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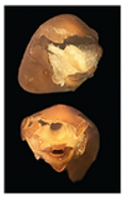

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Defluorination of per- and polyfluoroalkyl carboxylic acids (PFCAs) by wood decomposer fungi

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ABSTRACT

Large-scale manufacturing and disposal of fluorinated chemicals have led to global pollution by per- and polyfluoroalkyl substances (PFAS) that will require novel remediation techniques and investigation for their environmental fates. Fungi are dominant carbon nutrient recyclers in ecosystems, but their roles in responding to and degrading these persistent fluorocarbons remain largely untapped. Here, we investigated the fungal species' responses to perflouroalkyl carboxylic acid (PFCA) chemicals and their capacities in breaking down C–F bonds for defluorination (deF) by using the ion-selective electrode for quantifying free fluoride anions and the ^{19}F nuclear magnetic resonance (NMR) for monitoring PFAS removal in fungal cultures. Cytotoxicity assays showed that taxa within a unique class of fungi that cause "white rot" type of wood decay have developed an inherent defense mechanism for fluoride and fluorocarbon chemicals, setting off a basis for further investigating their deF phenotype. Although the current test did not evidence clear deF in legacy PFAS, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), it identified dehalogenated PFCA structures associated with an electron-attracting alkenyl group that provokes C–F cleavage. Our research, therefore, set a foundation for further unraveling the fungal deF mechanisms, and it also highlighted that future research should give sufficient attention to resident fungal communities in impacted environments due to their potential to recycle fluorinated compounds.

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Fluoride anion detection; fungal defluorination; fungal tolerance to PFAS; per- and polyfluoroalkyl carboxylic acids; PFAS; wood decay fungi

INTRODUCTION

Fluorinated carbon chemicals, particularly per- and polyfluoroalkyl substances (PFAS), which are widely used for consumable products and firefighting foams, are a growing source of pollution of concern to the U.S. Environmental Protection Agency (EPA) (Abunada et al. 2020). Traditional waste management facilities, including landfills and wastewater treatment plants, are facing difficulties treating these chemicals (Coggan et al. 2019; Lang et al. 2017; Liou et al. 2010), and their runoffs are leading to significant accumulations of PFAS in soil, water, and other environments (Anderson et al. 2016; Brusseau et al. 2020; Lang et al. 2017; Masoner et al. 2020). Structures of PFAS are considered exceptionally persistent and non-biodegradable in natural environments, mainly due to their C–F bonds, which have the strongest bond dissociation energy (130 kcal mol^{-1}) among the single carbon bonds (Bentel et al. 2019; Buck et al. 2011; Wang and Liu 2020). Being able to cleave the inert C–F bond, namely, defluorination (deF), is therefore recognized as the crucial step toward the biodegradation of PFAS (Kucharzyk et al. 2017). However, the deF capacity of microorganisms remains a rarely discovered phenotype (Wackett 2022). Recent studies have suggested that microbes have developed limited capacities to biotransform or defluorinate (deF) PFAS (Che et al. 2021; Colosi et al. 2009; Huang et al. 2022; Luo et al. 2018; Merino et al. 2018; Tseng et al. 2014), but the relevant studies are overall still limited in fungal species. Our research, therefore, aims to explore the deF capacities in a ubiquitous group of fungi that can cause wood decay and are crucial to carbon nutrient recycling in ecosystems, thus advancing our understanding of fungal roles in recycling fluorinated carbon compounds.

Wood decay fungi are primary plant carbon degraders in forest systems, and they evolved unique machinery to degrade lignin to expose polysaccharides embedded in plant cell walls (Andlar et al. 2018). These fungi adopted a set of oxidoreductases and hydrolases to break down the inert linkages of lignin, which meanwhile provides fungal cells the capacity to co-metabolize xenobiotic chemicals such as plastics, organic dyes, and halogenated pharmaceuticals (Zhuo and Fan 2021). Although it remains a debate whether or not the fungi can leverage their ligninolytic systems to degrade PFAS, studies have demonstrated that fungal species have the potential to degrade short fluorinated carbon chain chemicals. For example, a *Fusarium solani*

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strain isolated from "rodenticide 1080" contaminated soil can deF the C–F bond of fluoroacetate (Walker and Lien 1981), with fluoroacetate dehalogenase (FAcD) being characterized as the deF catalyst. The most recent catalytic model further suggested that FAcD not only can deF monofluoroacetate via a hydrolytic mechanism, but it also can catalyze the deF of poly- or perfluoroacetate to glyoxylate (Yue et al. 2021). However, whether a similar deF process has been adopted in other fungal species, especially in wood decay fungi, to deF the sturdier PFAS with longer carbon chains still requires further investigation.

Studies on fungal degradation of PFAS are still in their infancy, and research mostly focuses on investigating the aerobic degradation processes, as fungal metabolism primarily relies on respiration for gaining energy. Tseng et al. (2014) investigated the degradation of 6:2 fluorotelomer alcohol (6:2 FTOH) [$F(CF_2)_6CH_2CH_2OH$] by a "white rot" wood decay fungus, *Phanerochaete chrysosporium*. Their results suggested that this fungus has the biochemical pathways to aerobically transform 6:2 FTOH into a mixture of shorter-chain poly- and per- and polyfluoroalkyl carboxylic acid (PFCAs) [e.g., perfluoropentanoic (PFPeA) and perfluorohexanoic (PFHxA) acids] and other polyfluoroalkyl analogs/conjugates, with the majority product being 5:3 fluorotelomer carboxylic acid (5:3 FTCA) [$F(CF_2)_5CH_2CH_2COOH$]. Within this research, by referring to that of aerobic bacterial processes, the authors proposed that 6:2 FTOH can be firstly converted to 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA) [$F(CF_2)_5CF{=}CHCOOH$] via a series of oxidative steps, followed by β-elimination to remove fluorine to produce 5:3 FTUCA and then to 5:3 FTCA. Alternatively, 6:2 FTUCA can also be subjected to decarboxylation and α-elimination for deF, leading to the production of PFPeA or PFHxA. Merino et al. (2018) from the same research group then expanded this research and rediscovered the biotransformation of 6:2 FTOH with more wood-rotting fungal species, which repeatedly demonstrated that fungal degradation yields 5:3 FTCA (up to 51 mol% of initial 6:2 FTOH dosed) as the dominant transformation product. This research also claimed that 5:3 FTCA might be further transformed via the "one-carbon removal" cycle (Wang et al. 2012), despite that the process will likely be terminated at the step of 4:3 FTCA in fungi. Together, these works suggested that wood decay fungi are capable of transforming fluorotelomer alcohols to PFCAs.

