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Engineered nanomaterials and the microbiome: assessing disruptions in environmental and human microbial communities

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The rapid advancement and integration of engineered nanomaterials (ENMs) into consumer products, industrial processes, biomedical applications, and environmental technologies have revolutionized multiple sectors. However, their increased production and environmental release raise critical concerns about unintended interactions with microbial ecosystems. ENMs, including metal-based nanoparticles (silver, titanium dioxide, zinc oxide) and carbon nanomaterials (graphene, carbon nanotubes), possess unique physicochemical properties such as high surface area-to-volume ratios, tunable reactivity, and antimicrobial potential that allow them to interact directly with microbial cells or indirectly influence their habitats. This review critically examines the emerging evidence on ENM–microbiome interactions across human, aquatic, terrestrial, and agricultural systems. In human-associated microbiomes, especially the gut, ENMs can induce dysbiosis by disrupting microbial diversity, altering metabolite production (e.g., short-chain fatty acids), and impairing gut barrier integrity, contributing to inflammation and metabolic disorders. In environmental settings, ENMs influence key microbial functions like nitrogen fixation, organic matter decomposition, and biogeochemical cycling, potentially undermining ecosystem stability and agricultural productivity. Moreover, ENMs are increasingly implicated in accelerating antimicrobial resistance by promoting horizontal gene transfer and enriching resistance genes in microbial communities. The review highlights methodological advances such as high-throughput sequencing, meta-omics approaches, *in vitro* colon simulators, and *in vivo* models that have enhanced the assessment of ENM-induced microbiome alterations. Despite these advances, significant gaps remain in understanding long-term and low-dose effects, dose–response relationships, and ecological thresholds. Addressing these gaps through multidisciplinary research and regulatory frameworks is essential for ensuring the safe and sustainable deployment of nanotechnologies in a microbiome-sensitive world.

KEYWORDS

engineered nanomaterials, microbiome, dysbiosis, antimicrobial resistance, ecosystem health, meta-omics technologies

1 Introduction

The emergence and widespread integration of engineered nanomaterials (ENMs) into consumer products, industrial processes, environmental technologies, and biomedical applications has revolutionized multiple sectors of modern life (Bora et al., 2022; Rocco, 2025). These nanoscale materials, typically ranging from 1 to 100 nm in size, exhibit novel physicochemical properties such as high surface area-to-volume ratio, tunable reactivity, and enhanced mechanical or optical behavior (Kumar A. Y. N. et al., 2024). Common ENMs include metal-based nanoparticles (NPs), such as silver nanoparticles (AgNPs), titanium dioxide nanoparticles (TiO_2 NPs), zinc oxide nanoparticles (ZnO NPs), carbon-based nanomaterials (CNMs) like graphene, carbon nanotubes (CNTs), and composite nanostructures (Fritea et al., 2021). While these innovations have brought significant technological and economic benefits, their increasing production and environmental release have raised growing concerns about potential biological and ecological risks, especially concerning their interactions with microbial communities (Rajpal et al., 2025). Microbial ecosystems spanning human-associated microbiomes to those found in aquatic, terrestrial, and agricultural environments play fundamental roles in maintaining physiological and ecological equilibrium (Ma L. C. et al., 2023). In the human body, particularly within the gastrointestinal tract, the gut microbiota contributes to digestion, immune regulation, synthesis of essential vitamins and short-chain fatty acids (SCFAs), and defense against pathogens (See et al., 2025). Similarly, in natural environments, microbial communities regulate biogeochemical cycles, decompose organic matter, support plant growth through symbiosis, and influence soil and water quality. Any disturbance in microbial diversity, abundance, or metabolic function, termed as dysbiosis, can have cascading effects on host health and ecosystem resilience (Prasad et al., 2024; Zaman et al., 2025).

ENMs are increasingly recognized as potential disruptors of microbial homeostasis. When released into the environment through waste streams, or ingested or inhaled by humans and animals, ENMs can interact directly with microbial cells or indirectly influence their surrounding microenvironments (Ray et al., 2009; Kumar et al., 2022). Their mechanisms of action include generation or induction of reactive oxygen species (ROS), disruption of membrane integrity, interference with DNA and protein function, and modulation of microbial metabolic and signaling pathways (Kumar R. et al., 2024). These effects can lead to reduced microbial diversity, shifts in community composition, alterations in metabolite production, and perturbation of key functional processes such as nutrient cycling, energy metabolism, and host–microbe communication (Wang et al., 2017). In human systems, such alterations have been linked to inflammatory bowel diseases, metabolic disorders, immune dysregulation, and neurobehavioral conditions (Xuan et al., 2023). In environmental settings, ENMs can affect microbial-driven processes like nitrogen fixation, denitrification, and organic matter decomposition, ultimately threatening soil fertility, water quality, and ecosystem services (Hochella et al., 2019; Huang et al., 2024).

Furthermore, ENMs may play a role in accelerating antimicrobial resistance (AMR) by enhancing horizontal gene transfer, altering microbial stress responses, and promoting the persistence of resistance genes in various environmental matrices (Wang X. et al., 2024; Piergiacomo et al., 2022). This poses a dual risk: while ENMs are often employed for their antimicrobial properties, their indiscriminate or chronic use could inadvertently select for resistant strains, complicating both clinical and ecological health management (Alav and Buckner, 2024).

Given the breadth of these interactions, the current review aims to provide a comprehensive overview of the current understanding of ENM–microbiome dynamics, the effects of various NMs on the human gut microbiota, aquatic and soil microbial ecosystems, and the potential for ENMs to influence microbial resistance development. It also evaluates the methodologies used to study these effects, including high-throughput sequencing, omics technologies, and *in vitro*/*in vivo* models, while highlighting regulatory and scientific challenges in assessing long-term risks. Finally, key knowledge gaps are identified and future directions are proposed for ensuring that the deployment of nanotechnologies is conducted safely and sustainably in a microbiome-sensitive world.

2 ENMs as anthropogenic sources of environmental and human microbiome perturbation

ENMs, especially AgNPs, ZnO NPs, TiO_2 NPs, etc., are now ubiquitous across consumer, medical, agricultural, and industrial applications—ranging from antimicrobial coatings and textiles to water treatment, pesticides, cosmetics, and electronics (Figure 1). AgNPs are incorporated into food packaging materials for their antimicrobial properties, helping to extend shelf life and reduce spoilage (Martirosyan and Schneider, 2014). Titanium dioxide and silica (e.g., TiO_2, SiO_2 NPs) are common food additives or colourants (for example, TiO_2 as food colourant E171) or anticaking agents in powdered products (Villamayor et al., 2023). ENMs are also used in nano-encapsulation of bioactive compounds, improving bioavailability of vitamins or nutraceuticals, and in smart packaging systems (with sensors, diffusion barriers, or controlled antimicrobial release) (Martirosyan and Schneider, 2014). ENMs are widely used in cosmetic formulations to improve efficacy, texture, appearance, and UV protection. Common nanomaterials include nano-TiO_2 and nano-zinc oxide, which serve as UV filters in sunscreens because, at the nanoscale, they offer strong UV protection while remaining transparent on skin (Pastrana et al., 2018; Catalano et al., 2021). Other ENMs include nano-silica, used to improve spreadability, reduce greasiness, act as anti-caking agents in powders and lipsticks, and enhance pigment dispersion (Ferreira et al., 2023). ENMs are increasingly integrated into drugs and medical products due to their unique physicochemical properties, such as high surface area, tunable size, and surface functionality. In drug and vaccine delivery, ENMs like liposomes, polymeric nanoparticles, and metal-based nanoparticles enhance bioavailability, facilitate targeted drug delivery and sustained release to specific tissues, and reduce systemic side effects. Applications of ENMs in medical devices, diagnostic imaging, wound dressings, antimicrobial textiles/implants, etc., are also increasing exponentially (Cai et al., 2023; Kurul et al., 2025); ENMs are increasingly applied in agriculture to enhance nutrient

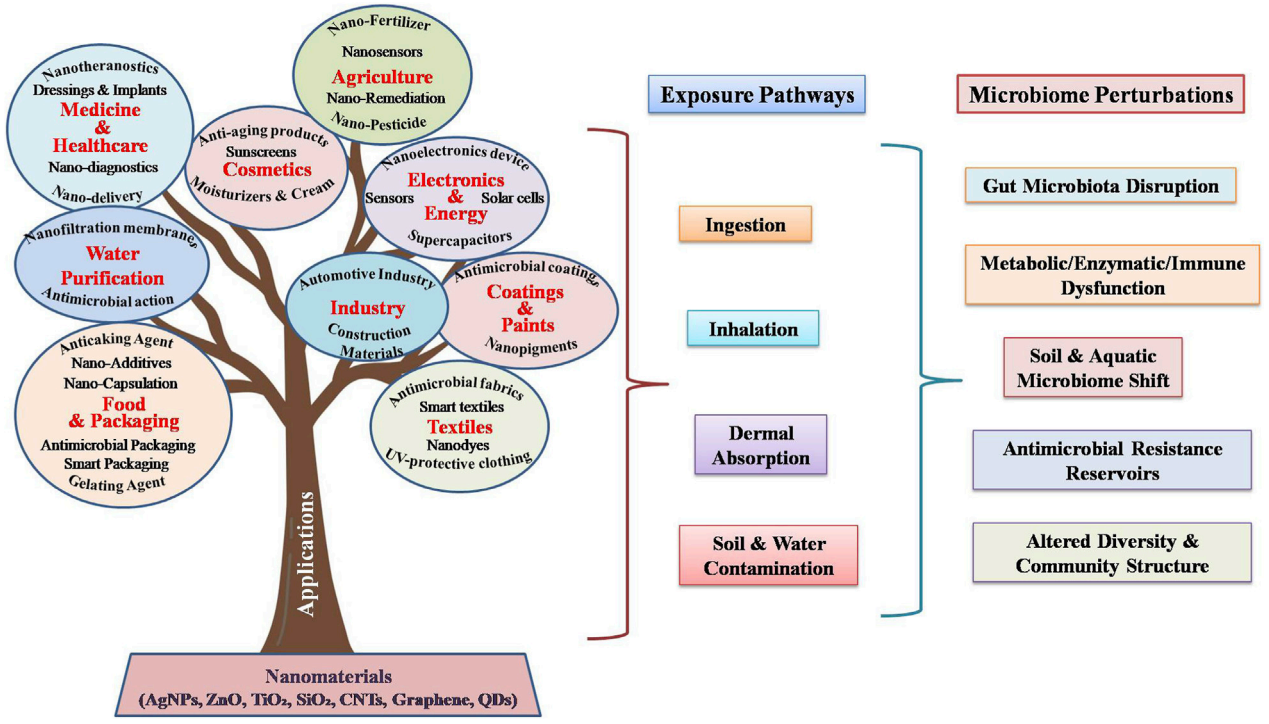

FIGURE 1
Applications of engineered nanomaterials (ENMs), exposure pathways, and associated microbiome perturbations. The schematic tree illustrates the diverse applications of ENMs across major sectors including medicine and healthcare, water purification, food and packaging, agriculture, electronics and energy, industry, textiles, and coatings and paints. Exposure through ingestion, inhalation, dermal absorption, or soil/water contamination can disrupt human and environmental microbiomes, causing functional, compositional, and resistance-related shifts.

efficiency, promote plant growth, and control pests. Nano-fertilizers using metal or oxide NPs (e.g., ZnO, Fe_3O_4) and carrier platforms (e.g., nanoclay, chitosan) help minimize nutrient leaching and increase uptake (N, P, K) in crops (Yin et al., 2018). Nanopesticides formulated with ENMs offer targeted delivery, lower chemical doses, and longer persistence against pathogens (Adisa et al., 2019). Beyond these applications, ENMs find widespread use in general industrial sectors such as coatings, electronics, energy, and construction. TiO_2 NPs are used in self-cleaning and UV-resistant coatings, paints, and solar panel coatings to improve durability and reduce maintenance (Moloi et al., 2021; Hussein, 2023). Graphene, CNTs, and hybrid nanocomposites enhance electrical and thermal properties in devices, lightweight materials, and structural composites (Cataldi et al., 2020). Silver, zinc oxide, and silicon dioxide ENMs are used in textiles, antimicrobial surfaces, filtration systems, and sensors, due to their unique optical, catalytic or antimicrobial functionalities (Moloi et al., 2021; Singh et al., 2023).

The ongoing and widespread use of ENMs brings them into frequent contact with environmental and human microbial communities, representing emerging anthropogenic sources of microbial disruption. ENMs in food (additives, packaging, nano-encapsulation), drugs or cosmetics are ingested or absorbed, while agricultural nano-fertilizers and nanopesticides enter soil and water, exposing soil and gut microbiota (Kah, 2015; Khan et al., 2021; Yadav et al., 2023). Improperly managed industrial nano-effluents serve as significant sources of toxicants to the environment and the food chain causing microbiome perturbations (Naz et al., 2024). These perturbations are dose-dependent, often manifest first in metabolic or enzymatic function and substrate utilization before major shifts in diversity or taxonomy (Avila-Arias et al., 2023). Given the environmental persistence and bioaccumulation potential of many ENMs, understanding realistic exposure levels (in water, soil, diet), their transformation (coating, aggregation, ion release), and their functional effects on microbial communities is critical for risk assessment and regulatory oversight.

