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Review Article

Ecotoxicological Assessment of Nanomaterials Used in Agriculture: Impacts on Soil Microorganisms and Non-Target Species

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Abstract: The Rapid Integration of Engineered Nanomaterials (ENMs) into agricultural practices particularly as nano-fertilizers, nano-pesticides, and soil amendments has raised critical concerns regarding their unintended ecotoxicological consequences. While nanotechnology offers substantial agronomic benefits, comprehensive understanding of its impacts on soil microorganisms and non-target species remains limited. This study systematically evaluates the ecotoxicological effects of agriculturally applied nanomaterials on soil microbial communities and representative non-target organisms across multiple trophic levels.

Using a combination of standardized ecotoxicity assays, soil enzyme activity profiling, microbial diversity analyses, and organism-level bioassays, the effects of commonly used nanomaterials, including metal-based, metal oxide, and carbon-based nanoparticles, were assessed. Results indicate that ENM exposure can significantly alter soil microbial biomass, enzymatic activities, and community structure, primarily through mechanisms involving oxidative stress, membrane disruption, and altered nutrient cycling. Non-target organisms such as earthworms, beneficial arthropods, and soil microfauna exhibited dose-dependent physiological and behavioral responses, highlighting potential risks to ecosystem functioning.

Importantly, the magnitude of ecotoxicological effects was strongly influenced by nanoparticle physicochemical properties, application rates, soil characteristics, and exposure duration. This study underscores the necessity for integrative, multi-scale risk assessment frameworks that bridge laboratory findings with field-level realities. The findings contribute critical insights toward the development of safer nano-enabled agricultural inputs and inform regulatory strategies aimed at minimizing ecological risks while sustaining agricultural productivity.

Keywords: Agricultural nanomaterials, Ecotoxicology, Soil microorganisms, Non-target species, Environmental risk assessment.

Introduction

Nanotechnology has emerged as a transformative tool in modern agriculture, offering innovative solutions to enhance crop productivity, nutrient use efficiency, and pest management. Engineered nanomaterials (ENMs), including metal-based, metal oxide, and carbon-based nanoparticles, are increasingly incorporated into agrochemicals such as fertilizers, pesticides, and soil conditioners due to their unique physicochemical properties [1,2]. These materials exhibit high surface area-to-volume ratios, enhanced reactivity, and tunable surface functionalities, enabling targeted delivery and controlled release of active ingredients [3]. While such advancements contribute to precision agriculture and resource optimization, they also raise concerns regarding the unintended ecological consequences of ENM release into agroecosystems.

Soil is a primary sink for agriculturally applied nanomaterials, where they interact directly with complex biotic and abiotic components. Soil microorganisms play a pivotal role in maintaining ecosystem functions, including organic matter decomposition, nutrient cycling, and soil structural stability [4]. Exposure to ENMs has been shown to alter microbial biomass, enzyme activities, and community composition, potentially disrupting these essential processes [5,6]. The extent of microbial response varies depending on nanoparticle characteristics such as size, concentration, surface coating, and solubility, as well as soil properties including pH, texture, and organic matter content [7,8].

These interactions underscore the need for systematic evaluation of ENM–microbe dynamics under environmentally relevant conditions.

Beyond microorganisms, agriculturally applied nanomaterials may pose risks to non-target soil-dwelling organisms that are integral to ecosystem functioning. Earthworms, nematodes, collembolans, and beneficial arthropods contribute to soil aeration, nutrient redistribution, and biological control of pests [9]. Studies have reported dose-dependent toxic effects of ENMs on non-target species, including reduced survival, impaired reproduction, oxidative stress, and behavioral alterations [10,11]. Such effects raise concerns regarding trophic transfer and bioaccumulation of nanoparticles within soil food webs, potentially extending ecological risks beyond the point of application [12].