Although wood decay fungi have shown their potential to transform fluorotelomer alcohols, free fluoride products were not detected in the previous research (Merino et al. 2018; Tseng et al. 2014), leaving a significant question of whether they can deF PFAS. Notably, the capability of these organisms to deF PFAS in the carboxylic acid format—PFCAs [e.g., perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS)] underscored by EPA regulations—requires further study, as this still remains a rare phenotype. Zhou et al. (2023) proposed that PFOA can be transformed via the combined cross-coupling and rearrangement processes catalyzed by free radicals during its degradation by *Phanerochaete chrysosporium*, the same white rot fungal species used by Tseng et al. (2014). Although this proposed model suggested a concurrent deF process, the detection of free F^- was not reported in the research (Zhou et al. 2023). Using a soil mucor fungus *Cunninghamella elegans*, Khan et al. (2023) found 20–30% deF of PFOA after 48 h of fungal treatment but detected a different set of transformation products than in *P. chrysosporium*. Alternative biochemical pathways of PFOA degradation were therefore proposed for *C. elegans*, suggesting that fungi may have developed various mechanisms to degrade PFAS, and broader investigation in alternative fungal species would be required. As a consequence, the missing information on deF in wood decay fungi has concurrently led to a major challenge in studying the corresponding biochemical pathways adopted for fungal degradation of PFAS. For instance, it complicated associating biocatalysts [e.g., cytochrome P450 (Merino et al. 2023), laccase (Luo et al. 2018), and ligninolytic peroxidase (Colosi et al. 2009)] with their functions in PFAS degradation. In this regard, more investigations into wood decay fungi for their capacities to deF PFCA chemicals are required.

In this study, we first monitored the radial growth of a collective of wood decay fungi on PFCAs to evaluate their responses to the toxicities of parent PFAS and of the possible defluorination products. By doing this, we expected to delineate the fungal response to PFAS toxicity across a broader taxonomic scale. Meanwhile, as the cytotoxicity of PFAS could be mediated by fluorosis, which impairs the functions of essential enzymes by competing with metal ions, such as Mg^{2+}, for binding sites (Strunecka and Strunecky 2020; Waugh 2019), fungal tolerance was used as an indirect indicator for screening deF potentials. Thus, although PFAS tolerance of a fungal strain does not necessarily prove its deF capacity, it will facilitate the rapid screening of the candidate species for further studying the fungal degradation processes of PFAS. Following this, we then adapted a potentiometer method using a fluoride ion selective electrode (F-ISE) to investigate their deF capacities. With appropriate optimizations in a previous work, we have confirmed that the F-ISE method demonstrated

reliability in quantifying free fluoride anions within the unique nutrient and metabolite backgrounds of fungal cultures (Ayers and Zhang 2025). Our results indicated that the fungal response to PFCAs is dependent on both the fungal clade and the type of wood decay. Notably, fungi that cause "white rot," particularly from the Agaricales and Polyporales groups, exhibited a distinct tolerance to the legacy PFCA chemical PFOA. This potential for PFAS detoxification was confirmed using the typical white rot species *Trametes versicolor*, along with eight PFCA chemicals and sodium fluoride (NaF). The screening of fungal deF capacities using F-ISE aligns with previous work demonstrating that fluorine attached to the unsaturated carbon atoms or those adjacent to unsaturated bonds [e.g., 4,4,4-trifluoro-3-(trifluoromethyl)crotonic acid ($(CF_3)_2C=CHCOOH$), 4,5,5-trifluoropent-4-enoic acid ($F_2C=CFCH_2CH_2COOH$), and 2-(trifluoromethyl)acrylic acid ($H_2C=C(CF_3)COOH$)] are susceptible to bacterial attack (Che et al. 2021; Yu et al. 2022). In contrast, fungal deF was not detected for the legacy PFCAs and fluorotelomer alcohols, such as PFOA, PFOS, GenX, and 6:2 FTOH, that lack unsaturated bonds. Overall, this research demonstrates a distinct biological response of a group of ubiquitous wood decomposer fungi to PFCA chemicals and provides fungal and chemical models for studying deF mechanisms in relation to various PFCA compounds.

MATERIALS AND METHODS

PFAS and fluoride chemicals.—Eight PFAS and sodium fluoride (NaF) were purchased from Sigma Aldrich (Milwaukee, Wisconsin) [PFOA, CAS no. 335-67-1, 98% purity; PFOS, 1763-23-1, 98% purity; 6:2 FTOH, 647-42-7, 97% purity; 2-(trifluoromethyl)acrylic acid, 381-98-6, 98% purity; vinyl trifluoroacetate, 433-28-3, 98% purity; NaF, 7681-49-4, 99.99% purity], Matrix Scientific (Columbia, South Carolina) [GenX, 13252-13-6, 97% purity; 4,4-trifluoro-3-(trifluoromethyl)crotonic acid, 1763-28-6, 97% purity], and Oakwood Chemical (Estill, South Carolina) [4,5,5-trifluoropent-4-enoic acid, 110003-22-0, 97% purity], and 10 mM stock solutions, except PFOS, which was prepared at 1 mM, were prepared aseptically in sterilized deionized water and stored at room temperature under constant agitation at 150 rpm until use. Polypropylene bottles containing the stock solutions were sonicated in Bransonic M Mechanical Bath 3800 (40 kHz) for 20 min to disperse micellar structures and homogenize PFAS solution prior to the spiking of fungal cultures. We have confirmed that under this low-intensity sonication, the PFAS chemicals tested here will not be defluorinated, although it has been reported by others that more intensive sonication (≥500 kHz for hours) may lead to the PFAS degradation (Awoyemi et al. 2024; Singh Kalra et al. 2021).

Fungal strains and culture conditions.—Wood decomposer fungal strains *Trametes versicolor* A1-ATF, *Stereum hirsutum* FP-91666, *Fomitopsis pinicola* FP105877R, and other mentioned species were obtained from the United States Department of Agriculture (USDA) Forest Products Laboratory (FPL; Madison, Wisconsin) or the Forest Mycology Collection at the University of Minnesota. The strains were stored on YMG (yeast extract 4 g/L, malt extract 10 g/L, glucose 10 g/L) agar slants at 4 C until being used for degrading PFCAs. Fungal mycelia were activated on YMG agar plates at 28 C for 6 days before being tested for their resistance to PFCA and fluoride chemicals and their deF capacities with either liquid or solid-state culturing conditions. The 15 g/L agar was used to make the agar slant or plate.