3 ENMs and human microbiome

The human gut microbiota is a complex community of trillions of microorganisms, primarily bacteria that live in the gastrointestinal tract, especially the colon. These microbes play a crucial role in maintaining human health by aiding in digestion, synthesizing essential vitamins, regulating the immune system, and protecting against harmful pathogens. The composition of the gut microbiota is influenced by various factors, including diet, antibiotics, age, and lifestyle. A balanced microbiota supports overall health, while disruptions, known as dysbiosis, have been linked to numerous conditions such as inflammatory bowel disease, obesity, diabetes, and mental health disorders (Thursby and Juge, 2017; Hou et al., 2022). ENMs, such as Ag NPs, TiO_2 NPs, ZnO NPs, and CNMs are increasingly used in food additives, packaging, pharmaceuticals, and cosmetics. When ingested, either directly

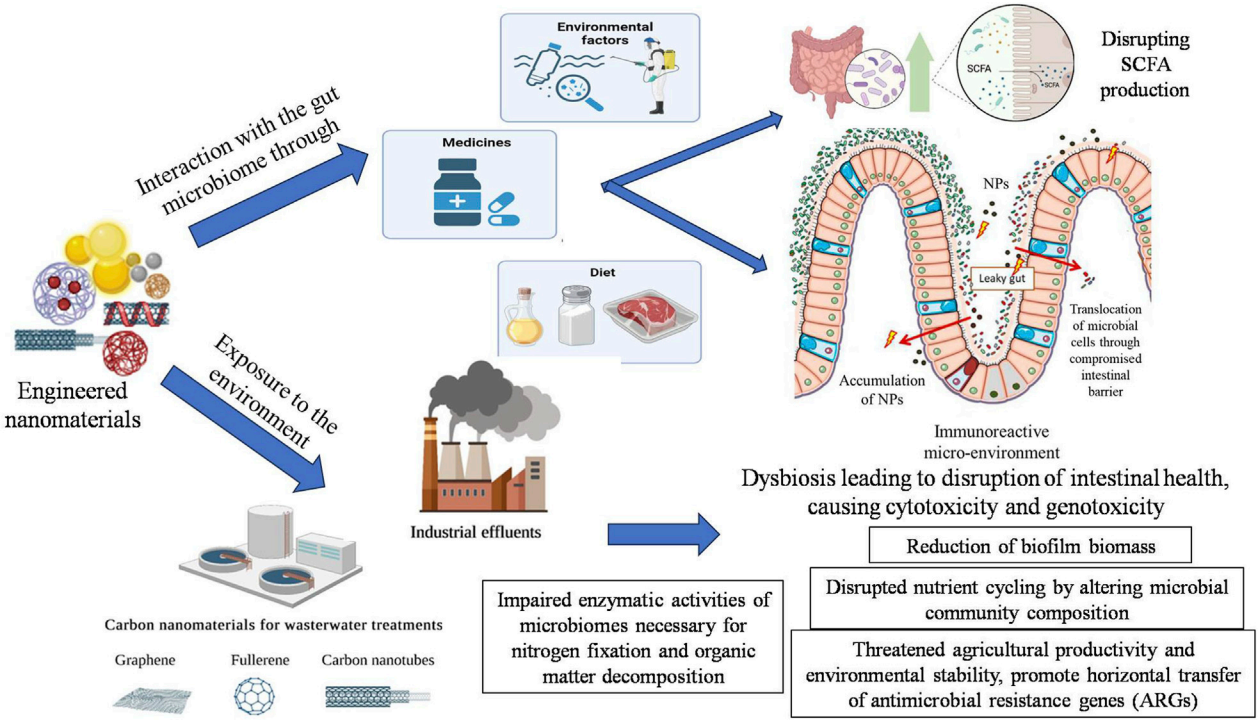

FIGURE 2
Effects of engineered nanomaterials (ENMs) on human gut microbiota and environmental microbial communities. This schematic illustrates the multifaceted impacts of ENMs, including metal-based nanoparticles and carbon nanomaterials, on microbial ecosystems. In the human gut, ENMs disrupt microbial diversity and composition, reducing beneficial taxa, altering short-chain fatty acid (SCFA) production, and compromising gut barrier integrity, which may lead to dysbiosis, inflammation, and metabolic disorders. In environmental systems (aquatic, soil, and agricultural), ENMs interfere with microbial-mediated biogeochemical processes such as nitrogen fixation, denitrification, and organic matter decomposition, potentially impairing nutrient cycling and ecosystem resilience.

through food or indirectly *via* environmental exposure, these NMs can interact with the gut microbiota and potentially disrupt gut health. Studies have shown that certain ENMs can alter the composition and diversity of the microbial community, leading to dysbiosis, inflammation, and impaired gut barrier function (Utembe et al., 2022). For example, Ag NPs and TiO_2 NPs have been associated with reduced beneficial bacteria (like *Lactobacillus* and *Bifidobacterium*) and increased pro-inflammatory species (Utembe et al., 2022; Ma Y. et al., 2023). Additionally, ENMs can affect microbial metabolism and the production of SCFAs, which are vital for maintaining intestinal health (Figure 2). While the long-term impacts on human health are still being investigated, emerging evidence suggests that chronic exposure to ENMs may contribute to gastrointestinal disorders, immune dysregulation, and metabolic disturbances by disrupting the delicate balance of the gut ecosystem (Table 1) (Tang et al., 2021; Wojciechowska et al., 2023).

3.1 Impact of titanium dioxide nanoparticles on gut microbiota and their mechanism of interaction

TiO_2 NPs appear to exert a limited effect on gut microbiota diversity; however, they tend to influence the overall abundance of gut bacteria more noticeably (Table 1). Notably, TiO_2 NPs have been found to affect specific bacterial groups, including *Lactobacillus*, as well as members of the *Firmicutes* and *Proteobacteria* phyla, while leaving overall microbial diversity at higher taxonomic levels relatively unaffected (Lin et al., 2014; Sohm et al., 2015; Burke et al., 2015; Zhang et al., 2022; Ma Y. et al., 2023). For instance, a total of 42 bacterial species were reported to undergo significant changes upon TiO_2 NPs exposure, suggesting a risk of dysbiosis (Zhang et al., 2022). These compositional shifts are accompanied by disruptions in key metabolic pathways, particularly those associated with oxidative phosphorylation and energy metabolism, which are critical for maintaining gut health (Figure 2). *In vitro* studies also indicated that TiO_2 NPs can inhibit the growth of beneficial bacteria like *Lactobacillus* and *Bifidobacterium*, through an exposure of diets containing 0.1% TiO_2 NPs for 90 days to 1 mg/kg TiO2 NP for 7 days respectively, further implicating them in adverse gut effects (Wu et al., 2023). Such microbial disturbances have been associated with health risks, including colitis and obesity (Gangadoo et al., 2021). Although some evidence suggests that TiO_2 NPs might enhance probiotic diversity under certain conditions, the prevailing data highlight their potential to negatively impact gut microbial communities (Kumar A. et al., 2024). Notably, these effects appear to be dose- and duration-dependent, underscoring the need for more comprehensive research to clarify the complex interactions between TiO_2 NPs and the gut microbiome.

TiO_2 NPs interact with gut microbiota primarily through mechanisms that induce dysbiosis linked to various health disorders, inflammation, immune modulation, and metabolic

TABLE 1 Effects of Engineered Nanomaterials (ENMs) on the human microbiota.

Nano-materials	Effects on microbiota	Mechanisms of action	Health implications	Key references
TiO_2 NPs	Altered specific bacterial groups, i.e., *Lactobacillus*, *Firmicutes*, *Proteobacteria*, etc., leading to dysbiosis, hindered the growth of beneficial bacteria like *Lactobacillus* and *Bifidobacterium*	*In vitro* alteration of tryptophan and arginine metabolism; *in vivo* downregulation of neuroprotective metabolites in urine; metabolic reprogramming	Gut dysbiosis; disrupted microbial-host metabolic signaling	Wu et al. (2023)
TiO_2-, Ag-, SiO_2-, iron oxides, ZnO- NPs	Exposure through food additive alters microbial composition; disrupts gut barrier; antimicrobial shifts; reduces commensals	Oxidative stress, barrier dysfunction, antimicrobial action	Colitis, obesity, immune dysfunction, metabolic shifts	Gangadoo et al. (2021)
AgNPs	Oral exposure altered gut microbiota composition: Decreases microbes in energy, amino acid, lipid metabolism	Ag accumulation; altered amino acid, purine, pyrimidine, lipid and energy metabolism	Dysbiosis- liver metabolic linkage; ~23% microbial shifts correlated with ~60% metabolite changes	Wang et al. (2022)
ZnO NPs	Alteration in microbial richness and diversity based on pre-existing health condition; alterations in plasma metabolites, indicating a complex and systemic metabolic impact; reduced SCFAs	Antibacterial activity, modulation of Nrf2 pathway, dose-dependent effects	Gut dysbiosis, immune effects, context-specific benefits	Yu et al. (2021)
CNMs (graphene, CNTs)	Affect microbial fermentation altering the production of key metabolites such as butyrate, inhibit probiotics whereas promoting the growth of opportunistic pathogens	Used as carbon source, fermentation into butyrate, oxidative stress	Gut dysbiosis, altered proliferation and differentiation of intestinal stem cells, long-term gut risks	Cui et al. (2023)
AgNPs	Airway inhalation altered airway microbiome; innate immune activation	Transcriptomic profiling showed upregulated innate immune pathways	Potential airway inflammation, dysbiosis-linked immune modulation	Zickgraf et al. (2023)
Carbon black + ozone	Amplified bacterial load in the gut, i.e., *Firmicutes*, *Bacteroidetes* and *Lactobacillus* along with decreased *Clostridiaceae*	Oxidative stress and immune–microbiome interaction	Gut dysbiosis, shifts in gut-lung axis balance	Zickgraf et al. (2023)
Ag-, SiO_2-, Ti-, TiO_2- NPs	Alteration in mollicutes, reduction in *Proteobacteria*, *Deltaproteobacteria*, and *Desulfovibrionales*; decreased the relative abundance of Eggerthellaceae family in gut microbiota	Downregulation of inflammatory cytokines in jejunum; modulation of cytokine and alarmin expressionin organoids	Micro-inflammation; increased susceptibility to dextran sodium sulfate-induced colitis; potential aggravation of inflammatory bowel disease (IBD)and immune-mediated disorders	Guilloteau et al. (2022)
AgNPs and silver nanowires	Inhibiting the proliferation of Gram-negative bacteriaand lessening the gut microbiotadiversity in mice after short-term (14 days) exposure	Enhanced 1H-indole-3-carboxylic acid and elevated levels of 5-HT in the gut and blood, oxidative stress, DNA damage, lipid peroxidation	Persistent metabolic reprogramming despite microbial recovery; potential risks for gut–brain axis modulation, dysbiosis, irritable bowel syndrome, inflammatory bowel disease, liver metabolic disruption	Wang X. et al. (2023)
ZnO NPs (25–100 mg/kg in hens, 9 weeks)	Dose-dependent reduction in microbiota richness; altered community structure (*Bacilli, Fusobacteria, Proteobacteria*); reduced *Lactobacillus* abundance	Disruption of gut microbial community balance; altered metabolism of glucose, amino acids; choline, lactate, methionine positively correlated with richness	Potential dysbiosis at higher doses; disturbance in nutrient metabolism, such as glucose, amino acids; links between reduced bacterial richness and metabolic imbalance	Feng et al. (2017)
TiO_2 NPs	Cytotoxicity, developmental alterations; immune response modulation in humans, mice, zebrafish, nematodes, plants	Changes in gene expression linked to stress, immune pathways, development; effects depend on intrinsic and transformed particle properties	Potential risks to gut and immune health; disruption of developmental and metabolic pathways	Wang S. et al. (2022)
ZnO NPs	Cytotoxicity and immune modulation - Developmental changes in model organisms	Transcriptomic signatures of oxidative stress, apoptosis, inflammation - Influenced by particle surface chemistry and transformations	Risks of cytotoxicity and immune dysregulation in humans - Possible contribution to inflammatory conditions	Wang S. et al. (2022)

(Continued on following page)

TABLE 1 (*Continued*) Effects of Engineered Nanomaterials (ENMs) on the human microbiota.

Nano-materials	Effects on microbiota	Mechanisms of action	Health implications	Key references
Nano-ZnO, 0.1–2.5 mg/L, *in vitro* colon simulator	Dose-dependent downregulation of microbiota diversity; altered community composition; reduction in SCFA production; shifts in functional metabolic pathways; alteration in antibiotic resistance genes (ARGs)	Direct toxicity to gut bacteria, altering growth and functional pathways; disruption of fermentation processes lowering SCFA levels; modulation of gut resistome through selective bacterial inhibition or enrichment	Reduced SCFA production may impair gut barrier and metabolic health; enrichment of ARGs at low dose raises risk of antimicrobial resistance; indicates potential long-term risks for gut health	Zhang et al. (2021)

pathway disruption (Figure 2). Studies have shown that oral exposure to TiO_2 NPs leads to significant shifts in microbial composition; for instance, decreasing beneficial bacteria like *Veillonella* while increasing genera such as *Lactobacillus gasseri*, *Turicibacter*, *Lachnospiraceae* NK4A136 group, etc., thereby impairing normal gut function and metabolism (Chen et al., 2019; Yan et al., 2020; Rinninella et al., 2021; Bianchi et al., 2024). These NPs also induce ROS within the gut, causing oxidative stress and inflammation that facilitate lipopolysaccharide (LPS) leakage from Gram-negative bacteria, further exacerbating gut inflammation-a process implicated in chronic metabolic diseases like obesity and diabetes. Additionally, TiO_2 NPs may directly engage with intestinal immune cells, altering the microbiota–immune system axis and perpetuating inflammatory responses (Lamas et al., 2023). Metabolically, TiO_2 NP exposure disrupts critical bacterial pathways including energy metabolism, detoxification, amino acid metabolism and SCFA production, which are vital for maintaining gut barrier integrity and regulating host energy homeostasis (Utembe et al., 2022; Wu et al., 2023; Pinget et al., 2019; Yang et al., 2022). Liquid chromatography-mass spectrometry (LC-MS/MS)-based untargeted metabolomics analysis demonstrated that TiO_2 NPs disrupt critical metabolic pathways in gut bacteria, particularly tryptophan and arginine metabolism, which are essential for maintaining gut and host health. *In vivo* studies showed that mice fed a diet containing TiO_2 NPs (0.1 wt% for 8 weeks) exhibited significant changes in urinary metabolites, with notable alterations in the tryptophan metabolism pathway. Furthermore, different neuroprotective metabolites such as tryptamine, N-Methyltryptamine, 6-Hydroxymelatonin, and N-Acetylserotonin, were significantly decreased in both bacterial cultures and the urine of treated mice (Wu et al., 2023). These interlinked mechanisms, i.e., microbial imbalance, oxidative-inflammation signaling, immune dysregulation, and metabolic impairment highlight how TiO_2 NPs can detrimentally reshape the gut ecosystem and contribute to metabolic health disturbances.

The interactions between NPs and gastrointestinal microbiota are governed by multiple parameters, including the surface charge and physicochemical properties of both NPs and bacterial cells, the electrostatic interactions with digested food matrices, the chemical composition and bioactivity of dietary components, as well as dynamic physicochemical conditions within the gastrointestinal tract, such as pH gradients, enzymatic activity, ionic strength, and the presence of bile salts and other metabolites (Siemer et al., 2018; Baranowska-Wójcik, 2021). Analysis of the impact of engineered NPs on both commensal and pathogenic microorganisms in a gastrointestinal study showed that food-grade and model NPs readily form stable complexes with both probiotic and pathogenic bacteria under simulated gastrointestinal conditions. NP size emerged as the key determinant of NP–bacteria interactions, with smaller negatively charged NPs binding more efficiently to bacterial surfaces than larger positively charged ones, regardless of charge similarity. Additionally, low gastric pH enhanced complexation, while polymer coatings on NPs inhibited binding *via* steric repulsion, highlighting factors influencing NP–microbe interactions in the GI tract (Siemer et al., 2018). Toxicity analysis of five TiO_2 NPs (10–50 nm) with different crystal phases using *Escherichia coli* revealed that antibacterial activity decreased with larger particle size and higher rutile content. Smaller anatase TiO_2 NPs induced greater ROS production, membrane damage, and internalization, while higher pH and ionic strength reduced their antibacterial efficacy (Lin et al., 2014). Further, it has been reported that oral TiO_2 NPs aggravated acute colitis by activating the NLRP3 inflammasome, leading to IL-1β and IL-18 release, ROS generation, and increased intestinal permeability. TiO_2 crystals accumulated in the spleen of treated mice, and elevated titanium levels were detected in UC patients with active disease (Ruiz et al., 2017). These findings suggest potential harm of TiO_2 NPs in individuals with compromised gut barrier and pre-existing inflammation, like IBD.

