



Universiteit
Leiden
The Netherlands

Irreversible immune dysfunction in earthworms from MoS 2 nanosheet exposure: implications for nanoenabled agricultural sustainability

Sun, K.; White, J.C.; Lynch, I.; Zhang, P.; He, E.; Peijnenburg, W.J.G.M.; Qiu, H.

Citation

Sun, K., White, J. C., Lynch, I., Zhang, P., He, E., Peijnenburg, W. J. G. M., & Qiu, H. (2026). Irreversible immune dysfunction in earthworms from MoS 2 nanosheet exposure: implications for nanoenabled agricultural sustainability. *Environmental Science And Technology*, 60(4), 3093-3106. doi:10.1021/acs.est.5c14710

Version: Publisher's Version

License: [Licensed under Article 25fa Copyright Act/Law \(Amendment Taverne\)](#)

Downloaded from: <https://hdl.handle.net/1887/4307967>

Note: To cite this publication please use the final published version (if applicable).

Irreversible Immune Dysfunction in Earthworms from MoS₂ Nanosheet Exposure: Implications for Nanoenabled Agricultural Sustainability

Kailun Sun, Jason C. White, Iseult Lynch, Peng Zhang,* Erkai He,* Willie J.G.M. Peijnenburg, and Hao Qiu*



Cite This: *Environ. Sci. Technol.* 2026, 60, 3093–3106



Read Online

ACCESS |

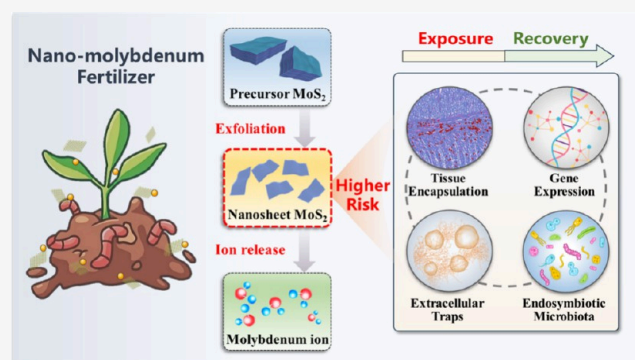
Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Nano-MoS₂ is emerging as a promising agricultural nanomaterial. However, nanoenabled agriculture must balance the proposed benefits with potential risks associated with material incorporation into soil matrices. This work establishes a full-process risk assessment profile for MoS₂ encompassing precursor particles, exfoliated nanosheets, and dissolved ionic species using the soil invertebrate *Eisenia fetida* under exposure-recovery cycles. The results showed that although precursor MoS₂ exposure at 5–50 mg Mo/kg for 7 days significantly activated ($p < 0.001$) earthworm lumbricinase (9.4%), antimicrobial peptide (10.2–14.0%), and hemolysin (11.9%) levels compared to the control, the biota exhibited transient immune memory and persistence upon another 7 days of recovery. Once exfoliated to nanosheet form at a consistent 5–50 mg Mo/kg dose range, more pronounced ion release (3.8–6.4%) and prolonged *in vivo* accumulation (43.9–55.4% excluded) of MoS₂ nanosheets mediated persistent immune-related gene initiation. Importantly, the filamentous extension of external DNA between immune cells and deterministic clustering of endosymbiotic microbial communities were shown to collaboratively establish the *in vivo* homeostasis after MoS₂ nanosheet exposure. These findings mechanistically demonstrate the persistent stress on soil invertebrate immune systems under MoS₂ nanosheet exposure and highlight the need to link nanoenabled agriculture to soil eco-sustainability.

KEYWORDS: nanoenabled agriculture, MoS₂ nanosheets, soil fauna, immune, risk



INTRODUCTION

Nanotechnology offers new and impactful opportunities for sustainable agriculture.^{1,2} However, a holistic perspective beyond simple crop incentives is needed to ensure that long-term sustainability and soil health are key endpoints for consideration.³ Two-dimensional layered molybdenum disulfide (MoS₂) nanomaterials, including multilayer precursor MoS₂ particles and few-layer MoS₂ nanosheets, are emerging as promising agricultural nanomaterials due to their antioxidant enzyme mimicking activity, large surface area for integration of micronutrients, and antibacterial properties compared to conventional ionic molybdenum fertilizers, enabling the promotion of crop stress resistance and disease resilience.^{4–7} The promotion of agricultural applications is anticipated to dramatically increase global MoS₂ production and market deployment beyond existing usage of 1.1×10^5 tons/year,^{8,9} thereby escalating incorporation into soil ecosystems as the ultimate sink. Importantly, various forms of molybdenum-based fertilizers (precursor particles, quasi-monolayer nanosheets, and conventional molybdenum ions) not only are

directly applied to soils but also undergo continuous transformation from multilayered to quasi-monolayer structures, eventually releasing ionic molybdenum species due to their inherent layered nature and soluble characteristics. Therefore, it is essential to transcend the conventional paradigm of ecological risk assessment based on any single molybdenum form. Instead, a full-process evaluation covering the continuous life cycle changes of nano-MoS₂ from exfoliation to ionic release is needed to understand the potential impacts of molybdenum-based fertilizers integration into the soil ecosystem on soil biota. This will advance the safe and sustainable application of these promising agricultural nanomaterials.

Received: October 16, 2025

Revised: January 1, 2026

Accepted: January 2, 2026

Published: January 16, 2026



Earthworms are a major component of animal biomass in soils, serving as representative models for assessing soil biota quality.¹⁰ *Eisenia fetida* is recommended by the Organization for Economic Cooperation and Development (OECD) and the International Organization for Standardization (ISO) as a standard test organism in soil ecotoxicology, owing to its short reproductive cycle, sensitivity to environmental pollutants, and ease of maintenance under controlled laboratory conditions.^{11,12} Although a large literature has documented the response of *Eisenia fetida* to exogenous stress with impacts on growth, reproduction, metabolism, and energy supply,^{13–15} these biological end points, which are linked to a broad range of biological functional components, do not always uniformly reflect their health status, particularly regarding exposure to sublethal doses. The earthworm immune system is a sensitive center that dynamically coordinates a broad range of other organs and represents a suitable system for a comprehensive evaluation of the health status in response to stressors. Importantly, many key questions regarding how the earthworm immune system recognizes and defends against different molybdenum forms have not yet been systematically evaluated in real soil and at potential environmentally relevant concentrations. For soil-inhabiting biota, heterogeneous contaminant distribution and earthworm mobility to avoid direct contaminant contact often result in complex and intermittent exposure scenarios. This pattern of alternating exposure and recovery cycles is not addressed by current hazard and risk estimation protocols. Consequently, understanding the recovery profile after stress is a critical component of assessing the system response under dynamic exposures.