Plate culture was performed to test the resistance of 18 fungal species to a variety of PFCA compounds and fluoride anions at different concentrations in Highley's minimum medium (HMM) (Highley 1973) that contains 10 g/L glucose and the following basal salts additions (per liter): essential nutrient mix I: 2 g NH_4NO_3, 2 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.1 g $CaC1_2{\cdot}2H_2O$; trace metal mix II: 0.57 mg H_3BO_4, 0.036 mg $MnC1_2{\cdot}4H_2O$, 0.31 mg $ZnSO_4{\cdot}7H_2O$, 0.039 mg $CuSO_4{\cdot}5H_2O$, 0.018 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.015 mg $FeSO_4{\cdot}7H_2O$; and 0.001 g thiamine hydrochloride. Mycelial growth on the plate was measured by monitoring the changes in the colony diameters over time.

Liquid cultures were conducted in five replicates in 125-mL Erlenmeyer polypropylene plastic flasks containing 50 mL of HMM spiked to 100 μM of the corresponding PFCA compounds to test fungal deF. HMM was supplemented with 1 g/L glucose and 10 g/L aspen wood sawdust as carbon sources. Ten 5-mm activated mycelial plugs were used to inoculate each flask, and the cultures were incubated at 28 C and 110 rpm for up to 3 weeks. Two milliliters of sample per flask were aliquoted every week and were centrifuged at 10 000 rpm and 4 C for collecting the supernatant for F^- quantification. The inoculated flasks without spiking PFCA, as well as flasks with PFCA but without fungal inoculum, were also prepared as the controls.

Solid-state fermentation (SSF) [moisture contents (MC) = 80–83%] in polypropylene Petri dishes in five replicates was used to test the deF of PFCAs by *T. versicolor*, whereas semisolid, slurry culture (MC =

91%) was in 125-mL Erlenmeyer polypropylene plastic flasks. To prepare the medium, each 100 mL HMM basal salts solution (~100 g) containing 100 µM PFCA compound of interest was added 25, 20, or 10 g aspen wood sawdust (*Populus* sp.; particle size 250–425 µm, i.e., from 40 to 60 mesh) to bring the MC [(HMM solution weight/total medium weight) × 100%] to 80%, 83%, or 91%, respectively. To facilitate the growth of wood decay fungi, the medium was also supplemented with 1% (*w*/*w*) maize meal. After thorough mixing, 20 g of medium was distributed into each dish/flask and was inoculated with five 5-mm activated *T. versicolor* mycelial plugs. SSF cultures were then incubated in a stationary-humidity chamber at 28 C and 70% humidity for up to 3 weeks, whereas slurry cultures were incubated in a shaking incubator at 110 rpm. After harvest, the entire SSF culture was oven-dried at 70 C and then transferred to a 50-mL Falcon tube containing 30 mL of distilled deionized (DDI) water for F^- extraction overnight with a horizontal agitator at 150 rpm and 4 C. Supernatants, collected by centrifuging the mixtures at 4000 rpm and 4 C for 10 min, were then used for measuring F^-. The inoculated plates without spiking PFCA were used as the controls, and five bioreplicates were used for each condition. The end-point MC of the SSF culture was measured by subtracting oven-dried weight from total wet weight to back calculate the F^- concentrations in the cultures. The F^- concentrations in slurry cultures were obtained by directly measuring the supernatants of the centrifuged cultures.

Fluoride Ion Selective Electrode (F-ISE) measurement in fungal cultures.—The potentiometric method for measuring fluoride ion was optimized using the Orion Star A214 pH/ISE benchtop meter (Thermo Fisher Scientific, Waltham, Massachusetts) and the Orion Fluoride Ionplus Sure-Flow solid-state combination electrode (CAS no. 9609BNWP) (Ayers and Zhang 2025). Two total ionic strength adjuster buffers (TISABs) were tested for their effects in eliminating the interference of fungal medium components. The low-level TISAB was prepared by dissolving 57 mL glacial acetic acid and 58 g of NaCl in DDI water, adjusting the pH with 5 M NaOH to 5.0–5.5, and finally fixing the total volume to 1 L with DDI water. TISAB II, with xxx 1,2-Cyclohexylenedinitrilotetraacetic acid (CDTA) and a stronger buffering capacity (CAS no. 940909), was purchased from Thermo Fisher Scientific. Results showed that the stronger TISAB II buffer can efficiently mitigate fungal culture interferences. Thus, it was used for the F-ISE measurement of fungal samples.

To measure F^-, TISAB II was mixed 1:1 with the F^- solution and incubated for 15 min at room temperature (20–22 C) before analysis. F-ISE measurement was done according to the manufacturer's instructions. After calibration in storage solution for 5 min, the probe was immersed in F^- solution for recording the millivolt (mV) reading in 5 min or until the reading was stable. The probe was rinsed and allowed to rest in a storage solution between different measurements. In this procedure, all solutions must be kept at the same temperature and pH (5–5.5), given that the changes in these two factors affect the electrode potentials. The mV readings were plotted against the F^- concentrations to test the effectiveness of the optimized F-ISE method.

The fungal extracts collected from liquid and solid fermentations, as above, were measured for fluoride concentrations using F-ISE. To do this, the TISAB II buffer with CDTA was mixed 1:1 with the extract in a 15-mL Falcon tube on the styrofoam holder and incubated for 15 min at room temperature prior to analysis. The mixture was then measured by F-ISE with a fluoride electrode, as described above. Along with samples, a series of F^- standards were also measured by preparing them in the context of corresponding PFAS compounds (100 µM) and control extracts from non-PFCA cultures to eliminate any possible interferences of fungal metabolites and F^- contamination in PFAS stocks. The mV readings were plotted logarithmically against F^- standard concentrations (0–124 µM) for calculating the calibration curve and for determining the F^- concentration in samples.

