample. PFHxA was recovered below quality control limits at 38.3% and 30.9% in the matrix spike and matrix spike duplicate samples, respectively, indicating possible matrix interferences. PFPeA and PFHxA from this sample were qualified as estimates (J-qualified) due to quality control results, and the results for PFPeA and PFHxA in the SFPR-State Bridge biofilm sample are interpreted with caution.

Results

PFAS were detected in all 28 water, biofilm, and sediment samples collected during this study. A summary of total PFAS concentration by sample matrix is provided in Table 2. The sections below describe the results by sample matrix.

Table 2. Summary statistics for total per- and polyfluoroalkyl substance (PFAS) concentrations in water, biofilm, and sediment samples collected during this study.

Matrix	Minimum	Maximum	Mean
Surface Water (ng/L), n=11	13.4	118	74.3
Biofilm (ng/g), n=11	0.11	3.6	1.6
Sediment, (ng/g), n=4	0.27	5.4	2.8
Pullman WWTP Influent (ng/L), n=1	—	—	27.6
Pullman WWTP Effluent (ng/L), n=1	—	—	76.0

WWTP = wastewater treatment plant.

Water Samples

In the surface water, total PFAS concentrations were 13.4 – 118 ng/L (Table 2). The lowest total PFAS concentrations were observed at SFPR-Stateline and SPFR-Bishop, the two upstream-most sites on the South Fork Palouse River (Figure 5). The highest total PFAS concentration was observed at SFPR-Colfax, the downstream-most site.

Total PFAS concentrations generally increased from upstream to downstream on the South Fork Palouse River. A large (340%) increase occurred between SFPR-Bishop and SFPR-State Bridge, located just downstream of the confluence with Paradise Creek. Both upstream and downstream sites on Paradise Creek (PC-Stateline and PC-QI) had among the highest PFAS concentrations in this study.

The most frequently detected analytes in the water samples were PFAAs. Six PFAA analytes were detected in 100% of the water samples: PFBA, PFPeA, PFHxA, PFOA, PFBS, and PFOS (Table 3). Short-chain PFCAs were the most frequently detected analytes and comprised a greater proportion of the total PFAS than other PFAS groups in the water samples (Figure 6). Twenty-three of 40 target PFAS analytes were not detected in any water samples, including several long-chain PFCAs (C12 – C14) and PFSAs (C9 – C11), ether-based PFAS, and several PFOS precursor and intermediary compounds (sulfonamides).

At the Pullman WWTP, the total PFAS concentration in the effluent sample was more than two times greater than in the influent sample (Table 2). Concentrations of almost all detected PFAS analytes increased from influent to effluent, except PFOS, which decreased by about 53%

(Figure 7). Most noticeably, the concentration of PFPeA increased by more than 10 times from influent to effluent. Concentrations of PFHxA and PFOA increased by more than 4 times from influent to effluent. In both influent and effluent samples, the concentration of 5:3 FTCA was about 7 ng/L; 5:3 FTCA was not detected in the surface water samples.

Downstream of the Pullman WWTP, total PFAS concentration remained about 76 ng/L from SFPR-Hayward to SFPR-Armstrong — similar to the Pullman WWTP effluent — then steadily increased towards Colfax (Figure 5). The analyte profile of PFAS samples collected downstream from the Pullman WWTP closely resembled that of the effluent sample (Figure 6). In particular, the dominant analyte was PFPeA, comprising 35% – 49% of the total PFAS in these samples. This was followed by PFHxA, which comprised 14% – 25% of the total PFAS. In contrast, PFPeA and PFHxA were less dominant upstream from the Pullman WWTP, comprising 9% – 19% and 5% – 11% of the total PFAS, respectively.

Biofilm Samples

Total PFAS concentrations in the biofilm were 0.11 – 3.6 ng/g (Table 2). The highest total PFAS concentration in biofilm was observed in Missouri Flat Creek (MF-State Bridge; Figure 5). Total PFAS concentrations in biofilm were lowest at the two most upstream sites on the South Fork Palouse River (SFPR-Stateline and SPFR-Bishop). Like water samples, a large (840%) increase in total PFAS occurred between SPFR-Bishop and SFPR-State Bridge. In general, total PFAS concentrations in biofilm increased along the river from the Washington-Idaho border, plateaued at SFPR-State Bridge, and then declined further downstream towards Colfax.

Twenty-seven of 40 target PFAS analytes were not detected in any biofilm samples. Of the biofilm results with detections, about 91% were found between the sample detection and reporting limits and, therefore, qualified J as estimated values.

Unlike the water samples, long-chain PFSAs (primarily PFOS) and long-chain PFCAs made up a greater proportion of the total PFAS in the biofilm samples (Figure 8). PFOS was the only analyte detected in 100% of the biofilm samples. Short-chain PFAAs made up a small fraction of the total PFAS in biofilm, except for the sample from SFPR-State Bridge (see Quality Assurance/Quality Control section).

Analytes from the fluorotelomer group were detected in three biofilm samples. 6:2 FTS was the dominant analyte in the Missouri Flat Creek sample (MF-State Bridge; Figure 8). 5:3 FTCA was the dominant analyte in biofilm at SFPR-Hayward, just downstream of the Pullman WWTP.

MeFOSAA was the only analyte from the sulfonamide group detected in biofilm (Figure 8). However, five of the eight biofilm samples with detections were NJ-qualified (tentatively identified based on mass-ion ratio outliers).

PFMBA (an ether-based PFAS) was also detected in the biofilm samples but not in the corresponding water samples. In the biofilm, PFMBA concentrations were generally low (non-detect to 0.46 ng/g), and 8 of 9 detected results were J-flagged as estimated values.

A significant correlation was not found between total PFAS concentration in water versus corresponding biofilm samples (Figure 10; $p>0.05$). However, significant correlations were found between concentrations of specific detected PFAS analytes (PFOS, PFOA, and PFDA) in water versus corresponding biofilm samples ($p<0.05$, R=0.69, 0.63, and 0.71, respectively).

Sediment Samples

Total PFAS concentrations in the four sediment samples were 0.27 – 5.4 ng/g (Table 2). Six analytes (PFHpA, PFOA, PFNA, PFUnA, PFHxS, and PFOS) were detected in all four samples (Table 3). Twenty of 40 analytes were not detected in the sediment samples, including PFBA and PFHxA, which were detected in 100% of the water samples.

Like the biofilm samples, sediment samples had a higher proportion of long-chain PFAAs and a small fraction of short-chain PFAAs (Figure 9). PFOS was the dominant analyte in three sediment samples (Figure 9). Analytes from the sulfonamide group were more frequently detected in the sediments compared to biofilm and water.

Ancillary Parameters

Results for ancillary parameters are provided in Appendices B – E.

In water samples, TOC and DOC were correlated with PFOS ($p<0.05$; R=0.60 and R=0.70, respectively) (Figure E1). DOC was also correlated with PFBA (R=0.69), PFNA (R=0.59), and PFBS (R=0.52) (Figures E1 and E2).

Sediment TOC was significantly correlated ($p<0.05$) with several long-chain PFCAs: PFDoA (R= 0.90), PFTrDA (R=0.94), and PFTeDA (R=0.95) (Figure E3). Sediment TOC was also correlated with PFBS (R=0.97; Figure E4).

Correlations between PFAS analytes and other ancillary parameters (temperature, dissolved oxygen, pH, conductivity, C:N ratio, and areal biomass) were generally not found.