Ecotoxicological responses to nanomaterials are often governed by complex mechanisms distinct from those of conventional chemicals. Nanoparticle-induced toxicity may arise from reactive oxygen species (ROS) generation, membrane damage, metal ion release, and interference with cellular signaling pathways [13,14]. Moreover, transformations of ENMs in soil such as aggregation, dissolution, and surface modification can significantly influence their bioavailability and toxicity over time [15]. These dynamic processes complicate hazard assessment and challenge the applicability of existing regulatory frameworks, which are largely designed for bulk chemicals rather than nanoscale materials [16].

Given the rapid expansion of nano-enabled agricultural products, a comprehensive ecotoxicological assessment that integrates effects on soil microorganisms and non-target species is urgently required. Current knowledge remains fragmented, with many studies conducted under laboratory conditions that may not accurately reflect field-level exposure scenarios [17]. This review synthesizes current experimental evidence from microbiological, ecotoxicological, and molecular studies to evaluate how agriculturally applied nanomaterials influence soil microbial communities and non-target organisms, thereby providing an integrated perspective on ecological risks associated with nano-enabled agriculture.

Agricultural Applications of Engineered Nanomaterials and Environmental Entry Pathways

The application of engineered nanomaterials (ENMs) in agriculture has expanded rapidly due to their functional advantages in improving nutrient delivery, pest control, and crop resilience. ENMs such as silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO_2), iron oxide, and carbon-based nanomaterials are widely incorporated into nano-fertilizers, nano-pesticides, and soil conditioners [1,3]. Following application, these materials are inevitably released into soil systems through direct amendment, foliar wash-off, irrigation runoff, and degradation of nano-formulations, making agricultural soils a primary environmental reservoir for ENMs [16].

Once introduced, ENMs undergo physicochemical transformations such as aggregation, dissolution, and surface modification, which influence their mobility and bioavailability in soil matrices (15). These processes determine the extent of exposure to soil biota and non-target organisms, thereby shaping ecotoxicological outcomes.

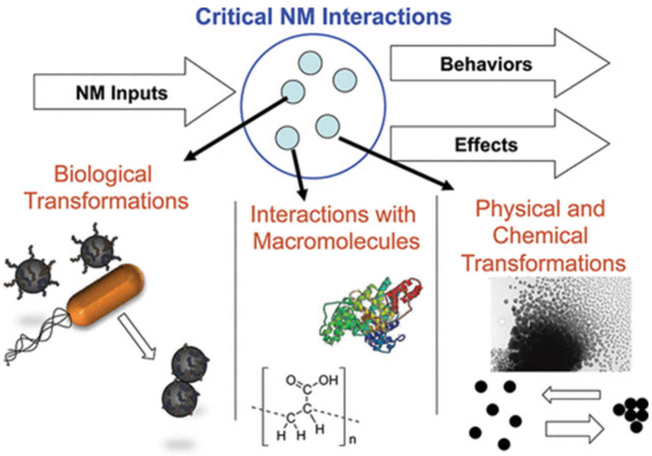

Figure 1: Nanomaterial transformations are critical processes affecting NM interactions. Transformations include physical and chemical transformations, biologically mediated transformations, and interactions with macromolecules and biomacromolecules (Lowry et al. (2012) and Cornelis et al. (2014)).

2. Ecotoxicological Effects of Nanomaterials on Soil Microbial Communities

Soil microorganisms form the foundation of terrestrial ecosystem functioning by regulating nutrient cycling, organic matter turnover, and soil structural integrity [4]. Exposure to ENMs has been reported to alter microbial biomass, enzymatic activity, and taxonomic diversity, often resulting in impaired soil functionality [5,6]. Metal-based nanoparticles, in particular, can induce oxidative stress through excessive reactive oxygen species (ROS) production, leading to membrane damage and metabolic disruption in microbial cells [14].

The sensitivity of microbial communities to ENMs is strongly influenced by nanoparticle concentration, size, surface charge, and soil properties such as pH and organic matter content [7]. These interactions can shift microbial community structure, favoring resistant taxa while suppressing beneficial functional groups involved in nitrogen fixation and phosphorus solubilization.

