

REVIEW

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Unlocking the potential of soil microbial communities for bioremediation of emerging organic contaminants: omics-based approaches

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Abstract

The remediation of emerging contaminants presents a pressing environmental challenge, necessitating innovative approaches for effective mitigation. This review article delves into the untapped potential of soil microbial communities in the bioremediation of emerging contaminants. Bioremediation, while a promising method, often proves time-consuming and requires a deep comprehension of microbial intricacies for enhancement. Given the challenges presented by the inability to culture many of these microorganisms, conventional methods are inadequate for achieving this goal. While omics-based methods provide an innovative approach to understanding the fundamental aspects, processes, and connections among microorganisms that are essential for improving bioremediation strategies. By exploring the latest advancements in omics technologies, this review aims to shed light on how these approaches can unlock the hidden capabilities of soil microbial communities, paving the way for more efficient and sustainable remediation solutions.

Keywords Bioremediation, Emerging contaminants, Omics, Soil microbial communities

Introduction

“Emerging contaminants (ECs)” are pollutants that have a new origin, alternate route to humans, or require new techniques for treatment [1]. These pollutants are divided into different categories according to their chemical properties and sources, including organic, inorganic, biological emerging contaminants, and other unknown compositions like micro and nanoplastics. Emerging inorganic contaminants include engineered nanoparticles, radionuclides, and nuclear wastes. Biological contaminants such as pathogenic bacteria, antibiotic-resistant bacteria and resistance genes, viruses, and protein contaminants [2]. Emerging

organic contaminants (EOCs) encompass a wide range of chemical compounds, including pharmaceuticals and personal care products (PPCPs), pesticides, endocrine disrupting compounds (EDCs), surfactants, flame retardants, plasticizers, and industrial additives, among others. Metabolites and intermediate degradation products of the original compounds are also part of the EOCs [3]. They can be produced and released from households, hospitals, laboratory wastewater, agricultural processes, construction, landscaping transportation, or the food industry [1, 4–6]. Due to its unique filtering and buffering characteristics, the soil can absorb and retain most anthropogenic substances causing their accumulation in inland areas [7].

There are various health risks associated with EOCs, both in the short-term and long-term. These risks include abnormal physiological processes, higher cancer rates, increased toxicity potential of chemical mixtures, endocrine-disrupting effects, birth defects, infant

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malformations, and diabetes [1, 8]. Moreover, frequent exposure to antibiotics as an emerging pollutant can lead to antibiotic resistance in specific pathogenic microorganisms, making their treatment challenging [7, 8]. Considering their hazardous effects on humans and other biota, finding a remedial solution for removing EOCs sounds urgent.

Among various conventional, non-conventional, chemical, and physical treatment processes [9, 10], bio-based remedial techniques (see Fig. 1 for more information) are known for being safer, more cost-effective, and require less energy than physicochemical techniques [11, 12]. Bioremediation and biodegradation methods have the added benefit of being eco-friendly as they involve the interaction and cooperation of microbial consortia to remove contaminants [13, 14]. However, these interactions are complex and unpredictable, since more than 99% of microbial cells are difficult to culture using traditional techniques [13]. To address this challenge, the systems biology approach that combines geochemical data with biologically pertinent measurements can be used. This approach can detect contamination sources and propose remedial methods. It relies on biological materials that act as "biosignatures," such as genomes, proteins, lipids, metabolites, and transcribed RNA [15, 16]. Omics-based studies that track these biosignatures are promising approaches giving a

broad vision of biodiversity in microbial communities and their potential to remove persistent pollutants. Based on their enzymatic profile and/or their expressing genes, these methods employ innovative tools for discovering the hidden worlds of microorganisms and their potential. This target would be achievable by taking advantage of next-generation sequencing techniques along with bioinformatics, and genomic/RNA/microbial metabolic pathways/proteins databases that are the main tools of omics approaches [17, 18]. The use of omics methods has grown in importance for understanding and improving microbial-facilitated remediation of environmental pollutants. Genomics, transcriptomics, proteomics, and metabolomics offer valuable insights into the microbial populations engaged in remediation and the underlying molecular processes [19]. These advanced techniques have empowered researchers to more comprehensively characterize the composition, operation, and changes of microbial communities in contaminated settings. Applying omics methodologies has facilitated the identification of new microbes and enzymes for bioremediation, as well as the enhancement of existing microbial communities for more effective contaminant elimination [20]. This, in turn, aids in pinpointing the crucial microbes and enzymes responsible for breaking down pollutants. Studies based on omics have revealed previously unknown microbial pathways

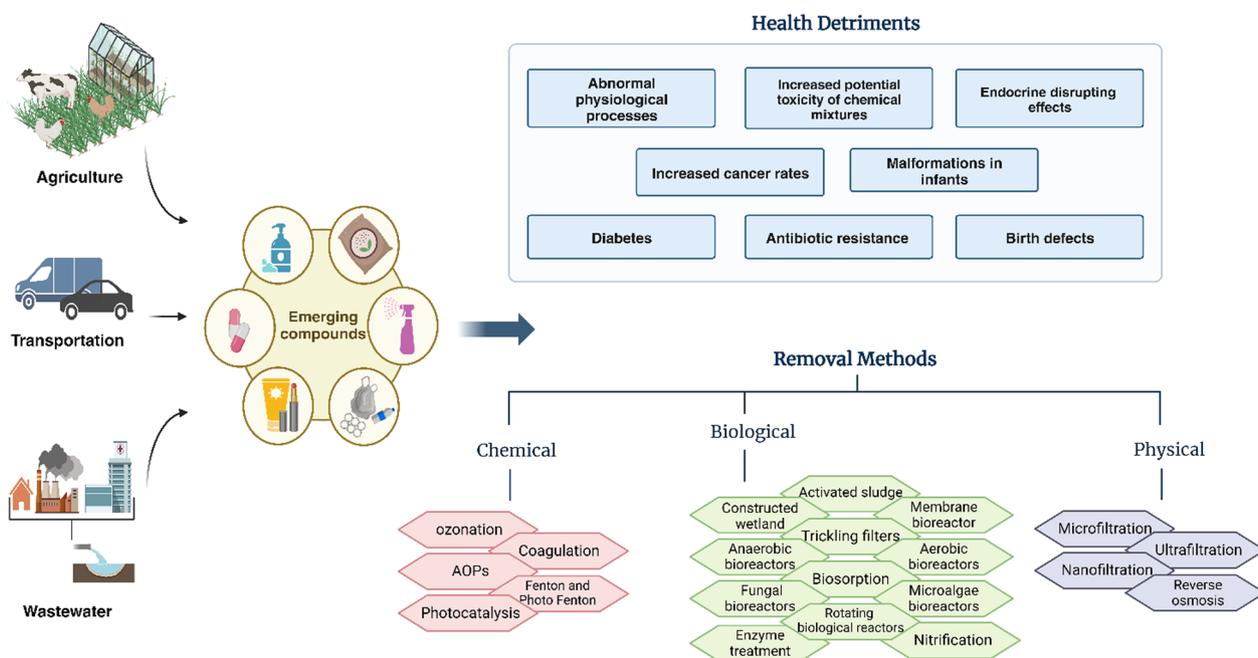


Fig. 1 Sources, hazards, and available removal methods of emerging compounds, including physical, chemical, and biological approaches. AOPs: Advanced oxidation processes. Created with BioRender.com

and genes crucial to the degradation of diverse environmental toxins such as pesticides and industrial chemicals [19, 21, 22].

Soil contains a wide variety of microbial communities with many potential remedial properties, some of which have been discovered while others remain unknown. The absorption of pollutants by plants from the soil can lead to harm in ecosystems as they move up the food chain [23–25]. The presence of different microorganisms in the soil drives the process of bioremediation, leading to the breakdown of contaminants through the interactions of a community of microbes. The effectiveness of bioremediation relies on the capacity of these microorganisms to adjust to changing environmental circumstances [26]. In this regard, culture-independent omics-based approaches are the key methods to unlock the undetected side of this potential. Despite the fact that there are reviews related to soil bioremediation, there is still a need to investigate the potential of soil microbial populations in breaking down emerging organic contaminants with the help of novel and more accurate omics approaches. So, in this review, we aim to address the lack of information on using omics approaches to remediate soils contaminated with emerging organic pollutants and the way through the future of this field.

