Environmental Science & Technology

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Biotransformation of 8:2 Fluorotelomer Alcohol in Soil from Aqueous Film-Forming Foams (AFFFs)-Impacted Sites under Nitrate-, Sulfate-, and Iron-Reducing Conditions

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Cite This: *Environ. Sci. Technol.* 2022, 56, 13728−13739

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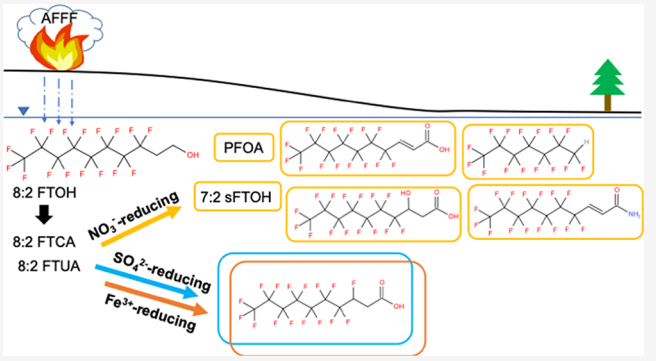
ABSTRACT: The environmental fate of per- and polyfluoroalkyl substances (PFAS) in aqueous film-forming foams (AFFFs) remains largely unknown, especially under the conditions representative of natural subsurface systems. In this study, the biotransformation of 8:2 fluorotelomer alcohol (8:2 FTOH), a component of new-generation AFFF formulations and a byproduct in fluorotelomer-based AFFFs, was investigated under nitrate-, iron-, and sulfate-reducing conditions in microcosms prepared with AFFF-impacted soils. Liquid chromatography−tandem mass spectrometry (LC−MS/MS) and high-resolution mass spectrometry (HRMS) were employed to identify biotransformation products. The biotransformation was much slower under sulfate- and iron-reducing conditions with >60 mol % of initial 8:2 FTOH remaining after ∼400 days compared to a half-life ranging from 12.5 to 36.5 days under nitrate-reducing conditions. Transformation products 8:2 fluorotelomer saturated and unsaturated carboxylic acids (8:2 FTCA and 8:2 FTUA) were detected under all redox conditions, while 7:2 secondary fluorotelomer alcohol (7:2 sFTOH) and perfluorooctanoic acid (PFOA) were only observed as transformation products under nitrate-reducing conditions. In addition, 1H-perfluoroheptane ($F(CF_2)_6CF_2H$) and 3-F-7:3 acid ($F(CF_2)_7CFHCH_2COOH$) were identified for the first time during 8:2 FTOH biotransformation. Comprehensive biotransformation pathways for 8:2 FTOH are presented, which highlight the importance of accounting for redox condition and the related microbial community in the assessment of PFAS transformations in natural environments.



KEYWORDS: *8:2 fluorotelomer alcohol, FTOH, AFFF, PFAS, redox condition, high-resolution mass spectrometry, biotransformation*

1. INTRODUCTION

Aqueous film-forming foams (AFFFs) are water-based chemical mixtures that have been used since the 1960s to effectively extinguish hydrocarbon-fuel fires at airports and military bases.[1,2] AFFFs contain various classes of per- and polyfluoroalkyl substances (PFAS),[3] and the repeated application of AFFF at fire training areas has resulted in high PFAS concentrations in soil and groundwater.[4,5] Recently, a few studies have identified a wide variety of perfluoroalkyl acid (PFAA) precursors (referred to hereafter as "precursors") as well as PFAAs in various AFFF formulations.[4,6−8] PFAAs are persistent compounds that can cause adverse effects on human health,[4,9,10] and they may come from the direct release of AFFFs or from the transformation of AFFF-derived precursors.[11−14] However, information on the environmental fate of precursors identified in AFFFs,[4,6−8] including their susceptibility to biotransformation under conditions representative of natural groundwater environments, is limited, which complicates the management and remediation of AFFF-impacted sites.

The biotransformation of some AFFF-derived precursors has been investigated previously, and most of these studies were conducted under oxic conditions, utilizing microorganisms from activated sludge,[15,16] river sediments,[17] and surface soils.[11,13,14,18] In contrast, limited studies were conducted under anoxic conditions.[12,17,19] The biotransformation of precursors under anoxic conditions was found to be distinctly different from that under oxic conditions. For instance, the half-life of 6:2 fluorotelomer alcohol resulting from the biotransformation under methanogenic conditions was re-

Received: May 22, 2022
Revised: August 31, 2022
Accepted: September 3, 2022
Published: September 20, 2022

ported as ca. 30 days,[19] which was much longer than that reported in aerobic activated sludge, sediment, and soil (i.e., 1−2 days).[20−22] The PFAA yields under methanogenic conditions were also at least 20 times lower than under oxic conditions.[19] The biotransformation of 6:2 fluorotelomer sulfonate occurred under oxic[16,23] and nitrate-reducing conditions,[24] with the most rapid transformation rate occurring in aerobic sediment,[17] whereas transformations did not occur under sulfate-reducing[17] and methanogenic[25] conditions. Another precursor, 6:2 fluorotelomer thioether amido sulfonate, was reported to be biotransformed much more slowly under sulfate-reducing conditions[12] than under oxic conditions,[11] with distinct biotransformation pathways observed between both conditions. These findings indicate that the redox condition plays an important role in precursor biotransformation.

Fluorotelomer alcohols [FTOHs, $F(CF_2)_nCH_2CH_2OH$] are primary raw materials used to manufacture surfactants and polymeric materials with water- and oil-repelling properties.[26] The unique properties have led to the use of FTOH-based products in a wide variety of applications including AFFFs.[26] Measurements of source fingerprints of new-generation AFFF formulations acquired in Norway[27] and commercially available AFFFs generated between 2012 and 2013 in Switzerland have detected 8:2 FTOH,[28] with the concentrations ranging from 8 to 26.5 mg/L. Furthermore, FTOHs are likely present as byproducts during the synthesis of the fluorotelomer-based precursors.[29] Therefore, the detection and prevalence of 8:2 FTOH at AFFF-impacted sites are anticipated to increase. Previous studies have demonstrated that 8:2 FTOH "readily undergoes" or are "susceptible to" biotransformation under oxic conditions in various environmental matrices, including activated sludge,[15,30] brackish water,[31] and pristine soils.[32,33] PFAAs, including perfluorohexanoic acid (PFHxA) and perfluorooctanoic acid (PFOA), were reported as the stable biotransformation products. In contrast, little information is available on the anaerobic biotransformation of 8:2 FTOH. Sáez et al.[34] did not observe the anaerobic biotransformation of 8:2 FTOH in municipal sewage sludge after a 9 week incubation. However, Zhang et al.[19] reported 8:2 FTOH biotransformation in digester sludge with a half-life of ca. 145 days. Moreover, Li et al.[35] found that 8:2 FTOH biotransformation in anaerobic activated sludge was rapid with a half-life of only ca. 5 days. All three studies investigated 8:2 FTOH biotransformation under methanogenic conditions, and the discrepancy observed among these studies was likely due to the differences between the microbial communities. To date, there is still a paucity of information on the biotransformation of 8:2 FTOH and potential pathways under other redox conditions representative of the natural aquifer environment (e.g., nitrate-, iron-, and sulfate-reducing conditions). Addressing this knowledge gap is essential to elucidate the fate and transformation of 8:2 FTOH in natural groundwater systems.