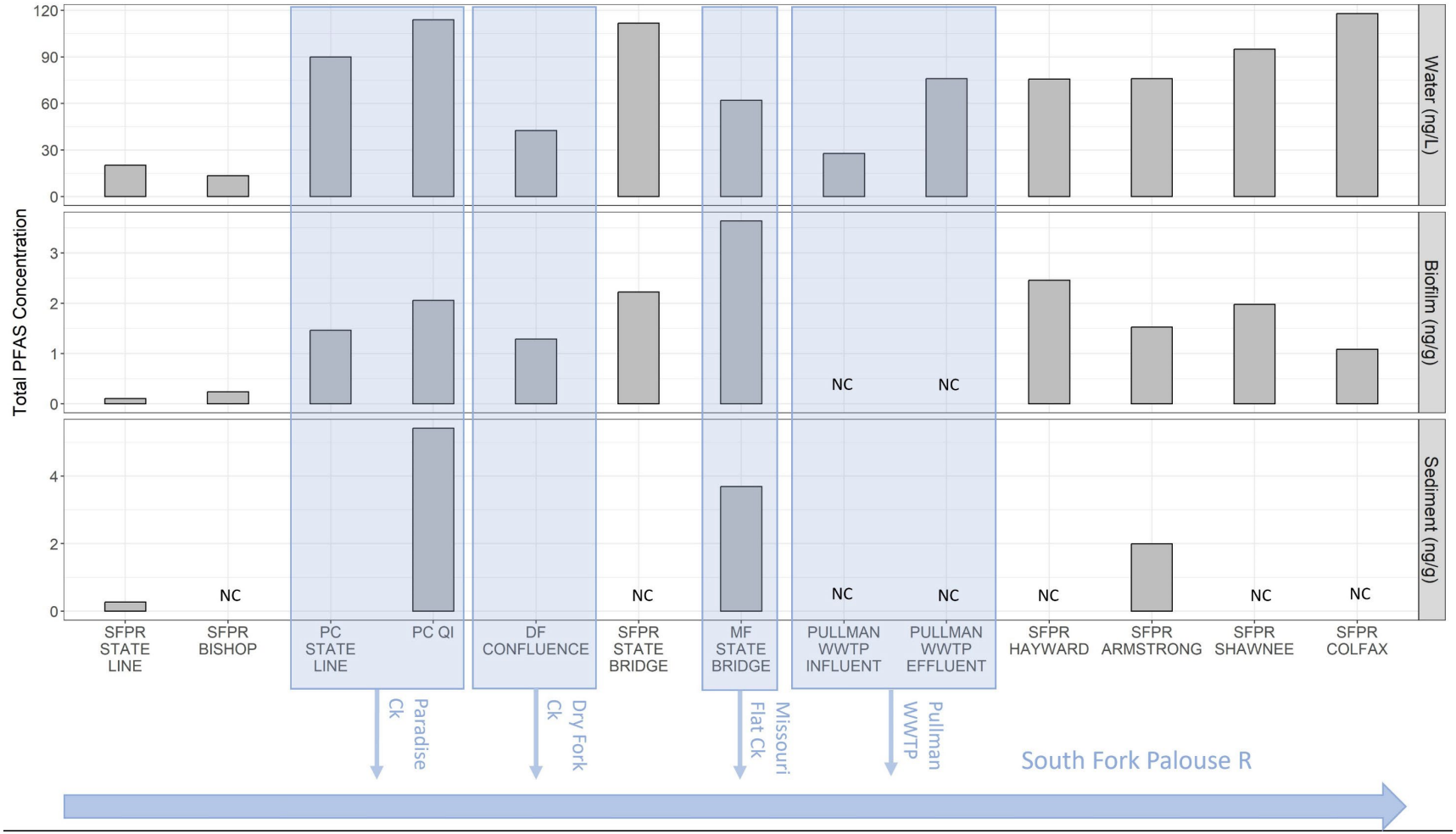

Figure 5. Total per- and polyfluoroalkyl substance (PFAS) concentrations in water, biofilm, and sediment samples collected at each site.

Blue-shaded sites represent discharges to the South Fork Palouse River from upstream (left) to downstream (right). “NC” denotes sample not collected.

Table 3. Detection frequencies of 40 target per- and polyfluoroalkyl substance (PFAS) analytes in water, biofilm, and sediment samples collected during this study.

PFAS Category	PFAS Analyte	CAS Number	Water (n=13) %	Biofilm (n=11) %	Sediment (n=4) %
PFCA	PFBA (C4)	375-22-4	100	0	0
PFCA	PFPeA (C5)	2706-90-3	100	27	75
PFCA	PFHxA (C6)	307-24-4	100	64	0
PFCA	PFHpA (C7)	375-85-9	92	0	100
PFCA	PFOA (C8)	335-67-1	100	82	100
PFCA	PFNA (C9)	375-95-1	92	0	100
PFCA	PFDA (C10)	335-76-2	92	82	75
PFCA	PFUnA (C11)	2058-94-8	8	0	100
PFCA	PFDoA (C12)	307-55-1	0	91	75
PFCA	PFTrDA (C13)	72629-94-8	0	0	75
PFCA	PFTeDA (C14)	376-06-7	0	18	75
PFSA	PFBS (C4)	375-73-5	100	0	75
PFSA	PFPeS (C5)	2706-91-4	15	0	0
PFSA	PFHxS (C6)	355-46-4	92	36	100
PFSA	PFHpS (C7)	375-92-8	0	0	0
PFSA	PFOS (C8)	1763-23-1	100	100	100
PFSA	PFNS (C9)	68259-12-1	0	0	0
PFSA	PFDS (C10)	335-77-3	0	27	75
PFSA	PFDoS (C12)	79780-39-5	0	0	0
Fluorotelomer	4:2 FTS	757124-72-4	0	0	0
Fluorotelomer	6:2 FTS	27619-97-2	23	18	0
Fluorotelomer	8:2 FTS	39108-34-4	0	0	25
Fluorotelomer	3:3 FTCA	356-02-5	0	0	0
Fluorotelomer	7:3 FTCA	914637-49-3	0	0	0
Fluorotelomer	5:3 FTCA	812-70-4	15	9	0

PFAS Category	PFAS Analyte	CAS Number	Water (n=13) %	Biofilm (n=11) %	Sediment (n=4) %
Sulfonamide	PFOSA	754-91-6	69	0	75
Sulfonamide	N-MeFOSA	31506-32-8	0	0	50
Sulfonamide	N-EtFOSA	4151-50-2	0	0	0
Sulfonamide	MeFOSAA	2355-31-9	31	73	75
Sulfonamide	EtFOSAA	2991-50-6	15	0	75
Sulfonamide	N-MeFOSE	24448-09-7	0	0	25
Sulfonamide	N-EtFOSE	1691-99-2	0	0	25
Ether	11Cl-PF3OUdS	763051-92-9	0	0	0
Ether	9Cl-PF3ONS	756426-58-1	0	0	0
Ether	ADONA	919005-14-4	0	0	0
Ether	HFPO-DA (GenX)	13252-13-6	0	0	0
Ether	NFDHA	151772-58-6	0	0	0
Ether	PFEESA	113507-82-7	0	0	0
Ether	PFMBA	863090-89-5	0	82	0
Ether	PFMPA	377-73-1	0	0	0

Note. Detection frequency is expressed as % of samples with detections.
CAS = Chemical Abstracts Services
PFCA = Perfluoroalkyl carboxylic acid
PFSA = Perfluoroalkane sulfonic acid

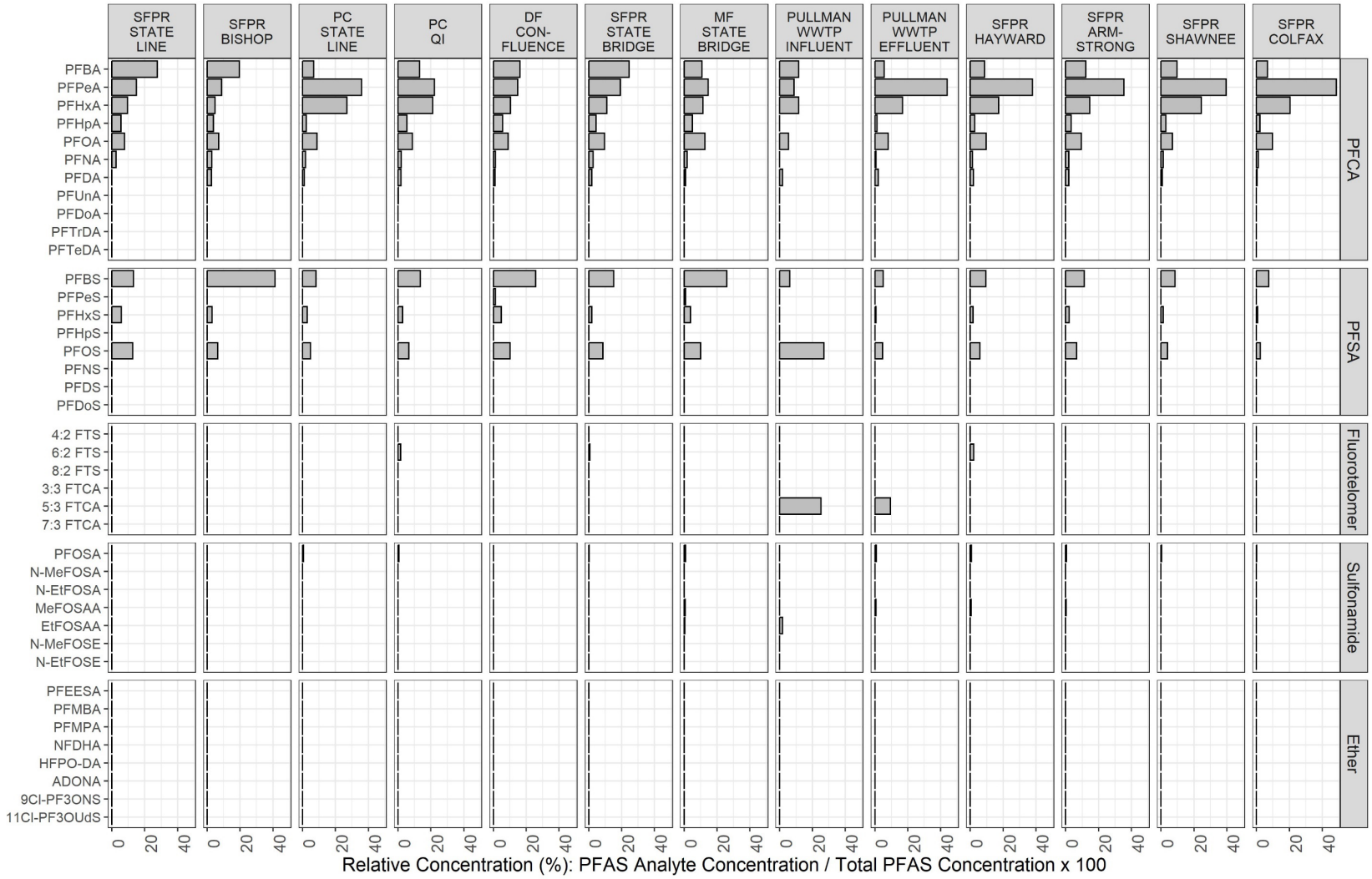

Figure 6. Concentration of per- and polyfluoroalkyl substance (PFAS) analytes relative to the total PFAS in water samples collected from each site.

