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Review Article

Exploring the Effect of Engineered Nanomaterials on Soil Microbial Diversity and Functions: A Review

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ABSTRACT

This review explores the impact of engineered nanomaterials (ENMs) on soil microbial diversity, function, metabolic pathways, and resilience. With the increasing application of ENMs in agriculture and industrial fields, understanding their interaction with soil microorganisms is crucial. We examine various types of ENMs, their physicochemical properties, and how these influence soil microbial communities. This review highlights the dual role of ENMs, demonstrating both beneficial and detrimental effects on microbial diversity and activity. Fundamental interaction mechanisms, such as altering metabolic pathways and microbial community structure, are discussed. Additionally, we address the implications of ENM-induced changes for soil health and agricultural productivity. Further, the review provides insights into the complex dynamics of ENMs in soil ecosystems and outlines directions for future research to optimize their use while minimizing environmental risks.

Keywords: Engineered nanomaterials; Soil microbial diversity; Microbial metabolic pathways; Soil ecosystem resilience; Nanomaterial-microbe interactions.

1. INTRODUCTION

The rapid growth of engineered nanomaterials (ENMs) has significantly revolutionized various scientific and industrial fields, including agriculture (Barhoum *et al.* 2022). These ENMs, distinguished by their small size, high reactivity, and substantial surface area-to-volume ratio, have found widespread applications, extending from medicine to enhancing agricultural practices (Sampathkumar *et al*. 2020). However, this very ubiquity in the environment, particularly in soil due to widespread agrarian use, wastewater irrigation, and industrial activities, has sparked a pressing need to understand their interactions with biological systems, most critically with microbial communities.

Notably, physical interactions of ENMs, such as adherence to microbial cell walls and potential membrane disruptions, alongside chemical interactions, including oxidative stress and cellular damage caused by toxic ions from metallic nanoparticles, showcase a complex interplay of effects (Gardea *et al*. 2014; Tong *et al*. 2007). The accumulation of nanoparticles within plant tissues can significantly alter the rhizosphere, impacting plant-microbe interactions essential for plant health and soil fertility (Judy *et al*. 2015a).

2. DIVERSE LANDSCAPE OF ENMs AND IMPACT ON SOIL MICROORGANISMS

The agricultural sector is experiencing a significant transformation with the introduction of ENMs, which are revolutionizing farming practices through their unique properties and applications. These nanomaterials, ranging from metal nanoparticles to carbon nanotubes, leverage their small size and extensive surface area to interact with microbes and plants, enhancing agricultural efficiency and sustainability. This transformation is characterized by diverse applications such as soil and water remediation, plant protection, and the delivery of agrochemicals, demonstrating the multifaceted impact of ENMs on agriculture (Carnovale *et al*. 2016; Farrow and Kamat, 2009; Gatoo *et al*. 2014; Tsang *et al*. 2017).

The physicochemical properties of ENMs, including particle size, shape, surface charge, and chemical composition, play crucial roles in dictating their interactions with soil microbial communities. Smaller particles, for example, penetrate microbial membranes more effectively, while the shape and surface charge influence aggregation and adhesion dynamics. These properties collectively contribute to the transformative impact of ENMs on agriculture, influencing everything from microbial diversity to the efficiency of agrochemical delivery (Gatoo *et al*. 2014; Walkey *et al.* 2012).

In addition to the direct applications, ENMs interact with soil microorganisms and their communities in intricate and profound ways, impacting key processes such as nutrient cycling, organic matter decomposition, and microbial metabolic pathways (Tables 1 and 2). These interactions can be synergistic or antagonistic, with certain nanoparticles (NPs) like, ZnO NPs enhancing the activity of nitrogen-fixing bacteria, while others like Ag NPs stimulate phosphate-solubilizing bacteria in the soil. Conversely, some ENMs can disrupt essential microbial processes, especially under acidic conditions or in the presence of environmental factors like, temperature and moisture levels (Bundschuh *et al*. 2018; Suazo *et al*. 2023; Suresh *et al*. 2013).