Emerging organic contaminants: a threat to the environment and biota

Emerging organic contaminants (EOCs) and their metabolites, which are frequently more toxic than their initial source, have been found in a variety of environments [27]. Emerging organic contaminants (EOCs) do not have established environmental monitoring or emission standards and can have harmful effects on ecosystems and human well-being. Because of their potential risks to the environment and human health, continuous release into the environment, and the challenge of fully removing these substances even with advanced wastewater treatment plants (WWTPs), EOCs may require future regulatory attention. Recent advancements in environmental analysis have resulted in frequent detection of these substances in various settings such as sewage, surface water, drinking water, and soil [28].

In vivo studies have shown that exposure to EOCs can cause hormonal imbalance, decreased aquatic organism survivability, reproductive issues, and a variety of health problems in humans, including cancer, diabetes mellitus, respiratory disorders, neurological disorders, metabolic diseases, and thyroid disease [29–31]. This is a cause of concern as some EOCs like Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonate (PFOS) have been found in wildlife, drinking water, human serum, and breast milk [29]. Additionally, EOCs can be harmful to

other living beings such as birds, fish, insects, and non-target plants [30]. Generally, based on scientific research, the immediate and long-term impacts of these environmental contaminants on ecosystems, natural resources, human health, and the environment have been proven [32] (Table 1).

Bioremediation: a convenient way to remove EOCs from the soil

For the contaminated soils, sediments, and water remediation, bio-based removal techniques such as phytoremediation and microbial-based bioremediation/biodegradation offer a financially advantageous and eco-friendly alternative to conventional physicochemical treatments that combined with biostimulation and bioaugmentation techniques speed up microbial activities in polluted sites [40, 41]. The type of pollutant, environmental conditions, and accessibility of phosphorus and nitrogen supplies all impact biodegradation efficiency [42, 43]. Moreover, factors including the type of microorganisms used, the screening situation, and the genetic profile of the organisms can affect microbial activity [44]. Even though biodegradation is considered a safe and eco-friendly technology to remove synthetic chemicals from the environment, it is important to consider that the process should not introduce more harmful substances into the environment than there originally were [45].

Microbial bioremediation refers to using microorganisms or their byproducts, such as enzymes (including cytochrome P450, laccases, hydrolases, dehalogenases, oxygenase, dehydrogenases, proteases, transferases, oxidoreductases, and lipases) [46–48], or their leftover biomass, to remove contaminants from the environment. Oxidoreductases and hydrolases are the two groups of enzymes with high biodegradation activity [49]. The former is responsible for detoxifying toxic organic compounds through oxidative coupling. This leads to the breakdown of chemical bonds and the transfer of electrons via oxidation–reduction reactions, resulting in the oxidation of contaminants to harmless substances. Oxidoreductases and peroxidases are present in bacteria, fungi, and higher plants [49, 50] and could play a role in the decomposition of lignin and the humification of phenolic and aromatic substances in soil [51, 52]. Additionally, they can detoxify toxic xenobiotics by polymerization, copolymerization, or binding to humic substances [50, 53]. Laccases belonging to oxidoreductase enzymes are powerful oxidizers of pesticides, PhACs, and hormones that could be purified from white-rot fungi [54, 55]. *Pleurotus ostreatus* as an example could remove bisphenol A. Their potential to degrade persistent compounds is mainly related to lignolytic enzyme

Table 1 A summary of emerging organic compounds list, their sources, and toxic effects [29, 30, 33–39]

Number	Name of compounds	Source	Toxic effects on living beings
1	Organochlorine (diazion, endosulfan, pentachlorophenol)	Pesticides	Diarrhea, blurred vision, respiratory disorder, blood problems such as aplastic anemia in humans, cardiovascular disease in rats and mice
2	Carbamates (carbofuran, thiourea)	Insecticides, nematocides, acaricides, rodenticides	Hypertension, vision defect, respirator disorder, bone marrow damage, carcinogenic effects in humans
3	Pyrethroids (tetramethrin, decamethrin)	Insecticide	Mutagenic effect, neurotoxin, paraesthesia in humans
4	Carbanilates (chlorpropham, diuron)	Herbicide	Kidney and liver failure, anemia, and bone marrow damage in humans
5	Acetamides (diphenamid)	Herbicide	Dermal irritation, inhalation toxicity in humans
6	Phenoxy allocates (2,4-dichlorophenoxyacetic acid)	Herbicide	Nervousness, headache, dizziness in humans
7	Triazines (cyprazine, propazine)	Herbicide	Constipation, nausea, dizziness, anemia in humans
8	Benzoic acid derivatives (dicamba)	Herbicide	Damage to CNS, heart failure in humans
9	Benzonitriles (bromoxynil)	Herbicide	Vomiting, urinary disorder in humans
10	Phthalimides (folpet)	Fungicide	Brain ailment, skin disease in humans
11	Detergents (alkylphenols)	Industrial and domestic	Respiratory, vision, and movement disorders in fish, disturbance in estrogen production and reproduction with increased number of eggs produced by minnows and vitellogenin level
12	UV filters (benzophenone-3 (BP-3), 3-(4-methylbenzylidene) camphor (4MBC), ethylhexyl methoxycinnamate (EHMC), and octadecene (OC)	Personal care products	Can bind to human hormone receptors and have binding or antagonistic effects on hormones, endocrine-disrupting influences (disruption of the hypothalamic-pituitarythyroid axis (HPT) and reproductive and developmental function) in lab animals
13	Endocrine-disrupting chemicals (EDCs) (steroid estrogens (SEs), PCBs, OCPs, dioxins)	Industrial chemicals, pharmaceuticals, personal care products, herbicides, and pesticides	Feminization in fish, reproductive abnormalities in birds, obesity, diabetes, various cancer types, cardiovascular risks, metabolic disorders, epigenetic alterations, autism in humans
14	Phthalates	Plasticizers	Obesity-related factors, glucose disturbances, hypertension in humans, reduced levels of thyroid hormones and progesterone in pregnant mothers
15	Bisphenol A (BPA)	Plasticizers	Estrogenic effects in rats, possible EDC in birds, hormonal effects in humans and animals, xenoestrogen, possible carcinogenic effect in humans
16	Quaternary ammonium compounds (QACs)	Disinfectants, fabric softeners, preservatives, and cosmetics	Reduced fertility in mice, respiratory issues such as asthma, increased risk of chronic obstructive pulmonary disease (COPD), neurological defects, decreased mitochondrial function in humans
17	Nonsteroidal anti-inflammatory drug (diclofenac)	Pharmaceuticals	Reduces the hematocrit values of fishes, and causes cytological changes in the liver, kidneys, and gills of fishes
18	β -blocker (propranolol)	Pharmaceuticals	Reduction of viable eggs of Japanese medaka (<i>Oryziaslatipes</i>), strong acute toxicity on benthos and zooplankton

Table 1 (continued)

Number	Name of compounds	Source	Toxic effects on living beings
19	Antibiotics (penicillin, sulfonamides, tetracyclines)	Pharmaceuticals	The development of resistance in bacterial pathogens
20	Acetaminophen	Pharmaceuticals	Disruption of the steroidogenic pathway in humans, chronic hepatotoxicity (cirrhosis and hepatocyte necrosis) in mice

production, making them xenobiotic-tolerant microorganisms. Monooxygenase as an oxidoreductase enzyme integrates oxygen atoms with the substrate in the reduction reactions and also performs hydroxylation, denitrification, ammonification, dehalogenation, and sulfurization of the substrate [50]. *Buttiauxella* sp. S19-1 as an example is a TNT-degrading bacterium with oxidoreductase activity [56]. In addition, hydrolytic enzymes play a role in disrupting chemical bonds of toxic compounds, and reducing their toxicity [49, 50]. This makes them effective agents for biodegrading oil spills, organophosphates, and carbamate pesticides. Hydrolases catalyze condensation and alcoholysis reactions. They are advantageous due to their availability, lack of cofactor stereoselectivity, and tolerance to water-miscible solvents. Lipase is an enzyme from the group of hydrolases that plays a role in the decomposition of organic oil pollutants, and its mechanism is the conversion of triacylglycerols into glycerol and free fatty acids [49]. Eventually, when contaminants reach the metabolic pathways for degradation and biotransformation, microorganisms can degrade them via their own or modified metabolic processes [49].