PFCA = Perfluoroalkyl carboxylic acid; PFSA = Perfluoroalkane sulfonic acid.

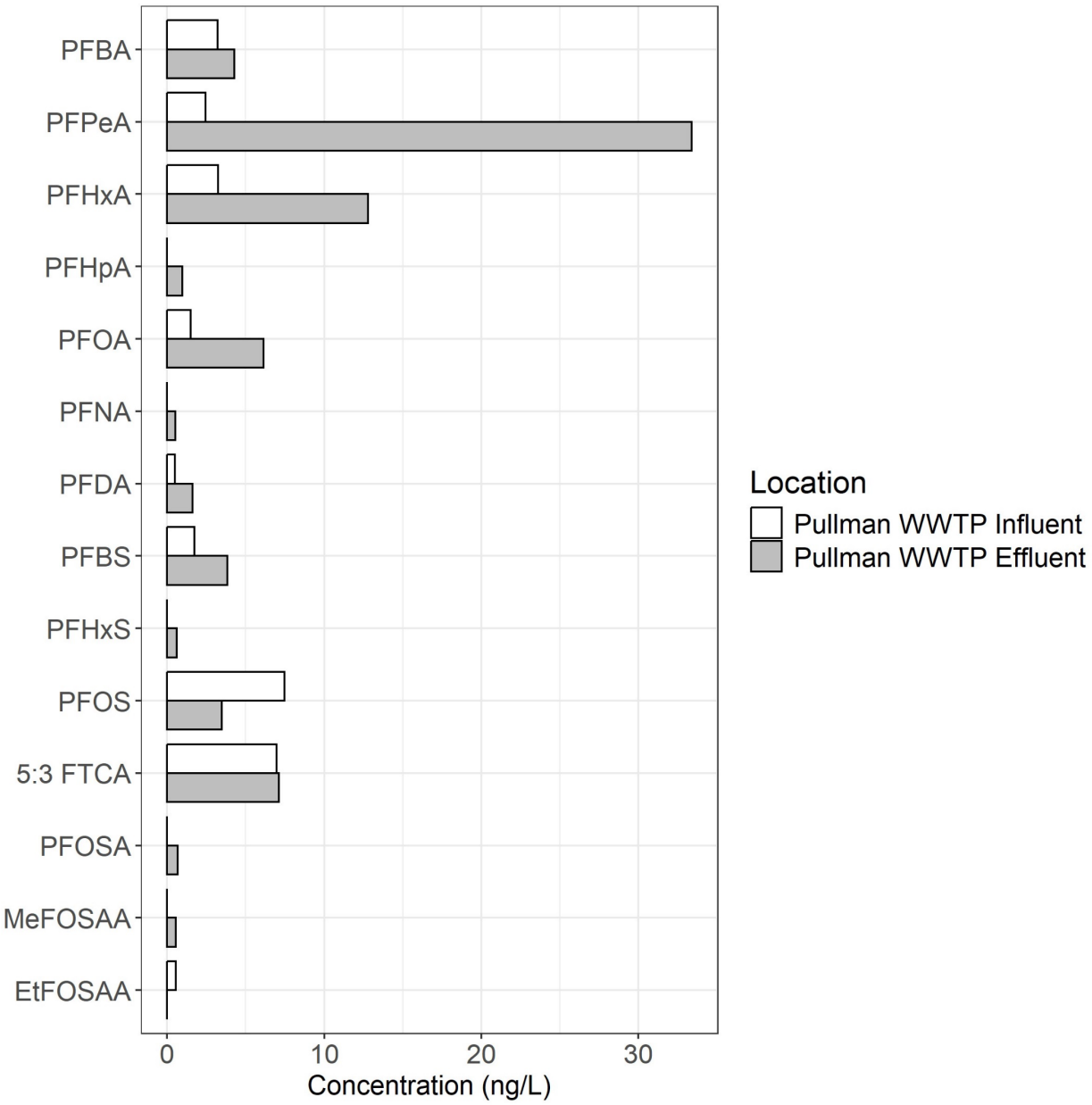

Figure 7. Concentrations of detected per- and polyfluoroalkyl substance (PFAS) analytes in influent and effluent samples collected from the Pullman Wastewater Treatment Plant (WWTP).

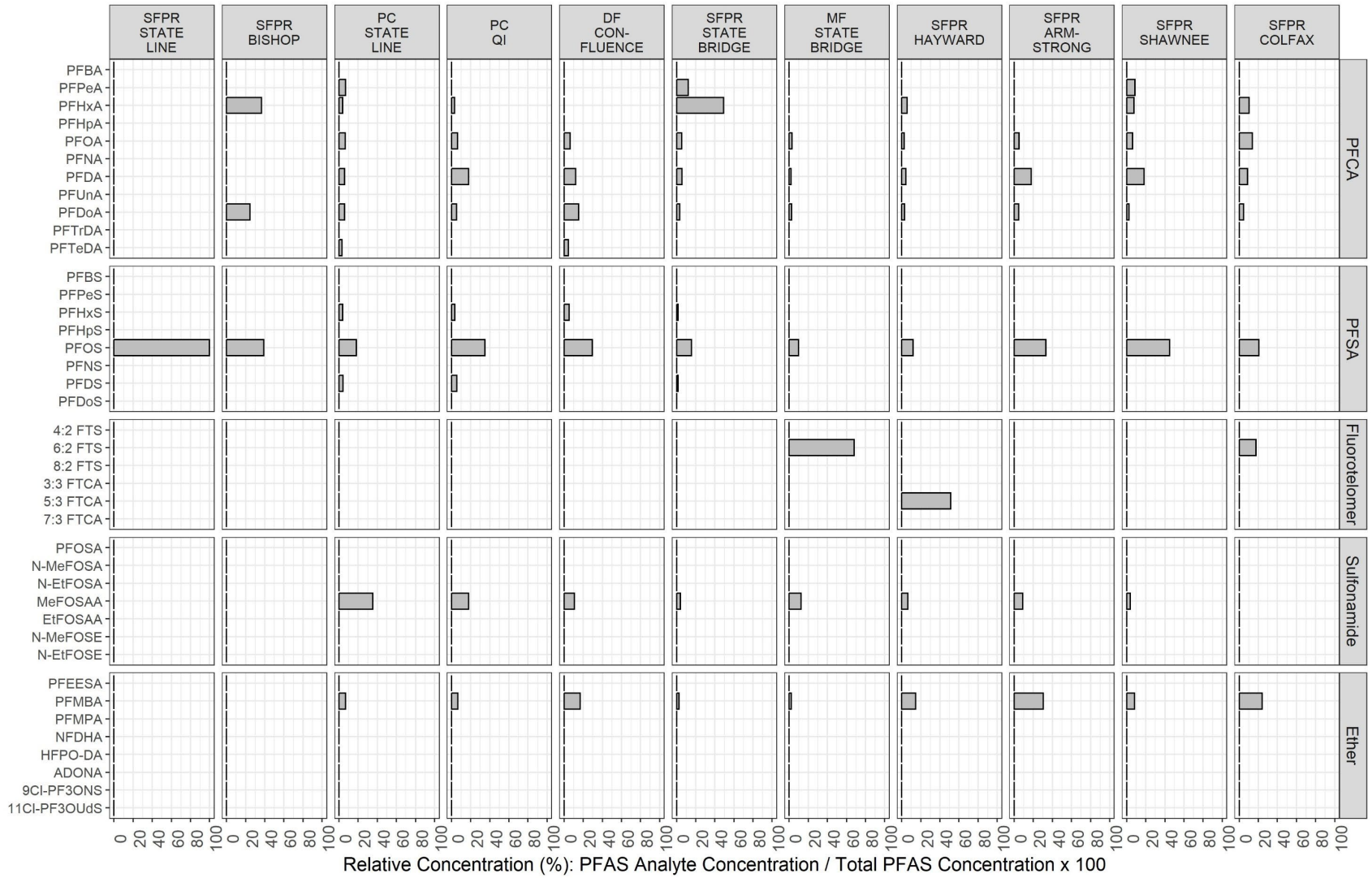

Figure 8. Concentration of per- and polyfluoroalkyl substance (PFAS) analytes relative to the total PFAS in biofilm samples collected from each site.