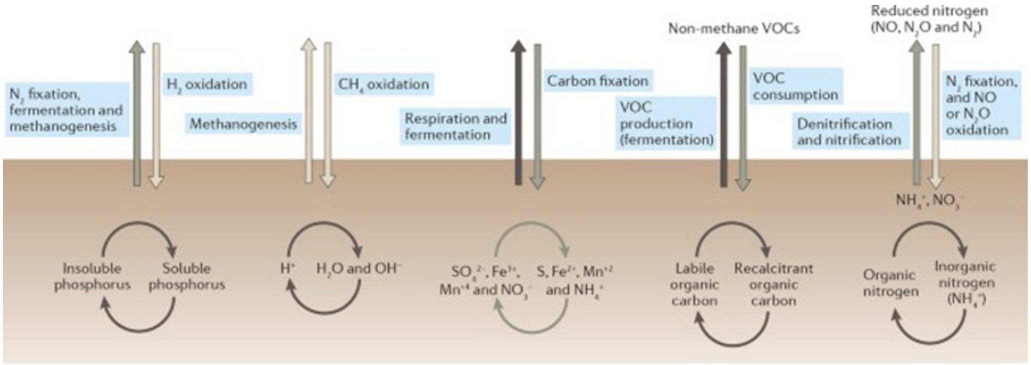

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Figure 2: Soil biogeochemical processes that can be modulated by the soil microbiome (Fierer (2017) and Simonin & Richaume (2015)).

Microbiological Evidence of Nanoparticle Effects on Soil Microbiota

Several experimental microbiological studies have investigated the impact of engineered nanomaterials on soil microbial communities using culture-based and molecular approaches. For instance, Ge et al. (2020) demonstrated that exposure to TiO_2 and ZnO nanoparticles significantly altered bacterial community composition in agricultural soils, with reductions observed in sensitive taxa involved in nutrient cycling [26]. Similarly, Simonin and Richaume (2015) reported that metal-based nanoparticles can suppress microbial biomass and reduce enzymatic activities associated with nitrogen mineralization and organic matter decomposition.

Studies employing high-throughput sequencing techniques have further revealed that nanoparticle exposure can lead to shifts in dominant microbial phyla such as Proteobacteria, Actinobacteria, and Firmicutes, suggesting selective toxicity toward sensitive microbial populations [27]. In addition, Cornelis et al. (2014) highlighted that soil physicochemical properties strongly influence microbial responses by regulating nanoparticle aggregation, dissolution, and bioavailability [28].

Laboratory microcosm experiments have also shown that silver and copper nanoparticles may inhibit nitrifying bacteria and phosphate-solubilizing microorganisms, thereby affecting key biogeochemical processes essential for soil fertility. These microbiological findings collectively indicate that nanomaterial exposure can lead to both structural and functional alterations in soil microbial ecosystems, reinforcing the need for long-term ecological monitoring of nano-enabled agricultural inputs [29].

3. Toxicological Impacts on Non-Target Soil Fauna and Trophic Transfer

Non-target soil organisms, including earthworms, nematodes, collembolans, and beneficial arthropods, play a critical role in soil aeration, organic matter fragmentation, and nutrient redistribution [9]. Numerous studies have documented dose-dependent toxic effects of ENMs on these organisms, including reduced survival, reproductive impairment, oxidative stress, and behavioral anomalies [10,8]. Such effects compromise soil ecosystem services and raise concerns regarding long-term soil health.

Additionally, ENMs may undergo trophic transfer through soil food webs, resulting in bioaccumulation and potential biomagnification [12]. These processes extend toxicological risks beyond the soil compartment, potentially affecting higher trophic organisms and agroecosystem stability.

4. Risk Assessment Challenges and Regulatory Implications in Nano-Agriculture

Assessing the ecotoxicological risks of agricultural nanomaterials remains challenging due to their dynamic behavior in soil environments and their distinct mechanisms of toxicity compared to conventional chemicals [13]. Standardized ecotoxicity testing frameworks often fail to capture nanoparticle-specific transformations such as dissolution and surface reactivity, leading to uncertainty in hazard characterization [17]. Moreover, most toxicity studies are conducted under controlled laboratory conditions, limiting their relevance to field-scale agricultural systems.