^{19}F NMR analysis.—Fungal samples were prepared with 90% aqueous sample and 10% D_2O for PFAS quantification with ^{19}F NMR (nuclear magnetic resonance) spectroscopy. Trifluoroacetic acid (TFA) was added to each sample at a final concentration of 100 µM as an internal reference. Samples were vortexed to mix, and 700 µL was transferred to thin wall, 5 mm, 7 inch length, 500 MHz precision NMR tubes (Wilmad 528-PP-7-5; Sigma Aldrich) using Pasteur pipettes for measurement. ^{19}F spectra were acquired using Bruker Avance III HD 400 MHz spectrometer with SampleXpress autosampler (University of Minnesota facility) without proton decoupling. A delay time of 3 s and 450 scans was experimentally determined to provide a sufficient signal-to-noise ratio and relaxation. MestReNova software was used for spectral processing (Mnova 15.1). Each spectrum was phase-corrected, baselined using Whittaker smoother, and referenced to TFA (−76.55 ppm). Integration and chemical shifts were

recorded for the $-CF_3$ groups of PFOA and 4,4-trifluoro-3-(trifluoromethyl)crotonic acid. Molar concentrations of PFAS were calculated using the following equation:

$$C_x = \frac{I_x}{I_s} \times \frac{N_s}{N_x} \times C_s$$

where C_x is the concentration of PFAS, I_x is the integral of $-CF_3$ group of PFAS, I_s is the integral of $-CF_3$ group of TFA, N_s is the number of the $-CF_3$ group of TFA, N_x is the number of the $-CF_3$ group of PFAS, and C_s is the concentration of internal control TFA.

Statistical analysis.—At least three bioreplicates were used in this work to allow the statistical analysis. Mean values and standard deviations were presented. Two-tailed paired *t*-tests were used to establish significant differences when required, given unequal variances among the various treatments. Logarithmic regression modeling was used for calculating the F^- calibration curves of ISE measurements.

RESULTS AND DISCUSSION

Tolerance to PFCA chemicals is a variable fungal trait influenced by taxonomic clade and decay type.—Given that a fungus's ability to resist chemical toxins can reflect its biodegradative capacity (Mao and Guan 2016; Nesse et al. 2023), we assessed the tolerance of different wood decay fungi to PFCAs, aiming to identify suitable species for further studies on deF. We tested species from six clades within the Agaricomycotina subdivision of the Basidiomycota that represent two primary decay types, brown rot and white rot (Zhang et al. 2019), for their mycelial growth in the presence or absence of PFOA, a legacy PFCA chemical (FIG. 1). Our findings revealed that the effects of PFOA on hyphal growth are species specific; some fungi, such as *Leucogyrophana mollusca*, experienced a significant reduction in growth rates by 80–100%, whereas others, such as *Ganoderma austral* and *Trametes versicolor*, showed no inhibition from PFOA exposure (FIG. 1A). Further analysis indicated that the fungal sensitivity to PFOA depends on both taxonomic classification and wood decay type. Generally, fungi from the Agaricales and Polyporales clades appeared more resistant to PFOAs. However, there was considerable variability within the Polyporales group, where wood decay types also contribute to the differences among intragroup species. Conversely, fungi from the Boletales clade and early-diverged Agaricomycotina, such as Russulales and Hymenochaetales, were more susceptible to PFOA toxicity (FIG. 1B). When comparing decay types, brown rot fungi were inhibited by PFOA by an average of 58%, which is significantly more (5 times, with a *t*-test *P*-value of 0.026) than the inhibition observed in white rot fungi. This higher resistance in white rot fungi may be linked to their unique

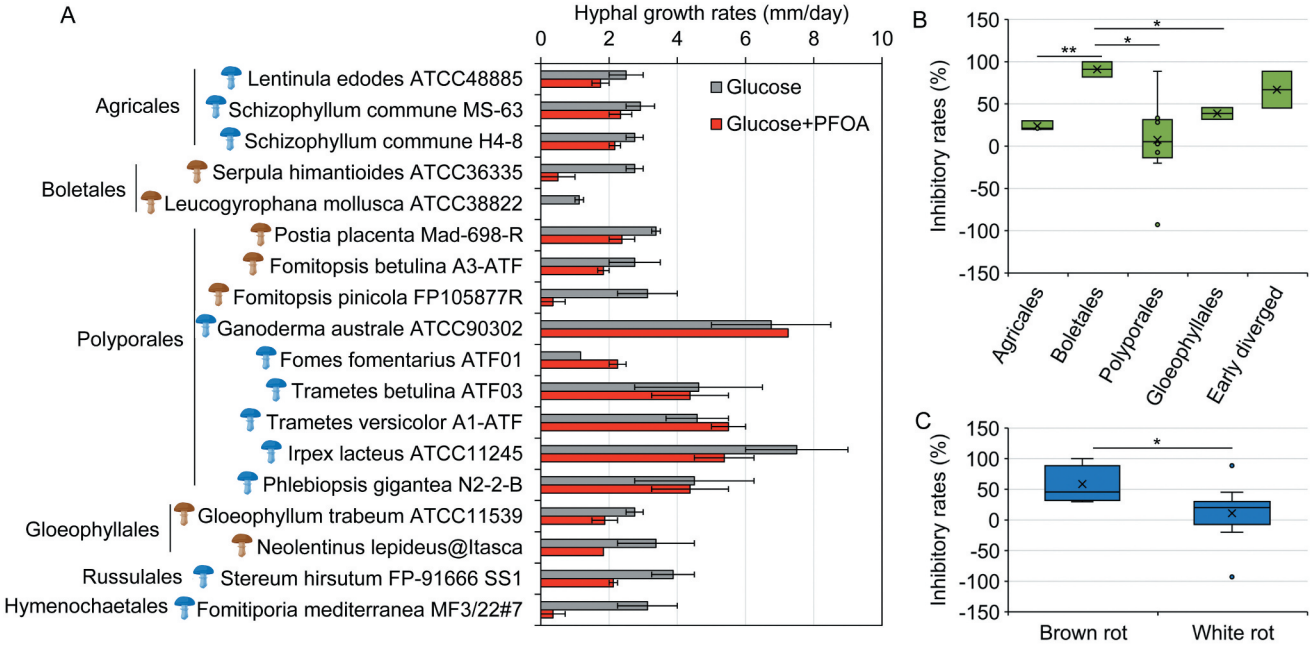

Figure 1. Comparison of growth rates of wood decay fungal species with PFOA supplementation. A. Eighteen wood decay fungal species distributed in six different Agaricomycota clades and possessing two primary wood decay types ("white-rot"—blue sporophore symbol; "brown-rot"—brown sporophore symbol) were cultured on Highley's minimum medium plates supplemented with either 10 g/L glucose or 10 g/L glucose + 500 mg/L PFOA (i.e., 1.2 mM PFOA) for monitoring the radial growth of hyphal colonies. Mean values ± standard deviations of three bioreplicates are shown. B, C. The inhibitory rates by PFOA were calculated relative to the growth on glucose and were compared among different fungal clades (B) and wood decay types (C). A two-tailed *t*-test was used to calculate the significant difference between defined fungal groups (*$P < 0.05$; **$P < 0.01$).

ligninolytic system used for lignocellulose degradation, which aligns with previous studies proposing that ligninolytic enzymes in white rot fungi play a role in PFAS transformation (Colosi et al. 2009; Luo et al. 2018; Merino et al. 2023). Overall, our results highlight that white rot species, particularly those in the Agaricales and Polyporales clades, show notable tolerance to the legacy PFAS PFOA. Although the specific detoxifying mechanisms remain unclear, these resistant patterns offer valuable insights for selecting candidate species for the mycoremediation of PFAS. Moreover, this finding could facilitate cross-species comparisons in the search for PFAS degradation genes and pathways.