Moreover, the development of nanocomposites and advancements in nanoemulsions, nanocapsules, and nanofertilizers mark significant strides in optimizing agricultural efficiency. These technologies enable slow-release of fertilizers and controlled agrochemical release, improving solubility, stability, and bioavailability and minimizing environmental impact (Umair *et al*. 2023; Usman *et al*. 2020).

Biosynthesized nanoparticles, derived from plants or microorganisms, present an eco-friendly alternative for pest control and plant growth, demonstrating the potential for sustainable and non-toxic production methods in agriculture. These biosynthesized ENMs offer a promising avenue for addressing environmental concerns while maintaining agricultural productivity (Changcheng *et al*. 2022; Ghidan and Al, 2020).

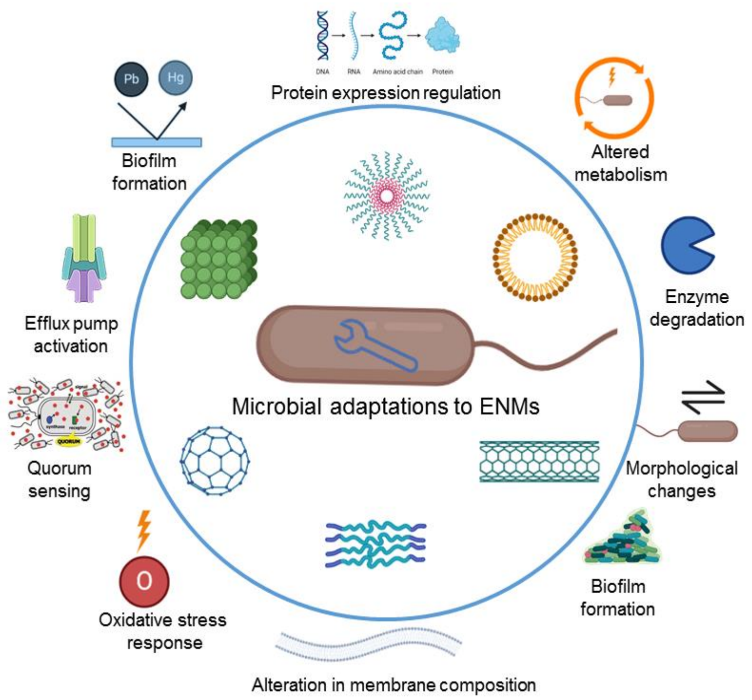

Fig. 1: Microbial adaptation strategies against ENMs

The long-term effects of ENMs on microbial communities, particularly under chronic exposure, remain a topic of ongoing investigation. It is crucial to understand the balance between the benefits and potential risks associated with ENMs in agriculture, as they can form within microbial cells or be involved in extracellular biomineralization, impacting biogeochemical cycling and environmental remediation. These dynamics highlight the complexity, significance of ENMs in environmental health, stability and their role in the future of sustainable agriculture (Carboni *et al*. 2021; Mansor and Xu, 2020; Moore *et al*. 2016a). Engineered nanoparticles (ENPs) exhibit remarkable antimicrobial properties against diverse microbes, including bacteria like *Escherichia coli* and *Staphylococcus aureus* (Yin *et al*. 2020).

2.1. Direct Physical Impacts on Microbes

2.1.1 Membrane disruption

Membrane disruption efficacy of ENPs can be attributed to their direct physical impacts on microbial membranes. For instance, the shape and size of ENPs like Ag NPs play a crucial role. Rod-shaped Ag NPs, with sharp edges, are more adept at puncturing and disrupting bacterial membranes compared to their spherical counterparts (Debashish *et al.* 2018; Urnukhsaikhan *et al.* 2021). This mechanism mirrors the process of receptor-mediated endocytosis, where nanoparticle size dictates cellular uptake efficiency (Shang *et al*. 2014).

While some ENPs directly damage membranes, others like TiO_2 NPs induce lipid peroxidation, indirectly weakening the membrane barrier (Erdem *et al*. 2015). This process disrupts normal metabolic activities and can even alter the metabolic profiles of entire microbial communities (Judy *et al*. 2015b). The multifarious impacts of ENPs extend beyond membrane disruption. Interestingly, some NPs like nanoscale zero-valent iron (nZVI) possess unique redox properties that influence microbial populations in complex ways (Hegde *et al.* 2016). Additionally, certain ENPs can activate efflux pumps in bacteria, specialized mechanisms for expelling toxins and contributing to antibiotic resistance (Modi *et al*. 2023).

