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Microbial and molecular approaches for PFAS transformation in soils: prospects and limitations

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Microbial and enzymatic strategies for per- and polyfluoroalkyl substance (PFAS) transformation are receiving increasing attention, but remain at an early stage of scientific development. This review evaluates the limited but emerging evidence for microbially mediated PFAS transformation, the putative enzymes implicated through multi-omics investigations, and the prospects and constraints of molecular engineering and synthetic biology. While computational modelling and omics approaches have proposed candidate mechanisms, no enzyme has yet been experimentally validated to catalyse efficient cleavage of perfluorinated C–F bonds, and most reported bio-transformations involve precursor compounds rather than terminal PFAS. Environmental factors, slow kinetics, and uncertain mechanisms further limit the practical application. Comparative analysis with established physicochemical technologies highlights that microbial strategies are best viewed as long-term, complementary tools rather than near-term solutions. Future work requires enzyme discovery, mechanistic elucidation, and careful integration of biological, computational, and engineering methodologies.

1. Introduction

Per-and polyfluoroalkyl substances (PFAS) are a large, chemically diverse class of synthetic organo-fluorine compounds widely used in industrial sectors and consumer products since the 1950s.[1] Their carbon chains substituted with fluorine confers exceptional chemical and thermal stability, as the carbon–fluorine (C–F) bond is among the strongest in organic chemistry.[2] These properties make PFAS useful in aqueous film-forming foams (AFFF), non-stick coatings, grease-resistant packaging, and waterproof textiles.[3] However, the same stability also drives their extreme environmental persistence, earning them the designation of forever chemicals.[4,5]

Widespread PFAS contamination has been reported in soils and groundwater, particularly near airports, military training sites, landfills, wastewater treatment plants, and industrial facilities where AFFF has been repeatedly discharged.[6,7] In soil system, PFAS can sorb to minerals and organic matter downward into aquifers, contributing to long-term human and ecological exposure.[8] Documented health risks include immunotoxicity, endocrine disruption, developmental effects, and carcinogenicity.[9] Conventional remediation approaches—chemical oxidation, excavation, incineration, thermal destruction, and adsorption using activated carbon or ion-exchange resins—are effective in some settings but are often energy-intensive, costly, and may simply transfer contaminants between phases rather than destroy them.[10,11]

These challenges have motivated interest in biological and molecular approaches capable of addressing PFAS contamination more sustainably. Multi-omics technologies now enable detailed examination of microbial responses to PFAS exposure. Genomics can identify organisms and genes potentially associated with PFAS transformation,[12] while transcriptomics and proteomics provide insight into stress-response pathways and proteins upregulated during PFAS exposure.[13,14] Metabolomics further helps identify intermediate compounds that arise during PFAS transformation, offering clues about biochemical pathways.[15] Together, these tools improve our mechanistic understanding of how microbes interact with PFAS, even when complete degradation is not observed.

Molecular engineering approaches—including enzyme engineering, synthetic biology, and CRISPR-based strain modification—are increasingly explored to enhance PFAS transformation. Enzyme engineering aims to improve the catalytic properties of oxidoreductases, hydrolases, or other enzyme families that may interact with PFAS, while synthetic biology seeks to design microbial systems or consortia with expanded metabolic capabilities.[16] Although these strategies remain largely conceptual, they offer promising frameworks for future innovation.

Importantly, microbial PFAS degradation is still in its infancy. No enzyme has been conclusively shown to catalyse the efficient defluorination of perfluoroalkyl acids, and most reported bio-transformations occur only for precursor PFAS. Many proposed mechanisms are inferred from omics correlations or computational predictions and require experimental

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validation. This review therefore distinguishes clearly between validated findings and hypothesized mechanisms.

Given the remarkable structural diversity of PFAS—varying in chain length, functional groups, and fluorination patterns—no single microbial or enzymatic pathway can be expected to function universally across all PFAS subclasses. Throughout this review, the terms transformation, biodegradation, and mineralization are used explicitly: (i) transformation refers to partial modification of PFAS molecules, often producing persistent intermediates; (ii) biodegradation refers to biologically mediated breakdown; and (iii) mineralization refers to complete conversion of PFAS carbon to CO_2 with full defluorination, which has not yet been demonstrated for perfluoroalkyl acids.

All abbreviations are defined at first use, including PFAS, perfluoroalkyl acids (PFAAs), perfluoro-octanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), AFFF, horseradish peroxidase (HRP), manganese peroxidase (MnP), density functional theory (DFT), *ab initio* molecular dynamics (AIMD), CRISPR, TEMPO, and ABTS.

This review integrates microbial adaptation, multi-omics-based pathway elucidation, enzyme engineering, synthetic biology, and systems-level modelling within the context of PFAS-contaminated soils. It critically examines both opportunities and limitations, situates biological strategies within the broader remediation landscape, and highlights the mechanistic gaps that must be addressed before scalable, field-relevant solutions can emerge.

2. PFAS properties and environmental behaviour

2.1 Organization and categorization of PFAS

Per- and polyfluoroalkyl substances (PFAS) comprises a vast and structurally diverse class of synthetic chemicals characterized by a carbon backbone in which hydrogen atoms are fully (perfluoro-) or partially (polyfluoro-) replaced by fluorine.[17] The carbon–fluorine (C–F) bond is the strongest in organic chemistry, conferring exceptional chemical and thermal stability and contributing to PFAS resistance to environmental and biological degradation.[18] The physicochemical properties—particularly C–F bond strength, amphiphilicity, and sorption-driven mobility—directly influence microbial accessibility and degradation potential in soils. Understanding these properties is essential for designing molecular bioremediation strategies. Perfluoroalkyl acids such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) contain fluorine substitutions along the entire carbon chain[19] (Table 1). Most PFAS possess an amphiphilic structure with a hydrophobic fluorinated tail and a hydrophilic ionic polar head group, a feature that underlies their widespread use as surfactants and repellents and shapes their environmental fate.[20,21]

2.2 Environmental fate and transport of PFAS

In soil systems, PFAS behaviour is strongly influenced by sorption dynamics, chain-length-dependent mobility, and interactions with mineral surfaces that ultimately determine microbial exposure and transformation potential.[22] Following environmental release through industrial discharge, use of firefighting foams, or leaching from consumer products and waste, PFAS display complex fate and transport processes.[6] Their persistence arises from the strength of the C–F bond, which render them resistant to hydrolysis, biodegradation, photolysis, and other natural degradation pathways.[23,24] PFAS can be transported long distances through both aqueous and atmospheric deposition, leading to their detection in remote regions such as the Arctic.[25] The mobility of PFAS in the environment is primarily dictated by long-chain PFAS (*e.g.*, PFOS, PFOA) exhibit higher affinity for sorption to soils and sediments, particularly those rich in organic matter, thus becoming more persistent and less mobile.[26,27] Short-chain PFAS and replacements like GenX and PFBS are more water-soluble and mobile, tending to leach into groundwater and consequently contaminate drinking water sources.[28,29] Anionic functional groups (carboxylates,

Table 1 Classification of PFAS

PFAS classes	General structure	Representative compounds	Major uses/sources	Environmental persistence toxicity	Relevance to remediation
Perfluoroalkyl carboxylic acids (PFCAs)	C_nF_{2n+1}–COOH	PFOA, PFNA, PFHxA	Surfactants, Teflon production, textiles	High persistence, bioaccumulative	Target for microbial degradation *via* oxidative and reductive pathways
Perfluoroalkyl sulfonic acids (PFSAs)	C_nF_{2n+1}–SO_3H	PFOS, PFHxS	Firefighting foams (AFFF), stain repellents	Strong C–F bond stability, endocrine disruption	Microbial defluorination under anaerobic conditions
Fluorotelomer alcohols (FTOHs)	C_nF_{2n+1}–CH_2CH_2OH	6:2 FTOH, 8:2 FTOH	Coatings, polymers, surface treatments	Precursors of PFCAs/PFSAs	Potential substrates for microbial oxidation
Perfluoroalkyl phosphonic/phosphinic acids (PFPAs/PFPiAs)	C_nF_{2n+1}–$PO(OH)_2$	PFPA, PFPiA derivatives	Specialty surfactants	Less studied, high mobility	Emerging targets in microbial and enzymatic studies
Perfluoroalkyl ether carboxylic acids (PFECAs)	C_nF_{2n+1}–O–CF(COOH)–	GenX, ADONA	Replacement for PFOA	Persistent, toxic	Limited microbial degradation reported
Other emerging PFAS	Variable (polymers, ionomers)	Side-chain fluorinated polymers, novel PFAS	Coatings, packaging, industry	High environmental load	Require new enzymatic/synthetic biology approaches