3.2 AgNPs and gut microbiota: exploring their impact and interaction mechanisms

The impacts of AgNPs on the human gut microbiota are complex and multifaceted, involving direct antimicrobial activity, disruption of microbial community structure, modulation of host metabolism, and impairment of gut barrier integrity (Table 1). AgNPs exert direct antimicrobial effects by interacting with bacterial cells, where they damage cell membranes, interfere with metabolic pathways, and inhibit DNA replication, ultimately leading to bacterial death (Bruna et al., 2021; Rodrigues et al., 2024). A key mechanism underlying these effects is the induction of oxidative stress within gut bacteria, characterized by increased nitric oxide production, lipid peroxidation, and DNA damage, all of which can destabilize the gut microbial ecosystem and promote dysbiosis (Li et al., 2019; Adeyemi et al., 2020; Manuja et al., 2021).

Such antimicrobial actions lead to significant alterations in the structural composition of the gut microbiota, with notable shifts in community diversity and abundance (Figure 2). AgNPs have been shown to selectively inhibit the growth of certain Gram-negative bacteria, reducing the prevalence of beneficial taxa like *Bacteroides* while favoring opportunistic and potentially pathogenic groups such

as *Enterococcus* (More et al., 2023; Xie et al., 2023). These compositional changes result in a measurable loss of microbial diversity, particularly after short-term AgNP exposure (Bi et al., 2020; Wang X. et al., 2023). This loss of diversity has been directly associated with dysbiosis and adverse health outcomes, including gastrointestinal disorders (Ghebretatios et al., 2021). Interestingly, some studies suggest that microbial diversity may partially recover over extended exposure periods; however, metabolic perturbations often persist, indicating enduring effects on host physiology (Wang X. et al., 2023). Structural alterations are particularly prominent among microorganisms involved in energy, amino acid, and lipid metabolism (Wang, X. et al., 2022). Notably, in studies where mice received oral gavage of AgNPs once daily for 120 consecutive days, there were significant alterations in gut microbial functions associated with amino acid, purine, pyrimidine, lipid, and energy metabolism, as well as downstream effects on liver metabolic pathways (Wang, X. et al., 2022). Further, AgNPs significantly impact bacterial adhesion to the intestinal epithelium, a critical step in colonization and biofilm development. AgNPs exert a dual role in adhesion dynamics—primarily inhibiting bacterial attachment and biofilm formation, yet potentially triggering compensatory virulent behaviors under certain conditions. These adhesion-disrupting effects are crucial for their application in gut environments, where they may influence both pathogen colonization and commensal biofilm stability (Saeki et al., 2021; Afrasiabi and Partoazar, 2024; González-Fernández et al., 2025).

Moreover, AgNPs can compromise the integrity of the intestinal barrier, which serves as a critical defense against the translocation of bacteria and toxins from the gut lumen into the systemic circulation (Figure 2). Disruption of this barrier has been linked to increased gut permeability, low-grade inflammation, and the potential development of systemic health issues (Shayo et al., 2024; Li W. et al., 2024). While AgNPs hold promise in controlling pathogenic bacteria, their ability to perturb microbial balance and host metabolic processes raises significant concerns regarding their widespread use in consumer products and medical applications. The inherent resilience of the gut microbiome may offer some degree of recovery from AgNP-induced dysbiosis; however, the persistence of metabolic and barrier-related disruptions highlights the need for cautious evaluation of AgNP exposure and its long-term implications for gut and overall health.

The biological impact of AgNPs on gut microbiota is strongly shaped by particle size, shape, surface charge, concentration, surface coating, and the existing microbial community. Smaller AgNPs (<10 nm) exhibit heightened toxicity due to their larger surface-area-to-volume ratios, accelerated release of Ag^+ ions, and greater ROS generation, which promotes oxidative stress in microbial cells (Zhang L. et al., 2018; Menichetti et al., 2023; Dinç, 2025; Sati et al., 2025). NP shape also affects efficacy; spherical, triangular, hexagonal, cubic or rod-shaped forms of AgNPs displayed variable antimicrobial activity. However, the anti-microbial potential based upon morphological attributes varied contrastingly according to the variable experimental conditions (Van Dong et al., 2012; Alshareef et al., 2017; Hanan et al., 2018; Vanlalveni et al., 2024). Therefore, a generalization may draw false narrative. Surface charge modulates interactions with bacteria: positively charged AgNPs strongly adhere to negatively charged bacterial membranes *via* electrostatic attraction, increasing membrane disruption and antimicrobial potency, whereas negatively charged or neutral coatings can reduce this effect (El Badawy et al., 2011; Alshareef et al., 2017). The concentration of AgNPs is critical—higher doses induce more pronounced disturbances in microbial diversity, metabolic function, and intestinal inflammation, while low or sub-threshold levels may have minimal or reversible effects; for instance, a dose-dependent alteration in gut microbiota α- and β-diversity has been reported in mice orally exposed for 28 days to food pellets supplemented with increasing doses of AgNPs (0, 46, 460, or 4,600 ppb). They also observed that higher doses shifted the phyla balance (*Firmicutes vs. Bacteroidetes*), but low doses had lesser or no overt toxicity (van Den Brule et al., 2015). Similarly, oral exposure to AgNPs (0, 1, 5, 10, 25, 50 mg/kg bw/day for 4 weeks) induced dose-dependent toxicity in mice, evidenced by body weight suppression and reduced intestinal dendritic cell numbers, confirming that both systemic and local immune effects scale with dose (Ren et al., 2023). However, contrasting results have also been reported documenting no changes in the *Firmicutes/Bacteroidetes* ratio in gut microbiota of rats and mice exposed to AgNPs at dose levels of 9 mg/kg bw/day and 10 mg/kg bw/day, respectively (Wilding et al., 2016; Hadrup et al., 2012). The duration of NP exposure may also have significant effects. In mice, administration of AgNPs and Ag nanowires (0.5 and 2.5 mg/kg) for 14 days reduced gut microbial diversity and altered community structure. After 28 days, partial recovery of the microbiota was observed, although metabolic disturbances, particularly in gut metabolites, persisted (Wang X. et al., 2023). Surface coatings, such as PEG, PVP, or chitosan, are routinely used to reduce toxicity by limiting dissolution and aggregation; PEG-coated AgNPs, for instance, exhibit reduced cytotoxicity and attenuated gut microbiota disruption (Caballero-Díazet al., 2013; Das et al., 2017; Menichetti et al., 2023; Vanlalveni et al., 2024). Finally, the baseline gut microbiota composition and host factors shape the response to AgNPs exposure—individual differences in microbial communities, and host-specific parameters determine susceptibility to dysbiosis and ecological shifts (Ren et al., 2023). Even, AgNPs exposure during critical developmental periods leads to enduring gut dysbiosis, neurobehavioral, and metabolic alterations as evidenced in mice (Lyu et al., 2021). Together, these factors underscore that the effects of AgNPs on gut health are not solely dependent on dosage but are the result of complex nanoparticle–microbiome interactions that must be addressed in designing safe, targeted applications.

3.3 ZnO NPs and gut microbiota dynamics

ZnO NPs exert multifaceted effects on the human gut microbiota, influencing microbial diversity, community composition, and metabolic activity (Table 1) (Figure 2). Research shows that these effects are highly dependent on factors such as concentration and the individual's health status. For example, ZnO NPs [0 mg/kg (control), 25 mg/kg, 50 mg/kg, and 100 mg/kg] were fed to poultry birds for 9 weeks; at the highest concentration (100 mg/kg), ZnO NPs tended to reduce microbial richness and diversity, particularly impacting beneficial bacteria

such as *Lactobacillus* (Feng et al., 2017). Exposure of the human intestinal microbiome to ZnO NPs at 0.1, 2.5, and 50 mg/L revealed clear dose-dependent effects. While low concentrations caused minimal changes, higher doses (especially 50 mg/L) significantly altered microbial composition, reduced diversity, and disrupted metabolic functions (Zhang et al., 2021). However, the response is not uniform across all populations. In children with Autism Spectrum Disorder, ZnO NPs actually increased gut bacterial diversity, whereas a decrease was observed in healthy children, suggesting that pre-existing health conditions may modulate the microbial response to ZnO NP exposure (Yu et al., 2021). Metabolically, ZnO NPs have been associated with a reduction in the production of SCFAs, which play essential roles in gut health and host metabolism (Zhang et al., 2021). Additionally, alterations in gut microbiota composition due to ZnO NPs have been linked to changes in plasma metabolites, indicating a complex and systemic metabolic impact (Feng et al., 2017). The effects of ZnO NPs also extend to microbial resistance traits. While medium concentrations were shown to reduce antibiotic resistance genes, low concentrations paradoxically enriched tetracycline resistance genes, revealing a nuanced influence on the gut resistome (Zhang et al., 2021). Interestingly, not all effects of ZnO NPs are negative. In certain contexts, such as in broiler chickens, ZnO NPs have been found to support the growth of beneficial bacteria, enhance gut health, and boost immune function (Qu et al., 2023). This duality highlights the complexity of ZnO NP interactions with the gut microbiome and underscores the need for further targeted studies to fully elucidate their health implications.

ZnO NPs influence gut microbiota through several mechanisms of action, with their effects varying depending on individual health status, such as in children with Attention-Deficit Hyperactivity Disorder or Autism Spectrum Disorder, as well as in various animal models. One of the primary mechanisms is their antibacterial activity. ZnO NPs have been shown to inhibit the growth of gut bacteria, particularly in healthy children, where they significantly reduced the number of live bacterial cells (Zhou et al., 2021; Yu et al., 2021). However, this response appears to differ in children with ASD, where ZnO NP exposure was associated with an increase in gut bacterial diversity, suggesting a more complex interaction with pre-existing microbial communities (Yu et al., 2021). Additionally, ZnO NPs are known to modulate microbial diversity by altering the overall community structure of gut bacteria. Higher concentrations, particularly 100 mg/kg, orally, for 9 days have been linked to a notable decline in beneficial bacteria such as *Lactobacillus* in poultry birds, with bacterial richness showing a negative correlation with ZnO NPs concentration indicating that excessive exposure may contribute to gut dysbiosis (Feng et al., 2017). Beyond their antimicrobial properties, ZnO NPs also possess anti-inflammatory effects. In conditions such as ulcerative colitis, they have been reported to help restore gut homeostasis by activating the Nrf2 signaling pathway and reducing levels of pro-inflammatory cytokines (Li et al., 2017). These findings suggest that while ZnO NPs can beneficially modulate gut microbiota and reduce inflammation under certain conditions, their overuse or inappropriate application may lead to harmful effects. Therefore, careful consideration of dosage and individual health status is essential when evaluating the therapeutic or dietary use of ZnO nanoparticles.

3.4 Carbon nanomaterials-gut microbiota interaction

CNMs, including single-walled carbon nanotubes (SWCNTs) and graphene oxide (GO), CNM-based nanozymes, quantum dots have a significant and multifaceted impact on the human gut microbiota, influencing both microbial composition and metabolic processes (Table 1) (Bantun et al., 2022; Zhao et al., 2024). These materials become integrated into the gut's carbon flow, where they affect microbial fermentation and alter the production of key metabolites such as butyrate (Cui et al., 2023). This interaction has the potential to influence the proliferation and differentiation of intestinal stem cells, raising concerns about the possible health risks associated with CNM exposure. One major impact of CNMs is on the microbial composition within the gut. Studies in rodents show that single-walled CNTs and GO, when ingested, alter microbial composition shifting the *Firmicutes/Bacteroidetes* balance and increasing pro-inflammatory taxa like *Alistipes* and *Lachnospiraceae* (Chen et al., 2018; Utembe et al., 2022). CNM exposure can inhibit the growth of beneficial probiotic bacteria while promoting the growth of opportunistic pathogens, leading to dysbiosis (Figure 2) (Ma Y. et al., 2023; Wojciechowska et al., 2023; Chen et al., 2025). Interestingly, CNMs may also lead to an increase in butyrate-producing bacteria, as some of these microbes can utilize CNMs as a carbon source. This shift in population dynamics can have downstream effects on gut health and homeostasis (Cui et al., 2023). Beyond compositional changes, CNMs also influence gut microbial metabolism. Through fermentation, CNMs are converted into organic metabolites such as butyrate, which plays a critical role in gut physiology. However, the excessive production of butyrate due to CNM metabolism can disrupt normal intestinal processes by affecting the proliferation and differentiation of intestinal stem cells (Cui et al., 2023). These metabolic shifts could have long-term implications for intestinal function and overall health. Although the adverse effects of CNMs on gut microbiota are becoming increasingly evident, the broader implications for human health are still being explored. Responses to CNM exposure appear to vary among individuals, likely due to differences in baseline microbiota composition and genetic predispositions. Therefore, more comprehensive research is necessary to understand the full scope of health risks and to identify potential strategies for mitigating the negative effects of CNMs on the gut microbiome (Wojciechowska et al., 2023; Zickgraf et al., 2023; Chen et al., 2025).