In this work, we hypothesized that the earthworm immune system would exhibit distinct response patterns and tolerance mechanisms toward molybdenum-based fertilizers encompassing precursor material (PrMoS₂), the exfoliated product (ExMoS₂), and the eventually released ions (Na₂MoO₄) under an exposure-recovery cycle consistent with natural scenarios (uneven distribution of contaminants) and earthworm habits (peristalsis and avoidance activity). To test this hypothesis, a mechanistic whole-chain risk assessment framework with *Eisenia fetida* was established, covering MoS₂ nanosheets from the precursor to exfoliation to dissolution. The orthogonal evaluation by machine learning enabled biological imaging, and high-throughput molecular techniques were employed to mechanistically understand the phenotypic and molecular responses of *Eisenia fetida* upon exposure to different molybdenum forms. 16S rRNA sequencing was applied to elucidate the critical role of the host's endosymbiotic microbiota in collaboratively mediating immune defense against xenobiotic exposure. This work enhances our understanding of soil invertebrate immune defense in response to agricultural nanomaterials under realistic exposure scenarios, and underscores the importance of dose threshold control and safety redesign protocols for any promising agricultural nanomaterial before widescale commercial applications.

MATERIALS AND METHODS

Exfoliation of MoS₂ Nanosheets from Bulk MoS₂ Particles

Bulk MoS₂ particles were first obtained by a high-temperature sulfidation approach using molybdenum trioxide (MoO₃) and sulfur powder as the molybdenum and sulfur sources, respectively. The monolayer or quasi-monolayer MoS₂ nanosheets were then exfoliated from bulk MoS₂ precursor particles, assisted by alkali metal ions and ultrasonic assistance.¹⁶ The specific exfoliation and characterization

methods are described in Text S1 and Text S2 in the Supporting Information. Details on the reagents used in this study are given in Text S3.

Experimental Design

The tested clean soil (sandy loam, pH = 7.47 ± 0.12, soil organic matter = 69.27 ± 2.1 g/kg d.w. (dry weight), background molybdenum concentrations = 0.037 ± 0.013 mg/kg d.w.; n = 3; see Text S2 for characterization methods) was taken from the plow layer (0–20 cm) of agricultural land located in Shanghai City, China, at 30°56′02″ N, 121°23′36″ E. The collected soil samples were air-dried and passed through a sieve with a pore size of 2 mm to ensure particle uniformity. A total of 7 nominal exposure concentrations were established: 1) clean soil (d.w.) as control; 2) 5 mg Mo/kg and 3) 50 mg Mo/kg PrMoS₂ in soil (d.w.); 4) 5 mg Mo/kg and 5) 50 mg Mo/kg ExMoS₂ in soil (d.w.); 6) 5 mg Mo/kg and 7) 50 mg Mo/kg Na₂MoO₄ in soil (d.w.) as ionic control to assess the toxicity contribution of molybdenum with different valence states. The established exposure concentration gradients fall within the predicted environmentally relevant levels of MoS₂ (model predictions ranging from ppb to ppm) as well as the dose range reported in existing toxicity assessments,^{9,16–20} and consistent with the sublethal doses suitable for earthworm stable survival as determined by preliminary experiments.

Before formal exposure, the test soils were digested (90 °C for 1 h + 145 °C for 45 min) with a mixture of HNO₃ and HCl in a volume ratio of 1:3. After the contents were cooled to ambient temperature, the solution was filtered and diluted to 10 mL with ultrapure water and then immediately quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES, iCAP-PRO, Thermo Fisher, U.S.A.) to determine the actual exposure concentrations; the detected concentrations indicate a satisfying agreement with nominal settings and confirm the successful establishment of effective and comparable concentration gradients (Table S1). As for the ion release content of PrMoS₂ and ExMoS₂ in the tested soils during 7 days, the soil leachate was isolated using gradient suction filtration after shaking incubation, followed by ultrafiltration treatment. The ionic molybdenum content was quantified by ICP-OES without digestion.

The earthworms, *Eisenia fetida*, were collected from a vermiculture facility in Jurong City, Jiangsu province, China. After acclimation under controlled conditions at 25 ± 1 °C, 50% humidity, and a 12 h/12 h light/dark cycle until stable survival, 10 earthworms with visible clitella and uniform weight (0.4–0.45 g) were depurated (24 h, using disposable Petri dishes with moist filter paper replaced every 12 h)²¹ and collectively introduced into each concentration treatment as a single exposure unit. Each treatment set had 6 independent biological replicates initially. Given that the sensitive immune system of earthworms typically exhibits defense responses after short-term exposure and can rapidly enter an adaptation period once exposure ends, we optimized the exposure protocols recommended by the OECD guidelines. Specifically, following a 7 day exposure period, earthworms from three replicates were collected for immediate analyses of immunological parameters at the tissue, cellular, and molecular levels. The remaining replicates were transferred to unamended soils and allowed to recover for an additional 7 days to evaluate the resilience of the induced immune response.

Earthworm Immune Response in Body Wall Tissues

Earthworms collected after the exposure and recovery phases were depurated and fixed in 4% paraformaldehyde for 24 h, and 3 μm sections were then cut from paraffin-embedded tissue. Tissue sections were specifically stained for brown bodies with iron dye (ferric trichloride: potassium ferricyanide: deionized water = 7.5:1:1.5), followed by differentiation with 1% acetic acid and counterstaining with neutral red. After dehydration in anhydrous ethanol, the stained sections were scanned using a digital slide scanner (Pannoramic-MIDI, 3DHISTECH). Simultaneously, the accumulation of different molybdenum forms in the depurated earthworm tissues was quantified by using the aforementioned ICP-OES method.

For image analysis assisted by machine learning, the Cell Detection module in QuPath was first run to define all cells in the given scanned