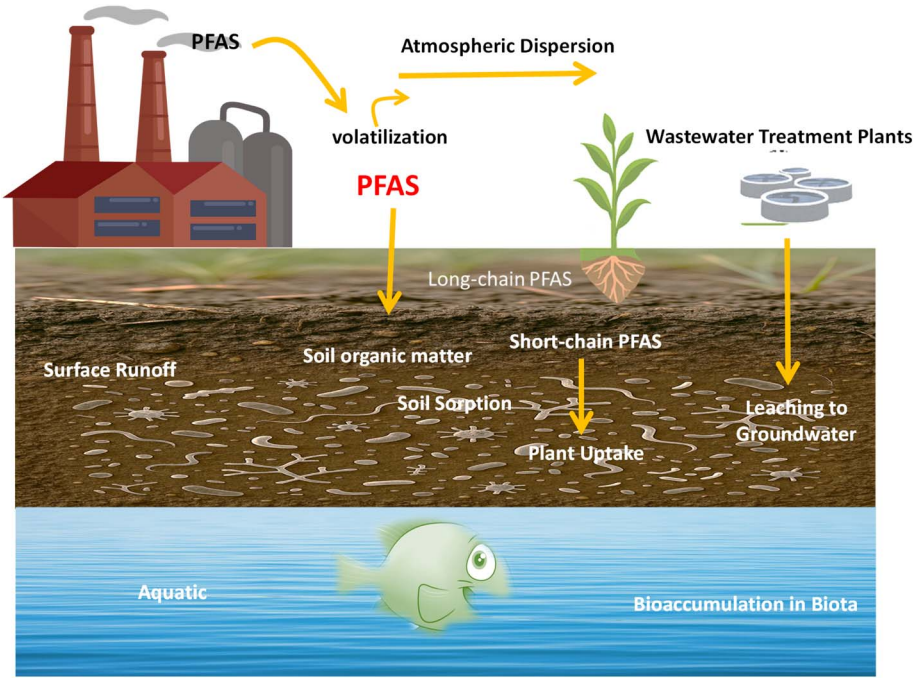

Fig. 1 Environmental fate and transport pathways of PFAS in terrestrial and aquatic systems: a comprehensive schematic depicting the environmental dynamics of PFAS, highlighting key processes such as atmospheric dispersion, soil sorption, groundwater leaching, surface runoff, plant uptake, bioaccumulation in biota, and entry into the human food web. The diagram illustrates the differential mobility of long- and short-chain PFAS, interactions with soil organic matter, and volatilization near emission sources such as industrial sites and wastewater treatment plants.

sulfonates) enhance aqueous solubility and play a central role in determining PFAS interactions with mineral surfaces.[30] Environmental conditions—including soil pH, texture, organic carbon content, temperature, redox potential, and the presence of natural or synthetic surfactants—further influence PFAS transport and retention in soils[31,32] (Fig. 1). Unlike many traditional persistent organic pollutants, PFAS bioaccumulate primarily through protein binding rather than lipid partitioning due to their amphiphilic character.[33] The combination of extreme chemical persistence, high mobility, and biological effects poses significant challenges for environmental management. Consequently, site-specific characterization and risk-based remediation strategies are essential for protecting ecosystems and public health.[34]

2.3 Comparative overview of PFAS remediation approaches

A wide range of physicochemical strategies have been developed for PFAS treatment, including high-temperature thermal destruction, electrochemical oxidation, advanced reduction processes, plasma-based degradation, and UV-based photolysis.[35] These methods can achieve measurable defluorination for some PFAS subclasses but often require high energy input, specialized infrastructure, or treatment under non-environmentally relevant conditions.[36] In contrast, biological strategies offer the potential for low-energy, *in situ* remediation but currently face major limitations due to slow kinetics, incomplete pathways, and lack of validated PFAS-active enzymes.[24] Microbial and enzymatic approaches therefore remain complementary to, rather than replacements for, established physicochemical technologies. Understanding this broader landscape is essential for assessing the realistic potential of microbial remediation.

3. Microbe-mediated degradation of PFAS: a molecular perspective

Building on the physicochemical context described above, this section evaluates current microbial responses to PFAS exposure and the extent to which these translate into measurable biotransformation.

3.1 Microbial adaptation to PFAS exposure

Although PFAS are highly persistent due to the strength of the carbon–fluorine bond, emerging studies show that some environmental microbes can physiologically and genetically adapt to PFAS contamination.[37,38] Most reported microbial activities target PFAS precursors rather than terminal perfluoroalkyl acids, and these reactions represent transformations rather than complete degradation. Chronically contaminated sites act as selective environment that enrich microbial communities capable of tolerating PFAS stress or mediating limited transformation reactions.[39] High-throughput metagenomic studies reveal enrichment of genes associated with oxidative stress tolerance, efflux systems, membrane transporters, and xenobiotic metabolism.[40,41] Putative oxygenases and reductases, and ATP-binding cassette (ABC) transporters, are frequently

detected and are hypothesized to participate in PFAS transformation, though none have been experimentally validated.[37] Microbial genera such as *Pseudomonas*, *Acidimicrobium*, and *Dehalococcoides* are consistently observed in PFAS-contaminated soils and are associated with the production of biosurfactants and extracellular polymeric substances (EPS).[32,41] These biomolecules are likely to increase bioavailability, suggesting but not confirming a role in enhancing PFAS transformation.[42] Transcriptomic studies similarly report modest upregulation of stress-response genes, oxidoreductases, and transporters in mixed cultures exposed to PFAS precursors.[43] While these responses point to possible biochemical pathways, current evidence remains correlative rather than causal, and no enzyme has been biochemically verified to cleave a perfluorinated C–F bond. Collectively, these adaptive traits highlight potential microbial capacities for PFAS