The mechanisms through which CNMs affect the human gut microbiome are complex, involving both direct interactions with microbial populations and indirect influences *via* metabolic pathways. Recent research has shown that CNMs, such as single-walled carbon nanotubes and GO, can be utilized by gut microbiota as a novel carbon source. This fermentation process results in the production of beneficial metabolites such as butyrate, a SCFA, essential for gut health and the regulation of intestinal cellular functions (Cui et al., 2023). CNMs enhance microbial fermentation by selectively supporting the growth of specific butyrate-producing bacteria, thereby increasing butyrate levels in the gut. This has important implications for maintaining intestinal health and promoting epithelial integrity (Cui et al., 2023). However, the impact of CNMs on microbial metabolism is concentration-

dependent. While moderate levels may support SCFA production, high concentrations of CNMs can suppress key metabolic pathways, disrupting SCFA synthesis and potentially leading to adverse physiological outcomes (Figure 2) (Cui et al., 2023; Wojciechowska et al., 2023). In addition to their metabolic effects, CNMs possess antimicrobial properties. They can compromise microbial cell integrity through oxidative stress, membrane disruption, and other toxic effects. The antimicrobial efficacy of CNMs including GO, CNTs, fullerenes, and carbon quantum dots is strongly governed by their physical and chemical attributes: size, shape, and surface functionalization. (Maksimova, 2019). Smaller GO sheets, for instance, exhibit higher oxidative stress–mediated killing when used as surface coatings, while larger sheets in suspension are more effective at entrapping and isolating bacteria, with smaller ones (~0.01 μm^2) showing a four-fold increase in antibacterial activity relative to larger sheets (~0.65 μm^2) in coatings (Perreault et al., 2015). The shape is equally decisive: sharp, high-aspect-ratio edges such as those in GO nanowalls or CNT tips mechanically disrupt bacterial membranes, significantly reducing survival rates of *E. coli* and *S. aureus* (Mohammed et al., 2020). Surface functionalization further modulates activity: oxygenated groups (–COOH, –OH), reduced forms (rGO), or conjugation with Cu^{2+} or Ag nanoparticles enhance dispersion, surface charge, ROS production, and targeted adhesion, with rGO–Cu hybrids achieving two orders of magnitude greater antibacterial effect compared to rGO alone (Tu et al., 2021). Thus, tuning CNM size, tailoring sharp morphologies, and engineering surface chemistries—especially *via* metal decoration or functional groups—synergistically optimize antimicrobial performance (Cobos et al., 2020; Khairol Anuar et al., 2021). Although CNMs hold promise for modulating gut microbial activity and enhancing certain health-related functions, their long-term impact on microbial diversity and gut health remains uncertain. Prolonged or high-level exposure may disrupt normal microbial communities and even promote the development of antibiotic resistance (Figure 2) (Xie et al., 2016). Therefore, while the therapeutic potential of CNMs is notable, careful evaluation of their safety and biological interactions is essential.

The interactions of ENMs with the human microbiome cannot be fully understood without considering their broader ecological footprint. ENMs released into soil, water, and air do not remain confined to the environment but can re-enter the food chain and drinking water, ultimately influencing human exposure. For instance, agricultural ENMs that alter soil or rhizosphere microbial communities may indirectly affect nutritional and microbial inputs to the human gut *via* crops (Mgadi et al., 2024; Dixit et al., 2024); similarly, ENM contamination of aquatic systems can influence water-borne microbiota that serve as reservoirs of antimicrobial resistance genes (Cui and Smith, 2022). Studies show that exposure to polluted or microbe-rich environments correlates with shifts in gut microbiome composition and diversity, suggesting a soil-plant-gut axis of microbial transmission and those environmental pollutants select for microbiome functionality in the gut (Ma et al., 2025; De Filippis et al., 2024). Thus, environmental and human microbiomes are interconnected through shared exposure pathways, bioaccumulation, and resistome exchange, highlighting the need to view ENM–microbiome interactions not as isolated domains but as part of a continuum linking ecosystem health and human health.

4 ENMs and environmental microbial communities

ENMs, such as nano-TiO_2 and various metal NPs, have a profound impact on water-borne microbial communities, influencing key ecological functions like nutrient cycling and overall ecosystem health (Table 2). Chronic exposure to ENMs can disrupt the structure and function of microbial communities, leading to significant changes in biogeochemical processes essential for ecosystem sustainability. For instance, long-term exposure to nano-TiO_2 has been found to reduce biofilm biomass and alter microbial community composition by decreasing the presence of taxa involved in nitrogen fixation and denitrification, the processes critical to nutrient cycling (Binh et al., 2016; Passarelli et al., 2020). Similarly, metal NPs such as silver and copper exhibit toxicity toward microbial populations, impairing enzymatic activities necessary for processes like nitrogen fixation and organic matter decomposition (Peyrot et al., 2014; Chhipa, 2021). ENMs also affect microbial activity by suppressing fundamental metabolic processes including respiration and photosynthesis, which are essential for maintaining ecosystem functions (Binh et al., 2016). Additionally, the presence of ENMs can lead to a shift in microbial community composition, favoring resistant taxa while reducing those crucial for nutrient cycling, thereby impairing key ecosystem services (Binh et al., 2016). These disruptions can have cascading effects on ecosystem health, such as reduced soil fertility and compromised ecosystem functionality, ultimately threatening agricultural productivity and environmental stability (Yadav and Yadav, 2024). Despite these concerns, some studies suggest that under certain conditions, ENMs may also enhance specific microbial functions or increase microbial resilience, highlighting a complex and context-dependent interaction between ENMs and microbial communities. As such, a comprehensive understanding of the dual roles of ENMs, both beneficial and detrimental is essential for evaluating their environmental impact and guiding their responsible use (Yadav and Yadav, 2024).

4.1 Impact of ENMs on aquatic ecosystems

ENMs, such as nano-TiO_2 and various metal NPs, profoundly affect aquatic microbial communities, disrupting ecological processes like nutrient cycling and ecosystem stability (Table 2) (Figure 2). In freshwater systems, chronic exposure to nano-TiO_2 can drastically reduce biofilm biomass and alter community composition, particularly diminishing nitrogen-fixing bacteria like *Azotobacter* and denitrifiers such as *Pseudomonas*, which play essential roles in nitrogen cycling (Binh et al., 2014). Short-term exposure further highlights species-specific effects: nano-TiO_2 has been shown to depress *Bacillus subtilis* and *Aeromonas hydrophila*, while paradoxically enhancing growth of *Arthrobacter* and *Klebsiella*, likely by photo-oxidizing organic matter into more bioavailable forms (Binh et al., 2014). Simultaneously, metal NPs like copper oxide (CuO NPs) disrupt critical microbial function such as nitrate reduction by damaging cell membranes, down-regulating enzymes like NADH dehydrogenase, cytochromes, nitrate and nitrite reductases in model denitrifier *Paracoccus denitrificans*, ultimately reducing denitrification efficiency by roughly 36%

TABLE 2 Effects of Engineered Nanomaterials (ENMs) on environmental microbiota.

Nanomaterial	System studied	Effects on microbiota	Mechanisms of action	Ecological implications	Key references
TiO_2 NPs	Aquatic ecosystems	Diminish nitrogen-fixing bacteria like *Azotobacter* and denitrifiers such as *Pseudomonas*, biofilm loss, altered community composition	Inducing reactive oxygen species (ROS) generation, inhibition of respiration or photosynthesis	Reduced nutrient cycling, risk of algal blooms	Binh et al. (2014)
CuO NPs	Aquatic ecosystems	Disruption of critical microbial functions—such as nitrate reduction; enzyme inhibition	Membrane damage, enzyme suppression	Reduced denitrification efficiency	Su et al. (2015)
AgNPs, ZnO NPs	Soil microbiomes	Lowering diversity indices (shannon, simpson); inhibited dehydrogenase, urease, phosphatase	Dose-dependent enzymatic inhibition	Reduced soil fertility and nutrient cycling	Asadishad et al. (2018)
ENMs (Ag, ZnO, Cu, CeO_2, CNMs)	Plant–microbe interactions	Suppress mycorrhizal fungi and nitrogen-fixers; sometimes enhance plant growth	Antimicrobial activity, altered signaling	Reduced nodulation, impaired symbiosis; but potential as smart fertilizers	Mortimer et al. (2020)
ZnO, Ag, CuNPs	Resistance traits	Increased horizontal gene transfer (HGT) & antibiotic resistance gene (ARG) transfer; plasmid conjugation	ROS, enhanced membrane permeability, SOS response	Spread of antimicrobial resistance	Markowicz et al. (2023)
TiO_2 NPs	Soil microbiomes and aquatic ecosystems	Changes in microbial community composition in soil and water; toxicity to aquatic organisms (e.g., reduced survival, growth inhibition in zebrafish and daphnia); disruption of photosynthesis and growth in plants and algae	Altered nutrient cycling via impact on microbial communities; bioaccumulation in aquatic food webs; photocatalytic activity under UV resulting in ROS generation causing oxidative stress in aquatic or soil organisms; physical adsorption onto cell surfaces altering permeability	Toxicity to aquatic organisms (zebrafish); developmental effects in nematodes; growth and stress responses in plants	Wang S. et al. (2022); Yamini et al. (2023)
ZnO NPs	Soil microbiomes	Toxic effects on soil microbes, altering nitrogen fixation and organic matter cycling	Dissolution to Zn^{2+} ions causes metal ion toxicity; ROS production leading to oxidative stress and DNA/protein damage; disruption of enzymatic and metabolic pathways in microbes, algae, and plants; particle aggregation influencing bioavailability and sedimentation in aquatic systems	Disruption of reproduction and growth in aquatic species; developmental and stress effects in plants and nematodes	Wang S. et al. (2022); Yamini et al. (2023)
AgNPs	Soil	Reduced microbial diversity; inhibition of nitrifying or denitrifying bacteria (*Nitrosomonas*, *Pseudomonas*); altered community composition	Release of Ag^+ ions results in enzyme inhibition; ROS generation; disruption of nitrogen cycle	Reduced N cycling, soil fertility loss, risk of resistant microbes	Choi and Hu (2009)
ZnO NPs	Soil microbiomes	Abundance of beneficial microbes (*Rhizobium*, *Bacillus*); reduced microbial richness at higher concentration	Dissolution to Zn^{2+} ions; ROS-mediated damage	Impaired plant-microbe interactions; reduced soil health	Ge et al. (2012)
TiO_2 NPs	Soil microbiomes	Depression in microbial richness and fungal/bacterial ratio shifts	Photocatalytic ROS under UV; adsorption onto microbial surfaces	Potential disruption of C and N cycling; context-dependent effects	Simonin and Richaume (2015)
CNTs	Soil microbiomes	Dose-dependent microbial inhibition; upregulating Actinobacteria, degrading proteobacteria	Physical piercing of membranes; ROS production; altered soil pH or micro-niches	Altered microbial balance leading to long-term shifts in nutrient cycling	Kang et al. (2008); Rodrigues et al. (2013)

(Continued on following page)

TABLE 2 (*Continued*) Effects of Engineered Nanomaterials (ENMs) on environmental microbiota.

Nanomaterial	System studied	Effects on microbiota	Mechanisms of action	Ecological implications	Key references
Graphene oxide	Soil microbiomes	Degrading the abundance of proteobacteria and firmicutes; disruption of soil enzyme activity	Strong adsorption to microbial cell walls; oxidative stress	Disturbance of enzymatic soil processes; long-term microbial shifts	Xiong et al. (2018)
Iron oxide nanoparticles (Fe_3O_4, Fe_2O_3)	Soil microbiomes	Low levels (1 and 10 mg/kg soil) stimulated microbial metabolic activity whereas higher concentrations (50–500 mg/kg) had variable or inhibitory effects	Released iron ion causes redox imbalance; enzyme interference	Potential stimulation of nutrient cycling at low doses; inhibition at high doses	Rui et al. (2016)
Aluminum oxide nanoparticles (Al_2O_3 NPs)	Soil microbiomes	Altered bacterial community composition; reduced fungal growth	Al^{3+} ion release; physical interaction with microbial membranes	Shifts in soil microbial balance; potential inhibition of plant-microbe symbiosis	Fajardo et al. (2014); Ansari et al. (2014); Sadiq et al. (2009)
AgNPs	Aquatic ecosystems	Reduced biofilm biomass; degrading nitrifying bacteria (*Nitrosomonas*); altered community composition; inhibition of denitrifiers (*Pseudomonas*)	Ag^+ ion release; ROS generation; enzyme inhibition (nitrification, denitrification)	Disruption of nitrogen cycling; reduced water quality; microbial dysbiosis	Das et al. (2012)
ZnO NPs	Aquatic ecosystems	Reduced bacterial abundance and diversity; shifts toward Zn-resistant taxa; altered algal–bacterial interactions	Zn^{2+} ion release; ROS-mediated toxicity	Impaired primary productivity; disruption of nutrient cycling; risk of resistance	Wu and Duncan (2020)
TiO_2 NPs	Aquatic ecosystems	Degraded biofilm biomass; altered microbial composition; reduced abundance of nitrogen-fixing bacteria (*Azotobacter*)	Photocatalytic ROS under UV; cell membrane damage	Impaired nutrient cycling; reduced ecosystem stability in freshwater	Cherchi and Gu (2010)
Graphene oxide	Aquatic ecosystems	Degradedbacterial richness; inhibition of cyanobacteria and green algae; shifts in aquatic microbial assemblages	Strong adsorption to cell walls; oxidative stress	Reduced algal–bacterial symbiosis; altered oxygen production	Li et al. (2020); Evariste et al. (2021)
CuO NPs	Aquatic ecosystems	Reducedmicrobial biomass; inhibition of nitrification and denitrification pathways; altered bacterial community	Cu^{2+} ion release; oxidative stress; enzyme inhibition	Reduced nitrogen removal capacity; eutrophication risk	Sielska and Skuza (2025); Wang Z. et al. (2024)
Iron oxide nanoparticles (Fe_3O_4, Fe_2O_3)	Aquatic ecosystems	Stimulation of some microbial groups at low levels whereas inhibition of diversity at higher concentrations	Iron ion release affecting redox balance; interference with microbial enzymes	Possible enhancement of nutrient cycling at low doses; inhibition at high doses	Gabrielyan et al. (2019); Caixeta et al. (2021)
Cerium oxide nanoparticles (CeO_2 NPs)	Aquatic ecosystems	Altered aquatic microbial community composition; inhibition of biofilm formation at high concentration	ROS scavenging at low doses; ROS induction at high doses	Potential dual role with antioxidant protection and microbial toxicity	Hoecke et al. (2009); García et al. (2012)

(from ~98% to ~62%) at concentrations between 0.05–0.25 mg/L (Su et al., 2015).

ENMs also impair microbial metabolic pathways integral to ecosystem function. Nano-TiO_2 and ZnO NPs suppress respiration and photosynthesis in primary producers; for instance, ZnO exposure lowers photosynthetic activity in cyanobacteria like *Microcystis aeruginosa*. Nano-TiO_2 inhibits metabolic activity in algae and bacteria, reshaping taxa distribution by reducing sensitive algal species and allowing more resistant cyanobacteria to thrive, raising concerns around harmful algal blooms (Chen B. et al., 2022). These disruptions carry serious ecological consequences (Figure 2). In agricultural runoff zones, ENMs can reduce microbial diversity and soil nutrient availability, decreasing fertility and crop yield. In wetlands, altered biofilm and microbial enzyme activity impairs organic matter decomposition, affecting carbon sequestration and water purification. However, not all effects are negative: low concentrations of iron oxide NPs have been observed to stimulate growth and denitrification in *P. denitrificans* and other denitrifiers, illustrating the nuanced, context-dependent nature of ENM–microbe interactions. Therefore, ENMs exert complex dual roles in aquatic ecosystems: they can both disrupt and potentially enhance microbial community structure and function. A deeper, context-sensitive understanding of these interactions is essential to assess environmental risks and guide responsible applications of nanotechnology.