2.1.2 Internalization

The internalization of ENPs by soil microbes through mechanisms like endocytosis and phagocytosis represents a complex process (Makvandi *et al*. 2021). Endocytosis is the primary route for ENP uptake in bacteria and fungi, involving membrane invagination to engulf the ENP into an intracellular vesicle (Fazel *et al.* 2020). The process includes different pathways, such as clathrin-mediated, caveolae-mediated endocytosis, and macropinocytosis, each with unique size and cargo preferences (Kou *et al*. 2013). Conversely, phagocytosis targets larger particles and occurs in specialized immune cells in certain soil organisms (Liu *et al*. 2020). Additionally, ENPs can adhere to microbial cell surfaces *via* adsorption, involving electrostatic attraction, van der Waals forces, and hydrophobic interactions, influencing cellular processes and potentially leading to secondary uptake mechanisms (Desmau *et al*. 2020).

2.1.3 Oxidative stress

Engineered nanoparticles with reactive surfaces pose a significant threat to biological systems due to their ability to generate reactive oxygen species (ROS) upon contact with cell membranes. This interaction unleashes a cascade of deleterious effects, primarily through oxidative stress. The resulting membrane damage, characterized by lipid and protein oxidation and leakage of cellular contents, is particularly concerning for metal oxide nanoparticles such as TiO_2. The mechanisms underlying this oxidative stress are complex, involving electron transfer, photocatalysis, and metal ion release, ultimately leading to cellular injuries like lipid peroxidation, protein oxidation, and DNA damage (Horst *et al*. 2013).

Studies on specific ENPs, such as ZnO and TiO_2, further highlight their capacity to induce oxidative stress in microbes, evidenced by increased ROS production (Laudadio *et al*. 2018). The oxidative stress wreaks havoc on cellular components like DNA, proteins, and lipids, disrupting critical enzymatic functions and vital processes like energy production and nutrient uptake (Kumar *et al*. 2011; Xiao *et al*. 2021).

2.1.4 Electrostatic interactions

Charged ENPs, particularly cationic particles, crucially interact with cell membranes, disrupting the electrostatic balance and increasing membrane permeability. Such interactions are influenced by factors like charge density, size, and shape of ENPs, affecting membrane-bound proteins and enzymes (George *et al.* 2023). Increased cellular permeability can lead to membrane deformation and pore formation, potentially causing cytotoxicity. Recent studies emphasize the importance of electrostatic features in modeling membrane-associated molecules and highlight the interdependence between mechanical and electrostatic properties of cell membranes (Lee *et al*. 2019). Understanding these interactions is critical for predicting nanoparticle behavior in biomedical applications (Zhang *et al*. 2021). Furthermore, nanoparticles like multi-walled carbon nanotubes can increase soil electrical conductivity, stimulating electrochemically active bacteria in denitrification and metal reduction (Baroja *et al*. 2021) processes.

2.2 Indirect Effect on Microbes through the Soil

Incorporating ENPs into soil ecosystems initiates a series of complex effects, significantly altering the soil's physical characteristics and the dynamic microbial communities within. Initially, ENPs impact the soil structure, particularly affecting porosity. This alteration, though seemingly minor, can have substantial implications. For example, increased pore space facilitates aerobic bacteria access to oxygen and nutrients, disrupting established microbial niches and enabling new microbial entities (Grün *et al*. 2019).