Table 2 Key microbial taxa involved in PFAS-degradation

Microbes involved	PFAS compound	Known function/adaptation	Evidence (technique used)	References
Pseudomonas plecoglossicida 2.4-D	Perfluorooctanyl sulfonate	Efflux pumps, oxidative stress resistance, biosurfactant production	Metagenomics, transcriptomics	149
Dehalococcoides mccartyi	Trichloroethane and tetrachloroethane	Reductive defluorination *via* electron transfer	Enrichment cultures, anaerobic reactors	150
Acidimicrobium sp. A6	Perfluoroalkyl acids (PFAAs)	Hypothesized defluorination capability, resilience to acidic environments	Shotgun metagenomics	61
Desulfovibrio	PFAS	Anaerobic metabolism, potential co-metabolism of fluorinated compounds	Sulfate-reducing bioreactors, proteomics	151
Pseudomonas putida	2,2-Difluoro-1,3-benzodioxole	Aromatic compound metabolism, stress-responsive pathways	Transcriptomics, gene expression profiling	152
Mycobacterium phocaicum MBWY-1	Fluoroglycofen ethyl	Hydrophobic compound assimilation, high cell wall tolerance	Genomic surveys, culture-based studies	153
Desulfomonile tiedjei	6:2 chlorinated polyfluorooctane ether sulfonate (6:2 Cl-PFESA)	Anaerobic dehalogenation, syntrophic metabolism	Metagenomics, stable isotope probing (SIP)	154
Sphingomonas	Fluorine	Organic contaminant degradation, membrane transporters	Whole-genome sequencing, proteomics	155
Geobacter metallireducens GS15	Different organic pollutants	Electron transfer chains, redox-active biofilms	Electrochemical enrichment, functional metagenomics	156
Labrys portucalensis F11	Sulfonic acid (PFOS), 6:2-fluorotelomer sulfonic acid (6:2 FTS), and 5:3-fluorotelomer carboxylic acid (5:3 FTCA)	Aerobic degradation, possible syntrophic interactions	Microcosms	157
Novosphingobium sp.	2-, 3-, or 4-rings aromatic hydrocarbons	Degradation of xenobiotic compounds, aromatic ring-cleaving dioxygenases	Whole-genome sequencing, culture-dependent assays	158
Thauera humireducens	Humus- and Fe(III)	Versatile anoxic respiration, fluorinated compound transformation	Denitrifying bioreactors, transcriptomics	159
Achromobacter sp. HZ01	Hydrocarbons	Biofilm formation, efflux mechanisms, alkyl chain cleavage	Microcosm studies, metabolomics	160
Variovorax sp.	Methylcyclohexane, *N*-hexadecane and cyclohexane	Aromatic degradation pathways, stress signaling	Genome-resolved metagenomics	161
Polaromonas		Psychrotolerance, potential PFAS adsorption	High-throughput sequencing from cold environments	162
Rhodococcus sp.	Monofluorinated alkane	Catabolic versatility, degradation of hydrophobic fluorinated compounds	Culture-dependent screening, GC-MS	163
Alcaligenes aquatilis	Sulfametoxydiazine	Niche adaptation to wastewater, biodegradation of persistent pollutants	Bioreactor studies, qPCR	164
Azoarcus sp. PA01	*o*-Phthalate	Anoxic metabolism, degradation of aliphatic fluorinated compounds	Anaerobic batch experiments, functional gene analysis	165
Comamonas trifloxystrobinivorans sp. nov.	Agrochemical contaminants (trifloxystrobin)	Versatile metabolism, perfluorinated acid transformation	Environmental genomics, chemical transformation assays	166

transformation, even though full mineralization remains rare. Understanding these responses provides a foundation for exploring how microbial communities or engineered systems might be leveraged in future bioremediation strategies. Table 2 summarizes microbial genera commonly detected in PFAS-impacted environments and their proposed adaptive mechanisms.

3.2 Functional metagenomics and microbiome profiling

Functional metagenomics enables discovery-driven screening of genetic potential directly from environmental DNA (eDNA), bypassing the need for cultivation and allowing identification of genes that may be involved in PFAS transformation.[39,44] By expressing eDNA libraries in heterologous hosts such as *E. coli*, researchers can screen for putative enzymes or activities associated with PFAS precursor transformation, even from low-abundance or unculturable microorganisms.[40] Complementary microbiome profiling techniques including 16S rRNA amplicon sequencing and whole-genome shotgun metagenomics have documented shifts in microbial community composition in PFAS contamination.[45] Enrichment of genera such as *Pseudomonas*, *Sphingomonas*, and *Dehalococcoides* has been frequently observed, suggesting potential roles in PFAS tolerance or interaction with PFAS substrates.[46] Genes encoding redox-active proteins, oxidoreductases, and hydrolases are often detected in these environments,[37,47] but their mechanistic relevance to PFAS transformation remains uncertain, as most findings are based on correlative omics associations rather than validated biochemical activity. Importantly, metagenomic studies have reported highly variable microbial responses: while some PFAS-amended systems show increased abundance of taxa such as *Desulfovibrio*, *Acidimicrobiaceae*, or *Pseudomonas*, other studies report no measurable PFOS or PFAS mineralization.[48] These inconsistencies highlight the early stage of knowledge and the need for integrative molecular approaches—combining functional metagenomics, transcriptomics, and biochemical validation—to identify candidate strains and catabolic gene clusters relevant to PFAS transformation and to develop site-specific remediation strategies (Fig. 2).

3.3 Transcriptomics and proteomics insights

Transcriptomic and proteomic analyses provide valuable insight into how microbial communities respond to PFAS exposure and help identify potential, though largely unvalidated, biochemical pathways involved in PFAS transformation.[41] Transcriptomic studies commonly report increased expression of genes encoding membrane transporters, efflux pumps, and oxidative stress regulators in microorganisms exposed to compounds such as PFOS.[49] Upregulation of ATP-binding cassette (ABC) transporters and major facilitator superfamily (MFS) proteins is thought to reflect cellular strategies for reducing intracellular PFAS accumulation and mitigating toxicity.[50] Proteomic analyses likewise detect elevated levels of oxidoreductases, hydrolases, and dehalogenase-like proteins—enzyme classes often proposed to participate in PFAS precursor transformation.[51] For example, increased abundance of certain cytochrome P450 proteins has been interpreted as a possible indication of oxidative attack on PFAS molecules, although this remains a hypothesis inferred solely from omics correlations.[40] Collectively, these omics

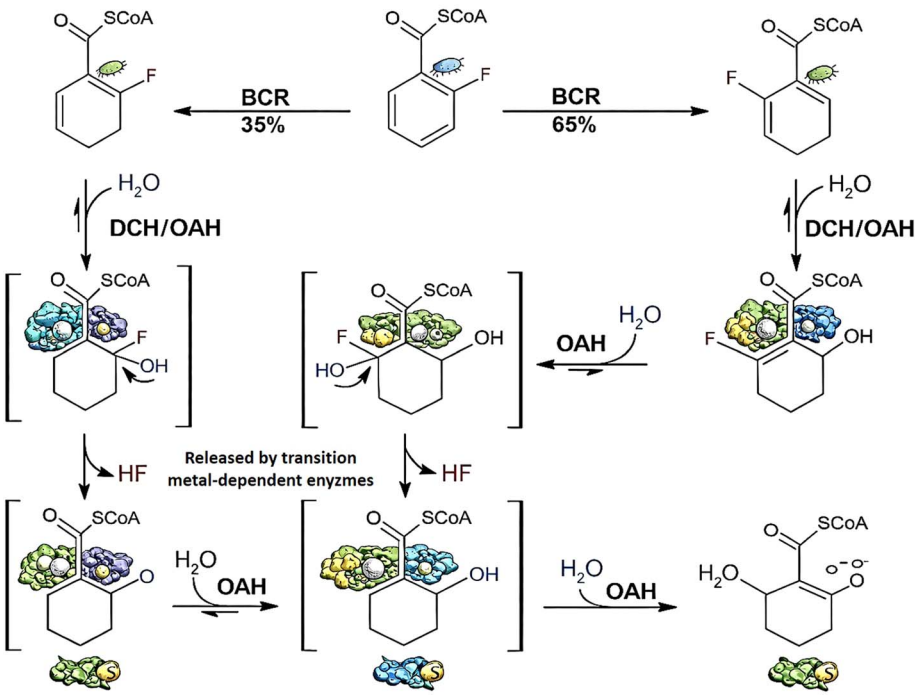

Fig. 2 Proposed mechanism for biotransformation of 2-F-BzCoA by the microbial enzymes BCR, DCH, and OAH. The chemicals shown in brackets are likely to be unstable intermediate degradation compounds. Redrawn from Shahsavari *et al.* (2021).[126]

approaches reveal consistent patterns of stress-response activation and highlight candidate proteins that may participate in limited PFAS biotransformation. However, transcriptomic and proteomic shifts should not be interpreted as evidence of catalytic activity. In many systems, upregulated cytochrome P450s, dehydrogenases, and redox-associated proteins likely represent generalized detoxification responses rather than direct metabolism of PFAS substrates. Experimental validation of enzyme function remains scarce, and the mechanistic significance of most identified proteins is still uncertain.