Despite several reports that focused on single strains' ability to biodegrade pollutants, microbial communities or consortiums of microbial strains are known to be more effective in remediation, especially in natural conditions. This is due to their co-metabolism that lessens cross-reactions and the metabolic pressure on single strains of the community [57]. Because of the cooperation of diverse species in consuming a compound as a substrate, microbial consortia frequently operate better and are more resistant in polluted settings; as a result, they are more efficient at bioremediation than particular single-strain microorganisms [17, 58]. Both bacterial and fungal intracellular and extracellular enzymes are being used to remediate the resistant contaminant [59, 60]. Due to the fact that bacteria grow more quickly and fungi produce stronger enzymes, fungal and bacterial consortia typically outperform single-strain cultures in their ability to break down resistant contaminants [61, 62]. As an example of the decolorization of a single dye, the bacterial and yeast consortium (*Brevibacillus laterosporus* and *Geotrichum candidum*) produced faster decolorization rates than the individual microbiological species [63]. Studies have shown that the joint activities of enzymes of mixed microbial cultures are much better in removing some pollutants compared to individual strains [63] which is why microbial populations in the consortia structures are promising organizations for biotreating degradation-resistant pollution.

However, it is important to ponder the drawbacks and risks associated with bioremediation for a more effective utilization of this method. These concerns include the comparably slow pace of the process in comparison to other remediation technologies, as well as the limitation in completely removing all amounts of contaminants. It is important to note that this method may not be suitable for cleansing certain mineral pollutants or organic compounds. Additionally, there are challenges in confirming the complete elimination of contaminants. Furthermore, during the decomposition of toxic compounds, there is a possibility of generating more toxic byproducts. On the other side, during bioremediation processes, there is a possibility that organic nutrients like animal manure and sewage sludge might carry antibiotic residues and resistant bacteria. Antibiotic resistance has the ability to disseminate globally through horizontal gene transfer, influencing both targeted and non-targeted microbial communities. The transfer has the potential to contribute to the rise of antibiotic-resistant strains and may impact human health by diminishing the efficacy of antibiotics in treating bacterial infections. These limitations are therefore crucial considerations when assessing the use of bioremediation for specific contaminated sites [64–66].

Some microorganisms and their effective enzymes in the biodegradation of emerging organic compounds are listed in Table 2.

The way through discovering the composition and function of EOC-degrading microbial populations: omics approaches

To understand the soil ecosystem, it is necessary to discover its microbial population, activities, and how they interact with the soil compartments [78]. This vision in a specific ecological niche has been given more weight by molecular approaches such as genomics, proteomics, transcriptomics, metabolomics, fluxomics, etc. [79]. The gathered data from different "omics" methodologies is then refined to offer adequate in-depth information to study microbial metabolism in bioremediation and provide full knowledge of the soil microbial population, their functional and key genes, mechanism of toxicity, and interaction details. [78, 80] (Fig. 2). Applying omics techniques, it is possible to investigate the changes in expression profiles associated with the degradation of compounds (proteomics), to identify and quantify specific metabolites that arise during degradation (metabolomics), and changes in the gene expression that accompany the exposure of microorganisms to pollutants (transcriptomics) [16].

Omics approach can reveal the specific genetic determinants, metabolic pathways, and regulatory

Table 2 EOC-degrading microorganisms and enzymes

Microorganism	Enzyme	Compound	References
<i>Schizophyllum commune</i> IBL-06	Lignin peroxidase	Diclofenac	[49, 67]
<i>Trametes versicolor</i> and <i>Pycnoporus sanguineus</i> CS43, <i>Pleurotus ostreatus</i>	Laccase	Bisphenol A, PCBs (polychlorinated biphenyls)	[49, 68–70]
<i>Nocardioides</i> sp. C190, <i>Pseudomonas</i> , <i>Rhodococcus erythropolis</i>	Atrazine dechlorinase, triazine hydrolase	Triazine herbicides	[68, 71, 72]
<i>Aspergillus niger</i> NCIM 563	Phytase	Organo phosphate	[68, 71]
<i>Pseudomonas</i> sp. LBr	Glyphosate oxidase (GOX)	Glyphosate (pesticides)	[43, 73]
<i>Pleurotus</i> sp.	Laccase, manganese peroxidase, lignin peroxidase	Degradation of Azo dyes	[74]
<i>Ganoderma lucidum</i>	Laccase, manganese peroxidase, lignin peroxidase	Degradation of phenanthrene and pyrene	[74]
<i>Stropharia coronilla</i>	Manganese peroxidase	biodegradation of benzo(a)pyrene	[74]
<i>Phanerochaete chrysosporium</i>	Peroxidases (Lignin peroxidase & manganese peroxidase)	Degradation of pesticides (atrazine & alachlor), nitroaromatic compounds	[71, 74]
<i>Trametes versicolor</i>	Laccase	Degradation of herbicide isoproturon, anthracene, and benzo(a)pyrene	[71, 74]
<i>Bacillus subtilis</i>	Laccase & Esterase	Biodegradation and detoxification of Cypermethrin (insecticide), Bisphenol A (BPA)	[74, 75]
<i>Trametes versicolor</i>	Laccase	Degradation of Carbofuran	[74]
<i>Candida antarctica</i> (CAL), <i>Candida rugosa</i> (CRL)	Lipases	Poly (bisphenol-A carbonate) (BPAPC)	[76]
<i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Chromobacterium viscosum</i> , and <i>Sphingobacterium</i> sp. strain S2	Lipase	PBSA, PLA, PCL, oil, and PBS	[77]
<i>Pseudomonas aeruginosa</i> PA1	Carboxylesterases	Malathion and parathion (insecticide)	[71]
<i>Humicola</i> sp.	Cellulase	Detergent and washing industrial contaminants	[71]

mechanisms that enable certain microbes to thrive in contaminated conditions. This knowledge can guide the selection and engineering of more effective inoculant strains [81, 82]. Omics can also elucidate how introduced inocula interact with the native microbiome, including competition, cooperation, and succession processes that impact colonization success. This informs strategies to better integrate inocula into the existing community [82, 83]. Omics can reveal the specific abiotic and biotic stressors in contaminated sites that limit inoculant survival and proliferation, guiding the development of more stress-tolerant strains [83].

Genomics and metagenomics provide insights into the genetic makeup and metabolic capabilities of microbial communities involved in bioremediation [84, 85]. This allows researchers to identify microbes with desirable traits for degrading pollutants, tolerating harsh conditions, and thriving in contaminated environments [86, 87].

Transcriptomics and proteomics reveal the genes and proteins that are actively expressed by microbes during bioremediation, shedding light on the molecular

mechanisms underlying pollutant degradation and stress responses. This knowledge can guide the engineering of more effective bioremediation strains or consortia [85, 88]. Metatranscriptomics and metaproteomics can track the activity and expression of target inoculant strains within the complex environment, allowing researchers to evaluate and optimize their performance [81, 89].

Metabolomics profiles the small molecule byproducts of microbial metabolism, tracking the flow of carbon, energy and nutrients during bioremediation. This helps optimize nutrient amendments, electron donors, and other environmental conditions to maximize microbial activity and pollutant removal [84].

Integrating multi-omics data provides a systems-level understanding of plant–microbe interactions, microbial community dynamics, and the complex biological processes involved in bioremediation. This enables predictive modeling and rational design of robust, efficient microbial inoculants and bioremediation strategies [85].