Table 1. Effect of engineered nanomaterials on microbial activities

Interaction Type	ENM Type	Microbial Species	Effect on Microbes	Interaction Mechanism in Cell	References
Synergistic	Fe_3O_4 NPs	*Rhizobium leguminosarum*	Enhanced nitrogen fixation	Improved iron availability for enzymatic processes	(Prasad *et al*. 2014)
	Ag NPs	*Escherichia coli*	Enhanced antibacterial activity	Disruption of microbial membrane integrity	(Adeleke *et al*. 2022)
	ZnO NPs	*Pseudomonas putida*	Increased metabolic activity	Nanoparticle-microbe surface interactions enhancing nutrient uptake	(Raliya and Tarafdar, 2013)
Antagonistic	Carbon nanotubes (CNTs)	*Nitrosomonas europaea*	Growth inhibition	Disruption of membrane integrity and oxidative stress	(Jin *et al*. 2013)
	CuO NPs	*Staphylococcus aureus*	Reduced cell viability	Release of ionic copper leading to protein and DNA damage	(Ren *et al*. 2009)
	Fullerene (C60)	*Escherichia coli*	Decreased bacterial growth	Lipid peroxidation and membrane disruption	(Lyon *et al*. 2006)
	TiO_2 NPs	*Bacillus subtilis*	Growth inhibition	Oxidative stress and damage to cellular components	(Adams *et al*. 2006)
	ZnO NPs	Marine phytoplankton *Thalassiosira pseudonana*	Toxicity	Zn^{2+} ions release from NP	(Miao *et al*. 2010)
	CuO, ZnO, TiO_2, silver and fullerene NPs	Recombinant *Escherichia coli* strain	Growth inhibition	Reactive oxygen species-related ecotoxicity	(Ivask *et al*. 2010)
	$CuSO_4$ and CuO NPs	Recombinant *Pseudomonas fluorescens*	Variation in bioavailable copper impacting bacterial response	Unknown	(Käkinen *et al*. 2011)
Resistance against NPs	Complex metal oxide NPs	*Shewanella oneidensis* MR-1	Rapid resistance development upon chronic exposure	Unknown	(Mitchell *et al*. 2019)

Table 2. Effect of engineered nanomaterials on microbial community

Microbial Community Type	Effect	Responsible Nanoparticle/	Reference
Soil bacterial community	Change in bacterial community composition	TiO_2 and ZnO NPs	(Ge *et al*. 2011)
Soil rhizosphere microorganisms	Altered metabolite profiles	SiO_2, TiO_2, Fe_3O_4 NPs	(Zhao *et al*. 2019)
Soil microbial community	Upregulation/downregulation of genes	TiO_2 NPs	(Simonin and Richaume, 2015)
Soil microbial community	Altered microbial community and metabolic profile	Ag NPs	(Zhang *et al*. 2020a)
Fungi and bacteria community involved in leaf litter decomposition	Negative effect on community structure and litter decomposition	Ag NPs	(Pu *et al*. 2019)
Soil microbial community	Redox reactions alteration	Iron in clay minerals	(Ilgen *et al*. 2019)

Moreover, the influence of ENPs is not limited to structural modifications. They can also modulate soil properties such as pH and cation exchange capacity (CEC). These changes are pivotal in determining bacterial viability. Alterations in pH can selectively favor certain bacterial groups, reorganizing the microbial community (Kibbey and Strevett, 2019). Variations in CEC, critical in nutrient and trace element availability, can further refine microbial interactions, introducing changes to the soil microbial composition.

2.2.1 Surface charge and interaction with microbial cell walls

The interaction between ENPs and microbial cells, especially bacteria, reveals the critical role of surface charge in their complex relationship. Positively charged ENPs are drawn to the negatively charged bacterial walls due to electrostatic attraction, originating from lipopolysaccharides and teichoic acids on the bacterial surface that contain negatively charged groups (Desmau *et al*. 2020). Such interaction leads to substantial changes in bacterial behavior, notably affecting quorum sensing, a key communication mechanism that controls biofilm formation, virulence, and antibiotic resistance (Zhou *et al*. 2020). Some ENPs can inhibit quorum sensing pathways, reducing biofilm formation and virulence. Moreover, ENP-bacterial interactions influence biofilm formation, with some ENPs enhancing it by acting as bridges between cells, while others disrupt it (Wang *et al*. 2022). These interactions have significant implications on the composition of microbial communities, affecting soil functionality, nutrient cycling, plant growth, and ecosystem health.