The influence of ENMs on bioaccumulation and ecotoxicological effects within keystone microbial species is profound and multifaceted. Silver and copper NPs, for instance,

readily accumulate in microbial cells, disrupting physiological functions and reshaping community structures based on concentration and particle type (He et al., 2014; Von Moos and Slaveykova, 2014). In freshwater sediments, exposure to citrate- and PVP-coated AgNPs at 0, 25, 50, 75, 100, and 125 mg/L caused dose-dependent inhibition of microbial functional diversity. At the highest concentration (125 mg/L), citrate-AgNP significantly reduced microbial catabolic activity by up to 80% and diminished both substrate richness and diversity, impairing organic matter degradation and broader nutrient cycling (Kusi et al., 2020). This bioaccumulation at lower trophic levels also poses a risk of trophic transfer, potentially leading to biomagnification in higher organisms.

Ecotoxicologically, ENMs can both generate ROS at their surfaces (e.g., *via* catalytic activity or metal ion release) and induce ROS production within organisms (e.g., *via* mitochondrial dysfunction or activation of NADPH oxidases) (Mendoza and Brown, 2019; Ge et al., 2019). This oxidative stress disrupts essential enzymatic and metabolic functions such as nitrogen fixation, respiration, and organic matter decomposition, all critical for ecosystem sustainability (Von Moos and Slaveykova, 2014; Zhai et al., 2018; Gambardella and Pinsino, 2022). In microbial and algal models (e.g., *Chlorella vulgaris*), exposure to CNTs and metal-oxide NPs triggered elevated antioxidant enzyme activity (e.g., superoxide dismutase) alongside ROS-induced damage and reduced cell viability (Pereira et al., 2014). In environmental contexts, even low levels of AgNPs reduced metabolic diversity in organic-matter-associated microbial communities, which in turn indirectly stunted invertebrate growth in aquatic food webs (Zhai et al., 2018; Guo et al., 2019; Bao et al., 2016; Kusi et al., 2020). The toxicity of these materials is closely tied to their physicochemical characteristics such as size, shape, surface chemistry, ion dissolution rates, all of which modulate interactions with microbial cells (Triboulet et al., 2013; He et al., 2014; Pereira et al., 2014; Von Moos and Slaveykova, 2014; Kusi et al., 2020). For instance, the ecotoxicity of silver varies significantly between metallic AgNPs and their sulfide-transformed forms (Ag_2S), with reduced bioavailability and toxicity in soils (Courtois et al., 2019). Copper NPs similarly trigger oxidative stress and metabolic shifts in bacteria, affecting glutathione levels and antioxidant enzyme pathways (Figure 2) (Rana and Kalaichelvan, 2013; Triboulet et al., 2013; Javurek et al., 2017).

Despite these adverse outcomes, there is emerging potential for ENMs in bioremediation applications, where targeted use of certain NPs can enhance microbial degradation of pollutants. This highlights a complex dual role: while ENMs may pose significant ecological hazards, they also offer novel opportunities for environmental management. However, addressing this duality requires nuanced and context-aware research to assess long-term impacts and guide responsible use of nanotechnologies.

4.2 Impact of ENMs on soil microbiome

ENMs significantly influence soil microbial diversity and enzymatic activities, thereby affecting soil health and agricultural productivity (Figure 2). Exposure to metal ENMs such as AgNPs, ZnO NPs and TiO_2 NPs can significantly alter microbial metabolic diversity and key enzyme functions in soils (Table 2) (Rajput et al., 2023; Islam, 2025). In one study, metal ENMs like AgNPs and ZnO NPs altered community-level physiological profiles of soil bacteria and significantly decreased the diversity indices, such as Shannon's diversity index, Evenness diversity index, and Simpson's diversity index while TiO_2 NPs had no detectable impact (Asadishad et al., 2018; Chavan and Nadanathangam, 2020; Yadav and Yadav, 2024). Further, soil amendments with AgNPs, ZnO NPs, and CuO NPs showed dose-dependent effects on enzymatic activity: ZnO and CuO either enhanced or had no effect at moderate doses (1–10 mg/kg), while AgNPs at higher concentrations (100 mg/kg) inhibited enzymes such as dehydrogenase, phosphatase, and urease (Tripathi et al., 2023). High levels of ZnO, TiO_2, and CeO_2 (around 1,000 mg/kg) also reduced populations of *Azotobacter* and other nutrient-solubilizing bacteria, along with suppressed enzyme activities. AgNPs are reported to suppress populations of essential nitrogen-fixing and nitrifying microbes, including *Rhizobium* and *Nitrosomonas*, which disrupts nitrogen cycling processes in soil ecosystems (Shah and Belozerova, 2009). Likewise, ZnO NPs have been found to impair microbial respiration and inhibit key enzymatic activities, such as dehydrogenase and urease, both of which are vital for organic matter breakdown and nutrient recycling (Parada et al., 2019). In another study, acute exposure to CuO NPs (10–500 mg kg^{-1}) through nano-pesticide markedly inhibited soil denitrification, with the highest dose (500 mg kg^{-1}) causing an 11-fold increase in nitrate accumulation and a 10.2%–24.1% reduction in N_2O emissions. This suppression was linked to decreased activities of nitrate reductase and nitric oxide reductase, along with inhibited electron transport system activity, altered expression of denitrifying functional genes, and shifts in bacterial community composition (Zhao et al., 2020). Similarly, only 90-day exposure of agricultural soils to TiO_2 NPs (1 and 500 mg kg^{-1}) significantly inhibited nitrification enzyme activities and reduced the abundance of ammonia-oxidizing microorganisms, as indicated by decreased *amoA* gene copies. This suppression cascaded to reduce denitrification enzyme activity, with declines in *nirK* and *nirS* gene abundances, alongside marked shifts in bacterial community structure, even at the lowest realistic NP concentration (Simonin et al., 2016). These disruptions can impair soil nutrient cycling. Specifically, enzymes critical for organic matter decomposition and nutrient release such as urease, phosphatase, and dehydrogenase show decreased activity upon ENM exposure, threatening soil fertility (Figure 2) (Asadishad et al., 2018; Chavan and Nadanathangam, 2019). Moreover, ZnO and CuO NPs may reprogram microbial metabolic pathways by upregulating nitrogen fixation genes (nifH, amoA) while downregulating denitrification genes (norB, nosZ), leading to imbalances in nitrogen cycling (Luche et al., 2016; Sun et al., 2022; Tripathi et al., 2023). Despite these negative impacts, low to moderate ENM levels could stimulate microbial resilience. Biogenic NMs like nanozeolite and nanochitosan, combined with plant probiotics, were found to enhance dehydrogenase, alkaline phosphatase, and fluorescein diacetate hydrolase activities—doubling or tripling enzyme performance under agricultural conditions (Upadhayay et al., 2023), a promising application in sustainable agriculture.

ENMs profoundly affect plant–microbe interactions, especially those involving mycorrhizal fungi and nitrogen-fixing bacteria, with outcomes that vary depending on material type, concentration, and microbial partners (Table 2). Arbuscular mycorrhizal fungi, essential for enhancing plant nutrient uptake, can be adversely impacted by

ENMs, which inhibit fungal growth and disrupt nutrient exchange between plants and soil (Yu M. et al., 2020; Vera-Reyes et al., 2023). Additionally, nitrogen-fixing bacteria such as *Bradyrhizobium diazoefficiens* are sensitive to ENM exposure: cerium oxide nanoparticles (CeO_2 NPs) and multi-walled carbon nanotubes (MWCNTs) can suppress their growth, reduce nodulation competitiveness, and interfere with plant–bacteria signaling critical for effective symbiosis (Mortimer et al., 2020). ENMs further reshape the soil microbiome, altering processes such as mineralization and nitrogen fixation (Figure 2). Their antimicrobial properties can decrease beneficial microbial populations, further disrupting plant–microbe symbioses. This disruption undermines soil functions that are key to plant health. However, context matters: in certain situations, NMs have been shown to enhance plant growth and mitigate abiotic stress by supporting beneficial microbial interactions and functioning as "smart fertilizers" (Ma et al., 2022; Berrios et al., 2023; Sodhi et al., 2025). At low concentrations (~5 mg/kg Ag and 50 mg/kg Zn and Ti), ENMs in biosolids did not adversely affect *Medicago truncatula* growth or metal accumulation in shoots. Instead, they enhanced symbiotic interactions with rhizobia (*Sinorhizobium meliloti*), as reflected by a higher nodule number, and significantly increased total soil microbial biomass. Furthermore, ENM exposure at low concentrations altered microbial community composition, increasing Gram-negative and anaerobic bacteria while reducing eukaryotic abundance. These findings suggest potential stimulatory effects of transformed ENMs on soil microbial activity and plant–rhizobia symbiosis (Chen et al., 2017). Exposure to ZnO NPs as nanofertilizer at 10 mg/kg and 100 mg/kg significantly reshaped the rhizospheric bacterial community structure, particularly altering the abundance of Cyanobacteria and key plant growth-promoting taxa, while alpha diversity remained stable. These compositional shifts were more pronounced at the higher dose (100 mg/kg), suggesting dose-dependent effects of ZnO NPs on soil microbial ecology. While 10 mg/kg ZnO NPs also coincided with enhanced lettuce biomass and photosynthetic rate, no additional plant growth benefits were observed at 100 mg/kg, despite the intensified microbial community changes (Xu et al., 2018). Therefore, the effects of ENMs on soil microbial communities are complex. High concentrations of metal-based NPs tend to inhibit diversity and enzyme function compromising nutrient cycling and soil fertility, while the use of certain nanobiofertilizers at controlled doses shows potential for improving microbial function and crop productivity (Zhang et al., 2024). Therefore, ENMs offer potential agricultural benefits such as improved nutrient delivery and stress resistance, while their adverse effects on crucial microbial partnerships raise concerns about their long-term ecological impact. This underscores the need for targeted research to balance ENM applications benefits against ecological risks.

5 Cross-talk between ENMs and microbial resistance

ENMs are increasingly implicated in exacerbating antimicrobial resistance among microbial communities by facilitating the persistence and dissemination of antimicrobial resistance genes (ARGs). Notably, metal oxide NPs such as ZnO NPs have been shown to promote horizontal gene transfer among bacteria. In laboratory and soil studies, ZnO NPs increased transformation frequency in *E. coli* by approximately 1.8-fold and enhanced the copy number of metal resistance–linked genes, indicating co-selection of resistance mechanisms (Markowicz et al., 2023; Otinov et al., 2020; Wang X. et al., 2024; Alav and Buckner, 2024). Furthermore, AgNPs and CuNPs stimulate the conjugative transfer of ARG-laden plasmids across bacterial genera; for instance, silver ions and AgNPs have been shown to facilitate plasmid-mediated resistance gene transfer, and CuNPs promoted multi-antibiotic resistance gene spread in environmental bacteria (Yu K. et al., 2020). The mechanisms underlying these effects include enhanced horizontal gene transfer among bacterial communities, particularly fostering the spread of ARGs through oxidative stress, increased membrane permeability, induction of the bacterial SOS response, and upregulation of conjugation-related genes (Figure 2) (Yu K. et al., 2020). Sub-lethal exposure to ENMs like nano-alumina (Al_2O_3) has been shown to induce ROS, which damage cell membranes and create pores that facilitate plasmid uptake; this also triggers the SOS DNA damage response that further promotes plasmid transformation and conjugation (e.g., *pBR322* into *E. coli* and *S. aureus*)—a striking increase in horizontal gene transfer efficiency linked directly to NP presence (Ding et al., 2016). Similarly, nanofullerene (nC_{60}) exposure increases ROS generation and membrane disruption, upregulating genes crucial for DNA transfer and conjugative machinery (e.g., *trbBp*, *korA/B*) in exposed bacteria (Ji et al., 2020; Amaro et al., 2021). ENMs also act as environmental ARG reservoirs. For example, certain NPs can adsorb plasmids or ARG-containing DNA, protecting them from degradation and increasing their environmental persistence. CeO_2 NPs, in particular, have been investigated both for their facilitation and inhibition of antimicrobial resistance genes propagation; while some studies identified increased conjugation, others found that CeO_2 can suppress antimicrobial resistance genes transfer by reducing ROS and downregulating horizontal gene transferring genes (Yu K. et al., 2020; Sharma et al., 2025). Additionally, ENMs impose selective pressure on microbial communities: resistant strains, better able to cope with metal-induced stress, proliferate while susceptible strains decline leading to enrichment of antimicrobial resistance traits (Neethu et al., 2022).

Comprehensive reviews reinforce the notion that ENMs in environments from various sources such as wastewater treatment plants exert selective pressure on microbial communities and enhance horizontal gene transfer by increasing membrane permeability through both direct interaction and ROS-mediated damage, concurrently inducing genetic changes linked to conjugation (Figure 2) (Cui and Smith, 2022; Li et al., 2025). Additionally, ENMs contribute to these effects by adsorbing extracellular ARGs and plasmids, protecting them from degradation and facilitating persistence and uptake in microbial populations (Ding et al., 2016; Cui and Smith, 2022; Xu et al., 2023; Kalli et al., 2023; Kaushik et al., 2023; Mosaka et al., 2023; Li et al., 2025). These processes lead to accelerated spread of resistance genes in microbial communities, with ENMs acting as vectors and catalysts for antimicrobial resistant gene propagation in wastewater and natural environments, a trend confirmed by increased antimicrobial resistant gene and mobile genetic element abundance following NP exposure (Ding et al., 2016; Cui and Smith, 2022; Li et al., 2025). However, research also

suggests a potential dual role: emerging NP designs with antioxidant or electrochemical properties may attenuate ARG transmission, indicating a path toward mitigating antimicrobial resistance spread *via* ENMs. The ENMs may also hold beneficial potential in antimicrobial resistance mitigation by their use in targeted drug-delivery systems for antibiotics and/or having synergistic effects reducing the overall required dosage, lowering selective pressure and slowing resistance development (Ribeiro et al., 2022; AlQurashi et al., 2025; Sharma et al., 2025). For instance, encapsulation of enrofloxacin in PLGA and lignin NPs mitigated its disruptive effects on the gut microbiome compared to free enrofloxacin, with lignin-encapsulated Enro showing minimal impact on microbial diversity. Notably, NP delivery delayed the rise in ARG expression between 24 and 72 h, suggesting a potential to reduce antibiotic-induced resistome shifts in the gut (Herrera et al., 2024). Nano-ZnO exposures have been reported to cause dose-dependent alterations in gut microbiota composition and diversity, suppressed SCFA production, and shifted key microbial functional pathways. While medium doses (2.5 mg/L) reduced several ARGs by inhibiting host bacteria, low doses (0.1 mg/L) unexpectedly enriched tetracycline resistance genes, highlighting potential risks to gut health and resistome stability (Zhang et al., 2021). Another study reported that during cultivation of leachate microbiota, ARG diversity and abundance dropped significantly (1.4–3.2 log), with NPs—especially metal oxides (CuO, ZnO)—enhancing this attenuation in an ARG-specific manner. The attenuation was driven by metal-induced bacterial growth inhibition, dissolved ion stress, and internalized NPs inducing oxidative stress (ROS), which together reduced horizontal ARG transfer and damaged resistance genes (Su et al., 2019). Altogether, ENMs may promote antimicrobial resistance—yet, with mindful design, they may also serve as tools to counteract microbial resistance dissemination in environmental as well as *in vivo* settings.