In the present study, the biotransformation of 8:2 FTOH was, for the first time, investigated under nitrate-, sulfate-, and iron-reducing conditions in laboratory microcosm reactors. The objective of this study was to evaluate the environmental fate of 8:2 FTOH under the conditions typical of AFFF-impacted sites. To this end, AFFF-impacted soil was collected from a former U.S. military base and used for the construction of the microcosms. The subsurface microbial communities in AFFF-impacted soil are expected to differ from those in activated sludge, river sediment, and aerated surface soil, and they may evolve to be more resistant to PFAS and more capable of transforming PFAS due to the historical exposure. Therefore, potentially distinct 8:2 FTOH biotransformation (e.g., rate, pathway, etc.) from those reported previously was hypothesized under these experimental conditions. The molar yields of known biotransformation products were determined in the microcosms under each redox condition. High-resolution mass spectrometry (HRMS) was also employed to identify potential unknown transformation products. Based on the findings of the current investigation and results from previous studies, the comprehensive biotransformation pathways for 8:2 FTOH under various redox conditions representative of natural environments were proposed.

2. MATERIALS AND METHODS

2.1. Microcosm Setup. The soil used in this study was collected from an AFFF-contaminated location at the former Loring Air Force Base (Limestone, ME). Detailed information on soil collection, taxonomic classification, and physical and chemical property characterization (pH, moisture content, organic matter content, cation exchange capacity (CEC), soil particle size distribution) is provided in the Supporting Information (SI) Section S1. To minimize the loss of volatile 8:2 FTOH and transformation products, a closed system using Wheaton glass serum bottles (60 mL) aluminum crimp-sealed with rubber septa was adopted for the microcosm setup. In each reactor, 30 mL of the growth medium[36] and 3 g (dry weight) of Loring soil were added. Sodium nitrate (20 mM), sodium sulfate (20 mM), and goethite (100 mM, mineral of Fe(III) oxide-hydroxide) were added in each batch of microcosms as the electron acceptor under nitrate-, sulfate-, and iron-reducing conditions, respectively. Each batch of microcosms included two sets of live-spiked treatments: one set of abiotic control and one set of positive control (see Table S1). For live-spiked treatments, each bottle was spiked with ∼170 μg/L of 8:2 FTOH prepared in diethylene glycol butyl ether (DGBE), which is a primary organic solvent in AFFF that has been shown to serve as a microbial electron donor and carbon source.[37] One set of live-spiked treatment mimicked natural attenuation (NA treatment) where 5 mM DGBE was introduced as the solvent for 8:2 FTOH and as the sole external potential electron donor, while the other set mimicked biostimulation where 20 mM sodium lactate was added as an additional electron donor and carbon source in conjunction with DGBE to enhance the microbial growth (ED treatment). Abiotic controls were prepared similarly to live-spiked treatments, but 1 g/L NaN_3 was added to inhibit the microbial activity (effective concentration was determined by preliminary experiments, data not shown here). Abiotic controls were used to evaluate the potential abiotic transformation of 8:2 FTOH and/or legacy PFAS originally in the Loring AFB soil. For the positive controls, only DGBE (without 8:2 FTOH) was dosed into the bottles, and all other procedures were identical to the live-spiked treatments. The positive controls were used to monitor the background levels of legacy PFAS in the Loring soil and their potential transformation products. All the microcosms were incubated at room temperature with 150 rpm shaking over ∼400 days.

2.2. Sample Collection and Preparation. At each sampling point, triplicate bottles from live-spiked treatments and duplicate bottles from abiotic and positive controls were sacrificed for sampling. The headspace of each bottle was

https://doi.org/10.1021/acs.est.2c03669
Environ. Sci. Technol. 2022, 56, 13728−13739

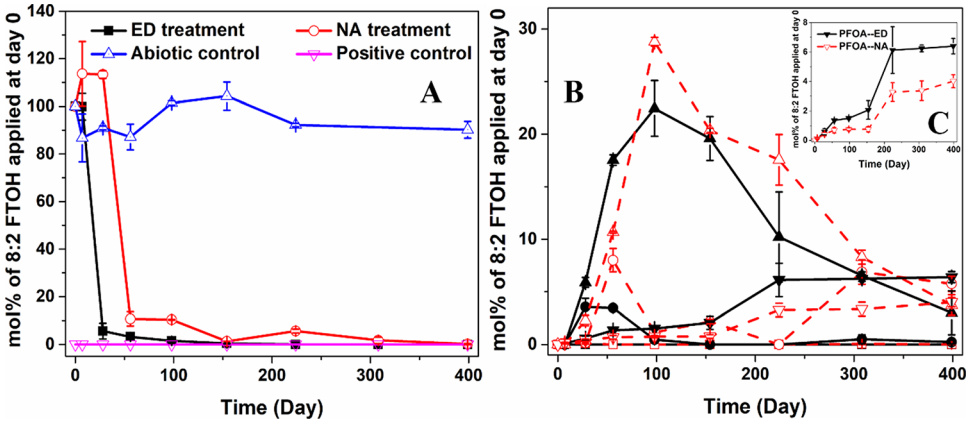


Figure 1. (A) Changes in molar ratios of residual 8:2 FTOH during biotransformation under nitrate-reducing conditions in the electron-donor (ED) treatment, natural attenuation (NA) treatment, abiotic controls, and positive controls microcosms. (B) Molar yields of 8:2 FTOH biotransformation products (squares: 8:2 FTCA, circles: 8:2 FTUA, up triangles: 7:2 sFTOH, down triangles: PFOA) in the ED (solid black lines and solid symbols) and NA treatments (dash red lines and open symbols). (C) Zoom view of panel B showing the time course trends of PFOA molar yields.

purged through a C_{18} cartridge (Maxi-Clean, Alltech, Deerfield, IL) by flushing N_2 to capture 8:2 FTOH and potential volatile transformation products. Each C_{18} cartridge was eluted with methanol (5 mL) for further PFAS analysis. The supernatant (0.5 mL) from each bottle was then collected and immediately mixed with 9.5 mL of methanol to avoid the potential loss of volatile PFAS. The diluted sample was filtered with a 0.2 μm Corning nylon syringe filter (Corning Inc., Corning, NY) into a 15 mL centrifuge tube for PFAS quantification of the microcosm aqueous phase. Subsequently, each bottle was shaken vigorously, and 1 mL of well-mixed slurry was collected for the measurements of geochemical parameters (e.g., SO_4^{2-}, NO_3^-, F^-, lactate, etc.; details in SI Section S1). All remaining slurry along with the rubber septum was transferred into a 50 mL centrifuge tube. The bottle was rinsed with 5 mL of ultrapure water (18.2 MΩ cm), and the rinse water was combined with the slurry. After centrifugation at 4000 rpm for 20 min, the supernatant was discarded, and the soil pellet was resuspended in 30 mL of methanol and vortexed for 30 min followed by sonication at 60 °C in a water bath for 30 min. The methanol extract was collected after centrifugation and further filtered with a 0.22 μm Corning nylon filter for quantification of sorbed PFAS from the septum and microcosm solid phases. The C_{18} cartridge eluent, methanol-diluted aqueous samples, and methanol extracts of soil and septum were stored at −20 °C before PFAS analysis.

2.3. Targeted and Nontargeted PFAS Analysis. Targeted LC−MS/MS analysis was performed using a Waters ACQUITY ultra high-performance liquid chromatograph coupled with a Waters Xevo triple quadrupole mass spectrometer (UPLC−MS/MS) (Waters Corporation, Milford, MA). Each of the three phases described above was analyzed by LC−MS/MS separately. The target PFAS in the LC−MS/MS analysis are listed in Table S2. PFAA analysis was performed following established methods.[38] Analysis of 8:2 FTOH and its polyfluorinated biotransformation products was performed following the method reported by Szostek et al.[39] with no ammonium acetate addition in the mobile phase, as FTOHs form adducts under negative electrospray ionization. In both methods, analyte separation was achieved using a Waters ACQUITY UPLC BEH C18 Column (130 Å, 1.7 μm, 2.1 × 50 mm). The solvent gradient and detailed instrumental parameters are specified in Tables S3−S6. The detection limits of target analytes are provided in Table S7. To quantify 1H-perfluoroheptane in each of the three sample phases, targeted gas chromatography (GC)−HRMS was performed using a high-resolution Thermo Q Exactive Orbitrap MS equipped with a Thermo Trace 1300 GC and a TriPlus RSH Autosampler. Operational details for GC−HRMS are described in SI Section S1.