2.2.2 Redox reactions and ROS generation

The complexities of ENP effects extend to the redox potential (Eh), a key factor in bacterial metabolism and electron transfer processes. Engineered nanoparticles can alter this balance, introducing novel challenges and competitive dynamics for bacterial species, especially those proficient in redox reactions (Khanna *et al*. 2021). Consequently, this can result in electrical conductivity (EC) changes, indicative of salt concentrations. Elevated EC levels, often resulting from ENP interactions, can impose osmotic stress on bacteria, necessitating a shift towards salt-tolerant species and adding diversity to the microbial community (Pangajam *et al.* 2020).

Cerium oxide nanoparticles (CeO_2 NPs), known as redox nanoparticles (RNPs), are characterized by their ability to undergo redox reactions, generating reactive oxygen species like hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-). These RNPs, particularly CeO_2, are effective in scavenging ROS due to the interchangeable oxidation states of cerium (Ce^{3+} and Ce^{4+}), making them regenerative. The synthesis methods, stabilizing agents, and Ce^{3+}/Ce^{4+} surface ratio of CeO_2 NPs significantly influence their biological effects, including prooxidant toxicity or antioxidant protective effects. The CeO_2 NPs mimic enzyme activities like catalase and superoxide dismutase and have applications in modulating intracellular oxygen environments, angiogenesis, and bacterial growth inhibition (Sadowska and Bartosz, 2018).

2.2.3 Dissolution and release of ions in soil

The dissolution and ion release of ENPs in soil, involving elements like Ag^+ and Zn^{2+}, are influenced by nanoscale properties and soil characteristics. Research indicates nanoparticle size, shape, surface charge, and coating significantly impact their dissolution rates. Smaller nanoparticles dissolve more rapidly, while coatings can impede the process (Meulenkamp, 1998). Soil factors, including pH, organic matter, and ionic composition, affect ENP stability and release (Rawat *et al.* 2018). Acidic soils can hasten metal ion release from ENPs (Suazo *et al.* 2023). Techniques like single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS) are pivotal in studying such dynamics (Wojcieszek and Ruzik, 2022).

2.2.4 Influence on soil organic matter

Engineered nanoparticles influence soil organic matter (SOM), essential for sustaining heterotrophic bacteria. By modifying the composition of SOM and affecting the bioavailability of nutrients, particularly phosphorus, ENPs can disrupt the complex cycle of nutrient acquisition and utilization managed by bacteria. This disruption can have cascading effects throughout the soil ecosystem, potentially undermining its health and capability to support plant growth (Grün *et al*. 2019). The extensive range of ENP effects, encompassing soil structure, pH, CEC, Eh, EC, SOM, and nutrient dynamics, highlights the complex network of interactions that characterize the soil microbiome.

3. RESILIENCE AND ADAPTATION OF MICROBIAL COMMUNITIES TO ENMs

Microbial communities, integral to environmental processes, display remarkable adaptability in the face of ENMs. These communities employ various strategies to mitigate the challenges posed by ENMs, encompassing genetic mutations, physiological variations, and horizontal gene transfer.

Mutation is a crucial adaptation mechanism, as seen in microbes exposed to Ag NPs, which develop resistance to counteract the nanoparticles' antimicrobial properties (Judy *et al.* 2015a). Besides genetic changes, physiological responses such as biofilm formation provide a protective barrier, while efflux pumps remove harmful ENMs from cells (Sobhanipoor *et al*. 2022).

Additionally, microbes combat oxidative stress induced by metal oxides through an enhanced antioxidant system (Mehla *et al.* 2021).

Horizontal gene transfer further boosts microbial resilience, allowing rapid sharing of resistance genes across communities effectively immunizing populations against ENMs (Chen *et al*. 2021). The agricultural use of ENMs, particularly in nanofertilizers and nanopesticides, significantly affects soil microbiomes, altering microbial abundance and diversity and necessitating an understanding of long-term effects (Khan *et al.* 2021; Zhang *et al*. 2020b). Concerns include the development of microbial resistance to nanoparticles, with microbes adapting through mechanisms like oxidative stress tolerance and membrane alterations (Kamat and Kumari, 2023).