Another crucial aspect is ENMs in combination with existing pollutants, such as heavy metals, can synergistically drive microbial resistance, posing a complex environmental hazard (Balta et al., 2025). ENMs like TiO_2 NPs and ZnO NPs interact with heavy metals to alter their bioavailability, which may enhance metal uptake by microbes and foster resistance *via* genetic mutations or horizontal gene transfer. This synergy not only increases toxicity to microbial populations but also pressures communities to develop resistance mechanisms such as efflux pumps and biofilm formation (Dickinson et al., 2019; Wu et al., 2021). The combined presence of ENMs and pollutants alters microbial community structures, often favoring resistant strains over susceptible ones and intensifying resistance traits through continuous stress. Moreover, co-contamination with ENMs and organic pollutants can result in synergistic toxicity effects that are typically underestimated in conventional environmental risk assessments (Tufail et al., 2022; Zhu et al., 2024; Olawade et al., 2024). This increases the overall health risks posed by resistant pathogens by creating niches that sustain and spread antimicrobial resistance (Balta et al., 2025). Despite these challenges, the interaction between ENMs and pollutants also presents opportunities for novel antimicrobial strategies. By understanding these synergistic effects, researchers hope to develop targeted nanoparticle-based interventions that can disrupt resistant microbial communities or enhance contaminant removal, offering a balanced approach to combating antimicrobial resistance.

6 Tools and techniques for assessing ENM-Microbiome interactions

Assessing the impact of ENMs on microbiome interactions requires a comprehensive, multi-tiered approach that integrates high-throughput sequencing (16S rRNA, metagenomics, metatranscriptomics), functional assays (enzyme activity, SCFA profiling, metaproteomics, metabolomics), resistome analysis (qPCR, metagenomics), microscopy techniques (TEM, confocal), and *in vitro*/*in vivo* models (organoids and organ-on-a-chip technologies, gnotobiotic mice, "Humanized" murine models) to evaluate compositional, functional, and genetic changes. Advanced tools like meta-analyses and machine learning further enhance the interpretation of complex omics data and support a holistic understanding of ENM–microbiome dynamics (Galloway-Peña and Hanson, 2020; Moreno-Indias et al., 2021; Mortimer et al., 2021). A global meta-analysis involving over 2,100 observations demonstrated clear negative effects of ENMs on soil microbial diversity, biomass, and functional enzyme activity, with machine learning models effectively predicting key determinants of impact, highlighting the power of these computational tools for ecological risk assessment. Beyond statistical modeling, mass spectrometry-based multi-omics approaches, including genomics, proteomics, lipidomics, and metabolomics provide essential systems-level insights into how ENMs alter microbial physiology and cellular pathways, revealing widespread adaptations and stress responses at multiple biological layers (Day et al., 2023). Community structure analysis further shows that ENMs, especially metal NPs, can profoundly disrupt microbial populations, diminishing critical functions like nutrient cycling and pollutant degradation by suppressing enzymatic activities.

Despite the robustness of these methodological frameworks, significant variability across studies underscores the need for standardized protocols. Differences in ENM types, doses, exposure durations, microbial communities, and omics platforms often limit comparability and reproducibility. Addressing this heterogeneity through harmonized experimental designs and cross-laboratory validation will be crucial to ensure consistent and reliable assessments of ENM-induced ecological risks across diverse environments.

6.1 Marker gene sequencing for microbiome analysis

Marker gene sequencing is a widely used and cost-effective approach in microbiome analysis that targets conserved genetic regions (e.g., 16S rRNA for bacteria and archaea, ITS for fungi, 18S rRNA for eukaryotes) to profile microbial community composition and diversity (Figure 3). This technique enables identification and relative quantification of taxa within complex microbial ecosystems by amplifying and sequencing these phylogenetic markers. It provides insights into taxonomic structure but has limited resolution for functional and strain-level analysis compared to whole metagenome sequencing (De la Cuesta-Zuluaga and Escobar, 2016; Douglas et al., 2018; Johnson et al., 2019; Galloway-Peña and Hanson, 2020; Bharti and Grimm, 2021).

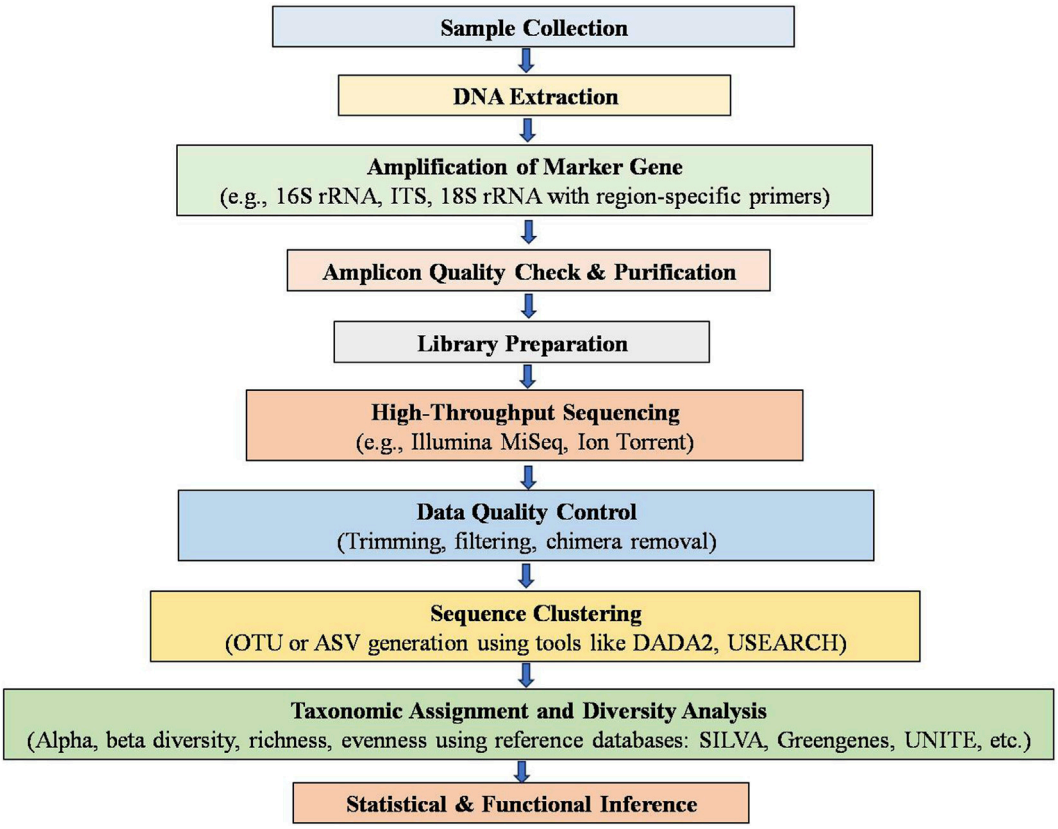

FIGURE 3
General workflow for marker gene sequencing in microbiome analysis. The schematic outlines the traditional metagenomics workflow using amplicon sequencing of marker genes (16S rRNA for bacteria/archaea, 18S rRNA for eukaryotes, and ITS for fungi). Key steps include DNA extraction, amplification of target regions, high-throughput sequencing, and bioinformatics analysis for taxonomic profiling and diversity assessment.

6.1.1 16S ribosomal RNA (rRNA) gene sequencing

16S rRNA gene sequencing is a cornerstone method for profiling bacterial and archaeal communities by targeting conserved and hypervariable regions (e.g., V1–V2, V3–V4, V4–V5) of the ~1,500 bp 16S gene. Primer choice such as V3–V4 for broad bacterial coverage or V1–V2 for higher species resolution is critical to capture the desired taxonomic groups accurately (Na et al., 2023; Combrink et al., 2023; Lee et al., 2023). Post-sequencing, raw reads undergo quality filtering, chimera removal, and demultiplexing using pipelines like QIIME 2 or Mothur (Galloway-Peña and Hanson, 2020; Combrink et al., 2023; Lewis et al., 2021). Feature tables can be generated as Operational Taxonomic Units (OTUs) by clustering at ~97% similarity which is effective for legacy comparisons and mitigating sequencing noise, or as Amplicon Sequence Variants (ASVs) *via* denoising tools like DADA2 for single-nucleotide resolution, improved reproducibility, and finer ecological insights (Galloway-Peña and Hanson, 2020; Lewis et al., 2021; Jeske and Gallert, 2022; Chiarello et al., 2022; Daly et al., 2024). Finally, taxonomic assignment is performed against curated databases (e.g., SILVA, Greengenes) and downstream analyses include diversity metrics and phylogenetic comparisons to reveal community structure and dynamics (Figure 3) (Johnson et al., 2019; Lewis et al., 2021; Combrink et al., 2023).

Using 16S rRNA gene sequencing targeting the V3–V4 region, a study revealed that long-term dietary exposure to Ag, SiO_2, and TiO_2 NPs significantly altered the gut microbiota composition and β-diversity in mice. ASV analysis using DADA2 method showed a dose-dependent increase in Cyanobacteria and a marked reduction in Tenericutes and *Turicibacter* with TiO_2 NPs, while SiO_2 and TiO_2 also suppressed SCFA production. These shifts in microbial composition and metabolic function were largely reversible after an 8-week recovery period without NP exposure, indicating transient but notable perturbations of the gut microbiome by dietary NMs (Perez et al., 2021). Another research employed 16S rRNA gene sequencing (targeting V1–V3 regions with 27f/534r primers) to investigate the influence of plant-derived nanoparticles on the gut microbiome of germ-free mice colonized with human fecal bacteria. Sequencing on the 454 Jr. platform and QIIME 2-based analysis revealed significant shifts in microbiota composition upon NP exposure, with notable enrichment of *Lachnospiraceae*, *Bacteroidaceae*, *Coriobacteriaceae*, and *Ruminococcaceae* families. OTUs were clustered at 97% similarity, and hierarchical clustering highlighted differences between *in vitro* and *in vivo* bacterial uptake of plant-derived nanoparticles, suggesting these NPs selectively modulate gut microbial communities and may alter functional pathways (Teng et al., 2025). Further, 16S rRNA gene sequencing revealed that oral exposure to SiO_2NPs in young mice significantly altered gut microbiota composition and diversity, with increased abundances of *Firmicutes* and *Patescibacteria* and distinct shifts in β-diversity

profiles. OTU-based analysis (97% similarity) and LEfSe identified 41 bacterial clades with differential abundance, suggesting SiO_2NP-induced microbiome dysbiosis, which was associated with neurobehavioral impairments *via* disruption of the gut–brain axis (Diao et al., 2021). Oral exposure to lead-based ($CsPbBr_3$) perovskite nanoparticles (CPB-PNPs) induced significant, dose-dependent alterations in gut microbiota composition as revealed by 16S rRNA gene sequencing. ASV-based analysis showed reduced alpha diversity indices (Chao1, Shannon, Simpson) and a marked shift in β-diversity, as evidenced by principal coordinate analysis (PCoA). High-dose CPB-PNPs increased pro-inflammatory taxa such as *Clostridia* and decreased beneficial *Muribaculaceae*, disrupting the *Firmicutes*/*Bacteroidetes* ratio. Differential abundance analysis further identified enrichment of microbial taxa linked to intestinal inflammation. These microbiota perturbations were associated with compromised gut barrier integrity and colitis-like phenotypes in exposed mice (Mei et al., 2023).

6.1.2 18S rRNA and internal transcribed spacer (ITS) sequencing

18S rRNA and ITS amplicon sequencing are pivotal techniques for profiling fungal and other eukaryotic communities in microbiome studies (Figure 3). The 18S rRNA gene, with its conserved and hypervariable regions (V1–V9), enables broad phylogenetic placement across diverse eukaryotes, though it typically resolves taxa only down to genus level. In contrast, the ITS regions (ITS1 and ITS2), located between 18S, 5.8S, and 28S genes, exhibit high variability and are therefore standard markers for species- and strain-level identification of fungi (Banos et al., 2018; Gao et al., 2021; Olivier et al., 2023). These amplicon data are processed through pipelines like QIIME 2, LotuS2, or ITSx, which include quality filtering, chimera removal, OTU/ASV clustering, and taxonomic assignment using curated databases such as SILVA for 18S and UNITE for ITS (Gao et al., 2021; Özkurt et al., 2022). This dual-marker approach offers comprehensive insights into eukaryotic microbiome structure and diversity. Using long-read sequencing of the nearly complete rRNA operon (16S-ITS-23S), a clear enhancement in species-level resolution of *Lactobacillaceae* has been reported compared to shorter amplicons. RibDif2 analysis revealed substantial allele overlap in V3–V4 (n = 43 overlaps) and whole 16S (~11), while complete 16S-ITS-23S showed zero predicted overlaps. Empirical MinION™ data confirmed these predictions: full-length operon sequencing identified 100% of target species with fewer misclassifications, outperforming both V3–V4 (80% correct) and single 16S (~95%), thus highlighting the combined power of 18S rRNA/ITS region (rRNA operon) for accurate microbiome profiling (Olivier et al., 2023).

Most of the available reports focused on 16S rRNA gene sequencing to track bacterial community changes, and responses of eukaryotic microbes (fungi, protozoa) *via* 18S/ITS remains largely unexplored in NP exposure contexts. Using 16S and 18S rRNA gene sequencing with PNA clamps and ITS qPCR, a study demonstrated that nanoscale sulfur (pristine and stearic acid-coated) modulated both bacterial and fungal communities in the tomato rhizosphere. While bacterial ASV diversity increased under nano-sulfur treatments, eukaryotic communities, particularly fungi and ciliates, showed resilience with minimal diversity shifts. ITS qPCR revealed no significant changes in total fungal abundance, but differential analysis indicated reduced relative abundance of *Fusarium oxysporum* in nano-sulfur treatments compared to controls. Enrichment of sulfur-oxidizing bacteria (*Thiobacillus*) and subtle shifts in fungal taxa suggest nano-sulfur may suppress soil-borne pathogens indirectly by altering microbiome composition and functional interactions (Steven et al., 2024).