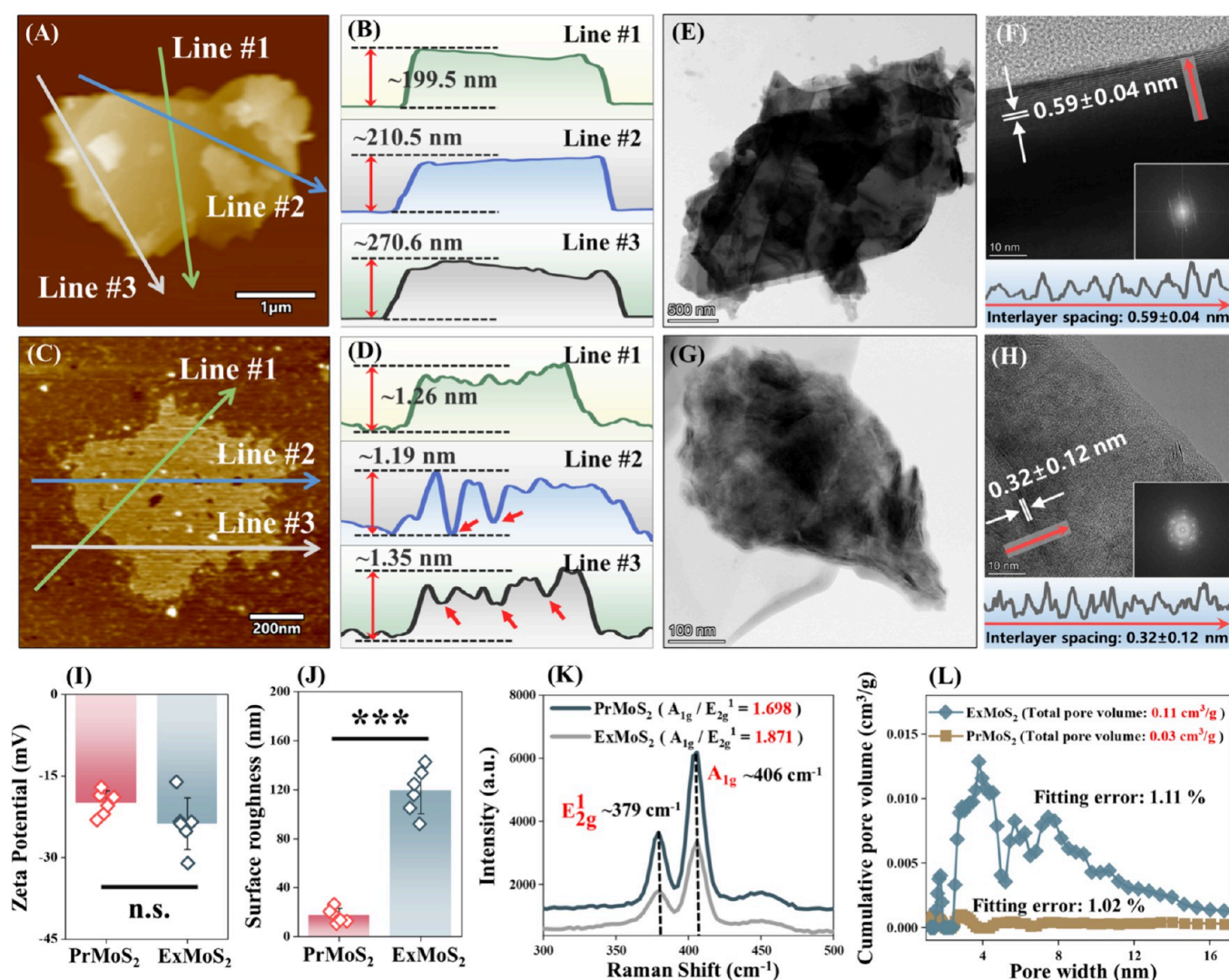


Figure 1. Characterization of precursor MoS₂ particles (PrMoS₂) and exfoliated MoS₂ nanosheets (ExMoS₂). Representative atomic force microscopy (AFM) images and height profiles of (A, B) PrMoS₂ and (C, D) ExMoS₂. The red arrows in the height curves highlight the intrinsic defect on the nanosheet lattice. Transmission electron microscopy (TEM) images and corresponding high-resolution patterns of (E, F) PrMoS₂ and (G, H) ExMoS₂. The electron diffraction patterns are inserted in the bottom-right of panels (F) and (H), and the data marked in the panel represent the mean spacing between 20 lattice points selected along the red arrow path (mean ± SD). (I) Zeta potential and (J) calculated surface roughness of PrMoS₂ and ExMoS₂; data are expressed as mean ± SD (*n* = 6); the distribution of the raw data is shown as individual data points in the panels. Statistically significant differences between two types of MoS₂ (PrMoS₂ and ExMoS₂) are expressed as (n.s.) *p* > 0.05 and (***) *p* < 0.001. (K) Raman spectra of PrMoS₂ and ExMoS₂. (L) Cumulative pore volume distribution and the total pore volume from Brunauer–Emmett–Teller (BET) analysis of PrMoS₂ and ExMoS₂.

image. Subsequently, customized regions of stained brown bodies were created, and a corresponding interactive random forest classification trainer was built. The custom annotated regions were trained for continuous classification and applied to all cells within the specified region to automatically quantify the proportion of tissue brown bodies.²²

Quantification of Whole-Animal Functional Immune Factor Levels

A double antibody sandwich system was used to quantify the whole-animal immunological factor levels in earthworms. Specifically, purified macromolecular functional immunoreceptors (lumbrokinase and polyphenol oxidase) and small molecular immunological factors (antimicrobial peptide, hemolysin, cytolytic factor, melanin, and lipofuscin) were used to coat enzyme-linked immunosorbent assay (ELISA) microplates and incubated overnight at 4 °C to prepare the corresponding solid-phase antibodies. Nonspecific binding was blocked using bovine serum albumin sealing buffer. The earthworm

tissue homogenate was subsequently added and conjugated with a horseradish peroxidase (HRP) labeled antibody. After thorough washing, the substrate 3,3',5,5'-tetramethylbenzidine (TMB) was used for color development upon HRP catalysis for 30 min at 37 °C. The absorbance at 450 nm was immediately recorded on a microplate reader (SpectraMax iD5, Thermo Fisher, U.S.A.).

Binding Mechanisms of Different Molybdenum Forms to Earthworm Macromolecular Functional Immunoreceptors

A mixture containing lumbrokinase (1 μmol/L), polyphenol oxidase (0.1 μmol/L), phosphate buffer solution (PBS, 0.2 mol/L), and different molybdenum dispersions/solutions (PrMoS₂, ExMoS₂, and Na₂MoO₄) at 0.1 mg Mo/L was incubated at 25 ± 1 °C for 40 min. The UV–visible spectra were acquired on a UV–visible spectrophotometer (V3100PC, MAPADA, China) at 190–380 nm. The resonance light scattering spectra (EX = EM = 200–600 nm) and synchrotron fluorescence spectra (EM = 265/310 nm, EX = 250–350 nm, Δλ = 15/60 nm) were recorded using a fluorescence