Aqueous samples and soil methanol extracts collected from each treatment at select sampling time points were pooled for nontargeted LC−HRMS analysis. Sampling time points were selected based on targeted LC−MS/MS analysis results to include the samples collected before and after the occurrence of 8:2 FTOH biotransformation. Nontargeted LC−HRMS analysis was performed using a Thermo QExactive HF-X Orbitrap MS equipped with a Vanquish UHPLC to provide high resolution, high mass accuracy, and high sensitivity over a large mass to charge (m/z) range. Detailed procedures for LC−HRMS are described in SI Section S1.

2.4. Microbial Community Analysis. To better understand the effects of the redox condition on the biotransformation of 8:2 FTOH, microbial community analysis was performed on samples from live-spiked treatments and positive controls under each redox condition. Duplicate soil samples were collected at the beginning and the end of incubation, and DNA was extracted using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Amplification and sequencing of soil DNA samples were performed at the Alkek Center for Metagenomics and Microbiome Research at Baylor College of Medicine. The V4 region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using barcoded primer sets (515F/806R) and sequenced on the MiSeq platform (Illumina, San Diego, CA) using a 2 × 250 bp paired-end protocol.[40] The read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090.[41] The 16S rRNA gene sequences were clustered into operational taxonomic units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm.[42] The generated sequences were mapped against the latest SILVA database.[43] The ATIMA (Agile Toolkit for Incisive Microbial Analyses) was used to analyze and visualize trends in taxa abundance,

https://doi.org/10.1021/acs.est.2c03669
Environ. Sci. Technol. 2022, 56, 13728−13739

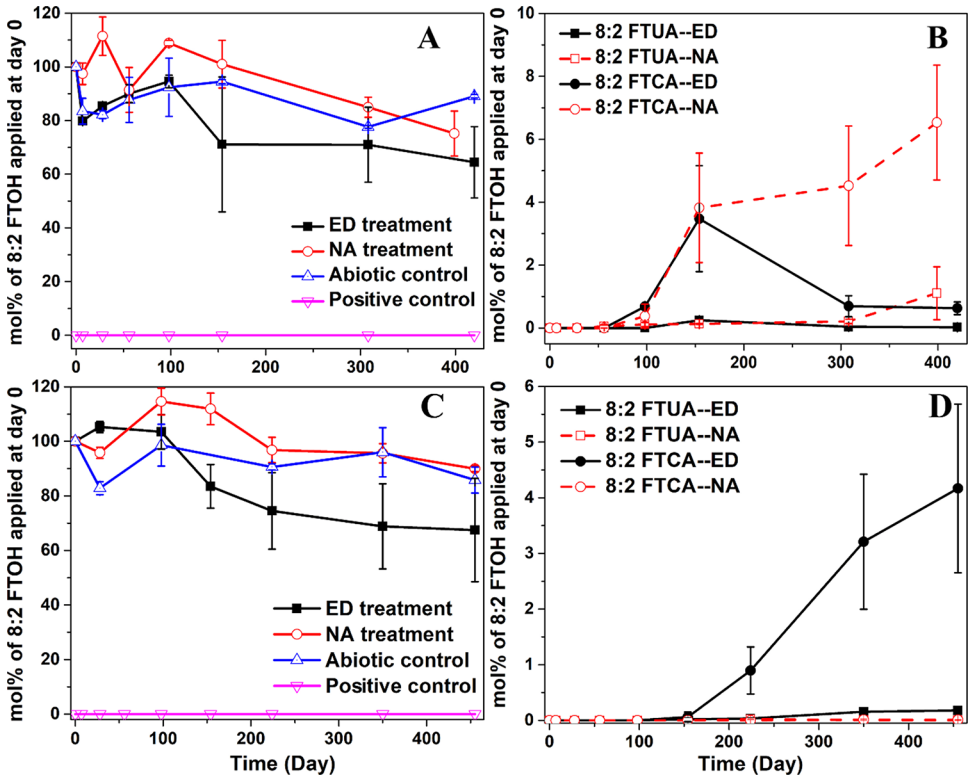


Figure 2. Changes in concentrations of targeted PFAS during 8:2 FTOH biotransformation under (A and B) sulfate-reducing and (C and D) iron-reducing conditions. Panels A and C show 8:2 FTOH in the electron-donor (ED) treatment, natural attenuation (NA) treatment, abiotic control, and positive control. Panels B and D show the biotransformation products of 8:2 FTOH in ED and NA treatments. Note that the *y*-axis scale in panel B is larger than the scale in panel D.

alpha diversity, and beta diversity as they relate to sample metadata.

3. RESULTS AND DISCUSSION

Throughout the experiments, nitrate-, sulfate-, and iron-reducing activities were confirmed in the respective microcosms by monitoring the consumption of the electron donor (i.e., lactate and/or DGBE) and electron acceptor (i.e., NO_3^-, SO_4^{2-}, or Fe(III)/Fe(II)) (Figures S1−S3). Additional information related to the establishment of nitrate-, sulfate-, and iron-reducing microcosms is provided in SI Section S2. A total of 19 PFAS compounds were quantified in all microcosms (Table S2). Several PFAS were detected in the day 0 samples; thus, the background levels of legacy PFAS in Loring soil were determined (Table S8). Additional discussion on the measurement of legacy PFAS in Loring soil is provided in SI Section S3. As shown in Figures 1A and 2A,C, 94.9 ± 7.8, 88.1 ± 7.4, and 90.7 ± 8.0 mol % of the initially spiked 8:2 FTOH remained in the abiotic controls of nitrate-, sulfate-, and iron-reducing microcosms, respectively, without significant changes ($p > 0.05$) throughout the experiments. These stable 8:2 FTOH concentrations under various abiotic experimental conditions verify the integrity of the experimental system and the efficiency of the extraction method applied in the present study.

3.1. Biotransformation of 8:2 FTOH under Nitrate-Reducing Conditions. *3.1.1. Biotransformation Rates.* In the live-spiked treatments (i.e., ED and NA treatments), substantial decreases in the spiked 8:2 FTOH were observed under nitrate-reducing conditions in the first 56 day incubations (Figure 1A). The half-life of 8:2 FTOH biotransformation in the NA treatment was calculated as ∼36.5 days as determined by fitting nine data points into a single first-order kinetic model (coefficient of determination R^2 = 0.847, Figure S4). Amendment of 20 mM lactate (i.e., ED treatment) greatly enhanced the biotransformation rate by reducing the half-life of 8:2 FTOH to ∼12.5 days (R^2 = 0.931, Figure S4). Thus, the biostimulation of nitrate-reducing microorganisms with lactate as a supplemental and/or favorable electron donor and carbon source could promote a more efficient biotransformation of 8:2 FTOH. Specifically, in the ED treatment, the residual 8:2 FTOH fraction decreased sharply from 99.9 ± 5.6 mol % at day 7 to 5.6 ± 3.3 mol % at day 28 and was further reduced to 0.4 ± 0.2 mol % by day 154 (Figure 1A). The residual 8:2 FTOH in the NA treatment, by comparison, did not decrease in the first 28 days. However, a rapid decrease was observed in the following 28 days with only 10.7 ± 3.0 mol % remaining by day 56, which was then gradually decreased to 0.2 ± 0.2 mol % by the end of incubation (Figure 1A). The relatively fast biotransformation of 8:2 FTOH under nitrate-reducing conditions in Loring soil, especially with lactate amendment, was comparable with those observed in aerobic soils (half-lives ranging from 1 to 4 weeks)[33,44] and was much faster than previously documented in digester sludge under methanogenic conditions (half-life of ca. 145 days).[19]