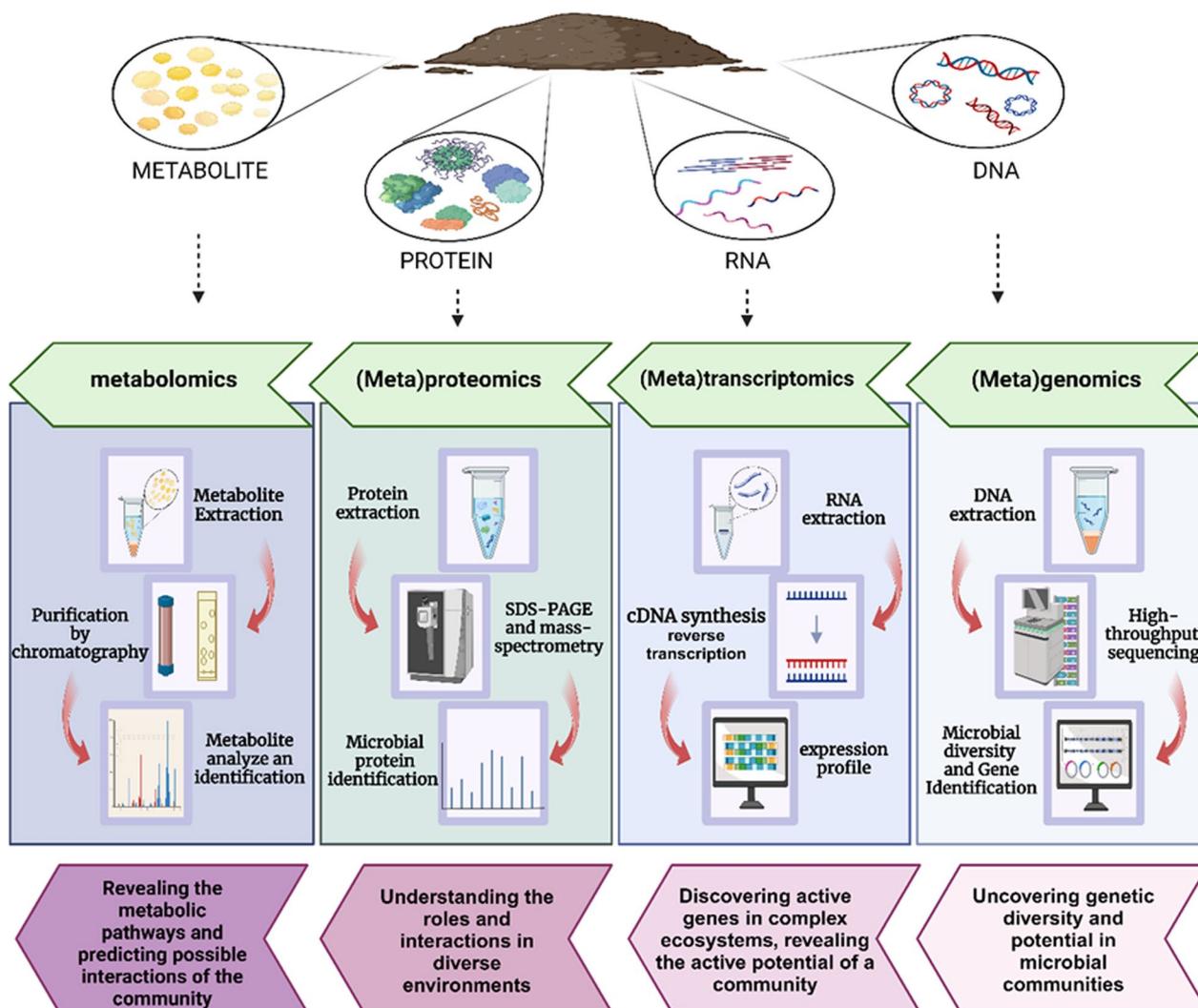


Fig. 2 Available omics-based methods to discover emerging pollutants’ remedial potential in the soil microbial communities. Each approach has its standard procedure and available techniques, giving different information from diversity to the metabolic potential of the microbial communities. Created with BioRender.com

Role of metagenomics in the identification of effective EOC-degrading microorganisms/genes

The systematic study of microbial communities at the genome level is called metagenomics. Over 99% of microorganisms that live in various natural habitats are either difficult or impossible to cultivate, leading to major limitations on processes that rely on culture. This method makes it possible to explore a sample’s entire genome sequence (genomics) and an enormous amount of genome that is straightly extracted from the environment (metagenomics) [90].

The following steps could be taken to acquire metagenomics data [91–93], including:

- I. Sampling and processing: Samples should be representative and appropriate DNA extraction methods should be used.
- II. Sequencing technology: Metagenomic sequencing has shifted from Sanger sequencing to next-generation sequencing (NGS) technologies, such as the 454/Roche, Illumina/Solexa systems, Pyrosequencing, PacBio, and Ion torrent sequencing.
- III. Assembly: Short-read fragment assembly is used to construct longer genomic contigs through co-assembly and de novo assembly methods.

- IV. Binning: Organizing assembled contigs into collections based on their probable taxonomic or functional classifications. This step is substantial for data analysis and finding key players and/or genes in the community.
- V. Annotation: Assigning functions to genes or gene products based on their similarity to known proteins or gene products in databases.
- VI. Statistical analysis: Measurement of alpha and beta diversity and the detection of differentially abundant taxa or functions, could be interpreted from metagenomic data outcomes using statistical analysis.
- VII. Data storage and sharing: To ensure that the findings of various investigations can be compared and contrasted and in order to develop metagenomic datasets, the extracted data should be deposited into databases.

The metagenomic approach with pure DNA can take two paths: (1) bioactive compounds screening in clones of the metagenomic library which is now obsolete (function-based) [94–97] and (2) complete genomic DNA sequencing (sequence-based). The extracted data of metagenomics contain information on the microbial communities, including their diversity and taxonomic characterization at the community level and their functional and metabolic potential. Predicting the community's functional capabilities and nutritional requirements provides the key insights needed to formulate selective media that will support the growth of these elusive microorganisms [94, 98–100].

Metagenomics has identified biomolecules such as antibiotics and microbial enzymes. It also helps to explore the hidden potential of bioremediation-relevant microbes [78]. Genomic-based identification of new promoters, genes, and degradative pathways has helped to develop more efficient pollutant-degrading strains for bioremediation [101]. Key bioremediant bacterial genera like *Pseudomonas*, *Shewanella*, *Deinococcus*, and *Dehalococcoides* have their whole genome sequences available, and detecting novel genes in such strains could provide insights into their degradation ability and substrate selectivity [102].

By analyzing the whole genome of a biomass sample, it is possible to identify the different levels of microbial taxonomy and pathways for breaking down xenobiotic compounds, both aerobically and anaerobically [103]. Garrido-Sanz, D. et al. (2018) isolated and analyzed a Polychlorinated Biphenyls (PCBs) biodegrading bacterial consortium through 16S rRNA amplicon and whole genome shotgun sequencing. *Pseudomonas* and *Rhodococcus* strains were abundant in this consortium,

harboring enzymes that catalyze biphenyl to benzoate and benzoate to Tricarboxylic Acid (TCA) cycle intermediates. The study demonstrated that metagenomic analysis can identify bacteria and their specific reactions and pathways involved in biodegradation processes [104]. The possible potential of *Bacillus*, *Krasilinkoia*, *Lysinibacillus*, *Rhodococcus*, *Sphingobium*, *Rubrivivax*, *Paenibacillus*, Nitrate Reducers, and *Enterobacter* species in remediation of azithromycin-contaminated soil was also another achievement of this approach [105]. *Pseudomonas*, *Achromobacter*, *Xanthomonas*, *Stenotrophomonas*, and *Cupriavidus* were found to be major players in atrazine bioremediation in the study of Bhardwaj et al. (2019) using whole metagenome sequencing [106]. *Streptomyces nigra* LM01 is a cornfield-isolated strain that also efficiently degrades atrazine and nicosulfuron. *atzA/trzN* were identified in its whole genome, indicating its possible potential to dechlorinate atrazine to hydroxyatrazine or convert it to cyanuric acid [107]. In another study based on a metagenomic fosmid library, the ability of soil microorganisms in polycyclic aromatic hydrocarbon degradation was assessed. Extradiol dioxygenases encoding genes and Rieske non-heme iron oxygenases were identified in a complex microbial network. These enzymes are responsible for activating aromatic compounds by substituting methyl groups to their side chains [108].

Microbial degradation is regarded as the most appropriate technique for degrading chlorimuronethyl, a common long-term residual sulfonylurea herbicide. *Rhodococcus erythropolis* D310-1 is a strain that harbors CarE, participating in the catalysis of chlorimuronethyl de-esterification through the catalytic action of carboxylesterase. This provides new insights into the process of sulfonylurea herbicide degradation as well as the theoretical datasets for enzymes [109]. Organophosphates (OPs) such as chlorpyrifos (CP), are another type of pesticide that impairs soil fertility and disturbs the biogeochemical cycle. *Pseudomonas aeruginosa* RNC3 and *Stenotrophomonas maltophilia* RNC7 are common soil bacteria that have significant CP breakdown potential. The genomes contain annotations for degradation processes and metabolism of aminobenzoate, chlorocyclohexane, chlorobenzene, toluene, and naphthalene. Organophosphorus hydrolase and 4-nitrophenol 4-monooxygenase are the major enzymes of the aminobenzoate degradation pathway which play important roles in CP breakdown. Furthermore, results demonstrated that phenolic compound oxidation is the most important step of CP biodegradation. Together with *opd* and *opch2* genes, a series of putative CP degradation genes were expressed in RNC3 and RNC7 including

metallophosphoesterase (OPCH2) and phosphotriesterase encoding genes [110].