PFCA = Perfluoroalkyl carboxylic acid; PFSA = Perfluoroalkane sulfonic acid.

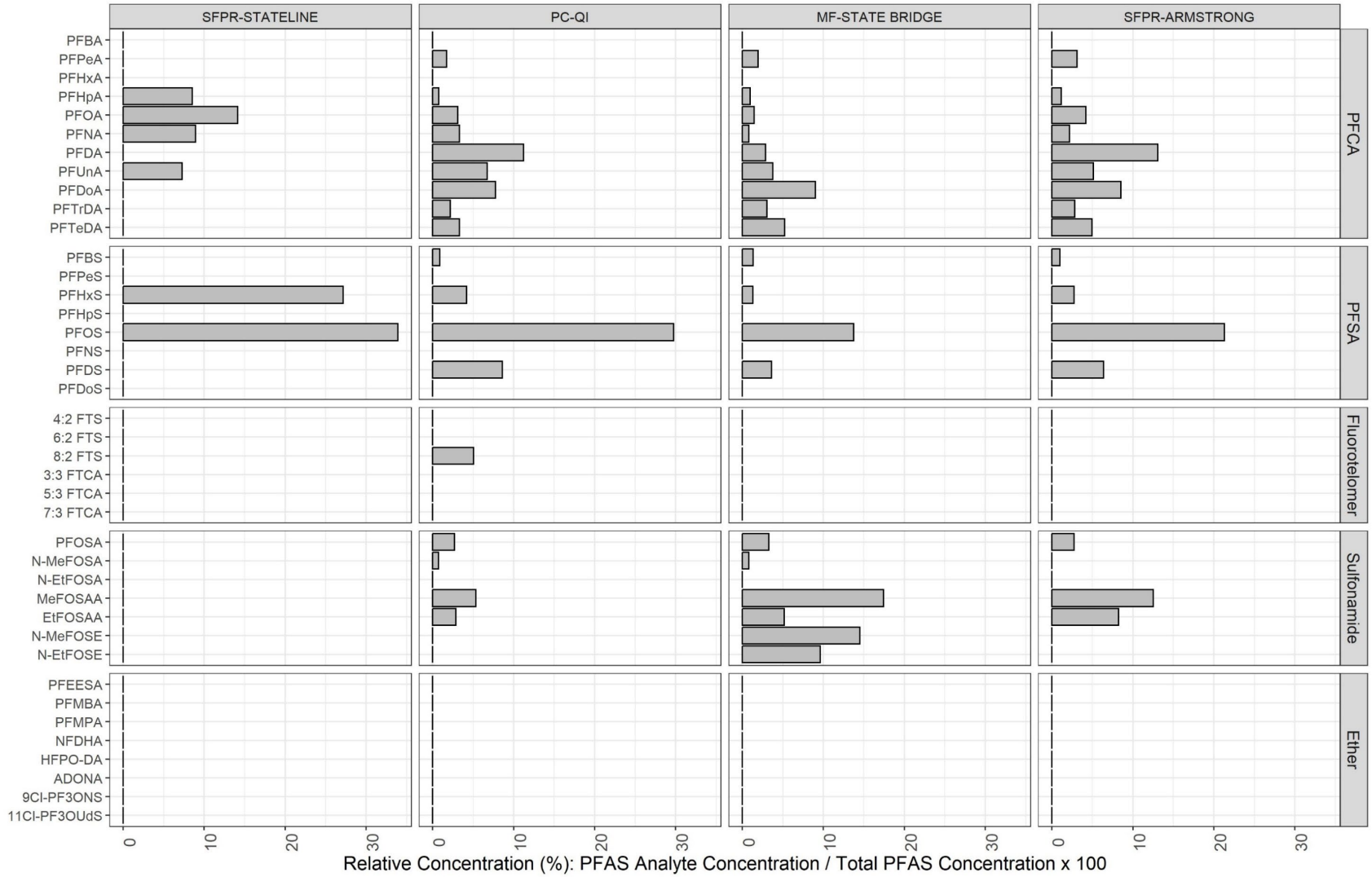

Figure 9. Concentration of per- and polyfluoroalkyl substance (PFAS) analytes relative to the total PFAS in sediment samples collected from each site.

PFCA = Perfluoroalkyl carboxylic acid; PFSA = Perfluoroalkane sulfonic acid.

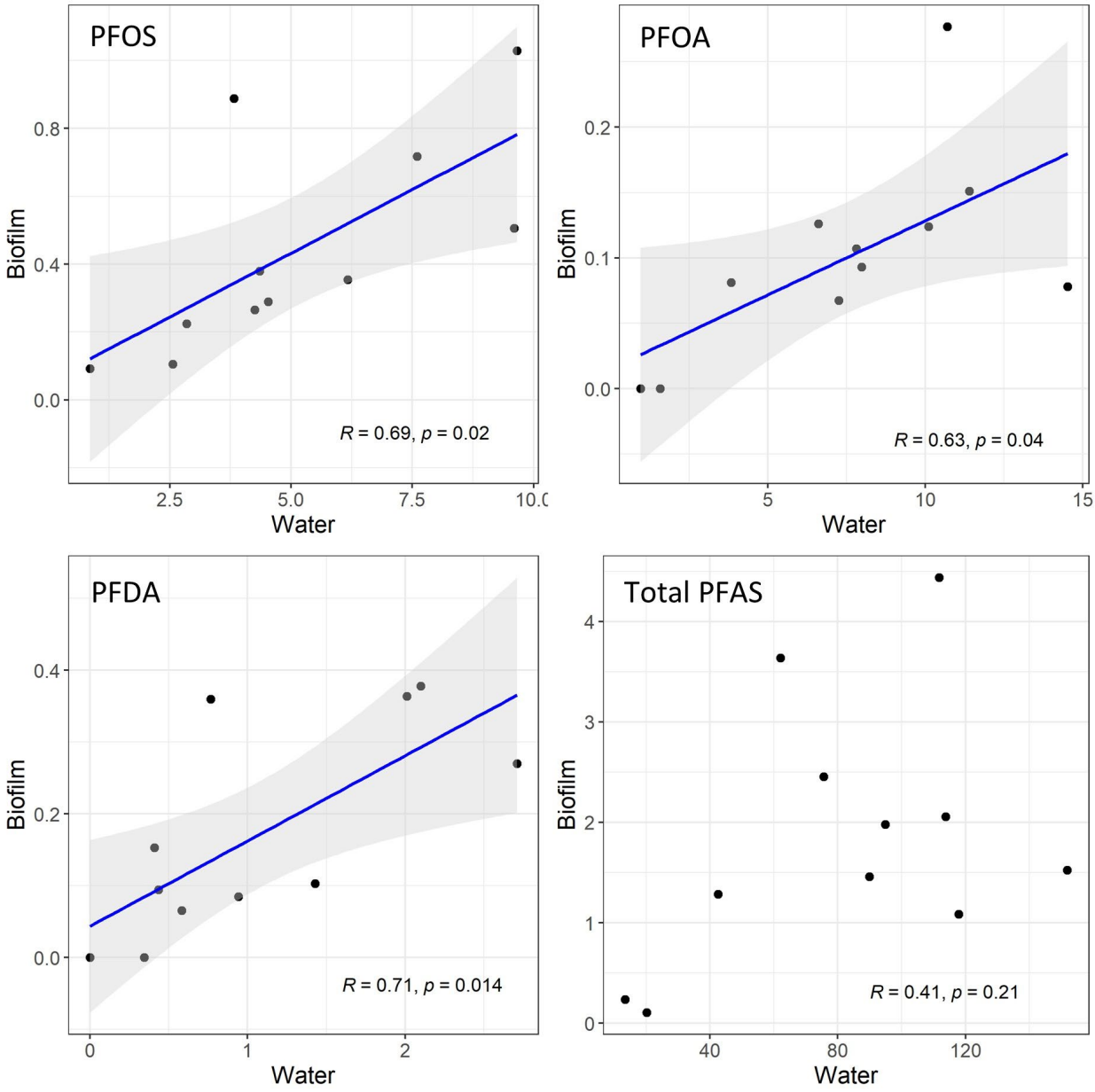

Figure 10. Scatterplots of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), and total per- and polyfluoroalkyl substance (PFAS) concentrations in water versus biofilm samples.
The correlation coefficient (R), p-value, and 95% confidence interval (gray) for each Pearson correlation test are shown.