3.4 Current limitations and challenges in microbial PFAS degradation

Despite recent advances, microbial PFAS degradation remains severely limited, and expectations for rapid or field-scale application must be tempered. Most reported transformations display extremely slow kinetics, typically yielding only partial defluorination over extended incubation periods, while complete mineralization of perfluoroalkyl acids has not been demonstrated. Microbial degradation of PFAS is generally insufficient under natural conditions, often leading only to transformation rather than complete breakdown.[52] Bio-transformations are generally restricted to precursor PFAS, producing terminal PFAS that remain highly persistent due to high bond strength and environmental stability.[53] A central barrier is the absence of thermodynamically favorable pathways for C–F bond activation because PFAS feature exceptionally strong covalent carbon–fluorine bonds that are rarely metabolized by microbes.[54] The perfluorinated C–F bond provides no growth-linked energetic benefit and cannot serve as an electron acceptor, rendering classical organohalide respiration ineffective for PFAS substrates. Microbial C–F bond cleavage mechanisms are barely understood, and most proposed enzymatic roles remain hypothetical.[55] In addition, PFAS molecules exhibit low polarizability and poor binding affinity to enzymatic active sites, limiting opportunities for catalytic attack. This is consistent with knowledge gaps in confirmed PFAS-defluorinating enzymes and low substrate specificity.[12] Microbial growth is further inhibited by fluoride release, surfactant behavior, and the inherent toxicity of some PFAS, all of which reduce transformation potential. The toxicity of released fluoride and PFAS impacts microbial viability and biodegradation capacity.[56] When compared with physicochemical technologies—such as thermal, electrochemical, or plasma-based treatments, which can rapidly destroy PFAS under engineered conditions—microbial strategies remain orders of magnitude slower and currently incapable of mineralizing terminal PFAS. Biological approaches may ultimately complement other technologies, particularly for low-concentration or *in situ* applications, but they should not be viewed as standalone solutions in their current state.

4. Enzymatic mechanisms in PFAS transformation

4.1 Reductive and abiotic defluorination pathways

Enzymatic activity reported in literature largely reflects transformation of precursor PFAS and should not be interpreted as evidence for universal biochemical pathways applicable to all PFAS subclasses. Reductive defluorination aims to break the strong C–F bonds through electron transfer reactions, typically mediated by reductive dehalogenases like system.[57] Early investigation focused on organohalide-respiring bacteria such as *Dehalococcoides*, though these organisms have shown only limited activity toward PFAS substrates.[58] More recent work has identified additional microbial taxa and abiotic reducing systems capable of partial defluorination; however, full mineralization remains rare, and intermediate products commonly accumulate. Abiotic analogs including UV/sulfite treatments, hydrated electrons, and metal-catalysed systems such as zero-valent iron with ferrate—tend to exhibit higher but laboratory-restricted defluorination efficiencies, often requiring elevated temperatures, strong reducing agents, or other non-environmental conditions.[59,60] Huang *et al.*[61] reported biodegradation of PFOA by *Acidimicrobium* sp. A6 under iron-reducing conditions in the presence of ferrihydrite. The Feammox process, in which ammonium acts as an electron donor for iron reduction, was linked to observed defluorination. Under optimized conditions, PFOA concentrations decreased by 37–68% after 150 days, accompanied by the formation of shorter-chain intermediates and fluoride release. The study further highlighted the critical roles of Fe(III) availability and microbial electron-transfer efficiency in enhancing defluorination rates. Overall, while reductive and abiotic approaches provide important insights into potential mechanisms of PFAS transformation, they remain constrained by slow kinetics, incomplete pathways, and dependence on conditions unlikely to be replicated in natural soils.

4.2 Hydrolytic enzyme in PFAS precursor transformation

Hydrolytic enzymes—including esterases and lipases have been investigated since the 2010s for their ability to cleave ester bonds in PFAS precursors, particularly polyfluoroalkyl phosphate esters.[62] These reactions have been observed in *Pseudomonas*, *Candida*, and mixed environmental consortia, and are often influenced by the structural features and surface properties of the precursor molecules. While hydrolysis represents an important initial transformation pathway, it typically generates more persistent perfluoroalkyl acids (PFAAs), limiting its effectiveness for complete PFAS degradation. Oxidative approaches have also been explored for PFAS transformation, but experimental evidence for full defluorination remains limited. Emerging chemoenzymatic strategies aim to integrate hydrolytic and oxidative processes to improve efficiency, though these remain at early stages of development.[54,60,63]

4.3 Enzyme engineering for enhanced PFAS transformation

Protein engineering approaches including directed evolution, site-directed mutagenesis, and computational design—are being explored to improve enzyme reactivity and substrate specificity toward PFAS.[64] Candidate target include dehalogenases, fluoroacetate dehalogenase (FAcD) variants, and multi-enzymatic pathways assembled through synthetic biology platforms. FAcD, one of the few enzymes known to cleave a C–F bond, is often considered

a starting point for engineering, although its natural activity is limited to small fluorinated substrates.[65] Efforts to expand turnover efficiency or broaden substrate scope remain preliminary. Synthetic biology enables engineered microbes to express designed pathways that may improve PFAS binding, tolerate fluoride toxicity, and support extended transformation.[66] Complementary *in silico* modelling and laboratory evolution techniques are used to optimize enzyme structure and host performance.[67] Major challenges include maintaining enzyme stability under environmental conditions and mitigating toxicity arising from fluoride release. Radical-generating enzymes and transition-metal-dependent systems have also been proposed as potential routes for C–F bond activation, based on chemical analogies in which fluorine can be displaced by transition metals.[68] However, no radical- or metal-dependent enzyme has been experimentally confirmed to cleave perfluorinated C–F bonds, and current studies remain conceptual. Extracellular peroxidases, such as horseradish peroxidase (HRP) and manganese peroxidase (MnP), have shown partial PFAS precursor transformation when paired with mediators like H_2O_2 or phenolic compounds. Similarly, laccase–mediator systems (*e.g.*, TEMPO, ABTS) have been shown to facilitate oxidative transformation of both PFAS precursors and perfluoroalkyl acids, including PFOA and PFOS, through enzyme-catalysed oxidative humification-like reactions.[69] These processes are analogous to natural organic matter humification occurring in soils and sediments. Although transformation rates are typically slow and defluorination efficiencies remain limited, such findings demonstrate that oxidative enzyme-mediated pathways can contribute to PFAS transformation under environmentally relevant conditions. Table 3 summarizes enzymes and functional genes implicated in PFAS transformation, along with the level of experimental evidence supporting their proposed roles. Importantly, enzymatic activity reported to date primarily involves precursor PFAS, and no engineered biocatalyst has been validated for defluorination of terminal perfluoroalkyl acids.[70] Overall, enzyme engineering for PFAS remains constrained by the absence of known natural PFAS-degrading templates, the lack of mechanistic understanding of C–F bond activation, and the high activation energy barriers associated with perfluorinated substrates. These factors limit the power of rational design and often place enzyme activities below detectable thresholds, making this field largely exploratory despite growing interest.

4.4 Critical comparison of enzymatic strategies

Although multiple enzyme classes—including dehalogenases, oxygenases, and oxidoreductases—have been explored for PFAS degradation, their efficiencies differ substantially. Reductive dehalogenases exhibit the highest potential for C–F bond cleavage but often show low turnover rates and narrow substrate specificity.[71] Oxidative enzymes such as laccases and peroxidases enable broader attack on PFAS precursors but require mediators or co-factors, limiting *in situ* applicability. Hydrolytic enzymes efficiently transform PFAS precursors but generally produce PFAAs, increasing long-term persistence.[66] Although there is interest in enhancing enzymatic PFAS transformation through protein engineering or synthetic biology, to date most studies have focused on native enzymes (*e.g.*, laccases, peroxidases) applied in oxidative humification-like reactions rather than engineered variants with demonstrably improved activity toward PFAS. Challenges such as enzyme instability, low turnover, and incomplete defluorination continue to limit applicability in environmental contexts.