In a recent study, Cao and colleagues (2024) discovered a novel *Klebsiella pasteurii* strain capable of degrading the herbicide mesotrione. This strain exhibited exceptional adaptability to environmental conditions. Through genomic analysis and RT-qPCR, the researchers identified the nitroreductase-encoding genes *nfsA* and *nfsB* as key players in mesotrione biodegradation, a finding that had not been reported before [111]. In another study, Sun et al. (2024) used diethyl terephthalate (DET) as a screening substrate

to discover a new amidohydrolase gene, AmiH52, in a soil metagenomic library. The recombinant enzyme, expressed in *E. coli*, demonstrated both esterase and amidohydrolase activities. It exhibited highly specific activity towards p-nitrophenyl butyrate and the ability to degrade various amide herbicides. AmiH52 was found to be particularly effective against the herbicide propanil, showing the most potent degradation activity [112].

Table 3 introduces the potentially EOC-degrading microbial cells and consortiums, discovered through

Table 3 EOC-degrading microbial communities that have been identified through genomic/metagenomic approaches

Microorganism/Microbial consortia	Method and Platform	Contaminant	References
<i>Sulfuricurvum</i> spp., <i>Pseudomonas</i> spp., and <i>Candidatus saccharibacteria</i>	16S rRNA/Illumina MiSeq	Hydrocarbons and their derivatives	[113]
<i>Rhodococcus</i> , <i>Sphingomonas</i> , and <i>Pseudomonas</i>	Shotgun metagenome sequencing/Illumina HiSeq	HCH, Endosulfan, and DDT pesticides	[114]
<i>Acinetobacter johnsonii</i> LXL_C1	Whole genome sequencing/Illumina HiSeq and PacBio	Cyprodinil	[115]
<i>Clostridium</i> , <i>Nocardioidea</i> , <i>Bellilinea</i> , <i>Anaerolinea</i> , <i>Longilinea</i> , and <i>Phycococcus</i>	16S rRNA/Illumina MiSeq	Cypermethrin, fipronil, imidacloprid, and sulfosulfuron	[116]
<i>Geobacter</i> , <i>Mycobacterium</i> , and <i>Sphingomonas</i>	16S rRNA/Illumina MiSeq	Polycyclic aromatic hydrocarbons (PAHs)	[117]
<i>Cellvibrio</i> bacteria, <i>Acidobacteria</i> (such as <i>Candidatus Koribacter</i> and <i>Candidatus Solibacter</i>)	16S rRNA/Illumina MiSeq	Acetaminophen (APAP)	[118]
<i>Pseudomonadaceae</i> , <i>Rhizobiaceae</i> , <i>Desulfobacteriaceae</i> , <i>Deinococcaceae</i> , <i>Bacillaceae</i> , <i>Sphingomonadaceae</i> , <i>Xanthomonadaceae</i> , and <i>Enterobacteriaceae</i> , <i>Panibacillaceae</i> , <i>Bradyrhizobaceae</i>	16S rRNA/IonTorrent	Azithromycin	[105]
<i>Pseudomonas mendocina</i> , <i>Brevundimonas olei</i> , <i>Serratia marcescens</i> , <i>Sphingomonas</i>	16S rRNA/Illumina MiSeq	Polycyclic aromatic hydrocarbons (PAH)	[119]
<i>Acinetobacter</i> spp.	Metagenomic libraries (BAC and fosmid clone DNAs)/Illumina HiSeq	Toluene	[120]
<i>Sphingobium fuliginis</i> ATCC 27551	Whole genome sequencing/Pacbio RSII and Illumina MiSeq	Neurotoxic organophosphate insecticides	[121]
<i>Nocardioidea carbamazepini</i> sp. nov	Shotgun metagenome sequencing/Illumina MiSeq	Carbamazepine and ibuprofen	[122]
<i>Thermobifida fusca</i> , <i>Pseudomonas mendocina</i> , and <i>Nocardia</i> sp.	Shotgun metagenome sequencing	Different types of plastic wastes	[123]
<i>Actinomycetales</i> , <i>Gemmatimonadetes</i> , <i>Proteobacteria</i> , <i>Acidobacteria</i> , and <i>Bacteroidetes</i>	Shotgun metagenome sequencing/Illumina HiSeq	Di(2-ethylhexyl) Phthalate (DEHP) (a plasticizer)	[124]
<i>Pseudomonas</i> , <i>betaproteobacteria</i> and <i>Rhodococcus</i> , <i>Bordetella</i> , <i>Stenotrophomonas</i> sp., <i>Achromobacter</i> and <i>Variovorax</i>	16S rRNA/Shotgun metagenome sequencing/Illumina MiSeq	Polychlorinated Biphenyls (PCBs)	[104]
<i>Proteobacteria</i> , <i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Chloroflexi</i> , <i>Firmicutes</i> , and <i>Gemmatimonadetes</i>	16S rRNA/Illumina MiSeq and Illumina HiSeq	Perfluorinated Compounds (PFCs)	[125]
<i>Bradyrhizobium</i> , <i>Mycobacterium</i> , <i>Rhodopseudomonas</i> , <i>Pseudomonas</i> , <i>Cupriavidus</i> , and <i>Streptomyces</i> , <i>Rhodococcus</i> , <i>Starkeya</i> , <i>Rhizobium</i> , <i>Sphingomonas</i> , <i>Ochrobactrum</i> , <i>Methylobacillus</i> , <i>Alicyciphilus</i> and <i>Stenotrophomonas</i>	Shotgun metagenome sequencing/Illumina HiSeq	Carbamazepine (CBZ), triclocarban (TCC), and triclosan (TCS)	[126]

genomic or metagenomic-based approaches in past years.

Transcriptomics and metatranscriptomics: identification of the active EOC-degrading genes

In order to understand the functional activities of the soil microbial communities, it is necessary to study transcription gene profiles, named "transcriptomics or metatranscriptomics" [17, 127]. The analysis of mRNAs allows for a clear understanding of gene expression in specific cells and tissues (Fig. 2). This includes determining the presence or absence of transcripts, evaluating alternative splicing to predict protein isoforms, and quantitatively assessing how genotype influences gene expression through the analysis of expression assessable trait loci or allele-specific expression [79]. While transcriptomics examines the gene expression profile of a single organism at a particular growth stage, metatranscriptomics examines this profile for the microbial community by extracting RNA from an environmental sample. The best procedures would involve complete mRNA extraction and enrichment, cDNA synthesis, microarray hybridization of cDNA, RNA-Seq, and reference mapping of sequence reads. The most important step is selective mRNA enrichment by rRNA depletion, followed by mRNA transcript sequencing [128, 129]. The two main techniques used nowadays to determine the transcriptional profile for virtually every biological sample under a wide range of circumstances are microarrays and RNA-Seq [130]. Using this approach and based on the Illumina HighSeq2500 sequencing method, Sharma et al. (2019) indicated that Archaea play a more substantial role in the nitrification process in metal and pesticide-contaminated soil than bacteria, demonstrating their active role in contaminated environments. This domain displays strong expression of transcripts for the glyoxalase and/or bleomycin resistance dioxygenases, 4-hydroxyphenylpyruvate dioxygenase, 2-nitropropane dioxygenase, metapyrocatechase, ring hydroxylating dioxygenases, and intradiol dioxygenase (from *Novosphingobium* spp.) genes related to aromatic hydrocarbon degradation in agricultural soil [131]. Understanding the key metabolic pathways involved in the biodegradation of xenobiotics and EOCs is a significant area of research in molecular biology. Studying the metabolic pathways in the biodegradation of chlorimuron-ethyl by *Rhodococcus erythropolis* D310-1 revealed toluene and aminobenzoate degradation as key pathways. During this process, essential genes like carboxylesterase, cytochrome P-450, and glycosyltransferase were identified through qRT-PCR experiments [132]. Brzeszcz et al. (2020) investigated the potential of seven non-pathogenic bacterial strains from the genera *Rhodococcus*, *Mycolicibacterium*, *Dietzia*,