3.1.2. Biotransformation Products Quantified by LC−MS/MS Targeted Analysis. As shown in Figure 1B, 8:2 fluorotelomer saturated carboxylic acid (8:2 FTCA), 8:2 fluorotelomer unsaturated carboxylic acid (8:2 FTUA), and 7:2 secondary fluorotelomer alcohol (7:2 sFTOH), which were previously reported as polyfluorinated intermediates of 8:2

https://doi.org/10.1021/acs.est.2c03669
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FTOH,[33,35,45] were quantified in both ED and NA treatments during 8:2 FTOH biotransformation under nitrate-reducing conditions. None of these compounds were above the limits of detection (LODs) in abiotic and positive controls. The most abundant transformation product was 7:2 sFTOH, which reached peak molar yields of 22.5 ± 2.6 and 28.8 ± 0.4 mol % of the initially applied 8:2 FTOH by day 98 in ED and NA treatments, respectively. Concentrations of 7:2 sFTOH then decreased to 10.2 ± 4.3 and 17.6 ± 2.4 mol % at day 224 and further decreased to 3.0 ± 2.1 and 3.7 ± 1.0 mol % at the end of incubation, respectively (Figure 1B). In contrast, 8:2 FTCA was only detected sporadically in the NA treatment (<0.2 mol % of initially applied 8:2 FTOH) and was below the LOD in the ED treatment (Figure 1B), likely due to its rapid conversion to other products (e.g., 8:2 FTUA). In the ED treatment, 8:2 FTUA was produced with a peak molar yield of 3.6 ± 0.8 mol % by day 28 and then later decreased to low levels (<0.5 mol %). In the NA treatment, 8:2 FTUA reached a peak molar yield of 8.0 ± 1.1 mol % by day 56 followed by some fluctuations and settled at 5.8 ± 0.7 mol % by the termination of the experiment (Figure 1B). The molar yields and time trends of these three polyfluorinated intermediates in the present study were consistent with 8:2 FTOH biotransformation reported in aerobic soils.[33] Another previously reported major polyfluorinated product of 8:2 FTOH was 7:3 acid;[19,33,35] however, it was not measured as a product in this study. Although 7:3 acid was detected in ED and NA treatments, it was also detected in abiotic and positive controls without significant differences ($p > 0.05$) throughout the incubations (Figure S5). This finding is in contrast to most previous studies of aerobic and anaerobic 8:2 FTOH biotransformation where 7:3 acid was a major stable transformation product.[19,23,33] Only one study found that 7:3 acid and its known direct precursor, 7:3 U acid (7:3 unsaturated acid, $F(CF_2)_7CH{=}CHCOOH$), were absent in the biotransformation of 8:2 FTOH by an alkane-degrading strain, *Pseudomonas oleovorans*.[26] The researchers assumed that *P. oleovorans* might lack microbial enzymes capable of defluorinating 8:2 FTUA to 7:3 U acid and then reducing the latter to 7:3 acid. Therefore, the formation of 7:3 acid was absent or extremely minimal during 8:2 FTOH biotransformation under nitrate-reducing conditions, which could be attributed to the lack of those enzymes responsible for 7:3 acid formation in the nitrate reducers in Loring soil.

As potential perfluorinated transformation products of 8:2 FTOH,[33,35] C4 to C9 perfluoroalkyl carboxylates (PFCAs) were detected in all treatments, indicating that these legacy PFAS were present in Loring soil (Figure S5). However, a significant increase ($p < 0.05$) was only observed for PFOA after comparing the concentrations of these PFCAs in ED and NA treatments to positive controls (Figure S5), demonstrating that PFOA was formed during 8:2 FTOH biotransformation under nitrate-reducing conditions. Although the concentrations of other PFCAs including perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA) in live-spiked treatments were not significantly different from those in positive controls, some PFCAs might be biotransformation products as reported previously under both oxic[23,32] and anoxic conditions[35,46] but with yields that were too low to be distinguished from the background levels in Loring soil. After subtracting the PFOA mass in positive controls at each sampling point, the molar yields of PFOA in both ED and NA treatments continued to increase during the incubations, reaching 6.4 ± 0.5 and 4.0 ± 0.4 mol % of the initially applied 8:2 FTOH at the end of incubation, respectively (Figure 1B,C). The PFOA yields measured here are much less than those previously reported in aerobic soils over ca. 7 months of incubation[33] but substantially higher than yields measured in digester sludge under methanogenic conditions over the 181 day study.[19] In addition, the higher molar yield of PFOA observed in the ED treatment compared to the NA treatment implies that the presence or amendment of additional and/or favorable electron donor and carbon source (e.g., lactate) at AFFF-impacted sites will likely result in more extensive and/or more rapid release of PFCAs to the environmental matrices under nitrate-reducing conditions.

3.1.3. Biotransformation Products Identified by LC−HRMS. In ED and NA treatments under nitrate-reducing conditions, the total mass recovery of 8:2 FTOH and the transformation products quantified by LC−MS/MS decreased sharply during the periods when 8:2 FTOH was rapidly transformed (Figure 1A and Figure S6). Total mass recovery then further gradually decreased to only ca. 10.0 mol % of the initially applied 8:2 FTOH by the end of incubation. Such a decrease was not observed in the abiotic controls (Figure S6). The irreversible binding of 8:2 FTOH and/or biotransformation products in the soil, which has been reported in previous 8:2 FTOH studies in soils, resulted in those compounds not being recovered.[32,33,47] This binding process, moreover, was likely catalyzed by microbial enzymatic activities,[48] which explain the overall satisfactory molar recovery (86.8−104.3 mol %) in abiotic controls. In addition, the formation of unknown 8:2 FTOH biotransformation products could contribute to the low mass recovery. Therefore, nontargeted LC−HRMS analysis was performed on the samples from each treatment under nitrate-reducing conditions, and a total of four potential biotransformation products of 8:2 FTOH were identified (Table S9).

Tentatively identified as a biotransformation product of 8:2 FTOH at confidence level 3 (assigned based on the Schymanski Scale,[49] detailed in SI Section S1) based on the MS^2 spectrum (Figure S7A), 7:3 U acid (m/z 438.9822) accumulated to a similar amount in ED and NA treatments after a 32 week incubation but remained at a very low level in abiotic and positive controls under nitrate-reducing conditions (Figure S8). In previous studies, 7:3 U acid has been recognized as a precursor to 7:3 acid under oxic and methanogenic conditions,[19,26,33,45] and the absence of 7:3 U acid was attributed to the fast biotransformation to 7:3 acid.[19,33] In this study, however, the formation of 7:3 acid was not observed in either ED or NA treatments under nitrate-reducing conditions in spite of the gradual accumulation of 7:3 U acid. This finding was likely due to a lack of microbial enzymes (e.g., reductase) capable of converting 7:3 U acid to 7:3 acid by the microorganisms in Loring soil under nitrate-reducing conditions.