Discussion

The range in total PFAS concentration in surface water samples from this study (13.4 – 118 ng/L) fit within the range of 7.36 – 170 ng/L found in a 2016 survey of 15 water bodies throughout Washington (Mathieu and McCall 2017). The lowest concentrations (13.4 – 20.1 ng/L) were found upstream of SFPR-Bishop, while the highest (75.6 – 118 ng/L) were found downstream of SFPR-Bishop. These results indicate the presence of major PFAS sources to the South Fork Palouse River downstream of SFPR-Bishop. Data from this study suggest at least three conveyances to the river downstream of SFPR-Bishop: Paradise Creek, the Pullman WWTP, and Missouri Flat Creek.

Influence of Wastewater Treatment Plants

The influence of WWTPs on PFAS concentrations in the South Fork Palouse River was evident in this study. The biggest increase in total PFAS concentration in the river occurs between SFPR-Bishop and SPFR-State Bridge (Figure 5). Paradise Creek is the main conduit to the river between these two sites. Both upstream and downstream sites in Paradise Creek had among the highest total PFAS concentrations in surface water samples, indicating that there are PFAS sources to Paradise Creek upstream of the Washington-Idaho border. The Moscow treatment plant, which discharges to Paradise Creek just east of the border and makes up most of its flow during summer (Pelletier 1993), likely provided a major pathway of PFAS to the creek and the South Fork Palouse River.

Effects from the Pullman WWTP were also evident. The total PFAS concentration in Pullman WWTP's effluent was 76.0 ng/L, which fit within the range of 42.1 – 125 ng/L found in effluent samples previously collected from five different WWTPs in Washington (Mathieu and McCall 2017).

Total PFAS concentration in the effluent was higher than in the influent (27.6 ng/L) and was attributed mostly to increases in PFCAs — most noticeably PFPeA, PFHxA, and PFOA (Figure 7). Increases in PFAS from influent to effluent, particularly for PFCAs, have been reported in other WWTP studies and attributed largely to the degradation of PFAS precursor compounds such as fluorotelomer sulfonates and fluorotelomer alcohols to PFCAs (Rodriguez-Jorquera et al. 2016; Coggan et al. 2019; Kim et al. 2022; Thompson et al. 2022; Moneta et al. 2023). In this study, increases in PFCAs from the treatment plant, especially PFPeA and PFHxA, are reflected in the surface water downstream from the Pullman WWTP (Figure 6).

While PFCAs increased from influent to effluent at the Pullman WWTP, the analyte PFOS decreased. The tendency for PFOS and other long-chain PFSAs to decrease or experience no change following treatment has been found in other studies and was primarily attributed to sorption onto sludge during the wastewater treatment process (Schultz et al. 2006; Guo et al. 2010; Bothfeld and Mathieu 2022; Kim et al. 2022; Thompson et al. 2022). This was likely the case with the Pullman WWTP, an activated sludge facility. Ancillary data support this

explanation, as the current study found a correlation between TOC and PFOS in water, and water sample collection at the Pullman WWTP showed a 92% removal of TOC from influent to effluent (Appendix B).

Another component of PFAS in the Pullman WWTP samples was 5:3 FTCA, which is commonly found in landfill leachate (Lang et al. 2017). Its presence in downstream biofilm suggests effects from the effluent, although it was not detected in downstream surface water.

Other Possible Sources

Results from the study indicate additional sources of PFAS to the South Fork Palouse River that were not influenced by WWTP discharges. The Missouri Flat Creek site (MF-State Bridge) had the highest total PFAS concentration in biofilm. This was represented mostly by the fluorotelomer, 6:2 FTS, a precursor of short-chain PFCAs. 6:2 FTS is an alternative PFAS compound used as a co-formulant in aqueous film-forming foams, as a chrome mist suppressant in the electroplating industry, and as a processing aid in the synthesis of fluoropolymers (Lu et al. 2017). The presence and magnitude of 6:2 FTS in the biofilm at this site differed from all other biofilm samples and may indicate a source to Missouri Flat Creek further upstream.

Dry Fork Creek represented another conduit of PFAS to the South Fork Palouse River, with surface water concentrations two to three times higher than concentrations in the river further upstream. However, field notes indicated that surface water levels in Dry Creek were near dry, so flows to the river were likely minimal during this time of year.

Other potential conduits include small tributaries within the watershed that were not sampled in this study. Contaminant pathways such as stormwater, urban runoff, and atmospheric deposition have also been shown to be important in the fate and transport of PFAS to receiving water bodies (e.g., Kim and Kannan 2007; Xiao et al. 2012; Shimizu et al. 2021). However, these were not investigated in the current study.

Matrix Comparisons

Analyte profiles in surface water, biofilm, and sediment samples collected during this study demonstrated that PFAS analytes partition differently in the different environmental matrices. Biofilm and sediment samples were composed mostly of long-chain PFSAs (primarily PFOS) and long-chain PFCAs, whereas surface water samples were composed mostly of short-chain PFCAs (Figures 6, 8, and 9). This finding is like previous studies that found higher sorption of long-chain PFAS in biofilm and sediments and greater partitioning of more-soluble, short-chain PFCAs in water (Qi et al. 2016; Munoz et al. 2018; Langberg et al. 2020; Zhang et al. 2022).

The only analyte detected in 100% of water, biofilm, and sediment samples was PFOS. Its pervasiveness in the environment and continued presence in the wastewater stream (treatment plant influent and effluent) suggest that this legacy contaminant continues to be problematic in the watershed despite being voluntarily phased out of production in the U.S. since 2002.

Sulfonamides were more frequently detected and at higher relative concentrations in sediments than in surface water or biofilm. This group of PFOS precursor chemicals has been associated with their use as surface treatment applications in the carpet, textile, paper, and packaging industries (Lee and Mabury 2011). Like long-chain PFAS, the propensity of sulfonamides to sorb onto sediments is likely due to their greater hydrophobicity (Langberg et al. 2020).

The polyfluoroalkyl ether, PFMBA (perfluoro-4-methoxybutanoic acid, often referred to as "PFMOBA"), was detected in 9 of 11 biofilm samples — with the exception being the two upstream most sites on the South Fork Palouse River. However, it was not detected in any water or sediment samples. PFMBA is a byproduct of GenX processing aids used in fluoropolymer manufacturing (e.g., plastics and adhesives; Woodlief et al. 2021) and has been reported in samples from other PFAS research and monitoring groups (Sun et al. 2016). Because of its low levels in the biofilm and lack of detection in water and sediments, the significance of its presence in biofilm samples from this study is uncertain.

Biofilms as a PFAS Monitoring & Source Tracing Tool

To some extent, biofilms were useful as a monitoring and source tracing tool for PFAS in the environment. Below is a discussion of where they were effective and ineffective.

Results from the study suggest at least three conveyances of PFAS to the South Fork Palouse River: Paradise Creek, the Pullman WWTP, and Missouri Flat Creek. The biofilms effectively picked up Paradise Creek as a conveyance to the river. Both surface water and biofilm showed a large increase in total PFAS between SFPR-Bishop and SFPR-State Bridge. Both surface water and biofilm also showed relatively high total PFAS concentrations in Paradise Creek.

Both surface water and biofilm results indicated effects from the Pullman WWTP, although in different ways. In downstream surface water samples, a primary indicator was an analyte profile resembling that of the effluent. This particular signature was dominated by PFPeA and PFHxA, which differed from upstream water samples. This signature was not evident in the biofilm samples, perhaps because of the tendency of short-chain PFCAs to prefer water. Another signature from the treatment plant, 5:3 FTCA, was evident in the downstream biofilm: The only detections of 5:3 FTCA in this study came from the treatment plant effluent and downstream biofilm.

Both surface water and biofilm results suggest Missouri Flat Creek, which is not affected by WWTP effluent, as a conveyance to the river. While 6:2 FTS was a major component of total PFAS in the biofilm sample from this site, it was not detected in the corresponding surface water sample. It is possible that bioaccumulation of this compound occurred in the biofilm over time from transient sources upstream; however, this is uncertain.

This study found no correlation between total PFAS in the surface water and total PFAS in the biofilm, which may be partly due to differences in the partitioning of PFAS analytes in the two matrices. However, correlations between surface water and biofilm were found for several long-

chain PFSAs (PFOS, PFOA, and PFDS), suggesting that biofilms may be effective for tracing sources of legacy PFAS contaminants such as PFOS and PFOA. This may especially be useful in situations where PFOS contamination in fish and other aquatic organisms is high (Mathieu and McCall 2017; Mathieu 2022).

While biofilms were effective in capturing long-chain PFAS, they were less effective in capturing short-chain PFAS, which were the main components of total PFAS in the surface water samples. For example, the short-chain PFCA signature in the Pullman WWTP effluent and downstream surface water was not picked up in the downstream biofilm. This may be important, especially as short-chain PFAS and their emerging precursors continue to be produced and used as alternatives to long-chain PFAS.