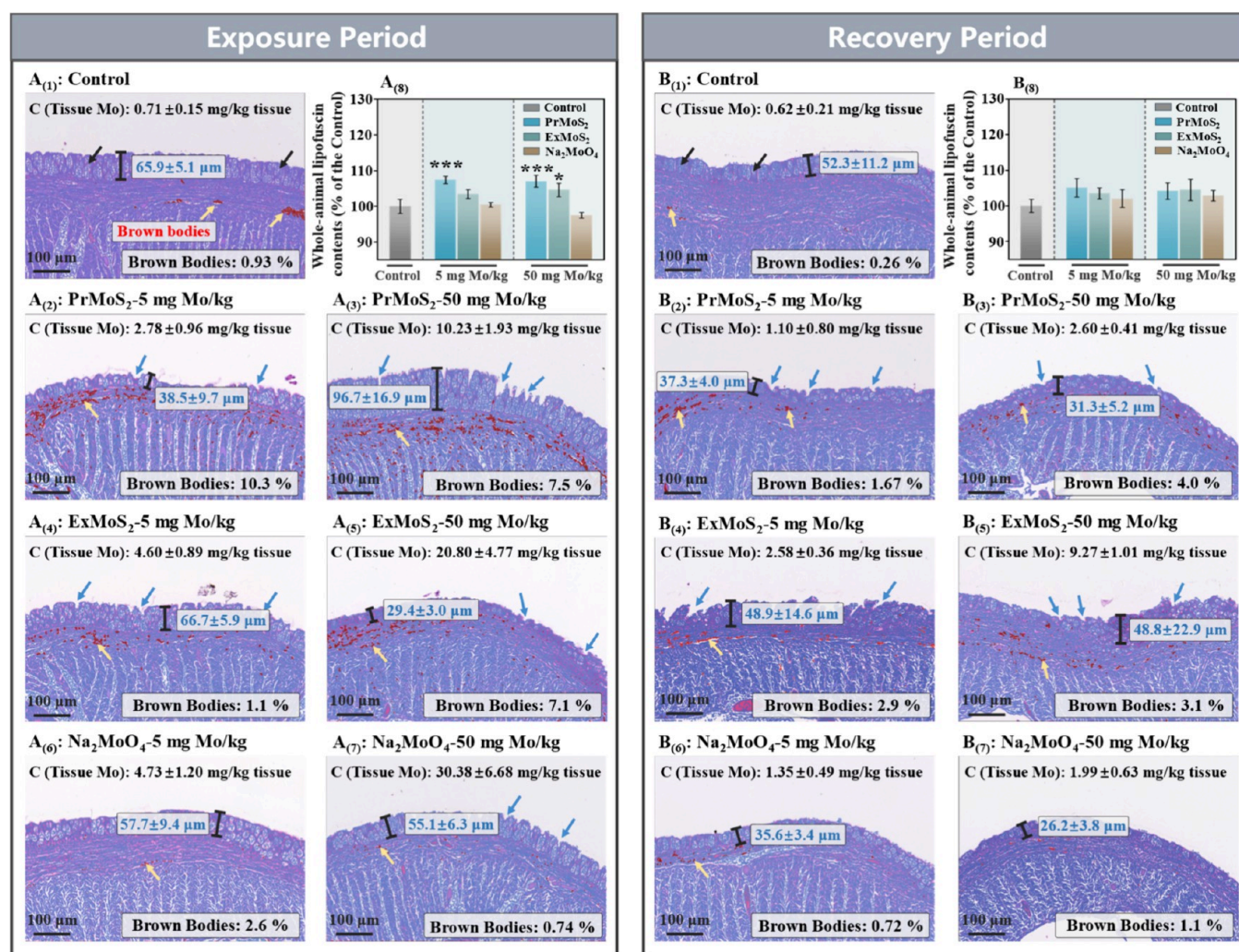


Figure 2. Barrier immune response profile of earthworms (*Eisenia fetida*) upon exposure to PrMoS₂, ExMoS₂, and their ionic counterpart (Na₂MoO₄) at concentrations of 5 mg Mo/kg soil (d.w.) and 50 mg Mo/kg soil (d.w.) for (A₁–A₇) 7 days followed by (B₁–B₇) another 7 days of recovery in clean soil. Black arrows indicate goblet cells, and the blue arrows indicate epithelium damage. Machine learning-based image recognition assists in the spatial distribution and relative quantification of tissue brown bodies (highlighted with yellow arrows), and the proportional area occupied by brown bodies was calculated and marked in the lower right corner of the panels. The blue numbers, expressed as mean $\pm$ SD, represent the statistical results of cuticle thickness measured using the Nano-Measure system (version 1.2.0) at 20 randomly selected locations within the layer. Panels (A₈) and (B₈) present the absolute quantification of whole-animal lipofuscin levels in earthworm tissues, as determined by enzyme-linked immunosorbent assay; the data are expressed as mean $\pm$ SD ($n = 3$).

spectrophotometer (F-7000, Hitachi, Japan). All fluorescence data were calibrated using our previously established method to ensure correct interpretation.²³

Laser Confocal Imaging of Filamentous Extracellular DNA

After each of the exposure and recovery periods, an optimized extraction medium was used to trigger spontaneous excretion of earthworm celomic immune cells.¹⁴ Isolated cells with satisfactory initial viability were coincubated with Sytox orange (5 μ M), a high-affinity nucleic acid dye incapable of penetrating cell membranes, for 5 min under dark conditions to label extracellular filamentous connective DNA. Fluorescence images were immediately acquired on a multiphoton laser confocal microscope under a 561 nm fluorescent channel (TCS-SP8-STED-3X, Leica, Germany), and the fluorescence intensity of extracellular DNA was quantified for supernatants of the staining system on a microplate reader.

Earthworm Transcriptomics Sequencing and Endosymbiotic Microbial Community Analysis

After the exposure and recovery periods, the earthworm surface was adequately washed using sterile PBS buffer in an ultraclean bench

until no visible particles or mucus residues remained, followed by rapid freezing of the earthworm tissues using liquid nitrogen. The total tissue RNA was extracted using a TRIZOL reagent, and the RNA meeting the quality control requirements (total RNA content >1 μ g, RNA concentration ≥ 30 ng/ μ L, RNA integrity number >6.5 , OD_{260/280} = 1.8–2.2) was used for library construction and sequencing on the Illumina Novaseq 6000 platform. Details of RNA extraction and sequencing library construction can be found in Text S4. The total DNA of the earthworm's intrinsic microbial community was extracted using an E.Z.N.A. soil DNA kit (D5625–02, Omega Biotek, U.S.A.). The V3–V4 region of the 16S rRNA gene was PCR amplified, and the products were quantified by a fluorescence quantification system (QuantiFluor-ST, Promega, U.S.A.). The NEXTFLEX Rapid DNA-Seq Kit (NOVA-5188, Revvity, U.S.A.) was used for library construction, and the sequencing was performed on an Illumina Miseq PE300 platform. Additional detailed methods are presented in Text S5.

Statistical Analysis

The significance of differences between treatments and the control group was tested in GraphPad Prism (version 7.0) software using one-

way analysis of variance (ANOVA) methods. All quantitative data are presented as mean values with variability assessed by standard deviation (SD).