In nitrate-reducing microcosms, 3-OH-7:3 acid ($F(CF_2)_7CHOHCH_2COOH$, m/z 456.9930) was identified as another biotransformation product of 8:2 FTOH (confidence level 2, based on MS^2 (Figure S7B)). The authentic standard of 3-OH-7:3 acid was not available for further structure verification. This product gradually accumulated in only ED and NA treatments under nitrate-reducing conditions, as indicated by the increase in peak area over time (Figure S9A). Only one previous study identified 3-OH-7:3 acid as a product

https://doi.org/10.1021/acs.est.2c03669
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during 8:2 FTOH biotransformation, and it was reported to form by the conversion of 7:3 U acid in aerobic soils.[33] This study represents the first reported identification of 3-OH-7:3 acid as a product during 8:2 FTOH biotransformation under nitrate-reducing conditions. During biotransformation, 3-OH-7:3 acid may be formed by the oxidation of 7:3 U acid by a hydratase type enzyme.

Nontargeted analysis also suggested the presence of a biotransformation product with a potential structure as 7:3 U amide ($F(CF_2)_7CH{=}CHCONH_2$, *m/z* 437.9975). However, the MS^2 fragmentation data of this product were not available, resulting in the identification at confidence level 4. It was reported that 7:3 U amide was a potential product during 8:2 FTOH aerobic biotransformation,[45] but this compound was not observed in subsequent aerobic or anaerobic 8:2 FTOH biotransformation studies.[19,32,33,35] In the present study, increases in the peak area of 7:3 U amide were observed only in ED and NA treatments under nitrate-reducing conditions (Figure S10), indicating its formation during 8:2 FTOH biotransformation under nitrate-reducing conditions. The maximum peak area of 7:3 U amide was much larger in the NA treatment than the ED treatment, peaking at week 22 (Figures S10A,A'). The formation of 7:3 U amide may result from the conversion of 7:3 U acid by a transaminase under nitrate-reducing conditions, and this reaction may be reversible.[45] In addition, decreases of 7:3 U amide in ED and NA treatments after week 22 indicated that it was likely an intermediate and was converted to downstream biotransformation products, such as PFHxA or PFOA as proposed previously.[45]

A novel biotransformation product, 1H-perfluoroheptane ($F(CF_2)_6$ CF_2H, *m/z* 368.9764), was identified during 8:2 FTOH biotransformation (confidence level 1 with a verified reference standard). Fragmentation analysis using the MS^2 spectrum indicated the presence of characteristic moieties, including the deprotonated molecule ion (*m/z* 368.9764) and several fragment ions (*m/z* 218.9860, 168.9893, and 118.9929) with one or multiple losses of $-CF_2$ groups (50 Da) (Figure S11A). The identification of this product as 1H-perfluoroheptane was further confirmed by the comparison with the compound standard (Figure S11B). The quantification of 1H-perfluoroheptane was then conducted using GC−HRMS. As shown in Figure S12, 1H-perfluoroheptane was formed rapidly in ED and NA treatments, with a molar yield of 56.1−81.1 mol % at day 28. Then, 1H-perfluoroheptane gradually decreased to below the LOD (1954 ng/L) by day 399 in the ED treatment, while in the NA treatment, its molar yield increased to 76.4 mol % at day 154 and then decreased to below the LOD at day 399 (Figure S12). In the abiotic and positive controls, 1H-perfluoroheptane was below the LOD during the incubation. As the concentration of 1H-perfluoroheptane measured in some solid and headspace samples was close to the LOD, caution should be taken regarding the quantification results; for example, the total mass of 1H-perfluoroheptane formed may be overestimated. Regardless, the substantial formation in the early incubation followed by the gradual decrease indicated that 1H-perfluoroheptane is a major intermediate during 8:2 FTOH biotransformation under nitrate-reducing conditions.

3.2. Biotransformation of 8:2 FTOH under Sulfate-Reducing and Iron-Reducing Conditions. *3.2.1. Biotransformation Rates and Products Quantified by LC−MS/MS Targeted Analysis.* The biotransformation of 8:2 FTOH under sulfate-reducing and iron-reducing conditions was also observed in live-spiked microcosms based on the detections of known biotransformation products (Figure 2B,D). The biotransformation under both redox conditions, however, was less complete and much slower than that under nitrate-reducing conditions. At the end of the incubation (>400 days), 64.4 ± 13.3 and 75.2 ± 8.4 mol % of the initially applied 8:2 FTOH remained in ED and NA treatments, respectively, under sulfate-reducing conditions (Figure 2A). Similarly, under iron-reducing conditions, 71.8 ± 20.6 and 90.0 ± 1.1 mol % remained in ED and NA treatments, respectively (Figure 2C). Among the known 8:2 FTOH biotransformation products reported previously[23,33] and those under nitrate-reducing conditions, only 8:2 FTCA and 8:2 FTUA were measured as transformation products in the sulfate-reducing and iron-reducing live-spiked microcosms (Figure 2B,D). No significant differences ($p > 0.05$) in 7:2 sFTOH, 7:3 acid, or C4-C9 PFCAs were observed among all treatments at each sampling event (Figures S13 and S14). Specifically, low concentrations of 8:2 FTCA and/or 8:2 FTUA were measured starting at day 56 in ED and NA treatment under sulfate-reducing conditions (Figure 2B). Both transformation products peaked at day 154 in the ED treatment with 3.5 ± 1.7 mol % of 8:2 FTCA and 0.2 ± 0.1 mol % of 8:2 FTUA and then subsequently decreased (Figure 2B). In the NA treatment, however, 8:2 FTCA and 8:2 FTUA increased continuously to the molar yields of 6.5 ± 1.8 and 1.1 ± 0.8 mol % at the end of incubation (Figure 2B). The slower biotransformation rate of 8:2 FTOH under iron-reducing conditions resulted in the later detection of transformation products, which were not detected until day 154 (Figure 2D). Only trace amounts of 8:2 FTCA and 8:2 FTUA (<0.1 mol %) were quantified throughout the incubation in the NA treatment (Figure 2D). Relatively more of 8:2 FTCA and 8:2 FTUA were formed in the ED treatment, continuously increasing to 4.2 ± 1.5 and 0.2 ± 0.1 mol % by day 450 (Figure 2D). Based on the residual fractions of 8:2 FTOH and the amounts of quantified transformation products (i.e., 8:2 FTCA and 8:2 FTUA) in ED and NA treatments during biotransformation under sulfate- and iron-reducing conditions (Figure 2), it is likely that the lactate amendment resulted in the faster biotransformation of 8:2 FTOH, similar to observations under nitrate-reducing conditions.

3.2.2. Biotransformation Product Identified by LC−HRMS Analysis. The molar recoveries were relatively higher in live-spiked treatments under sulfate- and iron-reducing conditions with 65.1 to 82.8 and 71.8 to 90.0 mol % at the termination of the experiment, respectively. These higher molar recoveries were likely due to the decreased extent of 8:2 FTOH biotransformation (Figure 2 and Figure S6B,C) relative to the nitrate-reducing conditions. Nontargeted LC−HRMS analysis was also performed on the samples from each treatment under sulfate- and iron-reducing conditions, and a novel biotransformation product of 8:2 FTOH, 3-F-7:3 acid ($F(CF_2)_7CFHCH_2COOH$, *m/z* 458.9885), was tentatively identified (confidence level 3, based on MS^2 (Figure S7C)) under both conditions (Table S9). The obvious increases in the peak area were observed in ED and/or NA treatments under sulfate- and iron-reducing conditions but not in abiotic and positive controls (Figure S15). One analogue of 3-F-7:3 acid, 3-F-5:3 acid ($F(CF_2)_5CFHCH_2COOH$), was previously identified as a product during 6:2 FTOH biotransformation in digester sludge, and 3-F-7:3 acid was proposed as a product of

https://doi.org/10.1021/acs.est.2c03669
Environ. Sci. Technol. 2022, 56, 13728−13739