5. Synthetic biology and molecular engineering approaches for PFAS bioremediation

Given the limited natural activity toward PFAS, molecular engineering and synthetic biology aim to expand or redesign

Table 3 Functional genes/enzymes implicated in the transformation of PFAS

Gene/enzyme	Function	Evidence source	Remarks	References
Dehalogenase	Catalyzes C–F bond cleavage (defluorination)	Proteomics, enzyme assays	Often inducible; substrate-specific	167
Oxidoreductase	Redox transformation of intermediates	Transcriptomics, qPCR	Requires cofactors such as NADH/NADPH	168
ABC transporters	PFAS efflux from microbial cells	Genomics, meta-transcriptomics	Upregulated under stress; supports resistance	169
Monooxygenase	Initial hydroxylation and ring cleavage	Whole-genome sequencing, enzyme assays	Often involved in co-metabolism	170
Peroxidase	Oxidative breakdown of organic PFAS	Biochemical assays, activity profiling	Requires H_2O_2; active under oxidative stress	59
Dioxygenase	Aromatic ring opening	Proteomics, enrichment cultures	May support PFAS co-metabolism with other organics	171
Hydrolase	Hydrolysis of PFAS precursors	Enzyme modeling, structure–function studies	May act on ester- or amide-linked PFAS forms	47
Glutathione-*S*-transferase (GST)	Detoxification *via* conjugation	Transcriptomics, protein expression	Enhances PFAS tolerance; antioxidant role	172
Fluoroacetate dehalogenase	Specific defluorination of fluoroacetate	Functional metagenomics	Active against short-chain fluorinated compounds	12
Superoxide dismutase (SOD)	Oxidative stress mitigation	Proteomics, RT-PCR	Indirect role in microbial resilience to PFAS	173

degradation capacity. This section integrates strain engineering, pathway construction, and synthetic consortia design within feasibility constraints.

5.1 Engineered strains and microbial consortia

Because natural microbial activity toward PFAS is extremely limited, synthetic biology and molecular engineering aim to expand or redesign metabolic capacity for PFAS transformation.[72] However, engineered systems cannot be assumed to function uniformly across all PFAS structures, as differences in chain length, head groups, and fluorination patterns strongly influence microbial accessibility and enzyme reactivity. Synthetic biology provides conceptual frameworks for constructing microbial strains or consortia with complementary metabolic functions.[73] In contrast to single-strain approaches, engineered consortia can perform sequential or parallel transformations, where each member contributes a specific role—such as initiating oxidative or reductive attack on PFAS precursors, processing intermediate metabolites, or mitigating toxic by-products.[47,74] Regulatory tools including inducible promoters, quorum sensing modules, and feedback control circuits help stabilize community composition and coordinate metabolic fluxes under variable environmental conditions.[75] Cross-feeding interactions can further enhance cooperative degradation, while stress-response circuits and biofilm-associated traits may improve resilience to pH fluctuations, nutrient limitation, or competition from native microbiota.[76] Several proof-of-concept studies illustrate the potential of engineered consortia. Zhang *et al.*[77] designed a three-member community comprising *Bacillus subtilis* (nutrient recycling and biofilm support), *Acinetobacter* sp. (intermediate degradation), and *P. putida* engineered for initial PFAS oxidation. This system achieved enhanced PFAS loss in soil microcosms relative to monocultures. Hernandez *et al.*[78] developed a synthetic quorum-sensing network in *E. coli* strains to regulate expression of PFAS-related enzymes, improving degradation rates and maintaining population balance. Despite these advances, current evidence remains preliminary.[79] CRISPR-enabled pathway editing and *de novo* pathway construction have been proposed for enhancing PFAS transformation, but no engineered strain has demonstrated sustained defluorination or mineralization of terminal perfluoroalkyl acids.[61] Many engineered pathways remain hypothetical or rely on computationally predicted reactions that lack experimental validation. Even for engineered consortia, reported PFAS losses are modest and inconsistent across studies, with several systems showing no measurable degradation under environmentally relevant conditions.

Overall, synthetic biology provides powerful conceptual tools for designing PFAS-responsive microbial systems, but these approaches remain exploratory and require substantial mechanistic elucidation before *in situ* deployment becomes feasible.

5.2 Limitations and feasibility of molecular engineering approaches

While molecular engineering offers exciting conceptual pathways for PFAS biotransformation, these approaches remain largely speculative and face substantial feasibility constraints. To date, no engineered enzyme has been experimentally verified to cleave perfluorinated C–F bonds in PFAS under environmentally relevant conditions—enzymatic C–F bond cleavage is largely unknown and remains a major challenge in PFAS biodegradation research.[70] Most proposed mechanisms rely on computational predictions, *in silico* design, or homology-based inference rather than validated catalytic activity for PFAS cleavage.[80] Pathway engineering is further constrained by the extreme strength and stability of carbon–fluorine bonds, which make PFAS highly resistant to biological attack.[81] Additionally, poor substrate binding, fluoride toxicity, and enzyme instability in fluorinated environments limit the development of effective biocatalysts.[82] Compounding these challenges is the absence of known natural PFAS-degrading metabolic pathways that could serve as scaffolds for rational enzyme or pathway design, since even microbial transformation of PFAS tends to stop at partial defluorination and not overall mineralization.[66] Similarly, designed microbial consortia have not yet demonstrated consistent or measurable PFAS mineralization in laboratory or field settings, with current microbial studies mostly reporting limited transformation of precursors under specific conditions rather than full degradation to non-toxic end products.[83] These limitations underscore the early stage of molecular engineering efforts for PFAS biotransformation and highlight the need for fundamental mechanistic discovery before predictive or rational design strategies can be realized at scale.

5.3 Environmental biotechnology using CRISPR–Cas systems

CRISPR–Cas systems provide precise, efficient, and multiplexable tools for microbial genome editing.[56] In principle, these tools can be used to enhance PFAS biotransformation by introducing candidate genes, modulating pathway fluxes, or improving microbial tolerance.[47] However, most applications remain conceptual, and no engineered strain has yet demonstrated sustained defluorination of terminal PFAS. CRISPR-mediated gene insertion has been used to introduce putative de-fluorinating enzymes—such as fluoroacetate dehalogenase and reductive dehalogenase-like genes—into adaptable hosts like *P. putida*.[84] These proof-of-concept studies report improved enzyme expression and modest increases in precursor PFAS transformation in controlled microcosms, though biochemical confirmation of enzyme function is still required. CRISPR interference (CRISPRi) enables targeted downregulation of competing metabolic pathways to redirect cellular resources toward engineered PFAS-related reactions. For example, Wang *et al.*[85] showed that repressing glucose catabolism in *Shewanella oneidensis* increased the intracellular electron availability, thereby enhancing reductive reactions relevant to PFAS precursor degradation.

Multiplex CRISPR editing has also been proposed for fine-tuning enzyme expression, cofactor biosynthesis, and stress-response pathways to improve microbial resilience under environmental conditions.[86] Lee *et al.*[84] demonstrated that a synthetic operon containing hydroxylase- and dehalogenase-like genes introduced into *P. putida* resulted in increased defluorination

Table 4 Multi-omics integration for system-level understanding of PFAS bioremediation

Omics approaches	Target insights	Tools/platforms	Output examples	Application in PFAS research	References
Genomics	Identification of genes, metabolic potential	Illumina, Oxford Nanopore, PacBio	Identification of PFAS resistance or degradation genes	Screening for natural PFAS-degrading microbes; identifying mobile genetic elements	77
Transcriptomics	Differential gene expression under PFAS exposure	RNA-Seq, qRT-PCR	Upregulation of efflux transporters, stress response genes	Understanding microbial stress adaptation and pathway regulation	174
Proteomics	Protein expression and pathway mapping	LC-MS/MS, 2D-GE	Detection of dehalogenases, oxidoreductases	Identifying functional enzymes involved in PFAS transformation	85
Metabolomics	Intermediates and end-products of metabolism	GC-MS, LC-MS, NMR	Identification of PFAS degradation products (*e.g.*, shorter-chain acids)	Validating transformation pathways and tracking metabolic flux	175 and 176
Metagenomics	Microbial community structure and functional genes	Shotgun sequencing, MG-RAST	Discovery of xenobiotic degradation gene clusters	Exploring non-culturable microbes with potential PFAS-degrading capacity	177
Meta-transcriptomics	Active genes in microbial communities	Total RNA sequencing	Expression of degradation-related operons	Capturing *in situ* functional responses in PFAS-contaminated soils	129 and 178
Meta-proteomics	Community-level protein expression	LC-MS/MS, iTRAQ	Functional enzyme and transporter identification	Linking taxa to specific functional proteins involved in PFAS response	44
Integrative omics	Systems-level understanding of microbial response	Bioinformatics pipelines (*e.g.*, Cytoscape, meta-omics)	Pathway reconstruction, network analysis	Designing engineered microbes and consortia	118

rates of precursor PFAS in soil microcosms, although complete mineralization was not achieved. While CRISPR–Cas systems offer powerful tools for customizing microbes, current applications largely involve hypothetical pathway construction or enhancements of precursor transformation.[87] Significant mechanistic validation, enzyme discovery, and environmental testing are still