Current regulatory frameworks, including REACH and OECD guidelines, are still evolving to address nano-specific risk assessment requirements [16]. Integrating microbial- and organism-level responses with physicochemical characterization is essential for developing robust safety thresholds and guiding the design of safer-by-design nanomaterials.

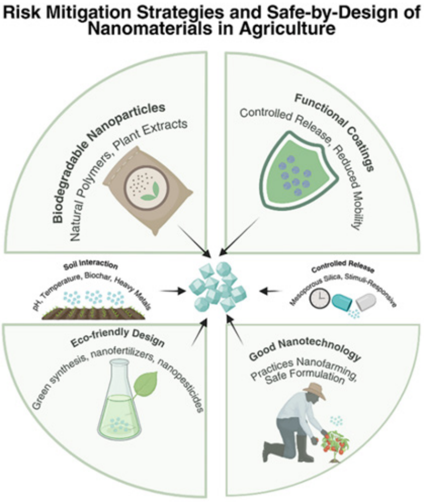

Figure 3: Integrated ecotoxicological risk assessment framework for agricultural nanomaterials (Holden et al. (2016) and Kah et al. (2019)).

Synthesis of Experimental Evidence on Nanomaterial-Induced Ecotoxicity

The studies summarized in Table 1 collectively demonstrate that engineered nanomaterials used in agricultural systems can exert measurable ecotoxicological effects across multiple biological levels. Early foundational work established oxidative stress and reactive oxygen species generation as central mechanisms driving nanoparticle toxicity, providing a mechanistic framework for subsequent organism- and ecosystem-level investigations [13]. Later experimental studies expanded this understanding by documenting significant alterations in soil microbial biomass, enzyme activity, and community structure following exposure to metal-based and metal oxide nanoparticles [5,6]. These microbial disruptions have direct implications for soil biogeochemical processes, including nutrient cycling and organic matter decomposition, thereby linking nanotoxicological effects to broader agroecosystem functioning.

Emerging Patterns, Knowledge Gaps, and Research Directions

Despite substantial progress, the comparative analysis presented in Table 1 reveals considerable variability in reported ecotoxicological outcomes, largely attributable to differences in nanoparticle properties, exposure concentrations, and experimental design [7,17]. Several studies highlight dose-dependent toxicity and trophic transfer potential in non-target organisms such as earthworms and soil invertebrates, raising concerns regarding long-term ecological resilience and food web stability [12,10]. However, the predominance of short-term laboratory assays limits the extrapolation of these findings to field-scale agricultural systems. Future research should prioritize long-term, multi-trophic, and field-relevant studies while integrating physicochemical characterization with biological endpoints to support robust risk assessment and safer-by-design nanomaterial development [1,16].

Table 1: Key Scientific Studies on Ecotoxicological Impacts of Agricultural Nanomaterials.

Scientist(s)	Nanomaterial Studied	Major Findings	Reference
Nel et al.	Various engineered nanomaterials	Identified oxidative stress and ROS generation as primary toxicity mechanisms at the nanoscale	[13]
Simonin & Richaume	Metal-based nanoparticles	Reported significant alterations in soil microbial biomass and enzymatic activity	[5]
Cornelis et al.	Metal oxide nanoparticles	Demonstrated soil-dependent bioavailability and toxicity of ENMs	[7]
Tourinho et al.	Ag, ZnO nanoparticles	Observed adverse effects on soil invertebrate survival and reproduction	[8]
Unrine et al.	Cu nanoparticles	Documented trophic transfer and bioaccumulation in soil food webs	[12]
Hu et al.	TiO_2 nanoparticles	Reported oxidative stress and reduced growth in earthworms	[10]
Ge et al.	TiO_2, ZnO nanoparticles	Identified shifts in bacterial community composition	[6]
Kah et al.	Nano-agrochemicals	Highlighted exposure pathways and potential ecological risks	[1]
Holden et al.	Multiple ENMs	Emphasized need for environmentally relevant ecotoxicity testing	[17]
Keller et al.	Global ENM releases	Estimated large-scale environmental release from agricultural use	[16]