RESULTS AND DISCUSSION

Characterization of PrMoS₂ and ExMoS₂

Atomic force microscopy (AFM) images demonstrate that PrMoS₂ possesses a maximum lateral size of $\sim 3\ \mu\text{m}$ (Figure 1A), with a thickness of $226.87 \pm 34.2\ \text{nm}$ counted along three random representative paths on the substrate (Figure 1B). The largest lateral dimension of ExMoS₂ exfoliated from PrMoS₂ was approximately 700 nm (Figure 1C), with a thickness of $1.27 \pm 0.07\ \text{nm}$, representing a quasi-monolayer structure (Figure 1D).²⁴ Transmission electron microscopy (TEM) images confirmed the size difference between microscale PrMoS₂ (Figure 1E) and nanoscale ExMoS₂ (Figure 1G) from AFM results, and also show the homogeneous distribution of molybdenum and sulfur on the nondefective lattice basal plane (Figure S1). High-resolution images (HR-TEM) further reveal a multilayer structure of PrMoS₂ (Figure 1F), confirming its suitability as a precursor for chemical exfoliation. The reduced interlayer spacing following exfoliation (from 0.59 ± 0.04 to $0.32 \pm 0.12\ \text{nm}$) further supports the transition of MoS₂ from a multilayer nature to a monolayer or quasi-monolayer structure.²⁵ Additionally, the lower zeta potential values of ExMoS₂ ($-23.8 \pm 4.5\ \text{mV}$) compared to PrMoS₂ ($-20.0 \pm 2.2\ \text{mV}$) (Figure 1I) indicated greater surface electrostatic repulsion and colloidal stability for the ExMoS₂.²⁶

Surface defects (red arrows in Figure 1D) and a heterogeneous electron density distribution (bottom-right panel in Figure 1F,H) associated with chemical exfoliation increased the surface roughness (PrMoS₂: $17.35\ \text{nm}$, ExMoS₂: $119.14\ \text{nm}$; $p < 0.001$) of ExMoS₂ (Figure 1J). Raman spectroscopy supports a highly disordered lattice for ExMoS₂; although no peak shifts corresponding to the in-plane (E_{2g}^1 ; $\sim 379\ \text{cm}^{-1}$) and out-of-plane (A_{1g} ; $\sim 406\ \text{cm}^{-1}$) lattice vibrations were observed, the increased Raman A_{1g}/E_{2g}^1 ratios (PrMoS₂: 1.698; ExMoS₂: 1.871) suggest a higher lattice distortion degree in the exfoliated ExMoS₂ (Figure 1K).^{27–29} Brunauer–Emmett–Teller (BET) analysis shows that the total pore volume of ExMoS₂ ($0.11\ \text{cm}^3/\text{g}$; Fitting error: 1.11%) was larger than that of PrMoS₂ ($0.03\ \text{cm}^3/\text{g}$; Fitting error: 1.02%). These pores, which are predominantly concentrated in the 2–8 nm range (Figure 1L), appear to partially originate from nanoscale defects such as nanoholes and disordered regions in ExMoS₂, as confirmed by AFM, HR-TEM, and Raman spectroscopy observations. Moreover, the molybdenum ion release content of ExMoS₂ in the tested soils (3.8–6.4%) was significantly higher than that of PrMoS₂ (2.6–4.0%) ($p < 0.001$; Table S2) within 7 days that consistent with the exposure period, which may be attributed to the higher surface roughness and total pore volume upon MoS₂ nanosheet exfoliation.^{30,31} Notably, the ion release profiles of both MoS₂ forms were significantly less than those reported in aqueous solutions, including water, culture medium, and soil solution,^{32–34} but does better represent the material transformation in the exposure scenario utilized herein.¹

Machine Learning-Facilitated Visualization and Quantification of Earthworm Somatic Immune Barrier Damage

The earthworm body wall is composed of epidermal keratin and a muscular layer that serves as the first defense against

xenobiotic exposure.²³ Both PrMoS₂ and ExMoS₂ caused a loss of epithelium integrity compared to the control, regardless of the dose (blue arrows in Figure 2A₂–A₅). This loss of integrity exhibited minimal healing after 7 days of recovery (Figure 2B₂–B₅). Earthworms exposed to 50 mg Mo/kg soil (d.w.) as PrMoS₂ ($96.7 \pm 16.9\ \mu\text{m}$) exhibited significant cuticle thickening ($p < 0.001$) compared to the control ($65.9 \pm 5.1\ \mu\text{m}$); this effect was not observed in the ExMoS₂ and Na₂MoO₄ exposure groups (Figure 2A₁–A₇). It has been reported that earthworms can accumulate surface keratins and polysaccharides through goblet cell proliferation and cornification, thereby regulating cuticle thickness as an active response to external environmental stress.^{35–37} The total molybdenum content in earthworm tissues was 4.60 ± 0.89 and $20.80 \pm 4.77\ \text{mg/kg tissue (d.w.)}$ upon 5 and 50 mg Mo/kg soil (d.w.) ExMoS₂ exposure, which is significantly higher than the accumulation in the PrMoS₂ exposed group at 2.78 ± 0.96 and $10.23 \pm 1.93\ \text{mg/kg tissue (d.w.)}$, respectively ($p < 0.01$) (Figure 2A₂–A₅). This increased molybdenum content is likely due to nanoscale ExMoS₂ penetrating the cuticle more easily than micrometer-sized PrMoS₂, as confirmed in previous studies involving other nanomaterials.^{38,39} Notably, Na₂MoO₄ exhibits higher tissue accumulation (4.73 ± 1.20 and $30.38 \pm 6.68\ \text{mg/kg tissue (d.w.)}$ at 5 and 50 mg Mo/kg soil (d.w.) groups; Figure 2A₆–A₇) than both MoS₂ forms and was significantly eliminated after a 7 day recovery period (71.5–93.5%; Figure 2B₆–B₇). This is attributed to ions more readily entering tissues through passive diffusion across intercellular spaces or via active uptake processes mediated by ion channels or transporters.⁴⁰

The formation of brown bodies, including lipofuscin and melanin, is an important immune response mechanism of the earthworm at the tissue level.⁴¹ Although less PrMoS₂ accumulated in earthworm tissues than ExMoS₂, automated image quantification based on machine learning showed a higher extent of brown body formation (yellow arrows in Figure 2) upon precursor exposure. This may be attributed to the larger lateral dimensions of PrMoS₂, which could be more readily recognized by the organism and rapidly trigger an immune response to facilitate the effective encapsulation and isolation of accumulated PrMoS₂.⁴² However, the muscular brown body content exhibited overt reduction after recovery, which was associated with a significant excretion or translocation of PrMoS₂ (60.4–74.6% excluded; Figure 2B₂–B₃). Conversely, the continuous secretion of brown bodies was observed after ExMoS₂ exposure (Figure 2B₄–B₅). Compared to PrMoS₂, nanoscale ExMoS₂ showed improved cuticle penetration and higher accumulation potential in muscle (43.9–55.4% excluded; Figure 2B₄–B₅). Moreover, ExMoS₂ possesses a quasi-monolayer structure with abundant surface defects, which was reported to enhance its reactivity.^{24,43} These properties may mechanistically promote the sustained formation of earthworm tissue brown bodies in the recovery period of the ExMoS₂ group.