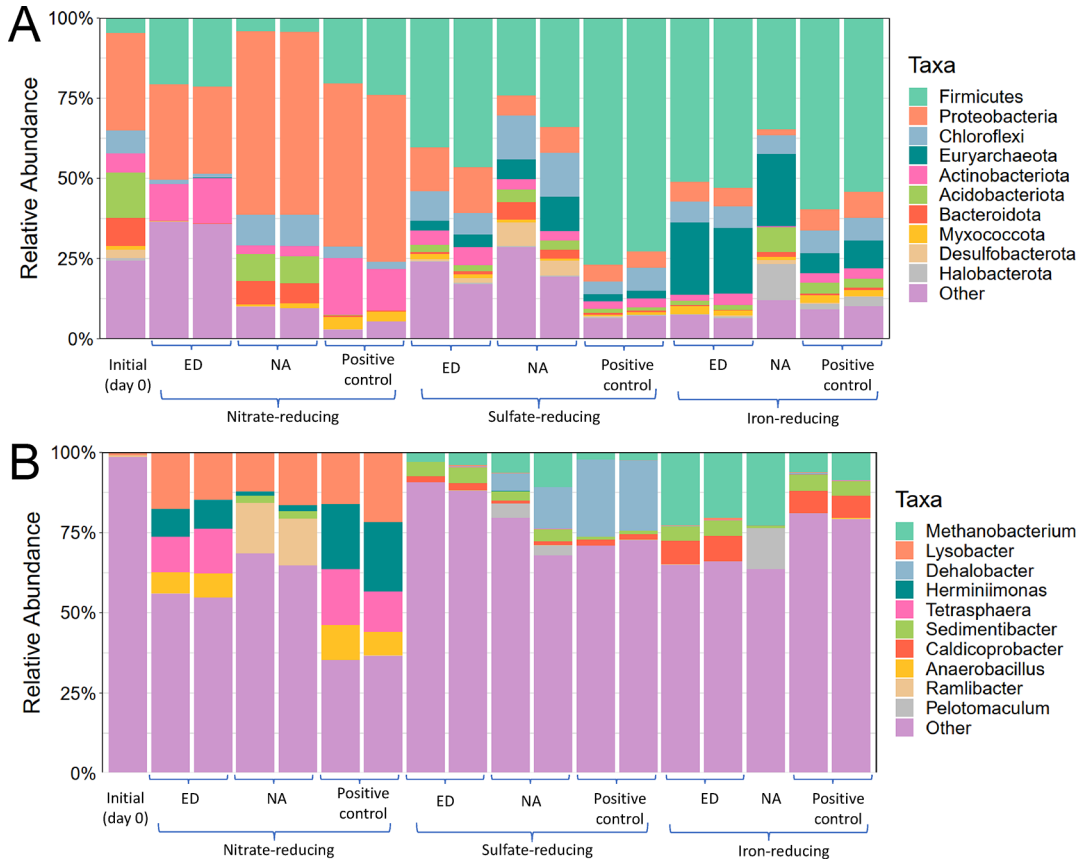

Figure 3. The relative abundance of microbial community composition at the (A) phylum level and (B) genus level in the initial Loring soil (day 0) and in the nitrate-, sulfate-, and iron-reducing microcosms at the end of ca. 400 days of incubation. Microcosms are classified as electron-donor (ED) treatment, natural attenuation (NA) treatment, and positive control.

8:2 FTOH in the same system.[19] In this study, 3-F-7:3 acid was likely formed by the reduction of 8:2 FTUA with a reductase under sulfate- and iron-reducing conditions. In the ED treatment under sulfate-reducing condition, 3-F-7:3 acid increased at week 22 and then decreased at week 44 (Figure S15B), indicating further biotransformation to other by-products or the possible conversion back to 8:2 FTUA as proposed previously.[19] The reverse reaction from 3-F-7:3 acid to 8:2 FTUA was less likely as the increase of 8:2 FTUA was not observed at week 44 in the ED treatment under sulfate-reducing condition (Figure 2B).

3.3. Distinct 8:2 FTOH Biotransformation Pathways under Different Redox Conditions. *3.3.1. Comparison of Biotransformation under Sulfate- and Iron-Reducing Conditions with That under Nitrate-Reducing Conditions.* The biotransformation of 8:2 FTOH observed in the sulfate- and iron-reducing microcosms was distinctly different from that in nitrate-reducing microcosms and also from those previously reported under oxic[32,33] and methanogenic conditions.[19,35] It has been proposed in prior studies that 8:2 FTOH was oxidized to 8:2 FTCA, which was further dehydrohalogenated to 8:2 FTUA.[19,32] Transformation from 8:2 FTOH to 8:2 FTUA was shown to occur readily under nitrate-reducing conditions, as well as under oxic and methanogenic conditions.[19,32] However, in the present study, 8:2 FTUA formation remained low (<1.1 mol %) during incubation under sulfate- and iron-reducing conditions. These results suggest that the conversion of 8:2 FTCA to 8:2 FTUA may be the rate-limiting step for 8:2 FTOH biotransformation under sulfate-reducing and iron-reducing conditions, likely associated with the lack of (unknown) microbial enzymes involved in the dehydrohalogenation reaction.

Moreover, the conversion of 8:2 FTUA to either 7:2 sFTOH or 7:3 U acid, which has been previously reported[26,33] and was also observed under nitrate-reducing conditions, did not occur under sulfate- and iron-reducing conditions. Instead, 3-F-7:3 acid was identified to be the likely transformation product of 8:2 FTUA under both conditions. Thus, under sulfate- and iron-reducing conditions, the hydrogenation pathway for 8:2 FTUA leading to 3-F-7:3 acid formation was preferred over the reductive defluorination pathway, which is required for the formation of 7:2 sFTOH or 7:3 U acid. This observation resonates with a recent study[50] that reported that reductive defluorination of fluorinated carboxylic acids with one fluorine substitution on the unsaturated carbon (e.g., 6:2 FTUA) was much less favorable than hydrogenation by an anaerobic microbial enrichment culture (82.9 and 17.1% of 6:2 FTUA underwent hydrogenation and reductive defluorination pathways, respectively). The preference of the hydrogenation pathway under sulfate- and iron-reducing conditions observed herein could also be associated with a lack/inefficiency of relevant enzymes responsible for defluorinating 8:2 FTUA to 7:3 U acid and decarboxylating 8:2 FTUA to 7:2 sFTOH. The similar enzymatic inefficiency has been reported in aerobic and methanogenic microorganisms.[19,26]

3.3.2. Comparison of Microbial Communities under Different Redox Conditions. Microbial community analysis of the samples collected from nitrate-, sulfate-, and iron-