Nanomaterial Aging, Transformation, and Long-Term Toxicity in Agricultural Soils

Following their application in agricultural fields, engineered nanomaterials undergo aging processes that significantly alter their physicochemical properties and toxicological behavior. Environmental transformations such as oxidation, sulfidation, dissolution, and interaction with soil organic matter can reduce or, in some cases, enhance nanomaterial toxicity over time [18,19]. For example, sulfidation of silver nanoparticles has been shown to decrease acute toxicity but increase persistence in soils, thereby posing long-term exposure risks to soil biota.

These aging-induced transformations challenge traditional ecotoxicological testing approaches that rely on pristine nanomaterials, which may not accurately represent real environmental conditions. Incorporating aged nanomaterials into toxicity assessments is therefore essential for realistic risk evaluation.

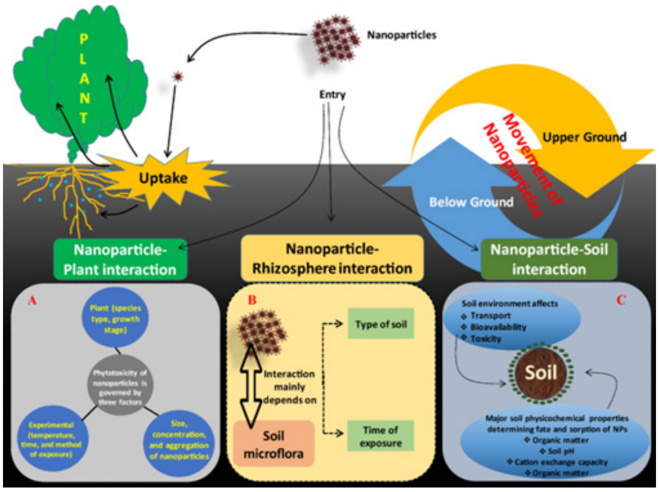

Figure 4: Nanoparticles in the soil-plant system (Miralles et al. (2012) and Rico et al. (2017)).

Omics-Based Approaches for Assessing Nanotoxicity in Agroecosystems

Recent advances in omics technologies, including metagenomics, transcriptomics, proteomics, and metabolomics, have revolutionized the assessment of nanomaterial-induced stress in soil ecosystems. These approaches provide high-resolution insights into molecular-level perturbations that precede observable physiological or population-level effects [20,21].

Omics-based studies have revealed that nanomaterials can alter gene expression related to oxidative stress response, membrane transport, and nutrient metabolism in soil microorganisms, even at sub-lethal concentrations. By linking molecular responses to functional outcomes, omics tools enable early detection of ecological disturbance and improve mechanistic understanding of nanotoxicity. However, the integration of omics data into regulatory frameworks remains limited due to challenges in data interpretation and standardization.

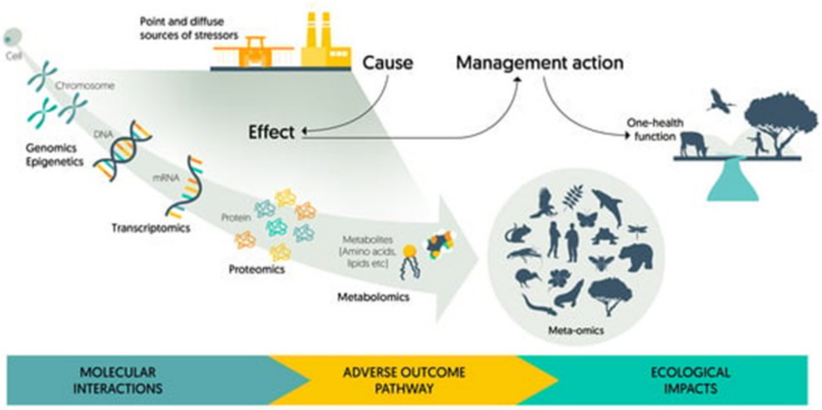

Figure 5: Omics-based framework for nanotoxicity assessment in soil ecosystems
(Chen et al. (2018) and Benelli et al. (2020)).