Pseudomonas, *Arthrobacter*, and *Gordonia* as bioaugmentation agents in soil historically contaminated with aliphatic and polycyclic aromatic hydrocarbons. They evaluated the effects of biostimulation and bioaugmentation on the transcriptomic profiles of the soil. The study found that *Gammaproteobacteria* and *Actinobacteria* classes were associated with alkane monooxygenase (AlkB) transcripts, with a significant proportion attributed to *Pseudomonas* and similarities to genes from *Mycobacteriaceae*, *Gordonia*, and *Arthrobacter* genera [133]. Comparing and analyzing the microbial community of biobed systems before and after a pesticide usage season in the field, Russell et al. (2021) showed a significant rise in the aromatic and xenobiotic degradation-related genes, including peroxidases, monooxygenases, and cytochrome P450. Metagenomic and metatranscriptomic analyses encourage approaches in which pesticides are removed in biobeds as the result of a complex network of interacting biodegrader activities that are highly enriched with bacteria: *Pseudomonas*, *Sphingobium*, and *Oligotropha*. Sharma et al. (2017) also conducted a metatranscriptome analysis on agricultural soil that has been exposed to chemical fertilizers and pesticides for many years, estimated to be polluted with heavy metals. It was discovered that the ecosystem contains a variety of organisms, with bacteria being the most prevalent, including *Achromobacter*, *Pseudomonas*, *Bacillus*, *Sphingobium*, *Micrococcus*, *Serratia*, and *Streptomyces* species. A high abundance of aromatic dioxygenase transcripts, related to breaking down Catechol, Benzoate ring, and Gentisate was also found in the sample [134]. Through metatranscriptomic research, it has been revealed that pesticide treatment and exposure to other EOCs will alter the microbial diversity of the soil. How this alteration will affect the functionality of the microbiota is the role of metatranscriptomic analysis [135]. Reducing the abundance of *Mesorhizobium*, *Rhodopseudomonas*; and *Stenotrophomonas*, a genus known to break down xenobiotics is one of the findings of this approach in this subject [136].

Metatranscriptomic could also be a tool to comprehend the microbial communities and their capacity to degrade organic contaminants in the soil. In this way, Singh et al. (2018) looked at the metatranscriptome data of wheat rhizosphere samples. According to their analysis, a total of 118 transcripts belonging to 47 distinct enzymes associated with 21 pathways involved in the breakdown of aromatic compounds. The abundance of aromatic amines degradation-related transcripts, and those related to carbazoles, benzoates, and naphthalene degradation, the keto adipate pathway, phenols, biphenyls, and xenobiotics removal in the soil samples suggest that these substances can be

degraded effectively. 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase is a specific enzyme, frequently found in various aromatic-degrading metabolic pathways, including carbazole, biphenyl, and central meta-cleavage pathway. The taxonomic analysis showed that the predominant communities that play a role in the degradation of aromatic compounds were bacteria, particularly *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Cyanobacteria*. These findings imply that the soil's aromatic contaminants are significantly removed by microbial communities connected to crop rhizospheres [137]. While being the key players, microorganisms are not the only sectors of the bioremediation and biodegradation process, especially in the soil with intricate interactions between microorganisms and plants. Metatranscriptomics in this regard, could be used to decode these interactions that are the driving force of resistant pollution removal. In other words, co-metabolism will increase the efficiency of bioremediation in many cases. Focusing on the collaboration between plants and microorganisms using tripartite metatranscriptomics, Tartaglia et al. (2022) conducted a mesocosm metatranscriptomic study on the rhizospheric soil containing *Festuca arundinacea* roots. The occurrence of extraction, degradation, and metabolism of xenobiotics detected in contaminated soil illustrates the phytoremediation process carried out through a tripartite activity involving plants, bacteria, and fungi. The presence of *Actinobacteria* and fungi has been found to be high, which could have an effect on the success of the remediation process. Some transcripts have been linked to PAH degradation including laccase, monooxygenase, and peroxidase. These are protocatechuate 3,4-dioxygenase, protocatechuate 4,5 dioxygenase, salicylate 1-monooxygenase, naphthalene 1,2 dioxygenase, and 4,5-dihydroxyphthalate decarboxylase. The majority of transcripts associated with protocatechuate 3,4-dioxygenase and protocatechuate 4,5-dioxygenase were from *Actinobacteria*, particularly the *Nocardioides* and *Streptomyces* genera [138]. deMenezes et al. (2012) discovered a grown concentration of transcripts linked to dioxygenase, stress response, and detoxification as a result of phenanthrene exposure in soil microbial communities [139]. Willow rhizospheres cultivated in contaminated soils were notably supplemented in transcripts related to PAH degradation, mainly belonging to the orders *Actinomycetales*, *Rhodospirillales*, *Burkholderiales*, *Alteromonadales*, *Solirubrobacterales*, *Caulobacterales*, and *Rhizobiales* [140, 141]. The response of *Arthrobacter* QD15-4, isolated from plastic-contaminated soils to dimethyl phthalate (DMP), a prevalent environmental pollutant, was studied by Wang et al. (2019). This strain demonstrated

the ability to break down DMP under the effect of the expression of specific genes associated with energy metabolism and ABC transporters in this bacterium. Notably, under DMP exposure, there was a notable rise in the intermediate metabolites pyruvic acid and citrate, indicating that *Arthrobacter* QD15-4 responded to DMP by modulating its metabolic pathways and transporters [142]. The bacterial strains *Burkholderia zhejiangensis* CEIB S4-3 and *Burkholderia cenocepacia* CEIB S5-2 can break down the pesticide methyl parathion (MP) and its byproduct p-nitrophenol (PNP) due to specific genes in their genomes (*mpd* gene and *pnp* gene cluster) [49] as well as those genes involved in sensing environmental changes, responding to stress, and degrading aromatics. qRT-PCR confirmed their importance in defense against MP and PNP toxicity. Genomic data shows CEIB S5-2 has genes for efficient PNP degradation via different pathways, making it a strong candidate for pesticide removal [143]. A transcriptomic study revealed gene expression changes during MP and PNP breakdown, highlighting roles in energy production, transport, metabolism, and stress response. Transporter genes play a key role in facilitating PNP entry and counteracting its toxicity during biodegradation. Overall, these *Burkholderia* strains possess the genetic tools to degrade MP and PNP effectively, with CEIB S5-2 showing promise for pesticide remediation [144].

Proteomics and metaproteomics: identification of the active microbial communities and their EOC-biodegradation progress

The profile of proteins, enzymes, and peptides that have different levels of expression in a particular circumstance is referred to as the proteome [128]. A thorough and adequate investigation is produced by proteomics, which offers a detailed analysis of the variations in protein composition as well as how they function and interact. Networks of protein–protein interactions, gene expression, and regulation are studied in proteomics research [145–147]. Predicting microbial functional activities has become feasible, due to the rapid advances of metaproteome and metatranscriptomics (Fig. 2). A new outlook for the biodegradation of toxins is opened by the proteomics approach, which allows to identification and characterization of new proteins that participate in a variety of metabolic pathways including stress response, transportation, energy metabolism, or transcription regulation [80]. Nowadays, proteome research is used directly on the microbial community (meta-proteomics) to understand how they function within a specific ecosystem. This can be very helpful in figuring out whether a microorganism has the ability to break down any compound that is

present in the sample [148]. Metaproteomics techniques like Mass Spectrometry (MS) and Two-Dimensional Electrophoresis (2-DE) have advanced our understanding of microbial biodegradation pathways by revealing the key catabolic enzymes involved [129]. For proteomic analysis, a biological specimen must first be prepared. Then, proteins must be extracted and separated using SDS-PAGE or 2D-GE. Next, experimental data must be generated, collected, and analyzed using software for gel image analysis, such as PDQuest (BioRad) and ImageMaster 2D/Melanie. Finally, proteins must be identified and characterized using microbial protein identification technology like MALDI-TOF mass spectrometry. Flex Analysis (v. 3.3) and BioTools may be used to visualize spectra and identify tandem-MS (MS/MS) proteins, and the findings could be connected to commercial proteomic search engines like MASCOT to search for the protein-sequence databases listed under the NCBI number [147, 149].

In a groundbreaking study, Pankaj et al. (2016) pioneered a proteomic approach to investigate the resistance of *Bacillus thuringiensis* SG4 to cypermethrin, a pesticide commonly found in agricultural soil. After a five-day incubation period, the researchers conducted a comparative proteomic analysis of the bacterium with and without exposure to cypermethrin. By extracting the differential whole-cell extracellular proteome from the active bacterial isolate SG4 and analyzing it using 2D electrophoresis, they identified the cypermethrin-resistant proteins in *Bacillus thuringiensis* strain SG4. The analysis also showed that cypermethrin-resistant bacteria exhibited decreased activity in certain dehydrogenase enzymes, including those that act on formate, glycerol-3-phosphate, isocitrate kinase, and phosphatase, as well as malate and ketoglutarate semialdehyde. Furthermore, the induction of cypermethrin led to the down-regulation of the translocase subunit Sec A protein in *Bacillus thuringiensis* SG4. Prominent proteins in the resistant bacteria included NAD kinase, ATPase pumps, ATP synthase, and transferases. The majority of the altered proteins were closely linked to various cellular processes, such as stress response, cypermethrin-degrading catabolism, protein synthesis and modification, gene regulation and transcription, energy production, and chemotaxis [150].