https://doi.org/10.1021/acs.est.2c03669
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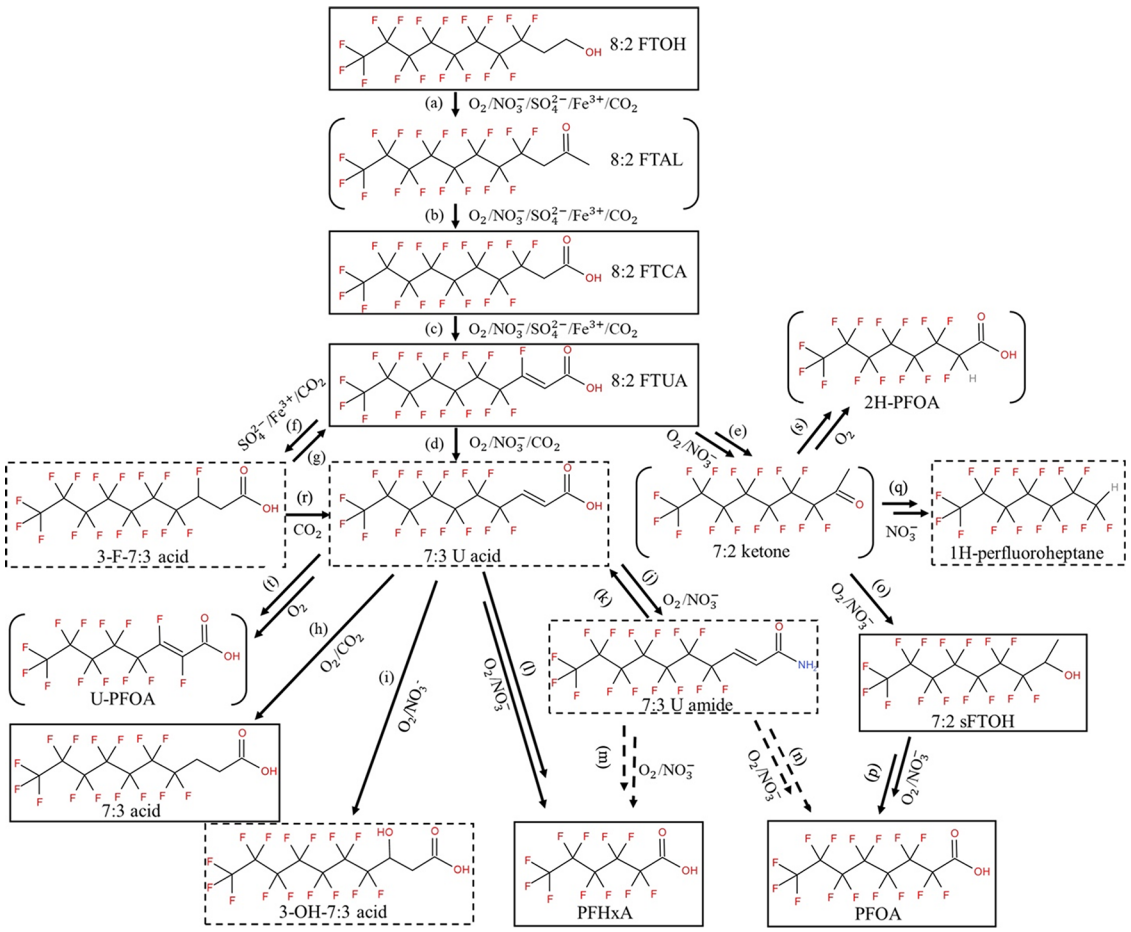

Figure 4. Proposed comprehensive biotransformation pathways of 8:2 FTOH under various redox conditions. O_2, NO_3^-, SO_4^{2-}, Fe^{3+}, and CO_2 represent oxic, nitrate-reducing, sulfate-reducing, iron-reducing, and methanogenic conditions, respectively. The pathways under oxic and methanogenic conditions were proposed in previous studies,[19,26,33,35,63] and they were observed in various environmental matrices (e.g., aerobic soil, activated sludge, digester sludge), which likely possessed different microbial communities from those in Loring soil (i.e., a historically AFFF-contaminated soil). The compounds in the solid rectangular boxes were quantitatively analyzed by LC−MS/MS, and the compounds in the dashed rectangular boxes were identified using nontargeted HRMS in the present study. The compounds in the brackets are the aerobic biotransformation products proposed and/or detected in previous studies but not detected in this study. The solid arrows indicate the biotransformation steps that would be expected to occur based on the current and/or previous studies. The dashed arrows indicate the potential reactions that may or may not occur. The double arrows indicate multiple enzymatic steps.

reducing microcosms provided further insight into the effect of redox condition on the biotransformation of 8:2 FTOH. Principal coordinate analysis (PCoA), an ordination technique used to visualize microbial community alternation,[51] revealed distinct clustering of microbial communities in Loring soil on day 0 compared to those at the end of ca. 400 days of incubation under each redox condition (Figure S16). At the end of incubation, microbial communities in the nitrate-reducing microcosms substantially separated from those in sulfate- and iron-reducing microcosms, while separation between communities from the sulfate- and iron-reducing microcosms was not apparent (Figure S16). These results indicate that the distinct biotransformation of 8:2 FTOH observed under sulfate- and iron-reducing conditions from that under nitrate-reducing condition was very likely due to the different microbial community compositions. As many sulfate-reducing bacteria are capable of using sulfate and iron(III) interchangeably as electron acceptors,[52,53] the relatively close microbial community structures may explain the similar 8:2 FTOH biotransformation observed under sulfate- and iron-reducing conditions.

The microbial community composition in nitrate-, sulfate- and iron-reducing microcosms was further analyzed at the taxonomic level by evaluating the OTUs and making comparisons to the SILVA database.[42,43] This additional analysis provided further support that distinctly different microbial communities were present under nitrate-reducing conditions compared to those under sulfate- and iron-reducing conditions (Figure 3). Proteobacteria was the most dominant phylum (relative abundance of 46.2 ± 12.2%) in nitrate-reducing microcosms, whereas it only accounted for 8.8 ± 3.7 and 5.4 ± 2.2% in sulfate- and iron-reducing microcosms, respectively (Figure 3A). Microorganisms belonging to Proteobacteria have been reported to degrade various hydrocarbon compounds,[54,55] as well as PFAS (e.g., FTOHs).[26,56−58] In this study, three genera in the Proteobacteria phylum, *Lysobacter*, *Herminiimonas*, and *Ramlibacter*, were exclusively found to be abundant in the ED and/or NA treatments under nitrate-reducing conditions (Figure 3B). Species in these genera may be responsible for the relatively rapid 8:2 FTOH biotransformation and transformation pathways observed under nitrate-reducing conditions. In

https://doi.org/10.1021/acs.est.2c03669
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sulfate- and iron-reducing microcosms, Firmicutes was the predominant phylum with relative abundances of 48.4 ± 19.1 and 51.0 ± 8.7%, respectively (Figure 3A). Firmicutes was previously found as a dominant phylum in PFAS-contaminated soils[59,60] and is also capable of biotransformation of chlorinated solvents.[61,62] In the present study, two genera in the Firmicutes phylum, *Caldicoprobacter* and *Sedimentibacter*, were found to be exclusively abundant at 1.6 ± 0.5 and 6.1 ± 3.0% in sulfate-reducing microcosms, respectively, and 3.0 ± 1.6 and 3.9 ± 1.7% in iron-reducing microcosms, respectively (Figure 3B). This prevalence suggests that the two genera may have contributed to the 8:2 FTOH biotransformation. However, the relevant enzymes for the transformation of 8:2 FTCA to 8:2 FTUA and further conversion of 8:2 FTUA to 7:2 sFTOH or 7:3 U acid may be absent in these genera.

Substantial differences between microbial communities under nitrate-reducing conditions and those under sulfate- and iron-reducing conditions described above likely resulted in the distinct 8:2 FTOH biotransformation (e.g., rate and pathways) observed in this study (Figure 4).

3.4. Comprehensive Biotransformation Pathways of 8:2 FTOH. Based on the biotransformation products detected by LC−MS/MS targeted analysis and by nontargeted HRMS under nitrate-, sulfate-, and iron-reducing conditions in this study, as well as the pathways proposed in previous studies on 8:2 FTOH biotransformation under oxic[26,33,63] and methanogenic conditions,[19,35] comprehensive biotransformation pathways for 8:2 FTOH were proposed under various redox conditions representative of most of natural environments (Figure 4). Although the pathways under oxic[26,33,63] and methanogenic conditions[19,35] were observed in various environmental matrices (e.g., aerobic soil, activated sludge, digester sludge), which likely possessed different microbial communities from those in Loring soil (i.e., a historically AFFF-contaminated soil), they were compiled here to compare with the pathways observed in Loring soil under nitrate-, sulfate-, and iron-reducing conditions to (1) provide insights into the role of redox condition and/or microbial community in 8:2 FTOH biotransformation and (2) give a holistic understanding of the 8:2 FTOH biotransformation pathways that have been observed to date.