6.2 Whole-genome shotgun (WGS) metagenomics

Whole-Genome Shotgun (WGS) metagenomics is an untargeted sequencing method that captures the entire genetic content of all microorganisms in a sample that include bacteria, archaea, fungi, viruses, and eukaryotes, providing comprehensive taxonomic and functional insights. Compared to 16S rRNA amplicon sequencing, WGS delivers higher species- and strain-level resolution, detects greater microbial diversity, and identifies functional genes such as antibiotic resistance and metabolic pathways (Ranjan et al., 2016; Jovel et al., 2016; Keepers et al., 2019; Brumfield et al., 2020). However, it is more expensive, requires deeper sequencing coverage, and demands advanced computational infrastructure due to large data volumes and complexity (Jovel et al., 2016; Fox et al., 2024). WGS metagenomics offers a powerful, holistic view of microbial communities and their functional potential, making it indispensable for studies that extend beyond taxonomic profiling into functional and ecological questions.

WGS metagenomics begins with sample collection and total DNA extraction, followed by random fragmentation and high-throughput sequencing (e.g., Illumina, PacBio, Nanopore). After quality control and contaminant removal (using tools like FastQC and Trimmomatic), reads are assembled *de novo* into contigs and binned into putative genomes (MAGs) using assemblers (e.g., MEGAHIT, metaSPAdes) and binning tools (e.g., MetaBAT, CONCOCT). These assemblies and unassembled reads are then annotated taxonomically (with tools like Kraken2, GTDB-Tk for MAGs, or Kraken2, MetaPhlAn for reads) and functionally profiled against databases such as KEGG, COG, eggNOG, and CARD to reveal metabolic pathways and resistance genes (Pérez-Cobas et al., 2020; Bharti and Grimm, 2021; Saenz et al., 2022). The final step involves comparing taxonomic and functional profiles across samples to assess microbial diversity, community structure, and functional potential (Figure 4).

WGS metagenomics and traditional metagenomics (e.g., amplicon sequencing of marker genes like 16S, 18S, or ITS) both aim to profile microbial communities, but they differ fundamentally in scope and resolution. WGS metagenomics sequences all DNA fragments randomly, enabling species- and strain-level identification, comprehensive detection of bacteria, archaea, fungi, viruses, and functional gene content including antibiotic resistance and metabolic pathways. In contrast, marker gene-based metagenomics targets specific loci (like 16S, 18S, ITS) to characterize community composition more cost-effectively and with simpler bioinformatics, though typically limited to genus-level resolution and offering limited insight into functional potential. While WGS metagenomics demands deeper sequencing, greater computational resources, and more complex analysis pipelines, it

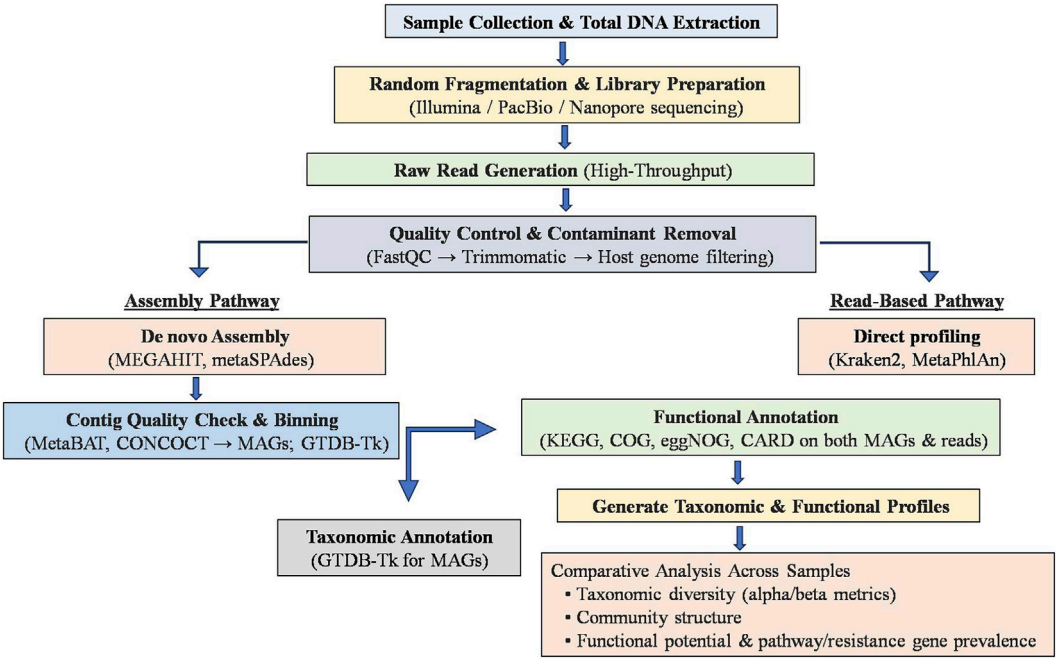

FIGURE 4
General workflow for whole genome shotgun (WGS) metagenomics in microbiome analysis. The schematic illustrates the WGS metagenomics workflow starting from sample collection and total DNA extraction, followed by random fragmentation, library preparation, and high-throughput sequencing (e.g., Illumina, PacBio, Nanopore). Quality control and contaminant removal (using tools such as FastQC and Trimmomatic) are performed before downstream analysis. Two pathways are depicted: the assembly-based pathway involves *de novo* assembly (MEGAHIT, metaSPAdes), binning (MetaBAT, CONCOCT), and taxonomic annotation (GTDB-Tk); the read-based pathway uses direct profiling tools (Kraken2, MetaPhlAn). Both approaches undergo functional annotation (KEGG, COG, eggNOG, CARD) to generate taxonomic and functional profiles, supporting comparative analyses of microbial diversity, community structure, and functional potentials across samples.

reveals richer diversity including rare taxa and accurate functional profiling, making it ideal for comprehensive ecological or clinical microbiome studies (Ranjan et al., 2016; Rausch et al., 2019; Brumfield et al., 2020; Pérez-Cobas et al., 2020; Wang Z. et al., 2023).

Whole-genome shotgun metagenomics revealed that ZnO NPs significantly altered soil microbial taxonomic and functional diversity, reducing gene abundance related to carbon degradation and nitrogen cycling while increasing genes for CO_2 fixation and sulfur metabolism. ZnO NPs also disrupted microbial network complexity by decreasing connectivity and modularity among taxa, with archaeal, fungal, and viral communities showing more pronounced responses than bacteria (Sun et al., 2025). Additionally, WGS metagenomic profiling of nano-ZnO–polluted soils revealed a dose-dependent enrichment of biofilm-related genes, particularly those involved in exopolysaccharide biosynthesis and cell attachment, with stronger effects seen for nanoparticles than bulk ZnO at moderate concentrations (50–500 mg/kg) (Dinesh et al., 2023b). Further, multidrug resistance genes and mobile genetic elements (MGEs) co-occurred with biofilm genes, indicating enhanced potential for horizontal gene transfer under nano-ZnO exposure (Dinesh et al., 2023a). At higher ZnO levels (≥500 mg/kg), nano-ZnO suppressed microbial biomass, respiration, and enzyme activity, reflecting microbial stress and community destabilization (Dinesh et al., 2023b). These findings underscore that nano-ZnO can reshape soil microbiome function and resistance dynamics *via* gene-level shifts in stress response and community interaction mechanisms. WGS metagenomic analysis of wastewater microbial communities exposed to gold NPs revealed that NP morphology (nanospheres vs. nanorods) strongly influenced both taxonomic composition and functional gene profiles. CTAB-coated nanospheres caused significant shifts in microbial structure and enriched antibiotic resistance genes, metal resistance genes, and plasmid-associated genes, whereas nanorods had subtler effects. These results highlight how NP design parameters can modulate microbiome diversity and functional potential in engineered ecosystems (Metch et al., 2018).

Therefore, both WGS metagenomic analysis and traditional marker-based metagenomics using next-generation sequencing provide culture-independent approaches to comprehensively characterize the human gut microbiome and environmental microbial communities. These methods enable the detection of dysbiosis, detailed taxonomic profiling, and the discovery of novel functional genes and metabolic pathways, including those associated with health, disease, and the resistome. Despite these advances, challenges remain. High-throughput sequencing (HTS) data quality can be compromised by sequencing errors, sampling bias, and variable 16S copy number across taxa—factors that skew abundance and diversity estimates (Kembel et al., 2012; Di Bella et al., 2013; Khachatryan et al., 2020; Beaudry et al., 2021; Fox et al., 2024). Moreover, the rapid growth of sequencing data demands continuous development of scalable, reproducible, and integrative bioinformatics frameworks to keep pace with analytical needs. Continued innovation in HTS technologies and computational tools will be critical to unlocking the full potential of microbiome science in both environmental and biomedical contexts.

6.3 Metaproteomics for microbiome analysis

Metaproteomics is a powerful, culture-independent approach for microbiome analysis that investigates the entire protein complement expressed by complex microbial communities in their native environment. Starting from biomass-enriched samples (e.g., feces, soil), proteins are extracted, digested into peptides (commonly *via* phenol extraction and FASP protocols), and then analyzed using LC–MS/MS (Tanca et al., 2014; Zhang et al., 2016; Heyer et al., 2019; Do et al., 2024). This workflow enables identification of active microbial species and their expressed enzymes, revealing metabolic pathways and biomarkers linked to community function such as over 600 microbial species and 250 protein families in gut samples (Tanca et al., 2014; Heyer et al., 2019; 2025). Bioinformatic tools like MetaProteomeAnalyzer, MaxQuant, and Unipept facilitate peptide-spectrum matching, taxonomic and functional annotation, and robust protein quantification (Starr et al., 2018; Kruk et al., 2024; Do et al., 2024; Nebauer et al., 2024). When combined with metagenomics or metatranscriptomics, metaproteomics offers a holistic view of microbial activity, host–microbe interactions, and ecosystem dynamics, advancing both research and diagnostic applications (Heyer et al., 2025). Metaproteomics, which analyzes the expressed proteins of microbial communities, provides a direct snapshot of active biological processes; improvements in mass spectrometry and bioinformatics have enhanced the ability to profile functional shifts within microbiomes (Jagtap et al., 2015; Young et al., 2015; Zhang X. et al., 2018; Do et al., 2024; Valdés-Mas et al., 2025).

While significant progress has been made in understanding NM–microbiome interactions using approaches such as 16S/18S rRNA amplicon sequencing for taxonomic profiling, WGS metagenomics for functional gene prediction, and culture-based assays for targeted microbial analyses, these methods offer only partial insights into microbial function. Notably, metaproteomics, a powerful tool capable of directly assessing protein-level responses, microbial activity, stress adaptations, and metabolic pathway alterations in complex communities, remains largely absent in this field. This represents a critical research gap, as integrating metaproteomics could provide a more comprehensive understanding of how NMs influence microbiome functionality beyond genetic potential, capturing real-time microbial responses to NP exposure. By integrating metagenomic sequencing with fecal proteomics, Valdés-Mas et al. (2025) applied metagenome-informed metaproteomics (MIM) to both mouse models and human IBD patients, enabling species-level resolution of host–microbiome–diet interactions. In IBD cases, they uncovered a dual signature of "compositional dysbiosis" *i.e.*, shifts in microbial taxa and "functional dysbiosis" *i.e.*, reduced protein activity from beneficial commensals in response to inflammatory signals. MIM also accurately reconstructed dietary exposure profiles and *in vivo* nutritional compliance using dietary-specific protein markers. Utilizing microbiome transfer experiments, the study revealed early-onset, species-specific microbiome and host proteomic responses, and identified candidate fecal host-microbiome protein biomarkers that outperformed traditional calprotectin (S100A8/S100A9) in predicting IBD (Valdés-Mas et al., 2025). These findings demonstrate that a combined dietary–microbial–host proteomic analysis can functionally dissect trans-kingdom interactions and offers advancements toward personalized diagnostics and therapeutics in gut-related diseases.

6.4 Metabolomics in microbiome analysis

Metabolomics in microbiome analysis employs techniques like LC–MS, GC–MS, and NMR to profile small molecules (metabolites) produced or transformed by microbial communities, offering a direct snapshot of microbial activity, biochemical interactions, and signaling. By illuminating changes in metabolic pathways such as SCFA production, amino acid metabolism, energy metabolism or xenobiotic transformations, metabolomics complements genomic and transcriptomic data to reveal functional and ecological impacts of microbiota under different conditions. Metabolomics begins with strategic experimental design followed by sample collection and rapid quenching to preserve metabolic states, then extraction of metabolites using solvents (e.g., methanol, acetone, chloroform) optimized for polarity range. Extracted metabolites are analyzed *via* LC–MS, GC–MS, or NMR, allowing separation and detection of wide-ranging compounds with sensitivity and structural resolution. Raw data undergo preprocessing including noise reduction, peak detection, retention time alignment, and normalization using tools like XCMS, MZmine, and MS-DIAL. Metabolite identification is achieved through spectral database matching (e.g., HMDB, METLIN), followed by statistical analysis (PCA, PLS-DA) and pathway enrichment (KEGG, MetaboAnalyst) to interpret biological significance (Kumar et al., 2020; Han et al., 2021; Chen Y. et al., 2022; Muhamadali et al., 2023).

Untargeted metabolomics revealed that TiO_2 NPs inhibited growth of key beneficial bacteria (*L. reuteri*, *L. gasseri*, *B. animalis*, *B. longum*) through membrane damage and disrupted metabolic pathways, including tryptophan and arginine metabolism *in vitro*. *In vivo*, mice fed with TiO_2 NPs showed altered urinary metabolite profiles, notably reduced neuroprotective metabolites and perturbed tryptophan metabolism, all indicative of gut microbiome–mediated host metabolic dysregulation (Wu et al., 2023). Studies integrating microbiome profiling with metabolomic analysis have shown that ZnO NPs (25–100 mg/kg in poultry) significantly disrupt gut microbial community structure and metabolism. Metabolomic shifts included altered levels of glucose, choline, lactate, methionine, indole derivatives, and pro-inflammatory arachidonic acid metabolites correlating with declines in beneficial taxa like *Lactobacillus* and *Bifidobacterium*, and increased *E. coli* and other opportunists. These multi-omics approaches reveal that NM exposure can rewire host–microbe metabolic interactions, with implications for inflammatory and metabolic health (Feng et al., 2017). In an *in vitro* digestion and fermentation model, ZnO, TiO_2, and Ag NPs induced significant shifts in gut microbial genera, particularly *Bifidobacterium*, *Sutterella*, *Escherichia*, and *Bacteroides* as determined by 16S rRNA sequencing. Metabolomic profiling revealed NP-specific modulation of metabolites, including indole derivatives, peptides, and protein metabolism intermediates, with TiO_2 notably increasing pro-inflammatory arachidonic acid pathway metabolites like prostaglandin E_2 and leukotriene B_4. These findings suggest that

metallic NPs exposure can disrupt both microbial composition and metabolic by-products linked to gut inflammation and disease pathways (Vaccari et al., 2023). Oral exposure to Ag NPs and silver nanowires (Ag NWs) at 0.5–2.5 mg/kg in mice significantly disrupted gut microbiota structure reducing diversity and suppressing Gram-negative bacteria within 14 days, while the community largely recovered by 28 days. Despite this recovery, fecal and systemic metabolomics revealed persistent increases in gut-derived 1H-indole-3-carboxylic acid and elevated gut and blood serotonin levels, linking NPs exposure to microbial metabolic shifts and neurochemical alterations (Wang X. et al., 2023). Therefore, for microbiome studies, metabolomics insights alone or preferably integrated with metagenomics, metatranscriptomics, or metaproteomics data can be useful to uncover mechanistic links between microbial composition, metabolic activity, and environmental or host interactions.