Combined Effects of Nanomaterials and Co-Occurring Agricultural Contaminants

In real-world agricultural settings, nanomaterials rarely occur in isolation but coexist with other contaminants such as pesticides, fertilizers, heavy metals, and emerging pollutants. Interactions between nanomaterials and co-contaminants can result in synergistic, antagonistic, or additive toxic effects, complicating ecological risk assessments [22,23]. For instance, nanomaterials may act as carriers for pesticides, increasing their bioavailability and toxicity to non-target organisms.

Understanding these combined effects is critical, as current regulatory testing frameworks typically assess single substances in isolation. Future ecotoxicological studies must therefore adopt mixture-toxicity approaches to reflect realistic agricultural exposure scenarios.

Linking Soil Ecotoxicology to Food Safety and Human Health

Nanomaterial-induced disruptions in soil ecosystems may have indirect yet significant implications for food safety and human health. Uptake and translocation of nanoparticles from soil to plants can introduce nanomaterials into the food chain, raising concerns about dietary exposure and chronic health effects [24,25]. Moreover, alterations in soil microbial communities can affect nutrient availability and crop quality, further influencing food system sustainability.

These findings highlight the necessity of adopting a “soil–plant–human” continuum perspective when evaluating the safety of agricultural nanotechnology. Integrating ecotoxicological data with food safety and human health risk assessments will be essential for holistic governance of nano-enabled agriculture.

Influence of Nanomaterial Physicochemical Properties on Ecotoxicological Outcomes

The ecotoxicological behavior of engineered nanomaterials in agricultural soils is strongly governed by their physicochemical characteristics, including particle size, shape, surface charge, crystallinity, and coating materials. Variations in these properties can significantly alter nanoparticle reactivity, dissolution rates, and interaction potential with biological membranes [13,7]. For instance, smaller nanoparticles with higher surface area-to-volume ratios tend to exhibit enhanced biological reactivity, increasing the likelihood of oxidative stress and cellular damage in soil microorganisms and invertebrates [14]. Surface functionalization, often employed to improve agricultural efficiency, may further modify toxicity by influencing aggregation behavior and bioavailability in soil environments.

Importantly, the same nanomaterial may exhibit contrasting toxicological responses under different environmental conditions due to transformations such as agglomeration, sulfidation, or organic matter coating [15]. These dynamic changes complicate hazard prediction and emphasize the need for standardized characterization protocols alongside biological testing. Understanding structure–activity relationships is therefore essential for linking nanomaterial design with ecological safety and for advancing safer-by-design strategies in nano-enabled agriculture [1].

Soil Properties as Modulators of Nanomaterial Toxicity

Soil physicochemical properties play a critical role in mediating the fate, transport, and toxicity of agricultural nanomaterials. Parameters such as pH, clay content, cation exchange capacity, and organic matter concentration directly influence nanoparticle aggregation, dissolution, and retention within soil matrices [7,8]. High organic matter content, for example, can reduce nanoparticle toxicity by promoting surface passivation and limiting direct contact with soil biota, whereas acidic conditions may enhance metal ion release and toxicity from metal-based nanoparticles [6].

These soil-mediated effects contribute to the observed variability in ecotoxicological outcomes across studies and limit the generalizability of laboratory findings to field conditions. Consequently, ecotoxicity assessments that fail to account for soil heterogeneity may either underestimate or overestimate environmental risks. Integrating soil-specific parameters into experimental designs and risk assessment models is therefore essential for accurately evaluating nanomaterial impacts in realistic agricultural scenarios [17].