Based on relative quantification assisted by image analysis, the absolute quantification of brown bodies further indicates that lipofuscin is a more sensitive biomarker for earthworm response to xenobiotics than melanin (Figures 2A₈ and 4F). Although significant tissue enrichment of molybdenum ions was observed ($p < 0.001$), this accumulation did not induce lipofuscin production, regardless of dose (Figure 2A₈,B₈). Unlike molybdenum ions, PrMoS₂ exposure increased earthworm tissue lipofuscin levels by 7.4 and 7.0% at 5 and 50 mg

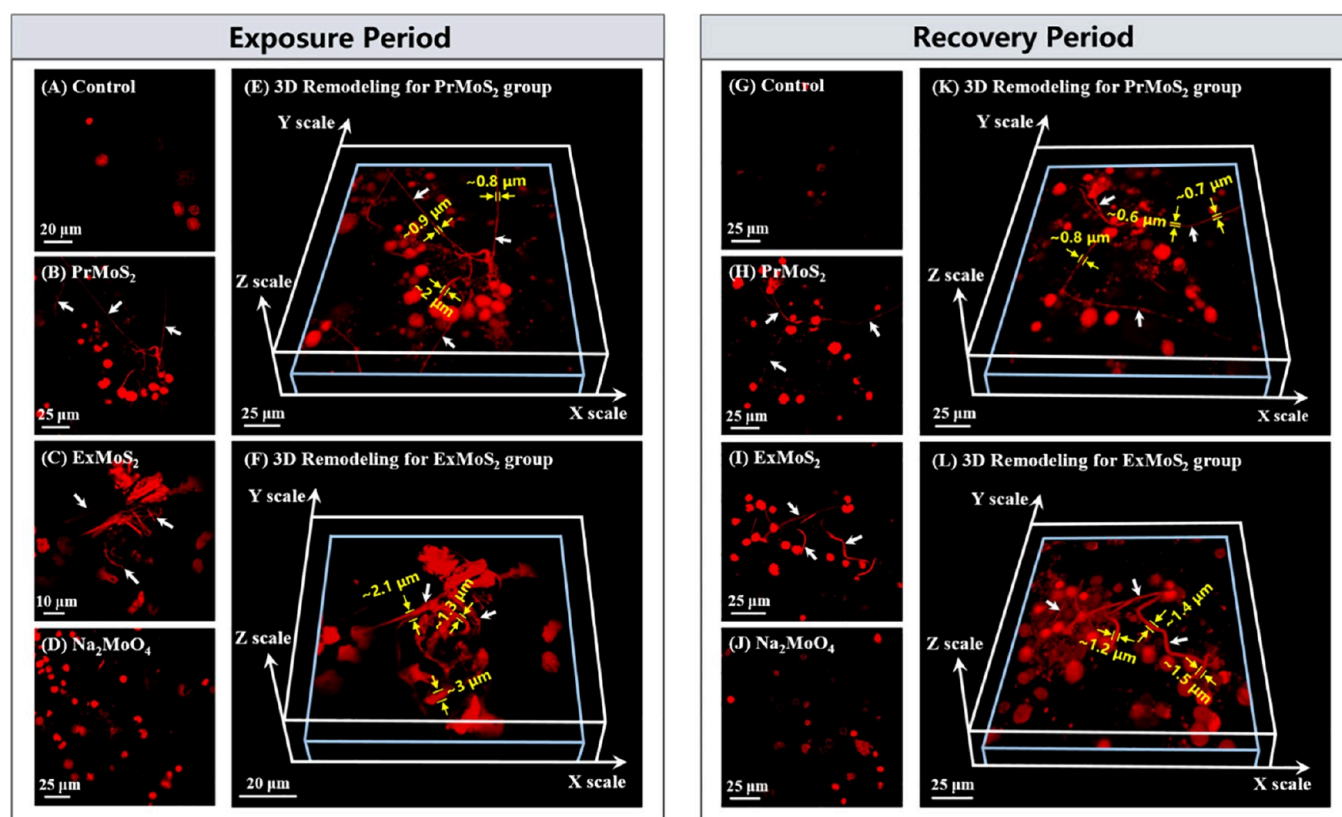


Figure 3. Laser confocal captures of extracellular NDA extrusion (white arrows) of functional immune cells isolated from earthworms (*Eisenia fetida*) exposed to PrMoS₂, ExMoS₂, and their ionic counterpart (Na₂MoO₄) at a concentration of 50 mg Mo/kg soil (d.w.) for (A–D) 7 days of exposure following (G–J) by another 7 days of recovery in clean soils. (E, F, K, L) Three-dimensional reconstruction patterns further show the spatial distribution and connectivity of extracellular NDA across cells.

Mo/kg, respectively, compared to the control ($p < 0.001$), whereas ExMoS₂ only increased lipofuscin content at high concentrations ($p < 0.05$). Importantly, this tissue-level immune response returned to near pre-exposure levels after 7 days of recovery, highlighting the dynamic transport and transformation mechanisms of earthworms against invasive xenobiotic particles.

Three-Dimensional Dynamic Imaging of ExMoS₂-Induced Irreversible Extrusion of Extracellular DNA

Upon penetration of the earthworm body and intestinal tissue, MoS₂ nanosheets (such as ExMoS₂) can further access the coelom and free-flowing functional immune cells. A selective SYTOX probe was used to visually identify the filamentous extracellular DNA with connectivity between different cells as a phagocytosis-independent immune mechanism.⁴⁴ Specifically, filamentous extension structures associated with immune cells were observed in the celomic content after exposure to 50 mg Mo/kg soil (dw) of PrMoS₂ and ExMoS₂ for 7 days (white arrows, Figure 3B,C); no external DNA secretion was evident in the control and Na₂MoO₄ groups (Figure 3A,D). By scanning along the Z-axis in the field of view and initiating three-dimensional remodeling, external DNA is observable, clearly extruded by individual cells, and extending outward, connecting multiple immune cells (Figure 3E–F). This establishment of intercellular connectivity mediated by filamentous extracellular DNA represents potential immune cell recruitment to enhance capture and phagocytosis of exogenous MoS₂.⁴⁵ Moreover, the filamentous extracellular DNA observed in the ExMoS₂-exposed group is thicker in

morphology than that in the PrMoS₂ group ($p < 0.05$), during both the exposure and recovery phases. Synchronous quantification of specific fluorescence intensity showed significantly elevated content of extracellular DNA in both MoS₂ groups compared to the control and Na₂MoO₄ groups during exposure (Figure S2A, $p < 0.05$), supporting the visual results. Extracellular DNA extrusion is a conserved mechanism from invertebrate evolution, responsible for capturing coelomic pathogens; this is effective as invertebrate coeloms connect to the exterior via dorsal pores and are rich in microorganisms.^{45,46} Interestingly, imaging and quantification results consistently demonstrate that coelomic extracellular DNA remained significant in earthworm tissues after 7 days of recovery from ExMoS₂ exposure; this was not evident with PrMoS₂ ($p < 0.05$, Figure 3G–L and Figure S2B). Overall, beyond previously reported pathogen defense, these findings demonstrate for the first time both visual and quantifiable earthworm immune cell DNA persistent extrusion that was unique to the nanoscale ExMoS₂ exposure, which may further impact the secretion of functional immune factors; this topic is discussed below. 3