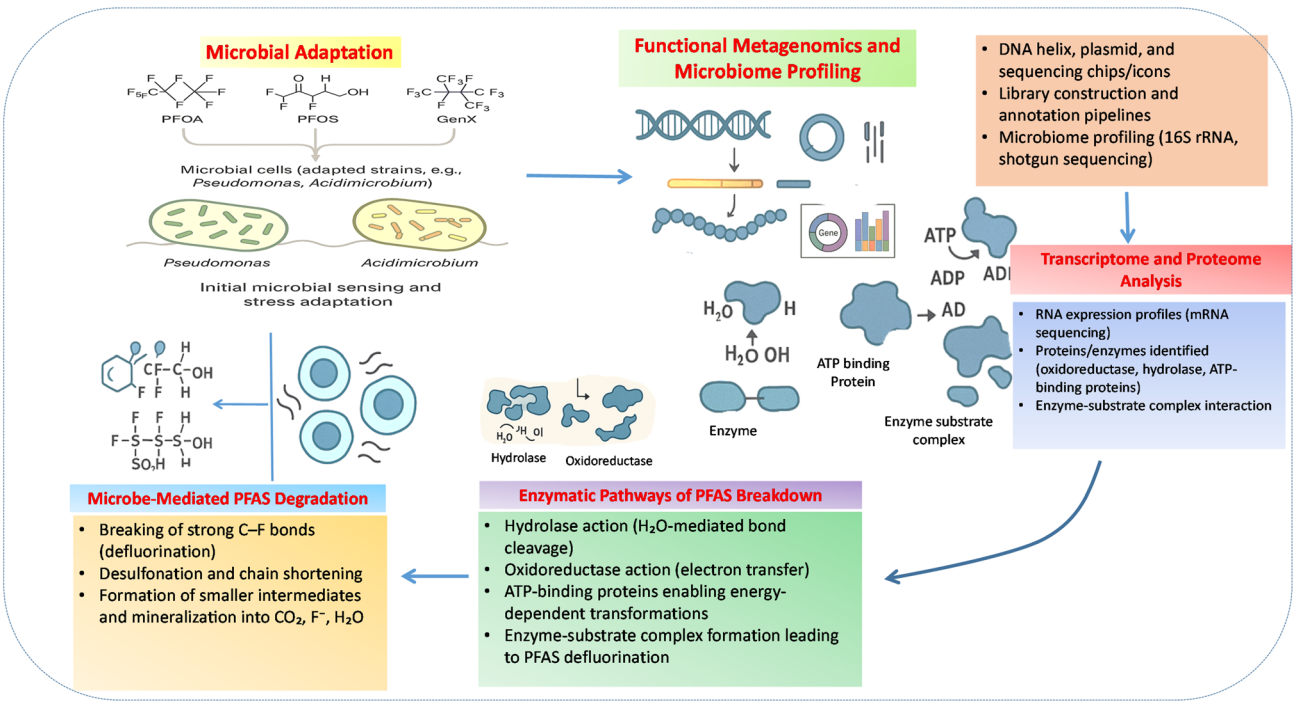

Fig. 3 Microbial and molecular mechanisms underlying PFAS degradation in soils. Microbial adaptation (*e.g.*, *Pseudomonas*, *Acidimicrobium*) initiates sensing and stress response to PFAS exposure. Functional metagenomics and microbiome profiling identify community structure and degradation potential. Transcriptome and proteome analyses reveal key enzymes (oxidoreductases, hydrolases, ATP-binding proteins) and enzyme–substrate interactions. PFAS breakdown proceeds through microbial defluorination, desulfonation, and chain shortening, supported by enzymatic pathways involving hydrolases, oxidoreductases, and ATP-dependent transformations, ultimately leading to mineralization into CO_2, F^-, and H_2O.

required before CRISPR-engineered strains can be considered deployable for PFAS bioremediation.

5.4 Development of biosensors for PFAS detection

Synthetic biology-engineered biosensors offer promising tools for real-time PFAS monitoring, supporting assessment and optimization of bioremediation strategies.[88] PFAS-responsive promoters and transcription factors can be coupled to reporter genes to produce measurable signals upon PFAS exposure.[89] Although engineering such promoters is challenging due to PFAS chemical stability and low reactivity, recent designs rely on cellular stress responses or PFAS-related metabolites to trigger reporter expression.[12] Common reporters include fluorescent proteins (GFP), luciferase, and chromogenic enzymes such as β-galactosidase.[90] Smith *et al.*[91] engineered an *E. coli* strain expressing GFP under the control of a PFAS-responsive transcription factor, enabling detection of PFOA at concentrations as low as 1 ppb within six hours. Similarly, An *et al.*[92] used luciferase reporters in *P. fluorescens* regulated by oxidative-stress-responsive promoters, allowing PFAS detection in complex soil matrices. Quorum-sensing (QS) modules and signal-amplification circuits can further enhance sensitivity by coordinating population-wide reporter expression.[93] Biosensors may also be co-deployed with degradative strains to correlate PFAS presence with microbial transformation activity, offering a potential framework for adaptive, *in situ* monitoring.[14]

While these systems demonstrate encouraging proof-of-concept capabilities, most biosensors remain laboratory-scale and rely on indirect detection mechanisms rather than direct PFAS binding.[94] Environmental stability, specificity across diverse PFAS structures, and biosafety constraints also limit field deployment.[95] Table 4 provides an overview of synthetic biology and genetic engineering strategies—including engineered consortia, CRISPR-enabled pathways, and microbial biosensors—along with their proposed roles in supporting PFAS bioremediation (Fig. 3).

6. Multi-omics integration for system-level understanding of PFAS bioremediation

To complement experimental observations, systems biology and computational approaches provide frameworks for predicting PFAS interactions, although their predictions require experimental validation.

6.1 Genomic and epigenomic insights

Whole-genome sequencing of microorganisms that tolerate PFAS contamination has provided important insight into the genetic traits that support adaptation in these environments.[45] Genomic analyses frequently identify gene clusters associated with xenobiotic metabolism, including oxygenases, reductive dehalogenase-like proteins, and efflux pumps that may help extrude PFAS or mitigate toxicity.[47] Genes involved in oxidative stress responses—such as catalases and superoxide dismutases—are also commonly enriched, reflecting microbial strategies to counter PFAS-induced cellular damage.[96] Mobile genetic elements, including transposons and plasmids, are often detected in PFAS-exposed microbial communities.[97] Their presence suggests that resistance- or tolerance-associated traits may disseminate through horizontal gene transfer, potentially accelerating microbial adaptation.

Epigenomic profiling provides an additional layer of insight by revealing dynamic regulatory mechanisms that modulate gene expression without altering DNA sequence.[98] DNA methylation, non-coding RNAs, and modifications of histone-like proteins can influence transcriptional responses to PFAS exposure.[99] For example, methylation of promoter regions in stress-response or transport-related genes may contribute to inducible expression under PFAS challenge, offering a flexible means of rapid adaptation.[100] Integrating genomic and epigenomic data can inform the design of engineered strains with both robust genetic potential and responsive regulatory networks suitable for PFAS transformation under varying environmental conditions.[101] Nguyen *et al.*[102] identified multi-gene clusters in *Acidimicrobium* sp. containing haloacid dehalogenase-like proteins and efflux pumps potentially associated with PFAS tolerance. Lee *et al.*[103] reported PFAS-dependent DNA methylation changes in *P. putida*, correlating with upregulation of oxidative stress genes. Smith *et al.*[104] documented the spread of conjugative plasmids carrying putative PFAS-related genes among soil microbes in contaminated sites, further supporting the role of horizontal gene transfer in microbial adaptation.