Based on the screening results, we selected a typical white rot fungus *T. versicolor* demonstrating a strong resistance of PFOA to test whether this fungal resistance could extend to other PFCAs and legacy PFAS (FIG. 2). We investigated eight PFCAs/PFAS with varying chain lengths and unsaturated carbons using plate assays, employing concentrations ranging from 100 to 300 µM (FIG. 2A and B). The results indicated that none of these PFAS significantly affected hyphal growth rates or colony morphologies on the agar plates at the selected concentrations. Microscopic visualization confirmed that the morphology of the hyphal cells was not altered (data not shown). These findings suggest that the white rot fungus may possess certain detoxifying mechanisms to cope with the diverse structures of fluorinated carbon chemicals.

To diagnose the potential resistance mechanisms, we further investigated the responses of *T. versicolor* to fluoride (NaF) (FIG. 2A and B), as the cytotoxicity of PFAS can be partially attributed to fluorosis, which impairs the functions of essential enzymes by competing with metal ions, such as Mg^{2+}, for binding sites (Strunecka and Strunecky 2020; Waugh 2019). Surprisingly, our assays revealed that *T. versicolor* was tolerant to F^- concentrations up to 1.5 mM (28.5 mg/L), significantly exceeding the EPA's maximum contaminant level of 4.0 mg/L designed to protect against skeletal fluorosis (Us Epa 2015a, 2015b). Moreover, rather than displaying an inhibitory effect, we consistently observed that the tested concentrations of F^- increased hyphal growth rates by 50–80% ($P < 0.05$, as determined by a paired, two-tailed *t*-test). This suggests that the fungus may combat fluoride toxicity by accelerating its polarized growth. It is known that fluoride, in the form of aluminofluoride complexes (AlF_x), can activate heterotrimeric G proteins, enhancing G protein signaling and speeding up phosphoryl transfer reactions (Strunecka and Strunecky 2020; Waugh 2019). These processes are important for cell metabolism, energy transduction, cytoskeletal protein assembly, cell proliferation, aging, and apoptosis. Meanwhile, genetic evidence indicates that the constitutive activation of G protein and small GTPase signaling leads to enhanced polarized apical growth of hyphal cells (Dautt-Castro et al. 2021; Leberer et al. 2001; Tisch et al. 2011; Zhang et al. 2012; Zhu et al. 2009). Therefore, it is likely that fluoride stimulates fungal growth through a mechanism similar to that in this research. Like F^-, other investigated PFAS also promoted fungal growth to varying degrees until the level of PFOA reached 10 mM, which completely inhibited growth (FIG. 2B). In summary, although the exact resistance mechanisms remain unclear, the evident tolerance of wood decay fungi to PFAS and fluoride makes these organisms strong candidates for exploring and utilizing relevant biodegradation processes for PFAS research.

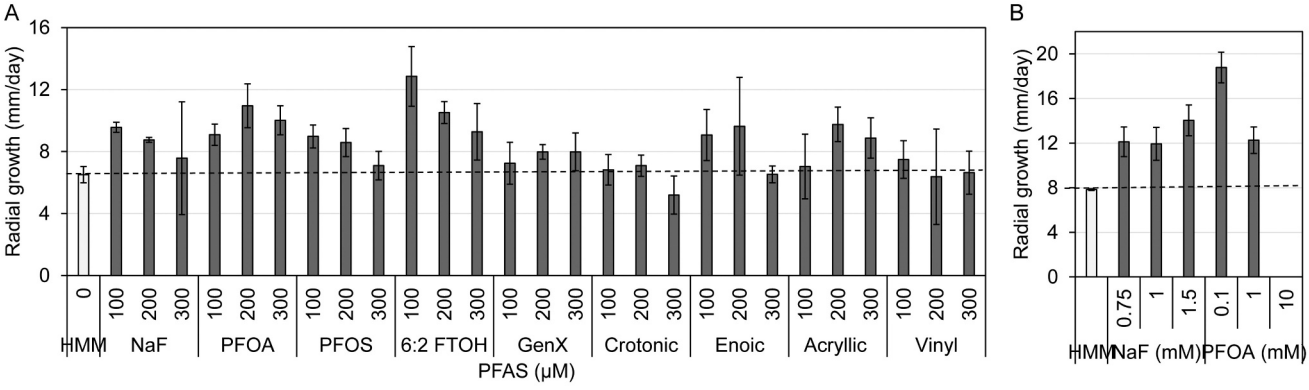

Figure 2. Tolerance of the wood decay fungus *Trametes versicolor* to fluoride and PFAS chemicals. A. Radial growth rates of the colonies of *T. versicolor* were monitored on agar plates that were supplemented with various PFAS and sodium fluoride at defined concentration gradients. The horizontal line represents the growth rate without any fluorinated carbon chemicals. B. Resistance of *T. versicolor* to fluoride and PFOA was confirmed by measuring hyphal growth rates at the extended range of concentrations. Mean values ± standard deviations from three bioreplicates are shown.

Wood decay fungi preferentially deF PFCA chemicals that possess alkenyl carbon structures. —After assessing the fungal tolerance to PFCA substances and fluoride anions, we investigated whether wood decay fungi could degrade PFAS by cleaving the carbon-fluorine (C–F) bonds. We first examined the ability of a white rot fungus, *Trametes versicolor*, to deF two legacy PFAS, PFOA and PFOS. We included a brown rot fungus, *Fomitopsis pinicola*, and an early-diverged fungus from the Agricomycotina group, *Stereum hirsutum*, for comparative analysis across different decay types and clades. Our results indicated that all three species showed negligible deF on both PFOA and PFOS over a period of 1 to 3 weeks (FIG. 3A and B). Similarly, tests conducted on two other commonly used commercial PFAS, 6:2 fluorotelomer alcohol (6:2 FTOH) and GenX [2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid)], yielded negative results as well. Prior research has indicated that certain wood decay fungal species can transform 6:2 FTOH into 5:3 FTCA and other transformation products; however, it also highlighted difficulties in detecting fluoride ions released into the culture supernatant (Merino et al. 2018, 2023; Zhou et al. 2023). In our study, considering a significant deF is detectable for the "dehalogenated" PFAS that are listed and discussed below, we conclude that wood decay fungi likely do not have the distinguished ability to deF PFAS with the long-carbon, perfluorinated alkyl structures, although they may transform these compounds into the formats of fluorinated aldehydes, alcohols, and aromatic ring, as detected by mass spectrometry (Merino et al. 2018, 2023; Zhou et al. 2023).