Response Pattern of Earthworm Immune Functional Receptors to Different Molybdenum Forms

The activity of lumbrokinase, which is the central macromolecule responsible for the catabolism of fibrin-containing organic matter, increased by 9.42 ($p < 0.001$) and 4.35% (n.s.) compared to the control as a function of 5 and 50 mg Mo/kg soil (d.w.) PrMoS₂ exposure, respectively, suggesting potential energy deprivation (Figure 4A). Conversely, ExMoS₂ signifi-

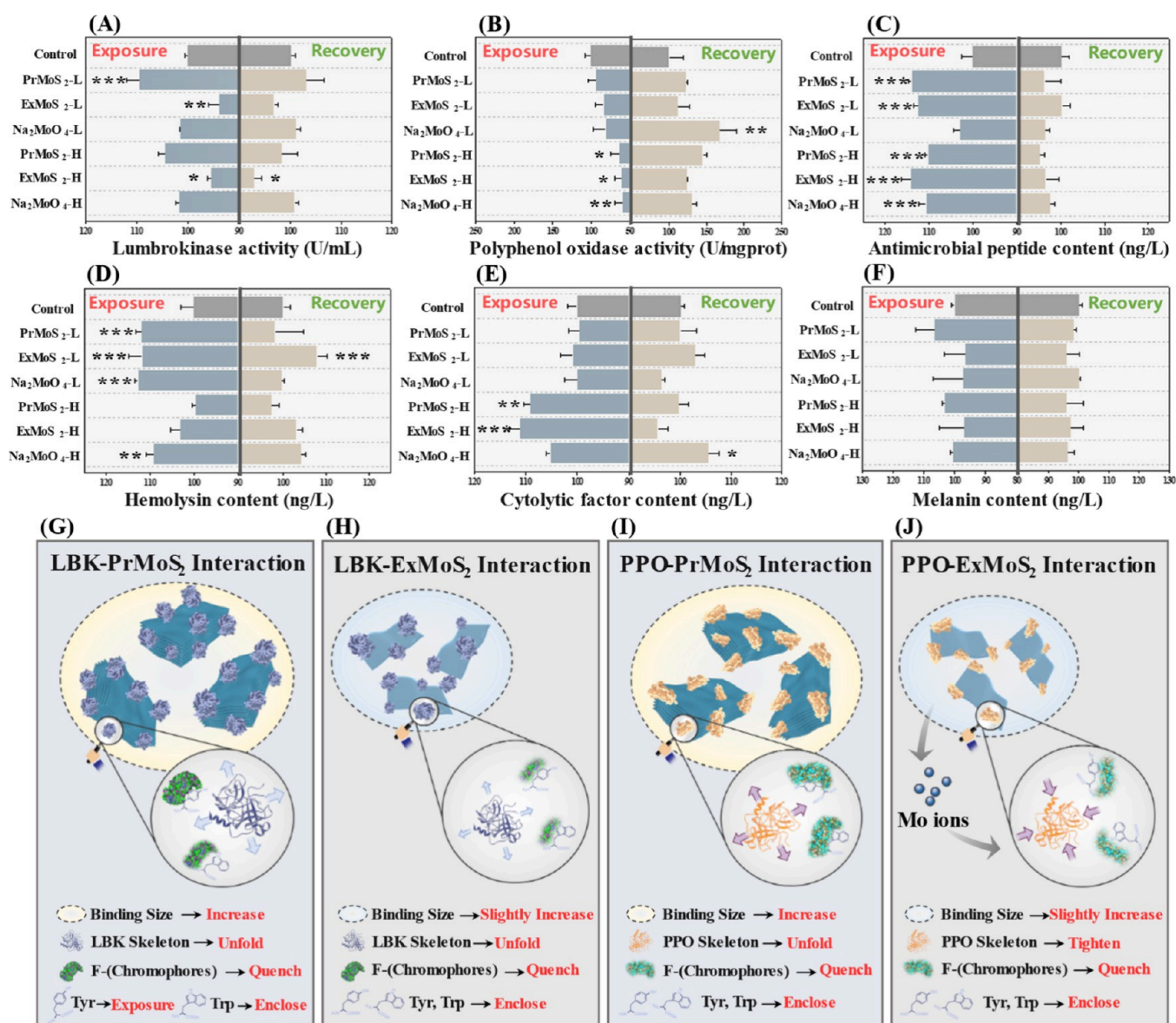


Figure 4. Macromolecular functional immunoreceptors (A for lumbrokinase and B for polyphenol oxidase) in the earthworm (*Eisenia fetida*) after exposure to different molybdenum forms (PrMoS₂, ExMoS₂, and Na₂MoO₄) at 5 mg Mo/kg soil (d.w.) (marked as L for low dose) and 50 mg Mo/kg soil (d.w.) (marked as H for high dose) for 7 days following another 7 days of recovery in clean soil; data are expressed as mean $\pm$ SD ($n = 3$). The small molecular immunological factors are characterized by (C) antimicrobial peptide, (D) hemolysin, (E) cytolytic factor, and (F) melanin contents. Statistically significant differences compared to the control are expressed as (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$. (G–J) Binding patterns of lumbrokinase and polyphenol oxidase to PrMoS₂ and ExMoS₂ characterized by multispectra technologies.

cantly inhibited lumbrokinase activity at both doses ($p < 0.01$). Lumbrokinase not only decomposes organic matter but also regulates cellular immune activity by repairing coelomocyte damage and reducing inflammation.⁴⁷ Comparing the results for the different molybdenum forms, ExMoS₂-induced lumbrokinase dysfunction was clearly specific to its nanoscale size, and this biomolecular impact may further disable the positive regulation of earthworm cellular immune activity and anti-inflammatory factor secretion. Quantification of immune cell metabolic activity based on CCK-8 probing confirms this hypothesis; 7 days of ExMoS₂ exposure at 5–50 mg Mo/kg soil (d.w.) inhibited immune cell activity by 10.4–11.7% compared to the control (Figure S3), consistent with the functional impairment of lumbrokinase. Interestingly, lumbrokinase activity recovered to pre-exposure levels upon

recovery from PrMoS₂ exposure, whereas ExMoS₂-induced lumbrokinase dysfunction ($p < 0.05$) was irreversible.