Together, these *meta-omics* technologies transcend traditional culture-based methods, enabling comprehensive analysis of microbial species, functional capacity, and metabolic activity in relation to health and disease. However, despite their power, challenges remain in data integration and interpretation. The sheer complexity of meta-omics datasets necessitates robust bioinformatics frameworks and harmonized analytical standards to fully leverage these approaches in microbiome-targeted therapies and precision medicine (Wang et al., 2015; Puig-Castellvi et al., 2023; Do et al., 2024).

6.5 Use of *in vitro* models and *in vivo* animal studies

In Vitro Colon Simulators (e.g., SHIME®, TIM-2, CoMiniGut, SIMGI) are dynamic, multi-stage fermentation systems that faithfully reproduce human colonic conditions and microbial ecosystems. For ENM–microbiome interaction studies, they offer unparalleled ability to simulate realistic dosing, track microbiota and metabolite alterations, and integrate with host-response models making them an essential tool in assessing NMs safety and biological impact in the gut. Using a dynamic, multi-stage *in vitro* colon simulator inoculated with human fecal microbiota, Zhang et al. (2021) demonstrated that exposure to nano-ZnO caused dose-dependent declines in SCFA production and significant perturbations in microbial composition and diversity. Following cessation of exposure, microbial diversity largely rebounded; however, SCFA levels remained suppressed in relation to ZnO concentration. Moreover, the study revealed contrasting effects on the gut resistome: a medium concentration (2.5 mg/L) reduced the abundance of many antibiotic-resistance genes, whereas a low concentration (0.1 mg/L) notably enriched tetracycline resistance genes (Zhang et al., 2021).

Intestinal organoids and gut-on-chip systems as *in vitro* models and *in vivo* animal models together provide a complementary and powerful framework for evaluating how ENMs interact with and impact the microbiome, with valuable insights for human health risk assessment. Human intestinal organoids, derived from stem cells and often cultured within biomimetic hydrogels or perfusable microfluidic platforms, offer improved physiological relevance over 2D cultures for investigating NM–microbiome interactions in the gut. These 3D structures faithfully recapitulate crypt–villus architecture and cellular diversity including enterocytes, goblet cells, Paneth cells, and M cells—enabling physiologically relevant assessments of nanotoxicity and uptake dynamics (Prasad et al., 2021; Bantun et al., 2022; Yuan and Liu, 2023; Shi et al., 2024). When co-cultured with microbiota, they reveal critical insights into cross-kingdom metabolism: for instance, gut microbes have been shown to enzymatically degrade CNMs (e.g., GO, SWCNTs), fermenting them into butyrate, which then modulates epithelial stem cell proliferation and barrier integrity (Cui et al., 2023). Moreover, exposure to nanoplastics or engineered inorganic nanoparticles triggers inflammatory signaling in organoids, particularly through M cell-mediated pathways and disrupts commensal composition by altering the *Firmicutes/Bacteroidetes* ratio, with downstream effects on mucus secretion and mucosal immunity (Hao et al., 2022; Qiao et al., 2024). A study aimed to elucidate how nano-sized food additives interact with commensal and pathogenic bacteria within the gastrointestinal tract utilized human gastric organoids to demonstrate that silica NP–*H. pylori* complexes retained bacterial adherence but significantly attenuated bacterial internalization and pathogenic signaling, including CagA phosphorylation and IL-8 secretion. These findings underscore the potential of ENMs to modulate host–microbiome interactions within the gastric niche (Siemer et al., 2018).

Gut-on-chip platforms are microfluidic devices that reconstitute key physiological and structural aspects of the human intestine such as epithelial villi, mucus layers, and peristaltic flow, while enabling co-culture with microbial communities under controlled, real-time conditions (Trujillo-de Santiago et al., 2018; Xiang et al., 2020; Thomas et al., 2023). These systems allow precise dosing of ENMs and measurement of outcomes like barrier integrity, cytokine production, microbial colonization, and metabolite exchange. Gut-on-chip models are faster and free from ethical issues than animal studies, making them ideal for high-throughput screening of nanoparticles and their acute effects on gut–microbe interactions.

In vivo animal models, particularly zebrafish and rodents, offer critical physiological and systemic context for assessing long-term effects of ENM exposure capturing microbiome perturbations, immune modulation, metabolic shifts, and organ-level responses. Zebrafish are especially valuable as high-throughput vertebrate models due to their conserved intestinal physiology, genetic tractability, and amenability to germ-free and fluorescent-transgenic approaches, enabling real-time visualization of host–microbe–ENM interactions (Xia et al., 2022). Rodent studies, while resource-intensive and ethically demanding, complement these findings by providing detailed insights into ENM-induced shifts in microbial communities, metabolite profiles, inflammatory markers, and histopathology across tissues. However, these *in vivo* approaches require careful design to balance throughput, ethical considerations, and translational relevance (e.g., dosing regimen, exposure duration), underscoring the need for integrated strategies that leverage both zebrafish and murine models (Ashammakhi et al., 2020; Xia et al., 2022; Zhong et al., 2022; Liu et al., 2024).

However, both models have limitations. Gut-on-chip systems lack full immune and multi-organ integration and are best suited for mechanistic, short-term investigations. Animal studies provide whole-body responses but are less amenable to mechanistic

dissection and suffer from inter-species variability. Thus, a hybrid strategy using gut-on-chip platforms for mechanistic hypothesis testing followed by targeted validation in animal models offers a robust pathway to translate findings to human-relevant insights. By standardizing ENM dosing, dynamic microenvironment conditions, co-culture complexity, and integrated omics readouts, the synergy between these approaches will enable safer ENM development and improved prediction of their microbiome-related effects.

7 Risk assessment and long-term implications

Assessing the risks and long-term implications of ENMs on microbiome interactions requires a robust, integrative approach combining cutting-edge tools, environmental and regulatory expertise. A global meta-analysis of over 2,100 observations has shown that ENMs negatively impact soil microbial diversity, biomass, and functional enzyme activities, with artificial intelligence models like random forests effectively predicting these outcomes based on ENM characteristics (Pietroiusti et al., 2016). To further elucidate these effects, multi-omics technologies such as metagenomics, metaproteomics, and metabolomics are increasingly being recommended by regulatory bodies, including the European Food Safety Authority (EFSA), to standardize microbiome assessments and identify biomarkers of disturbance under controlled exposure scenarios. For instance, mass spectrometry-based omics workflows enable comprehensive profiling of microbial functional responses to ENMs, revealing shifts in pathways linked to nutrient cycling and immune signaling (Mortimer et al., 2021).

Environmental and gut microbiome changes following ENM exposure evaluated through both acute (e.g., 90-day rodent studies) and long-term trials indicate persistent effects on gut barrier integrity, immune modulation, and SCFA production (Pietroiusti et al., 2016; Javurek et al., 2017; Tang et al., 2021; Utembe et al., 2022). Developmental exposure to AgNPs in mice resulted in persistent gut dysbiosis, characterized by reduced levels of beneficial taxa (*Bifidobacterium*, *Mucispirillum*) and enrichment of inflammatory-associated bacteria (*Prevotella*, *Enterococcus*). These microbiome alterations correlated with disruptions in metabolic pathways, reduced microglial counts in the brain, and behavioral and metabolic changes in offspring, highlighting the long-term impacts of early-life NMs exposure on the gut–microbiome–brain axis (Lyu et al., 2021). Moreover, emerging evidence indicates that CNMs can be fermented by gut bacteria into bioactive metabolites like butyrate, which then modulate host physiology, such as intestinal stem cell differentiation highlighting not only toxicological but also transformative microbial–host interactions (Cui et al., 2023). Animal models are essential for capturing chronic, systemic effects, but high-throughput *in vitro* systems such as gut-on-chip platforms are increasingly integrated into tiered risk frameworks to enhance hazard identification without excessive animal use. These tiered strategies align with emerging regulatory recommendations that prioritize microbiome endpoints and make use of NAMs (new approach methodologies), standardized sampling, and shared microbiome databases to improve cross-study comparability and regulatory confidence (OP EUROPA, 2027).

While the impacts of ENMs on microbiome include dysbiosis, impaired function of key microbial taxa, altered host-microbe interactions, chronic gut inflammation, immune dysfunction, metabolic and neurological disorders, their roles as environmental disruptors remain under-evaluated (Tang et al., 2021; Lamas et al., 2020; Li P. et al., 2024). EFSA's roadmap emphasizes establishing core microbiome definitions, bioindicator panels, harmonized analytical protocols, and cross-lab networks to resolve this gap (Debode et al., 2024). Overall, advancing ENM risk assessment necessitates a combined strategy linking global meta-analyses, multi-omics, sophisticated modeling, and tiered experimental designs supported by regulatory-driven standardization and data sharing. This holistic approach promises more reliable prediction, monitoring, and mitigation of ENM effects on microbial ecosystems and public health.

8 Regulatory and mitigation strategies

Regulation of ENMs currently leverages existing legislative frameworks rather than relying on bespoke NM laws. In the United States, the Toxic Substances Control Act (TSCA) empowers the Environmental Protection Agency (EPA) to require pre-manufacture notices and implement information-gathering mandates for new nanoforms, while the Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA), and Consumer Product Safety Commission regulate ENMs in food, drugs, cosmetics, and workplaces under broader statutes and hazard communication standards (Lin, 2001; Tang et al., 2024; El-Kalliny et al., 2023; Ghosh and Kumar, 2024). The European Union (EU) employs Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation, Classification, Labelling and Packaging (CLP) Regulation, and the Biocidal Products Regulation to enforce registration, hazard evaluation, and labeling of materials at or above one ton annually, and maintains transparency *via* national NM registries (e.g., France, Denmark, Norway, Belgium) and the EU Observatory for Nanomaterials (Environment EC, 2024b; Environment EC, 2024a; ECHA, 2025; Pavlicek et al., 2021). Standardization efforts led by international organizations like Organisation for Economic Co-operation and Development (OECD), International Organization for Standardization (ISO) Technical Committee (TC) 229 (ISO/TC 229), and World Health Organization (WHO) further support harmonized definitions, testing protocols, and "precautionary approach" workplace guidelines. Despite this multilayered oversight, challenges include fragmented global definitions, evolving physicochemical properties, detection limitations, and often a disconnect between innovation pace and regulation leading experts to call for adaptive, coordinated, and evidence-driven governance that leverages "Safe-by-Design" approaches and precautionary risk frameworks (Gottardo et al., 2021; Kraegeloh et al., 2018; Resnik, 2019; Salieri et al., 2021).

Mitigation strategies for ENM-associated risks follow a dual pathway: reducing hazard through material design and minimizing exposure across the life cycle. "Safe-by-Design" approaches actively tailor physicochemical properties such as size, shape, surface charge, encapsulation to improve stability and biocompatibility while limiting

harmful byproducts (Kraegeloh et al., 2018). These principles are implemented in EU projects (e.g., NANoREG, NanoReg2, SUN, caLIBRAte) that integrate risk pre-assessment, modeling (QSAR, AOP pathways), iterative stakeholder engagement, and green synthesis to align early-stage innovation with safety and regulatory preparedness (Kraegeloh et al., 2018; Isigonis et al., 2019; Schmutz et al., 2020). On the operational front, occupational controls adhere to the hierarchy of hazard mitigation: elimination/substitution, engineering controls (e.g., HEPA-filtered hoods, gloveboxes, sealed balances), administrative best practices, and personal protective equipment as per WHO and OSHA guidance (OSHA, 2025; McLean et al., 2024). Environmental surveillance and risk assessment rely on life-cycle analyses, environmental monitoring, and computational evaluation to forecast fate, transport, and ecological impacts, while AI-augmented characterization and risk modeling promise faster assessments and reduced animal testing. Together, these approaches couple proactive material design with rigorous exposure controls to manage ENM risks to environmental and human microbiomes.

9 Future directions and research gaps

Future research on the impact of ENMs on microbiome interactions points toward several critical directions. Firstly, the field must advance standardized life-cycle exposure models that track ENMs from synthesis through environmental release, transformation, and biological uptake—an area having significant research gap (e.g., bioaccumulation of transformation products) (Pietroiusti et al., 2016). Secondly, integrating *in vitro* and *in vivo* systems with multi-omics technologies (genomics, proteomics, metabolomics) and predictive machine learning that offers the promise of mechanistic insight and reliable ecological risk forecasting. For instance, recent meta-analyses and machine learning applications have effectively predicted ENM impacts on soil microbial communities and identified functional gene disruptions. Additionally, a One-Health research framework that encompasses soil, plant, animal, and human microbiomes is urgently needed to explore systemic ENM effects across ecological and host-associated networks. Furthermore, the identification of microbiome-based biomarkers such as shifts in SCFA profiles, immune-metabolite signatures, or specific community compositions could serve as early warnings of ENM-induced dysbiosis. Finally, establishing standardized protocols and NM research modules, shared data repositories, and inter-laboratory collaborations will be essential to ensure reproducibility, regulatory trust, and comprehensive understanding of long-term ENM–microbiome interactions.

10 Conclusion

ENMs are transforming numerous industries due to their unique physicochemical properties, yet their interactions with microbial ecosystems raise significant ecological and health concerns. This review highlights the profound effects of ENMs on human-associated microbiota and environmental microbial communities, revealing their capacity to alter microbial diversity, metabolic activity, and functional roles in nutrient cycling and host health. Metal-based NPs, such as Ag, TiO_2, ZnO, and CNMs, can induce dysbiosis, impair enzymatic activities critical for biogeochemical processes, and potentially accelerate the spread of antimicrobial resistance genes. While advanced methodologies like high-throughput sequencing, meta-omics approaches, and *in vitro*/*in vivo* models have deepened our understanding of ENM–microbiome interactions, challenges persist in standardizing protocols and assessing long-term risks. Given the dual nature of ENMs, offering both technological advantages and potential ecological hazards, it is imperative to adopt a precautionary approach toward their deployment. Future research should focus on elucidating dose- and context-dependent effects, exploring safer-by-design NMs, and developing regulatory frameworks that account for microbiome sensitivity. Only through such multidisciplinary efforts we can ensure the sustainable use of nanotechnologies while preserving microbial diversity and ecosystem resilience.

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