Microbes harness ENMs for beneficial purposes by forming biofilms on nanoparticles, aiding in biocontrol and crop management (Bhatia *et al*. 2021). They act as nanofactories, transforming heavy metals into elemental nanoparticles for environmental remediation (Bahrulolum *et al.* 2021). This adaptability extends to biotechnology and biomedicine, where microbes synthesize nanomaterials for various applications (Grasso *et al*. 2019). However, the risk of microbial resistance to these antimicrobial nanomaterials remains a concern (Zhang and Zhang, 2020).

To navigate these challenges, a comprehensive research strategy is essential. Studies should focus on mapping the impacts of nanomaterials, designing microbes for efficient nanomaterial synthesis, and understanding the ecological consequences of ENMs (Sun *et al*. 2022; Naughton and Boedicker, 2021).

Applications such as the combination of aptamers and noble metal nanomaterials for microbial toxin detection (He *et al*. 2020) and the control of microbial redox activity for synthesizing unique nanomaterials like magnetic oxides (McFarlane *et al.* 2015) demonstrate the broad potential of microbial interactions with ENMs.

4. NAVIGATING THE FUTURE: CHALLENGES, OPPORTUNITIES, AND RESPONSIBLE DEVELOPMENT

4.1 Harnessing the Power of Synergistic Interactions

Engineered nanomaterials interact synergistically with soil microorganisms, significantly impacting ecosystems and microbial communities. Such synergy is crucial in enhancing plant growth and nutrient uptake. TiO_2 NPs stimulate phosphate-solubilizing bacteria, increasing plant phosphorus availability (Kaur *et al*. 2022). In bioremediation, ENMs combined with soil microbes expedite contaminated soil remediation. Fe_3O_4 NPs aid and enhance organic pollutant degradation. Graphene oxide NPs effectively removes heavy metals from soils, aiding microbial adsorption and detoxification. In agriculture, ENMs are used as carriers for biocontrol agents, ensuring targeted delivery and sustained release for disease and pest control. Silica nanoparticles with *Bacillus subtilis* spores control fungal diseases, and neem-coated silver NPs combat agricultural pests.

4.2 Bridging Knowledge Gaps and Refining Risk Assessment

The study of the impact of ENMs on microbial communities is an emerging field that faces several significant challenges that hinder a complete understanding of its long-term ecological and health implications (Parani *et al*. 2016; You and Bonner, 2020). A critical issue in this field is the lack of long-term studies, as most existing studies are short-term and provide limited insight into the chronic effects and long-term ecological consequences of ENM exposure on microbial communities (Wu *et al*. 2021a; Wu *et al.* 2021b). This gap in research leaves many questions unanswered regarding the sustainability and safety of ENM use, especially in environmental and agricultural contexts (Nyberg *et al*. 2008; Ur *et al*. 2021). Another challenge in extensive application is the wide variability in the characteristics of ENMs. The size, shape, composition, and coating of ENMs vary greatly, making it difficult to generalize findings across different materials (Stegemeier *et al*. 2017; Suresh *et al*. 2013). Such variability complicates the understanding of specific characteristics that influence interactions between microbes and ENMs, adding complexity to the research (Tong *et al*. 2007; Zheng *et al*. 2016).

Additionally, while the effects of ENMs on microbial communities are increasingly documented, there is an inadequate understanding of the underlying mechanisms at molecular and cellular levels (Mendoza and Brown, 2019; Navya and Daima, 2016). The lack of detailed knowledge limits the ability to predict and mitigate potential adverse effects of ENMs on microbial ecosystems (Hegde *et al*. 2016; Judy *et al*. 2015a). The intricacies of environmental interactions further complicate this field of study. The interactions among ENMs, microbes, and various environmental factors, particularly under field conditions, are complex and poorly understood (Sun *et al*. 2022; Huali *et al*. 2019). These interactions can vary significantly depending on numerous environmental parameters, making it challenging to extrapolate laboratory findings to natural ecosystems (Judy *et al*. 2015a; Moore *et al*. 2016b). Lastly, there is a concerning lack of comprehensive studies on the potential impacts of ENMs on human health, especially those used in agriculture (Connolly *et al*. 2022; Fadeel *et al*. 2013). It is crucial to understand how ENMs affect human health through the food chain, given the increasing use of these materials in agricultural practices (Grasso *et al*. 2019; Naughton and Boedicker, 2021). Addressing these challenges requires a concerted effort from the scientific community to conduct long-term, detailed studies considering the variability of ENM characteristics, unravelling the mechanisms of interaction at molecular and cellular levels, and assessing complex environmental interactions and potential impacts on human health (Md *et al.* 2022; Tang *et al.* 2021). Such efforts are essential to ensure the responsible and safe use of ENMs in various applications (Kang and Mauter, 2009).