Sulfonamides were better captured in sediment than in biofilm or surface water samples in terms of detection frequencies and relative concentrations of target analytes. Their presence in surface water samples, albeit at low relative concentrations, suggests that there are still active sources of these PFOS precursor chemicals.

Method blank contamination in the biofilm appeared to be an issue for some PFAS analytes, especially given the relatively low concentrations found in the biofilm. Although the biofilms were useful for identifying trends in this study, method blank contamination could be problematic in similar cases where concentrations in the biofilms may be relatively low.

While biofilm alone was useful to some extent, the combination and analysis of multiple matrices — water, biofilm, and sediment — was most effective in providing a more complete picture of PFAS contamination in the environment and its potential sources.

Conclusions

The results of this 2021 study support the following conclusions:

- Based on surface water, biofilm, and sediment samples collected in this study, the main sources of PFAS appeared to be entering the South Fork Palouse River via two WWTP-influenced conveyances: Paradise Creek and the Pullman WWTP.
- Missouri Flat Creek, which is not influenced by WWTP discharges, also appeared to be a conveyance of PFAS to the river. Biofilm collected from Missouri Flat Creek indicated that there might be an upstream source of 6:2 FTS.
- PFAS analytes partitioned differently in the surface water, biofilm, and sediment samples. Short-chain PFCAs dominated the surface water samples, while long-chain PFAS dominated the biofilm and sediment samples. Sulfonamides had greater partitioning in the sediment compared to water and biofilm.
- Biofilms were effective as a PFAS monitoring and source tracing tool to some extent. Biofilms effectively captured long-chain PFAS, including PFOS, which are often the dominant PFAS contaminant found in fish. However, they were not effective in capturing short-chain PFAS.
- Analyses of a combination of environmental matrices—surface water, biofilm, and sediment—seemed to provide a more complete picture of PFAS contamination and potential sources in the South Fork Palouse River watershed compared to analysis of one matrix alone.

Recommendations

The results of this 2021 study support the following recommendations:

- Biofilms should be considered a potentially useful tool for monitoring or source tracing PFAS in aquatic systems, especially where PFOS contamination has been found in fish and other higher trophic levels.
- Future studies of PFAS in the environment should consider including multiple sample matrices, as it will allow for more robust characterizations of PFAS in the environment.
- It may be necessary to work with the analytical laboratory to address any potential method blank contamination issues with biofilm. It may also be helpful to know the expected range of PFAS analyte concentrations in biofilms within the study area to assess whether method blank contamination may be of concern.
- This small study confirmed WWTPs to be a conveyance of PFAS to the South Fork Palouse River during summer low flows. A more robust study would help assess the relative importance of various pathways to the river during different times of the year. This could include seasonal sampling, sampling of additional tributaries within the watershed, and consideration of other important pathways in other studies, such as urban runoff, stormwater, and atmospheric deposition.

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Glossary, Acronyms, and Abbreviations

Glossary

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

Effluent: An outflowing of water from a natural body of water or from a man-made structure. For example, the treated outflow from a wastewater treatment plant.

Parameter: Water quality constituent being measured (analyte). A physical, chemical, or biological property whose values determine environmental characteristics or behavior.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Pollution: Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare; (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses; or (3) livestock, wild animals, birds, fish, or other aquatic life.

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector, such as a stream, river, or lake at a lower elevation.

Acronyms and Abbreviations

CAP	Chemical Action Plan
DOC	Dissolved Organic Carbon
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
PBDE	Polybrominated diphenyl ethers
PFAA	Perfluoroalkyl acid
PFAS	Per- and polyfluoroalkyl substances
PFCA	Perfluoroalkyl carboxylic acid
PFSA	Perfluoroalkane sulfonic acid
RPD	Relative percent difference
RSD	Relative standard deviation
TOC	Total Organic Carbon
WRIA	Water Resource Inventory Area
WWTP	Wastewater treatment plant

Units of Measurement

°C	degrees centigrade
cm	centimeter
g	gram, a unit of mass
m	meter
mg	milligram
mg/L	milligrams per liter (parts per million)
mL	milliliters
mm	millimeters
ng/g	nanograms per gram (parts per billion)
ng/l	nanograms per liter (parts per trillion)
µS/cm	microsiemens per centimeter, a unit of conductivity

Appendices

Appendix A. Overview of Data Quality Based on Project Measurement Quality Objectives

Table A1. Summary of quality assurance/quality control results for this study.

Parameter	Field Duplicate[a]	Equipment Blank[b]	Lab Duplicate[a]	Method Blank[b]	OPR[c]	Surrogate Recovery[d]	Matrix Spike[d]	Matrix Spike Duplicate[a]
PFAS (Biofilm)	5%	NA	5%	5%	0%	0%	2.5%	0%
PFAS (Water)	0%	0%	0%	0%	0%	3%	0%	0%
PFAS (Sediment)	2.5%	NA	NA	3%	0%	8.5%	0%	2.5%
Total Organic Carbon (Water)	0%	NA	0%	0%	0%	NA	0%	NA
Dissolved Organic Carbon (Water)	0%	NA	0%	0%	0%	NA	0%	NA
Ash-Free Dry Weight (Biofilm)	NA	NA	0%	0%	NA	NA	NA	NA
Grain Size (Sediment)	—[e]	NA	—[f]	NA	NA	NA	NA	NA
Carbon & Nitrogen Isotopes	0%	NA	NA	NA	NA	NA	NA	NA

NA = not applicable; OPR = ongoing precision and recovery; PFAS = per- and polyfluoroalkyl substances; RPD = relative percent difference; RSD = relative standard deviation.

[a] % analyte results J-qualified due to RPD exceedance.

[b] % analyte results U-qualified due to blank contamination.

[c] % detected analyte results J-qualified due to recovery exceedances.

[d] % analyte results J-qualified due to recovery exceedances.

[e] RPD was 52% and 121% for gravel and clay, respectively. Sample results were J-qualified.

[f] RSD was 36.2% for gravel. Sample result was J-qualified.

Appendix B. Ancillary Parameter Results for Water and Sediment

Table B1. General chemistry results for water and sediment samples collected during this study.

Location ID	Sample ID	Sample Matrix	Total Organic Carbon-Water (mg/L)	Dissolved Organic Carbon (mg/L)	Total Organic Carbon-Sediment (%)	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	pH
SFPR-Colfax	2108067-12	Water	5.6	4.9	—	19.2	12.2	738	8.8
SPFR-Shawnee	2108067-13	Water	6.6	6.1	—	16.4	7.5	710	7.9
SFPR-Armstrong	2108067-14/2108067-25	Water/ Sediment	7.3	6.8	1.9	17.4	5.8	665	7.6
SFPR-Hayward	2108067-15	Water	7.3	6.8	—	20.2	6.3	699	7.3
SFPR-State Bridge	2108067-16	Water	10.9	11.1	—	16.1	8.3	498	7.8
SFPR-Bishop	2108067-17	Water	6.5	6.0	—	12.9	5.6	433	7.6
SFPR-Stateline	2108067-18/2108067-27	Water/ Sediment	11.7	8.9	1.0	13.1	5.1	500	7.3
PC-QI	2108067-19/2108067-28	Water/ Sediment	8.3	8.1	3.4	12.4	8.2	567	7.7
PC-Stateline	2108067-20	Water	5.6	5.4	—	17.5	5.3	772	7.3
MF-State Bridge	2108067-21/2108067-26	Water/ Sediment	4.1	3.8	4.7	14.2	6.8	514	7.7
DF-Confluence	2108067-22	Water	3.2	3.0	—	16.0	8.5	617	8.3
PULLMANWWTP-Influent	2108067-23	Water	80.7	63.1	—	—	—	—	—
PULLMANWWTP-Effluent	2108067-24	Water	6.0	5.9	—	—	—	—	—

Appendix C. Ancillary Parameter Results for Biofilm

Table C1. Areal biomass, carbon, and nitrogen results for biofilm samples collected during this study.

Location ID	Sample ID	Sample Matrix	Areal Biomass (mg/cm^2)	delta13C (permil VPDB)	delta15N (permil AIR)	Carbon: Nitrogen	% Carbon	% Nitrogen
SFPR-Colfax	2108067-01	Biofilm	—	-30.77	13.15	9.3	13.7	1.7
SFPR-Shawnee	2108067-02	Biofilm	0.024167612	-30.17	11.74	7	4.3	0.7
SFPR-Armstrong	2108067-03	Biofilm	0.023043523	-31.11	9.21	6.9	9.8	1.7
SFPR-Hayward	2108067-04	Biofilm	0.003894942	-29.93	9.9	7.2	11.2	1.8
SFPR-State Bridge	2108067-05	Biofilm	0.024895026	-28.85	9.13	9.7	5.3	0.6
SFPR-Bishop	2108067-06	Biofilm	0.006256847	-29.66	9.02	7.9	6.2	0.9
PC-QI	2108067-08	Biofilm	0.006811702	-31.57	10.56	8.2	4.2	0.6
PC-Stateline	2108067-09	Biofilm	—	-29.14	9.22	9.5	3.9	0.5
MF-State Bridge	2108067-10	Biofilm	0.001669816	-31.6	8.13	9.2	8.3	1.1
DF-Confluence	2108067-11	Biofilm	0.007118265	-25.44	7.35	10.4	11.2	1.3

Appendix D. Sediment Grain Size Results

Table D1. Results for sediment grain size samples.