It is not surprising that PFAS chemicals with longer chain lengths and perfluorinated alkyl carbons are not readily dehalogenated by fungi, as the C–F bonds in these structures possess higher dissociation energy. Therefore, we further investigated a variety of commercial PFCAs with alternative structures featuring shorter chains and unsaturated carbons, which may lower the energy required to break down a C–F bond. Our subsequent tests indicated that 4,4-trifluoro-3-(trifluoromethyl)crotonic acid (referred to as "Crotonic" in FIG. 3), 4,5,5-trifluoropent-4-enoic acid ("Enoic"), and 2-(trifluoromethyl) acrylic acid ("Acrylic") underwent defluorination readily, except for vinyl trifluoroacetate ("Vinyl") (FIG. 3). Overall, the white rot fungus Polyporales species *T. versicolor* exhibited higher deF rates than the other two species shown in FIG. 3A, and these rates increased with longer culture times. Variable deF degrees were observed across different culture batches, possibly due to differences in mycelium condition.

Analyzing the structures of these dehalogenated PFCAs revealed that they all share an alkenyl carbon bond that is directly linked to the fluorine (as seen in Enoic) or to the perfluorinated carbon (as in Crotonic and Acrylic). Through an inductive effect, the sp^2 hybridization of the C=C bond can attract electrons from the perfluorinated carbon, consequently destabilizing the C–F linkage. Similarly, in poly- or perfluoroacetate compounds (e.g., CF_3COOH), the carboxyl group attracts electrons from neighboring fluorinated carbons (Yue et al. 2021), which may impart characteristics allowing for dehalogenation to the compound. The corresponding biochemical pathways involved in fungal deF are not yet understood, and it is not known whether fungi share a similar aerobic deF mechanism proposed for activated sludge microbial communities

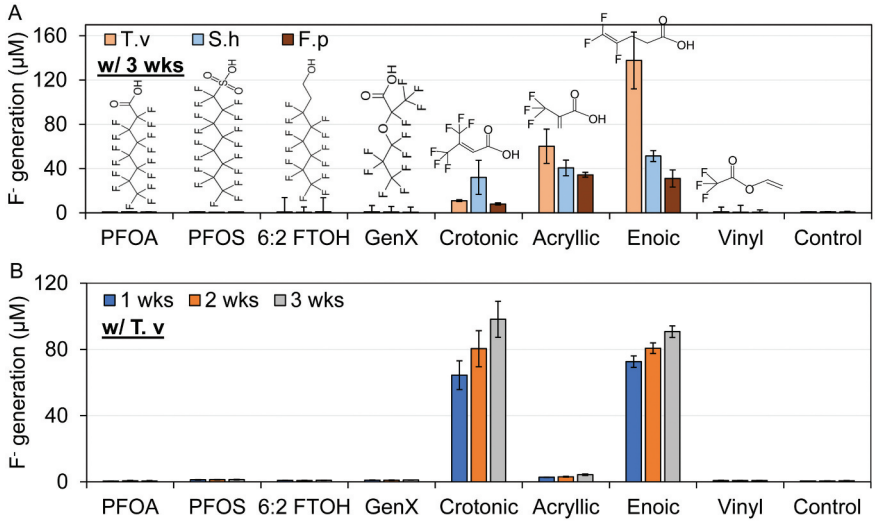

Figure 3. Fungal defluorination tests on various PFCAs and PFAS in liquid cultures. A. The 3-week end-point deF results for *Trametes versicolor* (T.v), *Stereum hirsutum* (S.h), and *Fomitopsis pinicola* (*mounacaea*) (F.p). B. The time course results of deF of *T. versicolor*. The mean values ± standard deviations of five bioreplicates are presented.