4.3 Shaping the Future with Responsible Governance

In the realm of agriculture, the advent of ENMs promises to revolutionize traditional practices through innovations like nanoparticle-based fertilizers, nanopesticides, and advanced soil and water remediation techniques (Jiang *et al*. 2022; Raliya *et al*. 2018). These groundbreaking advancements, offering targeted nutrient delivery and enhanced crop protection, have been made possible by the unique properties of ENMs (Kah *et al.* 2019; Li *et al*. 2023). However, alongside these benefits, deploying ENMs in agriculture introduces a spectrum of environmental challenges and ethical considerations, necessitating a comprehensive approach to governance and safety.

Addressing the potential impacts of ENMs on human health and societal equity is paramount. This challenge calls for ethical sourcing, fair labor practices throughout the ENM lifecycle, and transparent communication with stakeholders, including farmers, consumers, and policymakers. Such measures are crucial for building trust and guiding the ethical development of agricultural nanotechnology.

To effectively manage the long-term implications of ENMs, comprehensive policy frameworks that include environmental risk assessment, safety standards, labeling requirements, and responsible disposal practices are essential. Collaborative efforts between scientists, policymakers, and stakeholders can play a vital role in crafting adaptable and effective policies for the development, testing, and deployment of ENMs in agriculture (Karn *et al*. 2009; Predoi *et al.* 2020).

Long-term monitoring programs are indispensable in this context, as they provide critical insights into the environmental and health impacts of ENMs. These programs should encompass assessments of soil, water, and air quality, as well as the health of agricultural workers and consumers. The data gleaned from effective monitoring will inform adaptive management strategies, ensuring the responsible and sustainable use of ENMs.

The introduction of nanosensors in precision agriculture epitomizes the transformative potential of ENMs. Such technologies are reshaping farming practices by enabling accurate monitoring of soil health, moisture levels, and nutrient status (Kah *et al*. 2019). Further research is crucial to further our understanding of the interactions between nanomaterials and the soil-plant system, with a focus on developing eco-friendly, biodegradable nanomaterials that minimize ecological impact (Khan *et al*. 2021; Kumari *et al*. 2023).

4.4 Potential for Biotechnological Applications

The potential applications of ENMs in agriculture, environmental management, and disease control are particularly noteworthy for their revolutionary impact and biotechnological promise. Applying ENMs as nano-fertilizers, nano-pesticides, and nano-based biosensors is a groundbreaking agricultural development. Such ENMs are reforming the soil microbiome, altering the abundance and diversity of microbes. This advancement supports plant growth and conserves essential soil bacteria for nutrient transport, marking a significant leap in agricultural biotechnology (Salem and Husen, 2023). Furthermore, the complex interplay between soil, plants, microbes, and ENMs, governed by biotic and abiotic factors, opens up new avenues for research and application in soil science and plant biology (Vera *et al*. 2023).

In environmental management, the role of ENMs is equally transformative. Their impact on vital microbial processes like nitrogen fixation, mineralization, and plant growth promotion is a testament to their potential. The behavior of ENMs in soil, contingent on their properties and those of the soil, offers innovative strategies for environmental remediation and microbial management (Khan *et al.* 2021; Salem and Husen, 2023). The diverse applications of ENMs in soil remediation, particularly in modifying the environmental behavior of ENMs and their interactions with soil constituents, represent a significant advancement in environmental biotechnology (Lewis *et al.* 2019; Qian *et al*. 2020). However, the potential toxicity of ENMs to critical soil bacteria, which could indirectly affect plant growth, underscores the need for meticulous research and development in this area.