To understand how the strain *S. paucimobilis* 20006FA breaks down phenanthrene and accumulates intermediate metabolites, Macchi et al. (2018) analyzed its genome and compared predictions to experimental proteomic analyses. To investigate the strain's reaction to the phenanthrene, the proteome of *S. paucimobilis* 20006FA was analyzed by 2D-GE. The peptides were evaluated using an ultraviolet MALDITOF/TOF. Proteomic analysis

revealed several enzymes were related to transforming phenanthrene into TCA intermediates, which were upregulated by phenanthrene. Analyzing the distinct proteins revealed that they consisted of one NahA1f (alpha subunit of naphthalene dioxygenase), catechol dioxygenase, dihydroxybiphenyl dioxygenase, glutathione S transferases, and various enzymes involved in the lower metabolic pathway such as 2-hydroxyruyconic semialdehyde hydrolase and 4-oxalocrotonate decarboxylase, among others. The abundance of dioxygenase enzyme-coding genes in the genome suggested a sizable potential for aromatic biodegradation through the salicylate and protocatechuate pathways. Additionally, this strain was able to degrade other PAHs such as anthracene, dibenzothiophene, and fluoranthene. Through genomic analysis, they identified 126 potential genes that encode enzymes involved in all steps of phenanthrene degradation, which may also be involved in the breakdown of other PAHs [151].

Bastida et al. (2016) examined the effects of oil contamination on the potential of bioremediation using compost amendment. They used fatty acid and metaproteomics analysis to investigate the biomass, evolutionary relationships, and physiological responses of the microbial community in polluted semiarid soils. The fatty-acid analysis was performed by using a Trace Ultra Thermo Scientific gas chromatograph, and Mass spectrometry was used for proteome analysis. They revealed *Proteobacteria* dominated at the phylum level among bacteria with the abundance of *Rhizobiales*, *Sphingomonadales*, and *Caulobacteriales* families, while *Rickettsiales*, *Rhodospirillales*, and *Rhodobacteriales* made up no more than 10% of the *Proteobacterial* proteins. In the contaminated soils, there was a lower concentration of *Rhizobiales* proteins. As opposed to the corresponding control microcosms, *Caulobacteriales* significantly increased in the oil-spiked treatments. After compost treatment, *Sphingomonadale* abundance increased, and *Actinobacteria* made up to 17% of the identified bacterial proteins of the microbial community in addition to *Proteobacteria*. Oil pollution caused a decline in *Actinobacterial* proteins and regardless of the various treatments, *Burkholderiales* made up the majority of the identified *Proteobacterial* proteins, accounting for about 95%. *Pseudomonadales* and *Enterobacteriales* were the sources of up to 84% of the *Proteobacterial* proteins. *Actinomycetales*, which accounted for nearly all of the *Actinobacterial* proteins and increased in abundance in oil-spiked treatments and compost addition, dominated the *Actinobacteria* phylum. The addition of compost resulted in a significant reduction in PAHs and alkane concentrations that was primarily carried out by *Sphingomonadales* and uncultured bacteria, as their

catabolic enzymes' abundance such as catechol and cis-dihydrodiol dehydrogenases showed [152].

Williams et al. (2010) investigated the microbial community proteome in toluene-amended soil and its microbial inoculation cultures. They used a Proteomics Analyzer MALDI-TOF/TOF mass spectrometer for protein analysis. The two toluene-impacted groups shared multiple identical proteins, including Tuf (elongation factor Tu), glutamine synthetase, amino acid transporters, extracellular solute-binding proteins, outer membrane proteins, and cell surface-associated proteins like arginine deiminase (ArcA) and ornithine carbamoyltransferase (ArcB). The microbial communities in toluene-affected soil and cultures, but not in those affected by glucose, contained: GroEL (chaperonin), TolC (outer membrane protein), CspA (cold-shock protein), ArcA (arginine deiminase), SucC (succinyl-CoA synthetase), OmpF (outer membrane protein F), succinate dehydrogenase, ABC transporters, glutamate synthetase (Gln), extracellular solute-binding proteins, and outer membrane proteins (Omp). This suggests that these proteins may play a role in toluene removal. CspA and ArcA were present in toluene-amended cultures, while SodB and GroEL were found in microbial protein from toluene-amended soil. 16S rRNA gene analysis of the bacterial communities in toluene-added soil revealed a significant degree of dominance, with members of the *Bacillus* species accounting for 80% of the OTUs. Additionally, the toluene enrichment experiment identified ArcA and CspA, which showed a considerable increase in cultures of *Pseudomonas putida* DOT-T1E grown in the presence of toluene [153].

Metabolomics: identifying the metabolites during the biodegradation of EOCs

Metabolomics describes the response of microbial communities to particular biological factors, abiotic pressures, and their environment at a given moment. Production of a variety of metabolites or metabolomes in contact with natural environment stimulation is an important part of the study of metabolomics [154, 155]. The development of models that can be used for the prediction of microbiological activity in bioremediation strategies has been facilitated by these approaches [79] (Fig. 2). Researchers have conducted studies to investigate the metabolites produced during the biodegradation of pollutants via two main strategies: global untargeted metabolomics and targeted metabolomics. The first approach is preferred when there is no prior information available to identify the metabolites and generates large amounts of data that can be compared between samples. Using this approach, Keum et al., (2008) analyzed the

comparative intracellular metabolome of *Sinorhizobium* sp., including fatty acids, polyhydroxyalkanoates, and polar metabolites during phenanthrene degradation. Their study revealed an increase in the fatty acids profile, TCA and glycolysis intermediates, and accumulation of trehalose as the product of the phenanthrene breakdown in the cell. A few amino acids, such as glycine, homoserine, and valine, also exhibited a rise during the metabolism of phenanthrene. While presence of sulfur amino acids and nicotinic acid showed possible oxidative stress conditions during phenanthrene metabolism [21, 156]. The second metabolomics approach is called targeted metabolomics which is used to identify specific metabolites or metabolic pathways from known databases. The workflow for metabolomics involves selecting a biological sample, extracting and purifying metabolites through chromatography, and then analyzing the data using mass spectrometry or NMR spectroscopy to identify metabolites by comparing them with various databases and libraries [79, 157]. HPLC, GC-MS, and NMR spectroscopy were used by Moody et al. to investigate the metabolism of *Mycobacterium vanbaalenii* strain PYR-1, which degrades benzantracene. It has been demonstrated that benzantracene breaks down to generate dihydroxylated and methoxylated intermediates, which are then directed into the main carbon pathway [128, 158]. A frequently used pesticide is carbofuran. The internal and extracellular metabolites of the *Chryseobacterium* sp. BSC2-3 strain, which was chosen from a soil sample, was examined using an LC-MS-based metabolomics approach by Park et al. (2022). The BSC2-3 strain demonstrated the ability to convert carbofuran into 3-hydroxycarbofuran externally. Through intracellular metabolite analysis, it was observed that carbofuran primarily impacted the breakdown of aminobenzoate, the synthesis of ubiquinone and terpenoid-quinone, as well as the metabolism of arginine and proline. Furthermore, the strain was found to produce compounds that induce disease resistance and regulate plant growth. Moreover, the study identified the genes responsible for producing indole-3-acetic acid, a potent auxin [159]. In 2018, Tian et al. used stable isotope-assisted metabolomics (SIAM) to analyze soil contaminated with ¹³C-labeled fluoranthene, pyrene, and benzoanthracene. They identified metabolites and pathways, detected ring-cleavage products, and found sulfate conjugates of dihydroxy compounds as major metabolites, suggesting that fungi may contribute to the biotransformation of pyrene and benzoanthracene in soil [160].