6.2 Integrative analysis of transcriptomics, proteomics, and metabolomics

Multi-omics integration—linking transcriptomic, proteomic, and metabolomic data—provides a systems-level understanding of microbial responses to PFAS that cannot be obtained from individual datasets alone.[105] Transcriptomic studies commonly identify differential expression of genes involved in stress responses, membrane transport, and putative degradation activities, including reductive dehalogenase-like transcripts and oxidative stress regulators.[106,107] Proteomics complements these findings by confirming whether upregulated transcripts correspond to increased protein abundance and by identifying post-translational modifications that may influence enzyme activity or stability.[103] Metabolomics adds a functional layer by detecting PFAS intermediates, tracking metabolic fluxes, and identifying potential pathway bottlenecks or toxic by-products.[108] Integrating these datasets reveals coordinated networks of genes, proteins, and metabolites that help pinpoint regulatory nodes and candidate enzymatic steps relevant to PFAS transformation. Such combined analyses can also highlight rate-limiting reactions and potential synergistic enzyme combinations that warrant further mechanistic testing. For example, Park *et al.*[109] integrated transcriptomic, proteomic, and metabolomic data from *Shewanella* cultures exposed to PFAS and observed coordinated upregulation of electron transport and stress-response pathways, alongside measurable

fluoride release. Zhang *et al.*[110] used multi-omics analysis of engineered consortia to show that enhanced monooxygenase expression alleviated bottlenecks in hydroxylation steps associated with PFAS precursor breakdown.

6.3 Systems biology and computational modelling

Systems biology provides integrative modelling frameworks for predicting the biochemical and ecological dynamics of PFAS transformation in microbial systems.[111]

6.3.1 Metabolic network modelling. Genome-scale metabolic models (GEMs) incorporate genomic, proteomic, and biochemical data to reconstruct microbial metabolism. These models simulate metabolic fluxes under varying PFAS exposure and environmental constraints, helping identify pathway bottlenecks and potential enzyme targets for engineering.[112]

6.3.2 Dynamic community modelling. Agent-based and population models simulate interactions among microbial species, nutrient cycling, competition, and horizontal gene transfer. These models help predict the stability and performance of microbial consortia under PFAS stress and varying soil conditions.[113]

6.3.3 Computational chemistry (DFT and AIMD). Recent DFT and *ab initio* molecular dynamics studies have provided mechanistic insights into C–F bond activation that are difficult to obtain experimentally.[114–116] These simulations reveal possible radical intermediates, temperature-dependent activation pathways, and energetically feasible routes for PFAS breakdown. Such predictions complement microbial studies but should be viewed as hypotheses until experimentally validated.

6.3.4 Machine learning-supported analysis. Machine learning integrates multi-omics datasets with environmental variables (pH, moisture, redox state, co-contaminants) to identify patterns linked to PFAS transformation. Both supervised and unsupervised approaches can suggest optimal microbial consortia, enzyme combinations, or environmental conditions for improved degradation.[117]

Several studies illustrate the potential of these integrative approaches. Zhang *et al.*[118] developed a metabolic model for engineered *Pseudomonas*, predicting PFAS-related flux changes that aligned with metabolomic data. Wu *et al.*[119] combined machine learning with multi-omics data to optimize population ratios in synthetic consortia, improving PFAS degradation in soil microcosms. Kumar and Singh[120] linked PFAS sorption, degradation kinetics, and microbial growth within a stochastic model that accurately predicted long-term remediation performance. Integration of genomics, transcriptomics, proteomics, metabolomics, and epigenomics (Fig. 4) with machine learning and systems biology models enables holistic prediction and design of microbial pathways and consortia tailored for PFAS degradation. However, computational outputs remain predictive models, not confirmations of biochemical mechanisms, and require experimental validation.

6.4 Environmental and regulatory challenges in soil-based PFAS bioremediation

Environmental variables significantly constrain microbial and enzymatic PFAS remediation in soils. Soil heterogeneity—including pH, organic matter content, redox fluctuations, mineral surfaces, and competitive sorption—reduces PFAS bioavailability and disrupts microbial metabolism.[121] Moisture and oxygen gradients limit the stability and distribution of engineered pathways, while co-contaminants can inhibit microbial growth or enzyme activity. Ecological and regulatory factors also present major barriers.[122] Engineered microorganisms raise concerns regarding horizontal gene transfer, ecological displacement, and

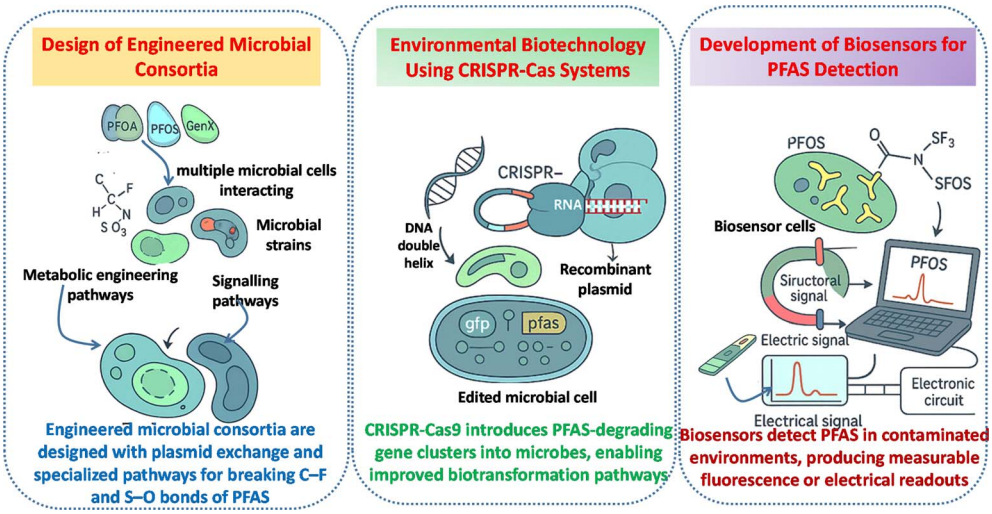

Fig. 4 Synthetic biology and molecular engineering approaches for PFAS bioremediation. The figure illustrates: (1) design of engineered microbial consortia, showing metabolic engineering, signaling pathways, and co-culture strategies for PFAS degradation; (2) environmental biotechnology using CRISPR–Cas systems, depicting targeted genetic modifications *via* recombinant plasmids to enhance PFAS-degrading capabilities; and (3) development of biosensors for PFAS detection, highlighting molecular recognition elements, signal transduction mechanisms, and electronic readouts for real-time monitoring. The diagram integrates PFAS chemical structures, engineered microbes, genetic tools, and biosensing devices in a detailed landscape layout.

persistence beyond treatment zones.[123] Stringent regulatory frameworks restrict deliberate environmental release of modified strains, and containment strategies (kill switches, auxotrophy, encapsulation) remain technically immature.[124] These constraints must be addressed before synthetic biology approaches can be deployed in real soil systems.

6.5 Advances and limitations in current computational modelling

While metabolic models and machine-learning frameworks have been applied to PFAS degradation, most studies remain conceptual and rely on limited experimental datasets.[125] Current models often lack validated kinetic parameters for PFAS–enzyme interactions, defluorination energetics, or soil-specific environmental constraints such as variable ionic strength and competitive sorption.[126] Furthermore, genome-scale metabolic models rarely incorporate PFAS-specific flux modules or fluoride toxicity feedback loops. Future efforts should integrate experimentally verified defluorination kinetics, enzyme turnover numbers, and multi-omics-informed reaction networks into dynamic models. Such integrative systems biology will allow robust prediction of degradation bottlenecks, rational design of synthetic consortia, and optimization of field-scale conditions.[55]

7. Field uses and real-world difficulties in PFAS bioremediation

7.1 Improving soil bioavailability

PFAS exhibit low bioavailability in soils due to strong sorption to clay minerals, organic matter, and metal oxides, which limits microbial access and reduces biotransformation rates.[8] Several strategies have been explored to overcome these constraints.