With pesticide-resistant and novel pathogenic microorganisms threatening global food security, ENMs stand out with their antimicrobial properties. These properties make ENMs an excellent candidate for managing plant diseases in agriculture, heralding a new era in plant pathology and microbial control (Avila *et al.* 2022; Hussain *et al.* 2023). The progress in nanodiagnostics for plant diseases further emphasizes the critical role of ENMs in the timely detection and management of plant diseases, crucial for maintaining agricultural sustainability and securing the global food supply (Li *et al.* 2020). Additionally, the advent of nanohybrid antifungals, showcasing improved efficiencies in pest and pathogen control, highlights the innovative steps in agricultural nanotechnology (Alghuthaymi *et al*. 2021).

4.5 Recommendations for Future Research

The advancement of our understanding of ENMs in agriculture requires a comprehensive and forward-looking research strategy. Emphasizing long-term field studies is essential to comprehend the persistent effects of ENMs on microbial communities and ecosystem functions. These studies are crucial for understanding the ecological consequences and sustainability of ENM use, particularly under natural conditions. To facilitate comparative research and foster a holistic understanding of ENMs in environmental contexts, standardizing research methods, especially in characterizing ENMs and assessing their microbial impacts, is imperative (Mortimer *et al*. 2021; Zhang *et al.* 2020b).

Advanced molecular biology techniques and omics technologies are critical in investigating the molecular mechanisms of microbial responses to ENMs. These approaches will help predict and manage ENM impacts on microbial ecosystems. Simultaneously, understanding the synergistic effects of ENMs with other environmental stressors and assessing potential human health risks in agriculture is vital to ensure food safety and public health. Developing comprehensive risk assessment frameworks considering ecological, human health, and socio-economic factors are also important for a balanced evaluation of ENMs in agriculture (Lewis *et al*. 2019).

Regarding the application of ENMs, their implications on soil microorganisms, crucial for soil health and ecosystem balance, necessitate a thorough investigation. This measure requires a multifaceted methodological approach, including advanced molecular techniques like metagenomics and proteomics, to study soil microbial communities. Understanding the functional implications of ENMs on soil microbes requires metabolic profiling and enzyme assays. Integrating laboratory and field studies in experimental designs will provide a more comprehensive understanding, with laboratory studies dissecting mechanical details and field studies assessing the impacts under natural conditions.

The development of standardized microbial toxicity tests is essential for the toxicological assessment of ENMs in soil ecosystems. These tests should account for the unique attributes of ENMs and their interactions with other agricultural inputs. A biological approach, coupled with predictive modeling is vital for synthesizing diverse methodological insights and forecasting the long-term effects of ENMs (Lewis *et al*. 2019).

Prioritizing the development of sustainable ENM applications is crucial, aiming to harness their benefits while minimizing ecological and health risks. Research should also focus on the environmental fate of ENMs, their influence on soil health, and their interactions with nutrient cycling, soil structure, and plant-microbe relationships. Informing regulatory policies with scientific progression and engaging various stakeholders, including farmers, industry experts, policymakers, and the public, is key to the responsible development and implementation of ENMs in agricultural systems (Bora *et al*. 2022; Leanne *et al.* 2020).

5. CONCLUDING PERSPECTIVES

The review highlighted the significant impact of ENMs on microbial communities, revealing substantial alterations in their diversity, structure, and metabolic functions. These changes can have far-reaching implications for ecosystem balance and agricultural systems. Microbial adaptation to ENMs, through genetic changes, biofilm formation, and efflux pump activation, showcases their resilience. Yet, the complexity of ENM-microbe interactions—spanning synergistic to antagonistic effects—is influenced by environmental factors like pH, temperature, and organic matter.

Current understanding of these interactions is evolving, with long-term ecological impacts and interaction mechanisms gaps. The potential of ENMs in environmental and agricultural applications necessitates a cautious approach, considering ecological and human health implications. Future research should focus on long-term field studies and molecular-level analysis to guide policy-making. As we advance in nanotechnology, a balanced approach is crucial in applying ENMs. Understanding their dual role as both beneficial and stressful to ecosystems is critical. Informed and responsible use of ENMs is vital for protecting ecological integrity and human health, ensuring that nanotechnology supports sustainable development without compromising natural system equilibrium.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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