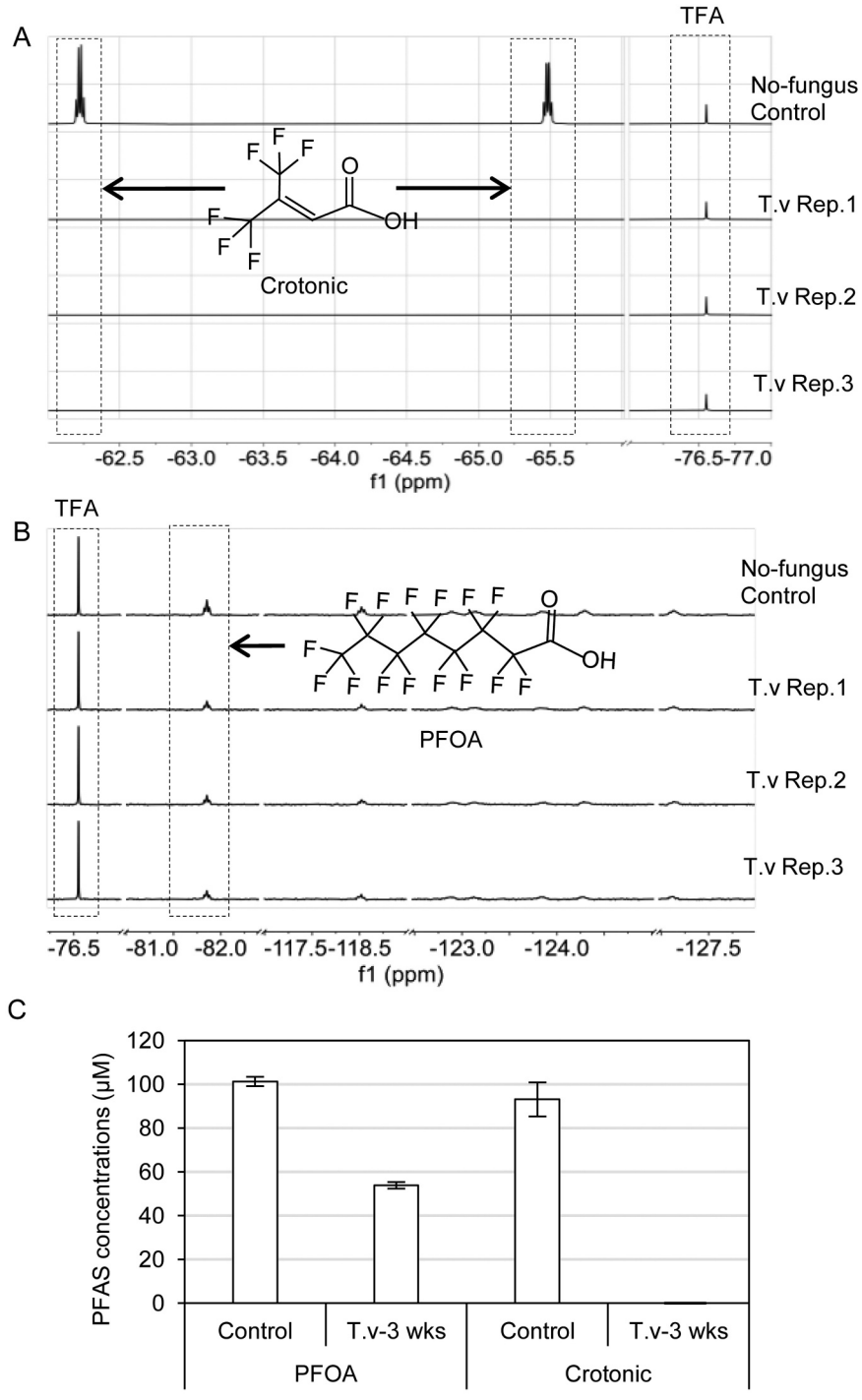

Figure 4. NMR quantification of PFCA chemical removal by liquid cultures of *T. versicolor*. The ^{19}F NMR quantifications of the residual Crotonic PFCA (A) or PFOA (B) chemicals after 3 weeks of culture by *T. versicolor* (T.v). ^{19}F spectra of the –CF3 groups of PFCA chemicals and internal control trifluoroacetic acid (TFA) are presented to indicate the removal of parent PFAS. Remaining concentrations of PFCA chemicals were calculated, as shown in C. Results from three bioreplicated are presented.

(Yu et al. 2022). Nevertheless, our finding supports the previously suggested structural model to pave the route for future exploitation of the underlying molecular mechanisms of fungal deF. Ongoing research will guide future attempts to create alternative formulations for PFAS.

Quantification of fungal removal of PFCA chemicals.—To assess whether fungal deF has led to the removal of the parent Crotonic PFCA chemicals, we quantified using ^{19}F NMR the residual concentrations of the PFCA after treating it with *T. versicolor* in liquid cultures for 3 weeks (FIG. 4).

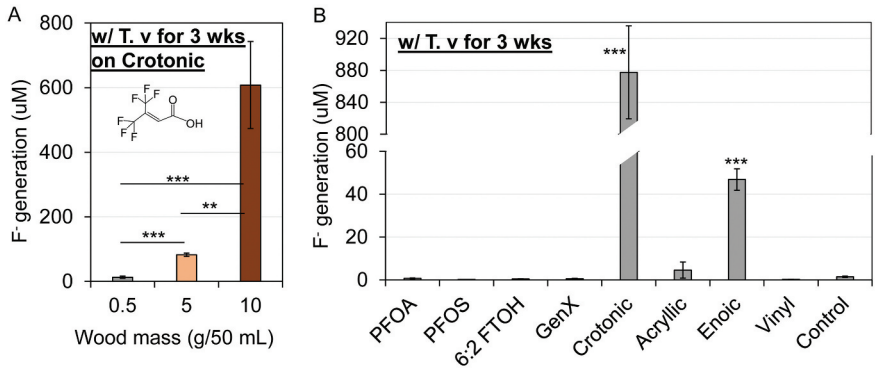

Figure 5. Fungal deF test under solid-state culture conditions. A. The deF of 4,4-trifluoro-3-(trifluoromethyl)crotonic acid (initial concentration = 100 μM) by *Trametes versicolor* (T.v) cultures that were supplemented with varying amounts of wood sawdust for creating the aqueous (10 g/L), slurry (100 g/L), and solid-state (200 g/L) conditions. B. The deF of PFCA chemicals at an initial concentration of 100 μM by *T. versicolor* in solid-state cultures containing 250 g/L wood sawdust. Mean values ± standard deviations were calculated across five biological replicates after 3 weeks of cultivation for all experiments. A paired, two-tailed *t*-test was utilized to determine significant differences between group pairs or to compare PFAS treatment with control conditions (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Both ours and others' research have demonstrated that ^{19}F NMR is a robust method for rapidly quantifying PFAS and alleviating potential errors introduced by sample processing and interferences caused by the background matrix of the fungal samples (Camdzic et al. 2021, 2023; Corcoran et al. 2001; Lewis et al. 2023; Moody et al. 2001). The results, as shown in FIG. 4A and C, confirmed that the Crotonic PFCA with an initial concentration of 100 μM can be completely removed by the fungal cultures. Our parallel research suggested that the sorption effect only contributes to less than 15% removal of Crotonic PFCA (unpublished data); the current NMR results thereby indicate that fungal deF has caused the significant removal of the parent compound. Meanwhile, we quantified the removal rates of PFOA by fungal cultures, although it is shown as not being readily defluorinable by the deF tests. The results showed a reduction of PFOA in its concentration by 45.6 ± 1.8% (FIG. 4B and C). Inferred from our sorption test that demonstrated the 30–40% PFOA removal rates by oven-dried killed fungal-wood sawdust material (unpublished data), we believe that the PFOA removal by the fungal cultures in the current setup is likely due to the sorption effect. However, we should be cautious of this conclusion, as the previous research conducted with *Phanerochaete chrysosporium* (Zhou et al. 2023) suggested that PFOA could be transformed into other products by fungal pathways. Since our current research primarily focuses on testing the fungal deF phenotypes, the transformation products and the relevant biochemical pathways that lead to PFCA degradation will be studied in future research.

Solid-state culture enhanced the deF of "Crotonic" PFCA.—Wood decay fungi are known to thrive as terrestrial organisms in less damp environments (Mali et al. 2017; Shimada et al. 1997). We, therefore, adjusted the amount of wood sawdust added to the medium and studied how culturing them on a solid substrate rather than in a liquid medium affected fungal deF (FIG. 5). For the readily dehalogenated Crotonic PFCA, we found that increased wood concentration (i.e., decreased moisture content [MC] of the medium) significantly enhanced the deF rates by the test fungus *Trametes versicolor* ($P < 0.01$, paired *t*-test; FIG. 5A). Notably, under solid-state fermentation (SSF) conditions (MC = 83%, *w*/*w*), we observed that 100% of the fluorine could be removed from Crotonic PFCA at the original concentration of 100 μM. In contrast, only a 13.7 ± 0.9% deF rate was achieved under semisolid, slurry conditions at MC = 91%. However, the SSF conditions did not improve the deF rates for the other two dehalogenated PFCAs, nor for the remaining five PFASs, which showed no deF in the liquid culture tests (FIG. 5B). Overall, our findings indicated that solid culture condition could affect fungal deF of certain PFCAs, but this impact is variable among different PFCA chemicals.