Particle Size Name	MF-STATE BRIDGE	PC-QI	SFPR-ARMSTRONG	SFPR-STATELINE
Larger than Sand (%)	5.4	0.8	56.9	20.2
Sand-Very Coarse (%)	9.8	2.1	10.5	19.4
Sand-Coarse (%)	10.8	2.4	5.5	22.0
Sand-Medium (%)	4.6	2.5	5.2	6.7
Sand-Fine (%)	2.8	3.5	3.5	4.4
Sand-Very Fine (%)	4.0	5.9	3.7	3.9
Silt-Medium (%)	52.1	68.5	25.2	26.3
Clay (%)	9.0	10.1	5.1	5.5

Appendix E. Scatterplot Matrices

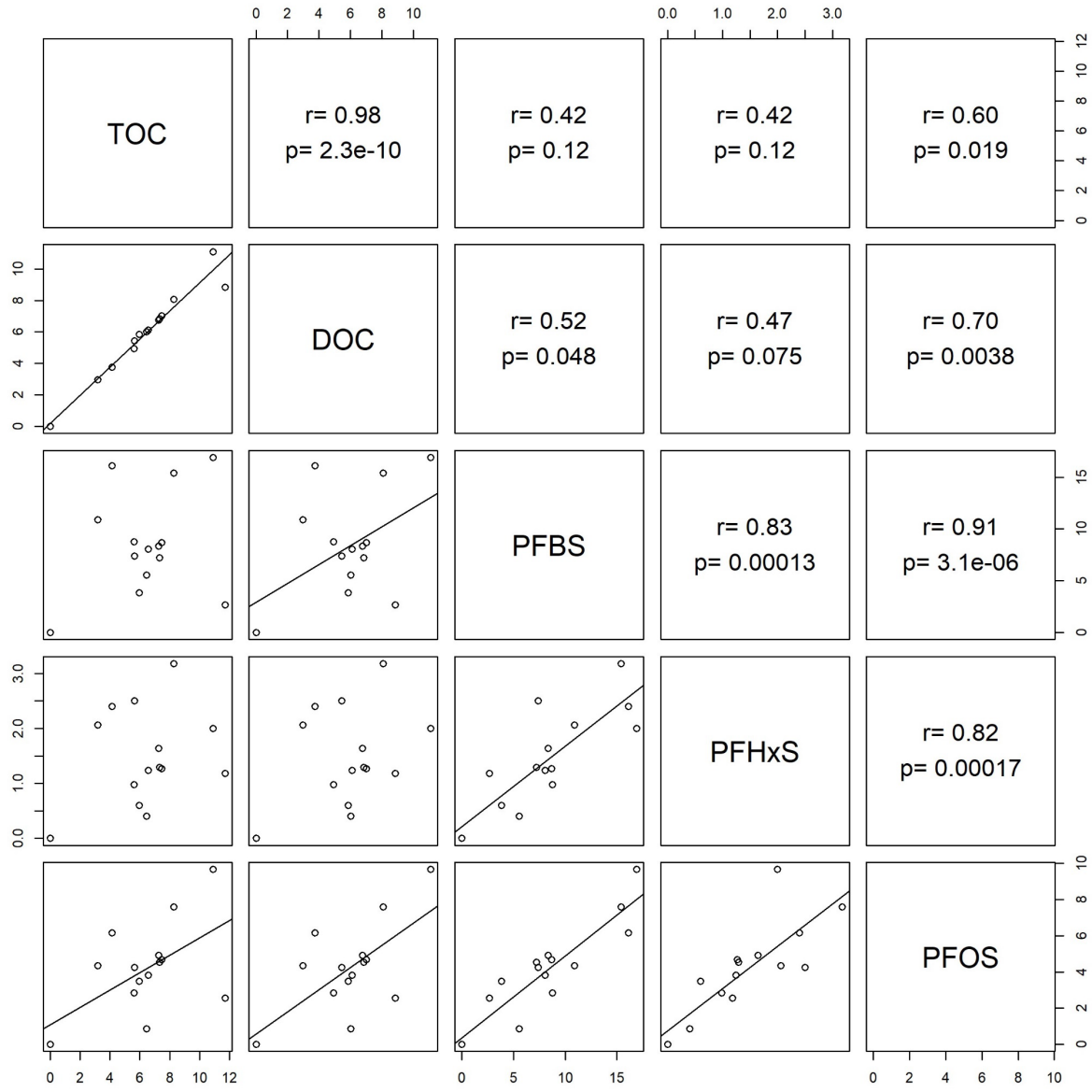

Figure E1. Total organic carbon (TOC) and dissolved organic carbon (DOC) versus perfluoroalkane sulfonic acid (PFSA) analytes in water.
Upper right panels: Pearson correlation (r) and p-value for each analyte pair.
Lower left panels: Scatterplot of each analyte pair, with a regression line shown for significant correlations .($p<0.05$).

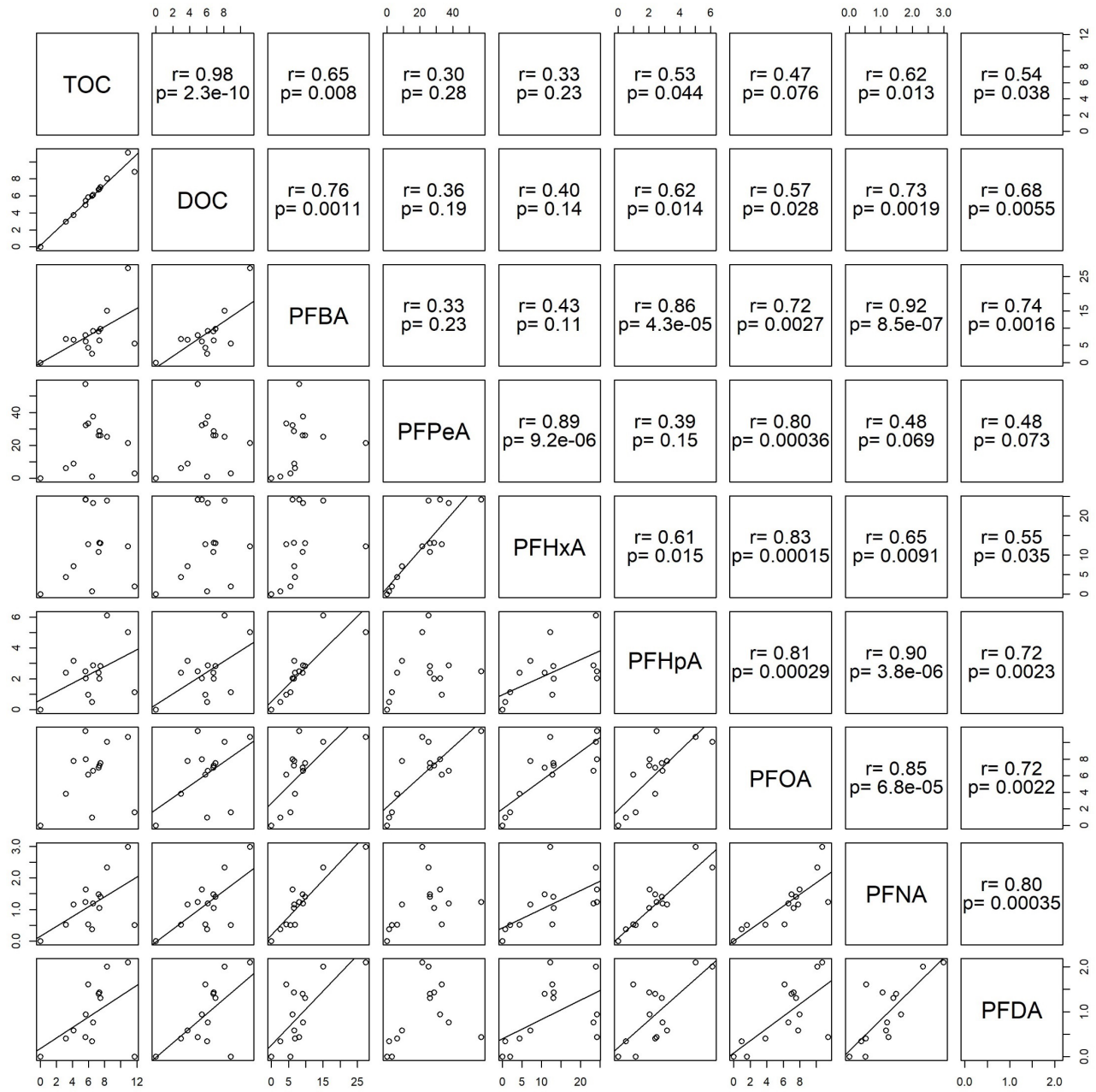

Figure E2. Total organic carbon (TOC) and dissolved organic carbon (DOC) versus perfluoroalkyl carboxylic acid (PFCA) analytes in water.