Fluxomics is a method used to measure and analyze the rates at which metabolic reactions occur and the changes

in these rates within a living organism. It involves studying the entire set of metabolic fluxes within a cell, which provides valuable information about various cellular processes. This collection of metabolic fluxes, known as fluxomics, is considered a unique characteristic of the cell. By comparing labeling patterns obtained through appropriate labeling distribution, fluxomics can effectively determine and describe the distribution of metabolic reactions within a cell [20, 21] and aims at capturing the dynamic nature of phenotypes and functional interactions between the genome and the environment [96, 161]. Being based on metabolite data, which is far less abundant than that from proteins and genes, fluxomics offers numerous benefits over proteomics and genomics [162, 163]. However, to the best of our knowledge and according to our searches, there is no report on using this approach in the case of biodegrading potential in the soil.

Multiomics analysis to unlocking the mechanistic biodegradation of EOCs

Thermophilic bacteria have shown great potential in remediating various pollutants, including azo dyes, DBG, and imidacloprid. Researchers have employed omics approaches, such as proteomics, metabolomics, and genomics, to gain insights into the biodegradation mechanisms employed by these microorganisms. Zhang et al. (2022) isolated a thermophilic bacterial strain, *Anoxybacillus* sp. PDR2, from soil to address azo dye effluent remediation. Through proteomic and metabolomic analyses, they identified crucial transport mechanisms, such as ABC transporters and two-component systems, involved in the stress response. The bacterium was found to self-synthesize a redox mediator, riboflavin, essential for the biodegradation process. *Anoxybacillus* sp. PDR2 utilized glucose as an energy source, employing the TCA cycle and pyruvate metabolism to generate energy in vivo, transferring NAD to the electron transport chain, and ultimately facilitating degradation. Overexpression of acetoacetate synthase and malate synthase G during biodegradation, as indicated by proteomic data, mediated energy supply [164]. An et al. (2020) investigated a thermophilic microbiota's ability to break down DBG using metagenomic sequencing and qRT-PCR to understand gene actions. Through quantitative metaproteomics, they identified specific DBG-degrading associated proteins, such as NADH ubiquinone reductase and NADH-quinone oxidoreductase subunit, linked to the TCA cycle and glycolysis pathways. These proteins produced reducing equivalents crucial for breaking down DBG. Their analysis also highlighted the role of FAD/NAD (P)-binding protein in the biodegradation process [165]. These studies demonstrate the power of omics approaches in unraveling the biodegradation mechanisms employed by

thermophilic bacteria. By identifying key enzymes, transport systems, and metabolic pathways involved in pollutant degradation, researchers can develop more efficient bioremediation strategies and optimize the use of these microorganisms in environmental remediation efforts.

Using a multiomics approach, Gautam and colleagues (2023) explored the imidacloprid biodegradation potential of *Agrobacterium* sp. InxBP2 through whole genome sequencing. Enzymes like FAD-dependent monooxygenase, amidohydrolase family protein, and ABC transporters were identified, showing similarity to known imidacloprid-degrading enzymes. Proteomics analysis revealed distinct metabolic processes in imidacloprid-treated samples compared to controls, a finding supported by metabolomics (GC-MS analysis) [166].

Conclusions and future perspectives

To successfully remove persistent pollutants like EOCs through biodegradation or bioremediation in their natural environments, it is crucial to understand and recognize the composition, structure, unrealized potential, and interactions between microbial communities and other inhabitants of the surrounding environment. In order to gain a deeper understanding of microbial populations, high-throughput metagenomics and metatranscriptomics would be preferable to traditional sequencing-based methods. However, these innovative methods and approaches are unable to properly address the range of microbes and pathways involved in bioremediation. This might be addressed by employing metaproteomics and metabolomics approaches to precisely identify the enzymes and metabolites present in contaminated areas. The fluxomics approach can also help determine the most significant and effective compounds produced during bioremediation by evaluating the flow and quantity of metabolites under certain conditions. As technology advances in sequencing, chromatography, and mass spectrometry, it is expected that identifying the biodegradation pathways in microorganisms become easier. Despite the developing advancement of omics approaches that led biological science to the "omics age", revolutionized investigation of microbial diversity and their undiscovered bioremediation abilities, uncovering the mysterious properties of this complicated and puissant network requires more multiapproach research to clarify the potential of microbial consortia. Integrated omics allows for the correlation of genome contents with proteins, enzymes, and final metabolites produced during bioremediation in microorganisms making it a standard method for analyzing microbial consortia. This insight enables metaomics to discover all biotechnologically relevant features within a natural consortium. Although omics approaches have significantly advanced

our understanding of microbial communities in bioremediation, there are still limitations and potential risks that need to be carefully considered and addressed for successful field-scale implementation. Although omics approaches give extensive insights into microbial communities, there are still gaps in our knowledge of their complex interactions and dynamics in contaminated locations. On the other hand, the analysis and interpretation of huge data sets generated by omics techniques can be complex, demanding specialized bioinformatics tools and knowledge. So, it might be challenging to anticipate unexpected ecological impacts when manipulating microbial populations using omics-guided bioremediation. Furthermore, bioremediation-based techniques may not always result in total pollutant removal, and leftover contaminants might still pose environmental and health concerns. Another gap is overcoming barriers to scaling up omics-based bioremediation technologies from the lab to the field. Soil heterogeneity, fluctuating environmental conditions, and complex pollutant mixes may restrict the direct implementation of lab-based omics findings. Furthermore, it is necessary to integrate omics data with other monitoring and modeling approaches in order to gain a broader view of bioremediation systems. Combining omics data with geochemical data and engineering concepts can result in better-informed soil remediation decisions. Creating numerical models and simulating omics data using sophisticated algorithms to improve the prediction of contamination reduction and microbial metabolism in polluted environments, as well as creating meaningful databases from the massive amount of omics data generated, in order to facilitate knowledge extraction and application in bioremediation may solve some of these limitations.

Abbreviations

TNT	2,4,6-Trinitrotoluene
2-ADNT	2-Amino-4,6-Dinitrotoluene
4MBC	3-(4-Methylbenzylidene) camphor
4-ADNT	4-Amino-2,6-Dinitrotoluene
AOPs	Advanced oxidation processes
AlkB	Akane monooxygenase
ArcA	Arginine deiminase
ARISA	Automated ribosomal intergenic spacer analysis
BPA	Bisphenol A
CAL	<i>Candida antarctica</i>
CRL	<i>Candida rugosa</i>
CBZ	Carbamazepine
carE	Carboxylesterase gene
CNS	Central nervous system
CP	Chlorpyrifos
COPD	Chronic obstructive pulmonary disease
Csp	Cold-shock protein
DEHP	Di(2-ethylhexyl) phthalate
DDT	Dichlorodiphenyltrichloroethane
DEPs	Differentially expressed proteins
DMs	Differently metabolites
DMP	Dimethyl phthalate
DBG	Direct black G

ECs	Emerging contaminants
EOCs	Emerging organic contaminants
EDCs	Endocrine-disrupting compounds
EHMC	Ethylhexyl methoxycinnamate
Gln	Glutamate synthetase
GOX	Glyphosate oxidase
HCH	Hexachlorocyclohexane
LiP	Lignin peroxidase
MnP	Manganese peroxidase
MS	Mass spectrometry
MALDI-TOF	Matrix-assisted laser desorption ionization time of flight
MBRs	Membrane bioreactors
MP	Methyl parathion
OC	Octadecene
OCPs	Organochlorine pesticides
OP	Organophosphate
OTs	Organotins
ArcB	Ornithine carbamoyltransferase
OPP	Ortho-phenylphenol
Omp	Outer membrane proteins
PacBio	Pacific biosciences
PFCs	Perfluorochemicals
PFOS	Perfluorooctanesulfonate
PFOA	Perfluorooctanoic acid
PCPs	Personal care products
PhACs	Pharmaceuticals
PAEs	Phthalate acid esters
PNP	P-nitrophenol
BPAPC	Poly (bisphenol-A carbonate)
PLA	Poly (lactic acid)
PCL	Poly(ε-caprolactone)
PBDEs	Polybrominated diphenyl ethers
PBS	Polybutylene succinate
PBSA	Polybutylene succinate-co-adipate
PCAs	Polychlorinated alkanes
PCBs	Polychlorinated biphenyls
PCBs	Polychlorinated biphenyls
PCNs	Polychlorinated naphthalenes
PAH	Polycyclic aromatic hydrocarbon
PDMs	Polydimethylsiloxanes
QACs	Quaternary ammonium compounds
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SIAM	Stable isotope-assisted metabolomics
SEs	Steroid estrogens
SodB	Superoxide dismutase
TCA	Tricarboxylic acid cycle
TCC	Triclocarban
TCS	Triclosan
2-DE	Two-dimensional electrophoresis

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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