This finding regarding solid culture has significant implications for future research aimed at exploiting PFAS-degrading fungal organisms in contaminated environments. Typically, liquid culture is used as the default method to assess the degradative capacities of environmental microbes. However, some fungal species might not thrive in aqueous conditions, which could lead to an underestimation of their roles in PFAS biotransformation. Therefore, our finding serves as

a reminder for researchers to consider how culturing conditions can influence the biodegradation of PFAS. Additionally, the growth of fungal mycelia on a solid substrate may enhance the capture of PFAS from the environment. Our recent sorption tests suggest that a system combining fungi and wood chips could be a promising sorbent for PFAS (data unpublished). Overall, SSF culture may offer the potential for a dual-function treatment approach that involves using low-cost lignocellulosic materials as sorbents for PFAS sequestration, followed by fungal degradation or other deconstruction methods to achieve sustainable remediation of PFAS in the environment.

Further discussions on the implications of the current research.—Fungi play a crucial role in nutrient cycles across various ecosystems. Some fungi have developed specialized systems to break down complex polymer structures, enabling them to co-metabolize and recycle man-made xenobiotics, such as plastics (Navarro et al. 2021). For example, wood decay fungi have evolved a complex array of hydrolytic and oxidative enzymes that possess broad substrate specificity, allowing them to degrade not only lignocellulosic materials but also xenobiotics (Byss et al. 2008; Chun et al. 2019; Dinakarkumar et al. 2024; Vipotnik et al. 2021). Our research indicates that wood decay fungi, particularly the "white rot" species, can tolerate and degrade perfluoroalkyl and polyfluoroalkyl substances (PFAS). Although the specific biochemical processes involved are not yet fully understood, this finding suggests that fungi may play a significant role in responding to and recycling fluorinated chemicals produced by industrial activities. Gaining a better understanding of these fungal processes could enhance our knowledge of how natural ecosystems respond to the disposal of PFAS.

In addition to their ecological implications, our studies offer valuable insights into the use of fungal organisms for the remediation of fluorinated carbon (FC) chemicals. Unlike bacteria, fungal mycelia can traverse air-water interfaces and efficiently colonize and transport nutrients through nonaqueous environments. This capability is especially relevant for treating contaminated soils or solid wastes, which often present challenges for bacterial or physicochemical treatment methods (Abunada et al. 2020; Bentel et al. 2019; Kucharzyk et al. 2017). Furthermore, fungal enzyme systems typically exhibit low substrate specificities, allowing them to initiate the degradation of toxic compounds (Navarro et al. 2021). This process can enhance fungal tolerance to external toxins. In contrast, bacteria must be acclimated to develop tolerance and improve their proliferation in the presence of pollutants before they can effectively conduct degradation experiments. Therefore, fungi are particularly well suited for addressing xenobiotic pollutants, including FC chemicals, in solid matrices.

With the endorsement of our current findings, several important scientific questions warrant further exploration in the continued research. First, our study showed that both F^- and PFAS at concentrations of 200 μM or more, including PFOAs at concentrations up to 1 mM, can potentially enhance the polarized hyphal growth of some white rot fungi. This observation seems counter-intuitive, given the common perception of PFAS/F^- as toxic substances. Therefore, it will be worth further investigating the underlying mechanisms that fungi use to respond to PFAS to enhance polarized growth. Second, the features of fungal tolerance do not entirely align with their capacities to degrade PFAS. This is evident from their strong tolerance but limited ability to degrade legacy, non-defluorinated PFAS. Therefore, it would be interesting to explore the relationship between these two behaviors—fungal tolerance and the degradation of PFAS. Third, given the structures of the PFAS that fungi can defluorinate, it is worthwhile to investigate the functional enzymes and biochemical pathways involved in this defluorination process. Although we found no evidence of fungi being able to defluorinate legacy PFAS (such as PFOA, PFOS, GenX, and 6:2 FTOH), which are known for their inert and long-chain carbon-fluorine (C–F) bonds, we did identify some unique functional groups. For instance, the presence of alkenyl bonds or other electron-attracting groups may reduce the dissociation energy of nearby C–F bonds, thereby enabling fungal processes to conduct defluorination. These findings provide valuable model fluorinated carbon structures that can be used to investigate the mechanisms of defluorination necessary for deciphering the processes involved in the fungal degradation of PFAS. Addressing this question could help to revisit the ongoing debate regarding the potential roles of fungal-derived P450 enzymes and ligninolytic enzymes in the biotransformation of PFAS (Luo et al. 2018; Merino et al. 2023; Steffens et al. 2023).

CONCLUSIONS

The biodegradation of fluorinated carbon chemicals is receiving significant attention due to the environmental issues caused by PFAS pollutants. However, the role of fungi in degrading and recycling these compounds remains largely untapped. In this research, we utilized an optimized fluoride ion selective electrode (F-ISE) method to investigate the defluorination (deF)

capacities of various fungi on a set of PFAS, which differ in carbon chain length, degree of fluorination, and functional groups. We did not find any significant deF activity for legacy PFAS, such as fluorotelomer alcohols (FTOH) and perfluorooctanoic acid (PFOA), which had previously been reported as degradable by wood decay fungi or their enzymes in other studies. However, evidence of fungal tolerance to various PFAS and fluoride chemicals was observed, although the exact defense mechanism remains to be determined. Our screening of 18 different wood decay fungal species suggested that this tolerance is most likely linked to white rot fungi, which have developed oxidative systems capable of mineralizing the resistant structures of lignin. Further testing on different PFCA variants identified the fungal dehalogenated structures rendered by the electron-attracting groups, such as the alkenyl bond near C–F bonds, which supports an intriguing "structure-reactivity" paradigm of PFAS. These insights could aid in the development of novel PFCA compounds that are less resistant and less environmentally harmful than currently used chemicals. Furthermore, our findings highlight the potential of fungal organisms to degrade fluorinated compounds and suggest that future research on microbial communities should focus on the resident fungal communities found in affected environments.

DISCLOSURE STATEMENT

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