Upper right panels: Pearson correlation (r) and p-value for each analyte pair.
Lower left panels: Scatterplot of each analyte pair, with a regression line shown for significant correlations ($p<0.05$).

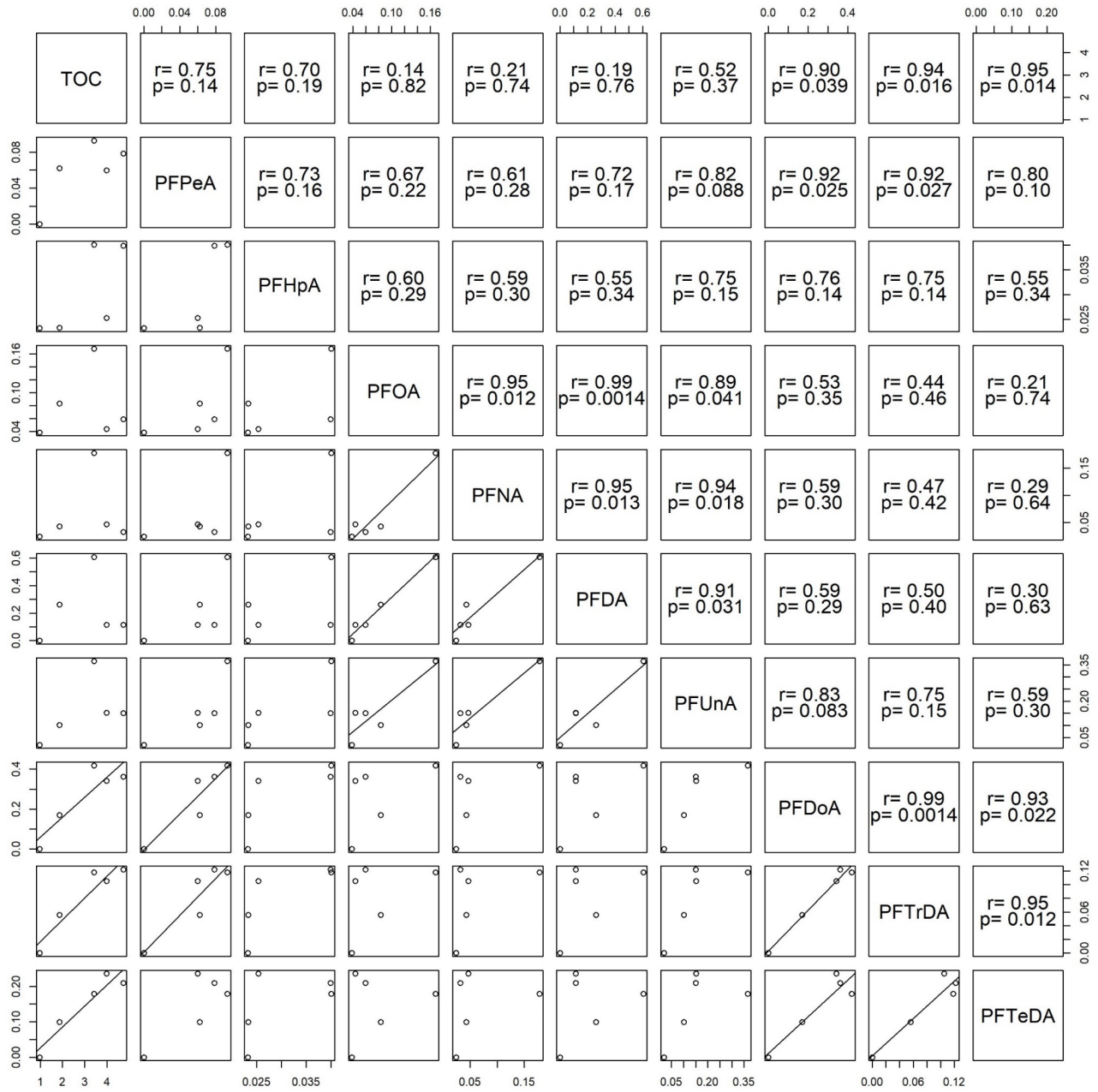

Figure E3. Total organic carbon (TOC) versus perfluoroalkyl carboxylic acid (PFCA) analytes in sediments.

Upper right panels: Pearson correlation (r) and p-value for each analyte pair.
Lower left panels: Scatterplot of each analyte pair, with a regression line shown for significant correlations ($p<0.05$).

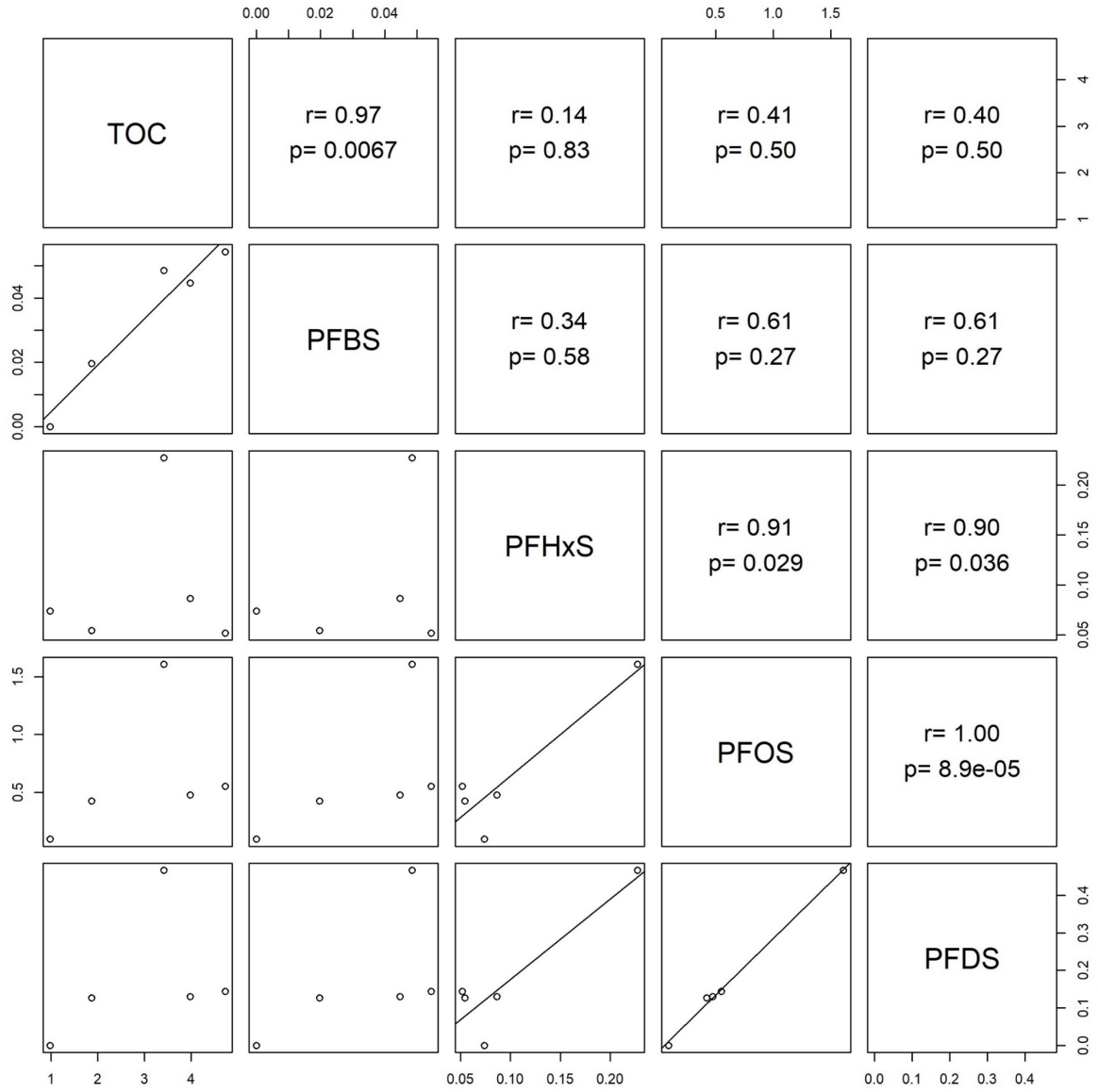

Figure E4. Total organic carbon (TOC) versus perfluoroalkane sulfonic acid (PFSA) analytes in sediments.

Upper right panels: Pearson correlation (r) and p-value for each analyte pair.

Lower left panels: Scatterplot of each analyte pair, with a regression line shown for significant correlations ($p<0.05$).