(i) Biosurfactants: microbial biosurfactants such as rhamnolipids, surfactin, and sophorolipids can decease surface tension and enhance PFAS desorption from soil particles.[127] *Bacillus*-derived surfactin has been shown to mobilize PFAS in contaminated soils, improving microbial uptake. Biosurfactants are generally preferred over synthetic surfactants because they are biodegradable and less toxic.[128]

(ii) Chelators: chelating agents such as citrate and EDTA can disrupt PFAS–metal interactions and release sorbed PFAS into the soil solution, increasing mobility and potential microbial availability.[129]

(iii) Co-metabolism: supplying organic co-metabolites (*e.g.*, lactate, acetate) can stimulate native microbial communities by providing energy sources and promoting co-metabolic transformation of PFAS intermediates.[130]

(iv) Plant–microbe system: plants influence rhizosphere chemistry through root exudates that modify pH, metal availability, and microbial activity.[131] Engineered or selectively chosen plants that exude higher levels of organic acids, sugars, or enzymes can enhance PFAS desorption and support microbial growth.[132] Coupling these plants with PFAS-tolerant or engineered rhizosphere microbes has shown potential for *in situ* remediation. Plants and associated rhizosphere microbes can synergistically enhance remediation of various contaminants, including pollutants such as hydrocarbons, metals, and organic compounds, by stimulating microbial degradation and increasing bioavailability in the rhizosphere.[133] Together, these approaches aim to increase microbial access to PFAS, accelerate transformation processes, and maintain soil ecological stability.

7.2 Biosafety and engineered microbe containment

The use of genetically modified microorganisms (GEMs) in environmental applications requires strict biosafety and containment strategies due to potential ecological risk. Several synthetic biology tools have been developed to limit unintended spread and ensure safe deployment.

(a) Kill-switch circuit: engineered genetic circuits can trigger cell death when specific environmental conditions are absent such as required specific synthetic molecules thereby preventing uncontrolled proliferation after remediation activities.[134]

(b) Nutritional and metabolic dependencies: GEMs can be designed to rely on synthetic nutrients or cofactors not found in natural environments, ensuring that survival is restricted to controlled settings.[84]

(c) Genetic isolation strategies: approaches such as orthogonal genetic codes or modified nucleotide bases reduce the likelihood of horizontal gene transfer to native microorganisms, helping prevent dissemination of engineered traits.[135]

(d) Regulatory and ethical considerations: standardized biosafety frameworks and risk assessment protocols are essential to evaluate persistence, potential gene flow, ecological impacts, and interactions with native microbiomes. Public transparency and engagement further support informed decision-making and societal acceptance.[136] Together, these measures help ensure the responsible development and deployment of synthetic biology tools for PFAS bioremediation.

7.3 Transitioning from lab to the field scale

Converting laboratory-scale PFAS biodegradation accomplishments into useful field applications is fraught with challenges. Because field soils vary in texture, organic content, moisture content, and contaminant distribution, their effects on PFAS bioavailability and microbial activity are unpredictable.[8] Variations in pH, temperature, oxygen concentrations, and competing ions can impact the viability of engineered microorganisms, enzyme activity, and degradation kinetics.[137] By either outcompeting or inhibiting introduced strains or by breaking down their metabolites, native microbiomes may lessen the efficacy of engineered microbes.[138] Optimized amendments are required because microbial growth and metabolism may be restricted by insufficient carbon (C), nitrogen (N), or cofactors.[139] Pilot-scale research and mesocosm experiments mimic field complexities to test formulations of microbial consortia, track persistence, evaluate biotic and abiotic influences, and improve application techniques like bioaugmentation or bio-stimulation.[140] The studies conducted by Yang *et al.*,[141] show that PFAS (*e.g.*, fluorotelomer sulfonates) transformation can be enhanced under mesocosm-relevant conditions through combined bioaugmentation and nutrient/plant-driven stimulation, but sustained removal depends strongly on soil microbiome interactions and supportive amendments. To

enable large-scale PFAS bioremediation deployments, successful scale-up also entails customized site-specific strategies based on in-depth site characterization and iterative feedback from monitoring data.

8. Prospects and research avenues for molecular PFAS remediation

Advances in molecular biology, systems science, and synthetic biology offer promising avenues for addressing the extreme persistence of PFAS. Key research priorities include enzyme discovery, microbial chassis development, integrated multi-omics analysis, standardized field protocols, and stronger stakeholder engagement.[142]

8.1 Enzyme discovery and engineering

Future work should expand the search for natural and engineered enzymes with improved defluorination capabilities. Mining metagenomes from PFAS-contaminated sites and extreme environments may uncover novel reductive, hydrolytic, or oxidative enzymes with broader substrate ranges or higher catalytic efficiency.[47] Protein engineering approaches—including directed evolution, computational design, and machine learning-assisted modelling—can help enhance enzyme stability, turnover rates, and selectivity across diverse PFAS chemistries.[143] Designing multi-enzyme systems or chimeric proteins capable of performing sequential transformation steps may increase the likelihood of deeper defluorination.[54]

8.2 Microbial chassis and synthetic consortia

Effective PFAS bioremediation will depend on environmentally resilient microbial hosts that maintain engineered functions under fluctuating soil conditions, including pH shifts, nutrient limitation, co-contaminants, and competition from native microbiomes.[144] Enhancing tolerance to fluoride and other toxic by-products—using efflux pumps, stress-response elements, and regulatory circuits—will be important.[145] Synthetic consortia that divide metabolic labor and communicate through engineered signalling networks may offer more robust *in situ* performance than single-strain approaches.

8.3 Integrative systems biology and computational tools

Combining genomics, transcriptomics, proteomics, metabolomics, and epigenomics with environmental metadata will help reveal previously unknown pathways and regulatory networks linked to PFAS transformation.[146] Machine learning and artificial intelligence can support the prediction of enzyme targets, optimal pathway configurations, and community-level interactions.[147] Iterative cycles that integrate modelling and experimentation will accelerate the refinement of engineered strains and consortia.

8.4 Standardized protocols and monitoring tools

Progress will require robust methods for environmental sampling, PFAS quantification, and molecular tracking of engineered microbes and enzymes. Biosensors and other real-time monitoring tools can support adaptive management by providing rapid feedback on PFAS concentrations and biotransformation progress.

8.5 Regulation, governance, and public engagement

Deployment of engineered organisms in open environments raises ecological and ethical considerations. Frameworks are needed to assess risks such as gene transfer, ecological disruption, and long-term stability.[148] Collaboration with regulators, policymakers, and community stakeholders will be essential to ensure transparency, build public trust, and support adaptive regulatory pathways for emerging biotechnologies.

Overall, the future of molecular PFAS remediation depends on coordinated advances in enzyme and microbial engineering, systems biology, and responsible field implementation. Interdisciplinary collaboration will be crucial for developing scalable, effective, and safe biotechnologies capable of mitigating PFAS pollution and protecting environmental and human health.

9. Conclusion

Molecular approaches provide promising long-term avenues for addressing PFAS-contaminated soils by combining multi-omics, synthetic biology, and microbial ecology to identify candidate enzymes, pathways, and engineered systems capable PFAS transforming. These strategies remain exploratory and currently operate far below the efficiency of established physicochemical treatments. Their most realistic near-term role is integration into treatment trains—such as thermal, electrochemical, or adsorptive systems—where microbial processes may support precursor transformation or polishing of low-concentration residues. Progress toward field-ready applications will require discovery of natural PFAS-active enzymes, clearer mechanistic understanding of C–F bond activation, and iterative validation of engineered strains and consortia in soil microcosms. Advances in AI-assisted pathway design, high-throughput enzyme evolution, and genome-scale modelling may accelerate development, but robust biosafety and regulatory frameworks will be essential for any future deployment. Overall, molecular engineering offers substantial potential but should be regarded as a long-term research direction rather than an immediate remediation solution.

Author contributions

Mohammad Shahid: conceptualization; data curation; formal analysis; software; writing – original draft; writing – review & editing. Zaryab Shafi: data curation; formal analysis; software; writing – original draft; writing – review & editing.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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