Under all redox conditions, 8:2 FTOH biotransformation pathways shared the first three biotransformation steps before diverging into different paths. The first step was the oxidization of 8:2 FTOH aerobically or anaerobically to 8:2 fluorotelomer aldehyde (8:2 FTAL) by an alcohol dehydrogenase as described previously (pathway ″a″; refer to Figure 4).[19,33] Next, 8:2 FTAL was oxidized aerobically or anaerobically to 8:2 FTCA catalyzed by an aldehyde dehydrogenase (pathway ″b″). Further, 8:2 FTCA was dehydrohalogenated to 8:2 FTUA with hydrogen fluoride (HF) elimination involved (pathway ″c″). In the present study, 8:2 FTAL was not quantified by LC−MS/MS analysis due to the unavailability of an authentic standard and was not identified by HRMS analysis, indicating that this product was either unstable or rapidly oxidized under the tested conditions, as previously reported in 8:2 FTOH biotransformation under oxic[15,45] and methanogenic[35] conditions.

A key branch point for further biotransformation under the various redox conditions was 8:2 FTUA, after which the pathways diverged (i.e., pathways ″d″, ″e″, and ″f″). On pathway ″d″, 8:2 FTUA was converted to 7:3 U acid possibly via reductive defluorination, which was demonstrated for unsaturated PFAS by a commercially available microbial culture.[64] This step has been commonly reported in the studies on 8:2 FTOH biotransformation under oxic and methanogenic conditions;[19,23,26,33,35,45] however, it was only observed in the nitrate-reducing microcosms in the present study. Although previous studies showed that 7:3 U acid could be further transformed to 7:3 acid by reductases under oxic or methanogenic conditions (pathway ″h″),[19,26,33,35,45] transformation to 7:3 acid was not observed in the nitrate-reducing microcosms. Instead, 7:3 U acid was likely transformed to 3-OH-7:3 acid (pathway ″i″) and 7:3 U amide (pathway ″j″) by a hydratase and a transaminase, respectively, under nitrate-reducing conditions, and the latter reaction might be reversible (pathway ″k″). The conversion of 7:3 U acid to PFHxA (pathway ″l″) and 7:3 U amide to PFHxA/PFOA (pathways ″m″ and ″n″) was proposed and/or demonstrated previously under oxic conditions.[26,33,45] The reactions may also happen under nitrate-reducing conditions, and the rate of PFHxA production might be too slow to be appreciated.

Biotransformation of 8:2 FTUA to 7:2 ketone proceeded via multiple enzymatic steps involving defluorination and decarboxylation (pathway ″e″).[26,33] Then, 7:2 ketone was converted to 7:2 sFTOH (pathway ″o″), which was further transformed to PFOA through some unknown enzymatic steps (pathway ″p″). The sequence of pathways ″e″ to ″o″ and ″p″ has been reported as a major aerobic biotransformation pathway for 8:2 FTOH in the soil,[33] landfill leachate,[23] and mixed bacterial cultures.[26] In this study, 7:2 ketone was not targeted in LC−MS/MS analysis due to the lack of an authentic standard and was not detected in nontargeted analysis possibly due to its rapid conversion to downstream products. However, the substantial formations of 7:2 sFTOH and PFOA indicate that the pathways ″e″, ″o″, and ″p″ were likely prevailing during the 8:2 FTOH biotransformation under nitrate-reducing conditions. Moreover, 7:2 ketone might also be metabolized via multiple enzymatic reactions to form 1H-perfluoroheptane (pathway ″q″), a major intermediate product during 8:2 FTOH biotransformation under nitrate-reducing conditions, though the transformation products of 1H-perfluoroheptane are unknown at this point.

Under sulfate- and iron-reducing conditions, 8:2 FTUA was potentially converted to 3-F-7:3 acid by a reductase (pathway ″f″), which could be converted back to 8:2 FTUA (pathway ″g″). These reactions were proposed previously in digester sludge (i.e., under methanogenic conditions),[19] where 3-F-7:3 acid was also proposed to be transformed to 7:3 U acid via dehydrohalogenation (pathway ″r″). Two other biotransformation products identified in previous aerobic biotransformation studies of 8:2 FTOH,[33,63] 2H-PFOA (F-$(CF_2)_6$CFHCOOH) and unsaturated PFOA (U-PFOA, F-$(CF_2)_5$CF=CFCOOH) through pathways ″s″ and ″t″, respectively, were not detected in nontargeted HRMS analysis under tested conditions in the present study.

4. ENVIRONMENTAL IMPLICATIONS

This study demonstrates that the biotransformation rates and pathways of 8:2 FTOH are highly dependent upon redox conditions. Biotransformation was much slower under sulfate- and iron-reducing conditions than under nitrate-reducing conditions, while the fastest biotransformation were reported under oxic conditions.[32,33] The PFCAs, which were found as the terminal products of 8:2 FTOH biotransformation under oxic conditions, were produced with less yield under nitrate-

https://doi.org/10.1021/acs.est.2c03669
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reducing conditions and were not formed under sulfate- and iron-reducing conditions. The production of two polyfluorinated acids (i.e., 8:2 FTCA and 8:2 FTUA), however, was observed under all redox conditions investigated in the current and previous studies.[19,23,26,32,33,35] Biostimulation (e.g., lactate amendment) was shown to increase 8:2 FTOH biotransformation rates and product yields (e.g., PFOA) in the anaerobic microcosms tested in this study, implying that caution and considerations might be needed when the bioremediation is applied to treat other contaminants (e.g., chlorinated solvents) at sites where PFAS are also present.

The environmental fate of the newly identified products such as 1H-perfluoroheptane and 3-F-7:3 acid is not yet understood; therefore, future research is needed to elucidate the potential biotransformation and pathways of these compounds. In addition, genera potentially responsible for 8:2 FTOH biotransformation under each redox condition were identified including *Lysobacter*, *Herminiimonas*, and *Ramlibacter* under nitrate-reducing conditions and *Caldicoprobacter* and *Sedimentibacter* under sulfate- and iron-reducing conditions. Future studies with isolated pure cultures would help to better assess the biotransformation of 8:2 FTOH with species from these genera. Overall, the presented research findings provide an improved understanding of the impact of microbial communities on 8:2 FTOH biotransformation rates and pathways under various redox conditions, which have implications for contaminant fate in multiple environmental matrices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c03669.

Details regarding chemicals, microcosm setup and preparations, analytical methods, LC−MS/MS and HRMS analysis, anaerobic microcosms establishment, PFAS concentrations and mass balance in microcosms, MS/MS spectra and fragmentation data, and plotted peak areas of products identified in HRMS analysis (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the Strategic Environmental Research and Development Program (SERDP) under contract W912HQ-18-C-0014 for Project ER18-1149, "Development and Laboratory Validation of Mathematical Modeling Tools for Prediction of PFAS Transformation, Transport, and Retention in AFFF Source Areas". The LC-HRMS used to identify reaction byproducts was partially funded by NSF Major Research Instrumentation (MRI) award CBET-1919870. We acknowledge Naji Akladiss at Maine Department of Environmental Protection (MDEP) for providing access to collect Loring soil. Peng-Fei Yan and Sheng Dong also acknowledge the financial support from the China Scholarship Council and Auburn University Graduate School and Office of International Programs.

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https://doi.org/10.1021/acs.est.2c03669
Environ. Sci. Technol. 2022, 56, 13728−13739