Implications for Soil Ecosystem Services and Agricultural Sustainability

Disruptions to soil microbial communities and non-target organisms induced by nanomaterial exposure can have cascading effects on ecosystem services critical to sustainable agriculture. Microbial-mediated processes such as nitrogen mineralization, phosphorus solubilization, and carbon sequestration are particularly vulnerable to shifts in microbial diversity and functional potential [4,5]. Similarly, declines in soil fauna populations may impair soil structure formation, aeration, and organic matter turnover, ultimately reducing soil fertility and crop productivity [9].

These findings challenge the perception of nano-enabled agricultural inputs as inherently sustainable and highlight the importance of balancing agronomic benefits with ecological integrity. Long-term field studies and ecosystem-level assessments are needed to evaluate whether short-term productivity gains may lead to unintended degradation of soil ecosystem services over time [16,1].

Toward Safer-by-Design Nanomaterials for Agricultural Applications

The concept of safer-by-design nanomaterials has gained increasing attention as a proactive approach to minimizing environmental and ecological risks while maintaining functional performance. By modifying nanoparticle composition, size, or surface chemistry, it may be possible to reduce bioavailability and toxicity without compromising agricultural efficacy [13,17].

Incorporating ecotoxicological endpoints early in the material development process can facilitate the identification of low-risk formulations suitable for agricultural deployment.

However, implementing safer-by-design strategies requires interdisciplinary collaboration among material scientists, ecotoxicologists, agronomists, and regulatory agencies. Aligning innovation with environmental safety will be critical for ensuring the responsible integration of nanotechnology into sustainable agricultural systems and for strengthening public and regulatory confidence in nano-enabled solutions [1,16].

Conclusion

The present review consolidates current knowledge on the ecotoxicological impacts of engineered nanomaterials used in agriculture, with particular emphasis on their interactions with soil microorganisms and non-target species. Evidence from laboratory and mesocosm studies clearly demonstrates that agriculturally applied nanomaterials can alter microbial community structure, enzymatic activity, and key soil biogeochemical processes, as well as induce physiological and behavioral changes in soil fauna. These effects are primarily driven by nanoparticle-specific mechanisms such as oxidative stress generation, membrane disruption, and metal ion release, underscoring that nanomaterials exhibit toxicological behaviors distinct from their bulk counterparts. While nano-enabled agricultural inputs offer agronomic advantages, their ecological implications necessitate careful evaluation to ensure long-term soil health and ecosystem stability.

A critical insight emerging from this review is the strong influence of nanomaterial physicochemical properties and soil characteristics on ecotoxicological outcomes. Particle size, surface chemistry, and solubility interact dynamically with soil parameters such as pH, organic matter content, and texture, resulting in highly context-dependent toxicity responses. This variability explains the inconsistent findings reported across studies and highlights the limitations of generalized hazard assessments. Consequently, ecotoxicological evaluations that integrate material characterization with soil-specific factors are essential for improving the reliability and environmental relevance of risk assessments in nano-agriculture.

From an applied perspective, the reviewed literature emphasizes the need to balance innovation with environmental safety through the adoption of safer-by-design approaches. Incorporating ecotoxicological endpoints early in nanomaterial development, along with improved regulatory frameworks tailored to nanoscale materials, can mitigate unintended ecological risks. Moreover, harmonization of testing protocols and alignment with international guidelines will be crucial for translating scientific evidence into actionable regulatory policies that support sustainable agricultural practices while safeguarding soil biodiversity.

Despite significant advances, several critical research gaps remain. Most existing studies rely on short-term laboratory assays that fail to capture chronic, low-dose exposures and long-term ecological effects under realistic field conditions. There is a notable lack of multi-trophic and ecosystem-level studies that examine trophic transfer, bioaccumulation, and functional impacts on soil ecosystem services. Additionally, limited attention has been given to the combined effects of nanomaterials with other agricultural stressors such as pesticides, microplastics, and climate variability.

Addressing these gaps through long-term, field-based, and interdisciplinary research will be essential for developing robust risk assessment frameworks and ensuring the responsible and sustainable integration of nanotechnology into agricultural systems.

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