Polyphenol oxidase is central to the prophenoloxidase system, which is crucial for invertebrate humoral immune response and catalyzes the oxidation of phenolic compounds to quinones, mediating local inflammatory immune stress initiation.⁴⁸ PrMoS₂, ExMoS₂, and the ionic counterpart significantly reduced polyphenol oxidase activity in a dose-dependent manner (Figure 4B, $p < 0.05$). This functional inhibition was restored to levels beyond the control in all tested groups after the 7 day recovery period. The relative inactivation of polyphenol oxidase was likely the result of the local immune function being disabled, mediated by antimicrobial and inflammatory pathways.⁴⁹ Significant upregulation ($p < 0.01$) of key immune factors in earthworms upon exposure across all treatments, including antimicrobial peptides,

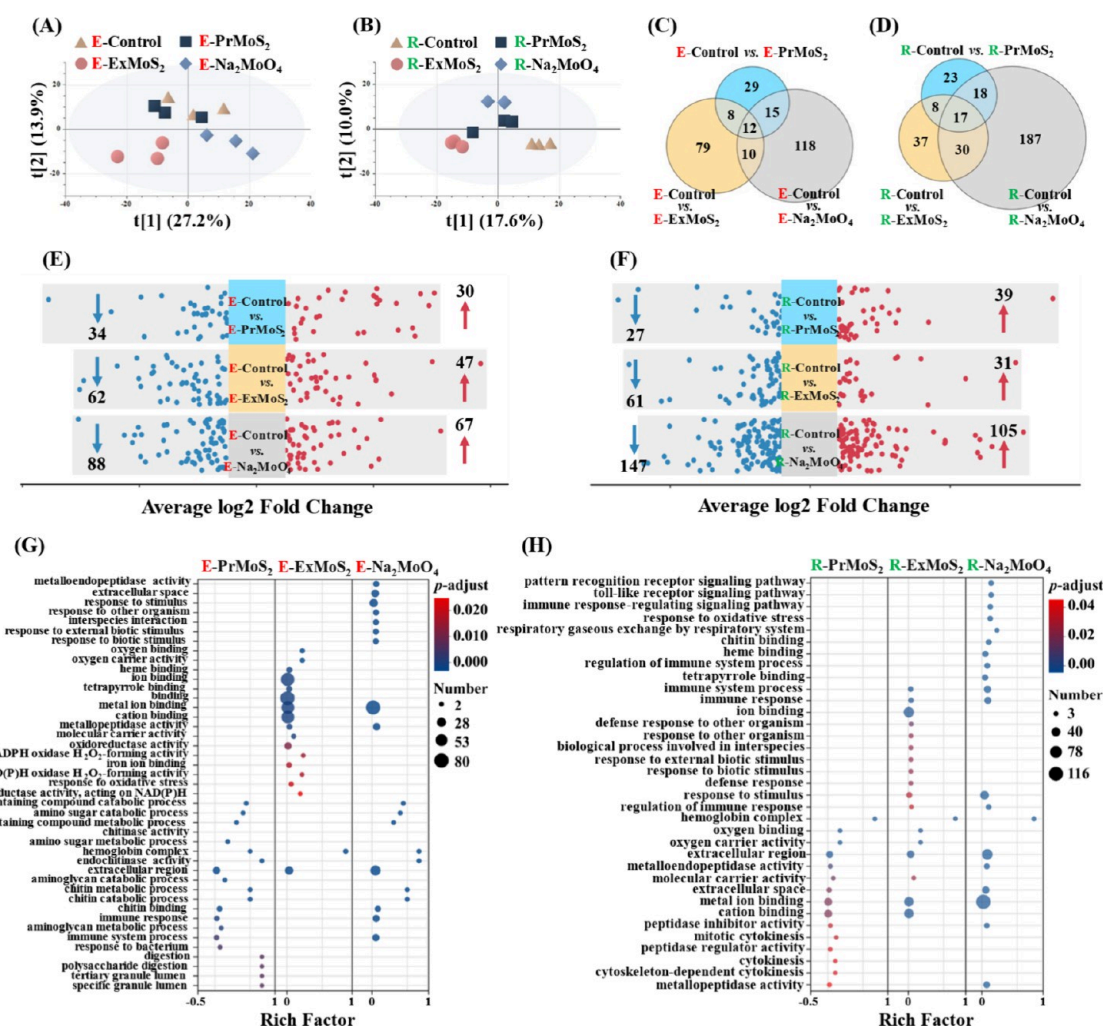


Figure 5. Immunity-specific gene identification in the earthworm (*Eisenia fetida*) after exposure to different molybdenum forms (PrMoS₂, ExMoS₂, and Na₂MoO₄) at a concentration of 50 mg Mo/kg soil (d.w.) for 7 days (marked as E for exposure) followed by another 7 days of recovery in clean soils (marked as R for recovery). Differentially expressed genes (DEGs) were defined as adjusted *p*-value (*p*_{adjust}) < 0.05 and |log₂ (fold change)| > 1. (A, B) Partial least-squares discrimination analyses performed using SIMCA software (version 13.0). (C, D) Venn and (E, F) volcano plots involving DEGs. (G, H) Gene ontology (GO) enrichment analyzed by Fisher exact test in Goatoools software (version 0.6.5) and the clusterProfiler package in R (version 4.2.3), and significant enrichment of the corresponding functional entry was defined only when *p*_{adjust} < 0.05.

hemolysin, and cytolytic factor, confirms the polyphenol oxidase dysfunction and demonstrates the activation of compensation mechanisms to overcome the impairment of antimicrobial and inflammatory immune functions (Figure 4C–E). The levels of these trace immune molecules (ng/L) in earthworms are highly dynamic; except for persistent hemolysin secretion with 5 mg of Mo/mL ExMoS₂, all the other tested immune factors recovered to control levels within 7 days of exposure cessation. Collectively, compared to PrMoS₂ and Na₂MoO₄, ExMoS₂ exposure disabled earthworm lumbrokinase function in a nanoscale-specific manner, disrupting the positive regulation from molecular to cellular levels in phagocytic immune cells within the earthworm coelom. Importantly, this immune toxicity was unique to ExMoS₂ and was irreversible, likely weakening earthworm tolerance in a persistent fashion that could compromise the organisms' response to other stressors and intermittent exposure scenarios.

Binding Mechanisms of PrMoS₂ and ExMoS₂ to Functional Immune Macromolecules at the Single-Molecule Level

As indicated by UV–visible spectra, the intense absorption peak near 190 nm observed in Figure S4 corresponds to the π – π^* electron transition of the C–O bond in the peptide chain of earthworm lumbrokinase and polyphenol oxidase.⁵⁰ Upon exposure to PrMoS₂, the absorbance of lumbrokinase and polyphenol oxidase at 190 nm decreased by 1.64% and 1.43% compared to the control group (Figure S4B–E), suggesting a loosening and unfolding of the protein backbone structure.⁵¹ Unlike PrMoS₂, the absorption peak of lumbrokinase decreased by 1.30%, while the corresponding peak of polyphenol oxidase increased by 0.87%, compared to the control after ExMoS₂ treatment. This indicates lumbrokinase skeleton loosening and polyphenol oxidase backbone contraction. Comparing the results between molybdenum forms, the ExMoS₂-induced shrinkage of the polyphenol oxidase molecule may be associated with the effect of soluble ions. The resonance light scattering spectral intensity of lumbrokinase and polyphenol oxidase in the PrMoS₂ exposed group increased by 156.8% and 130.0% compared to the control,

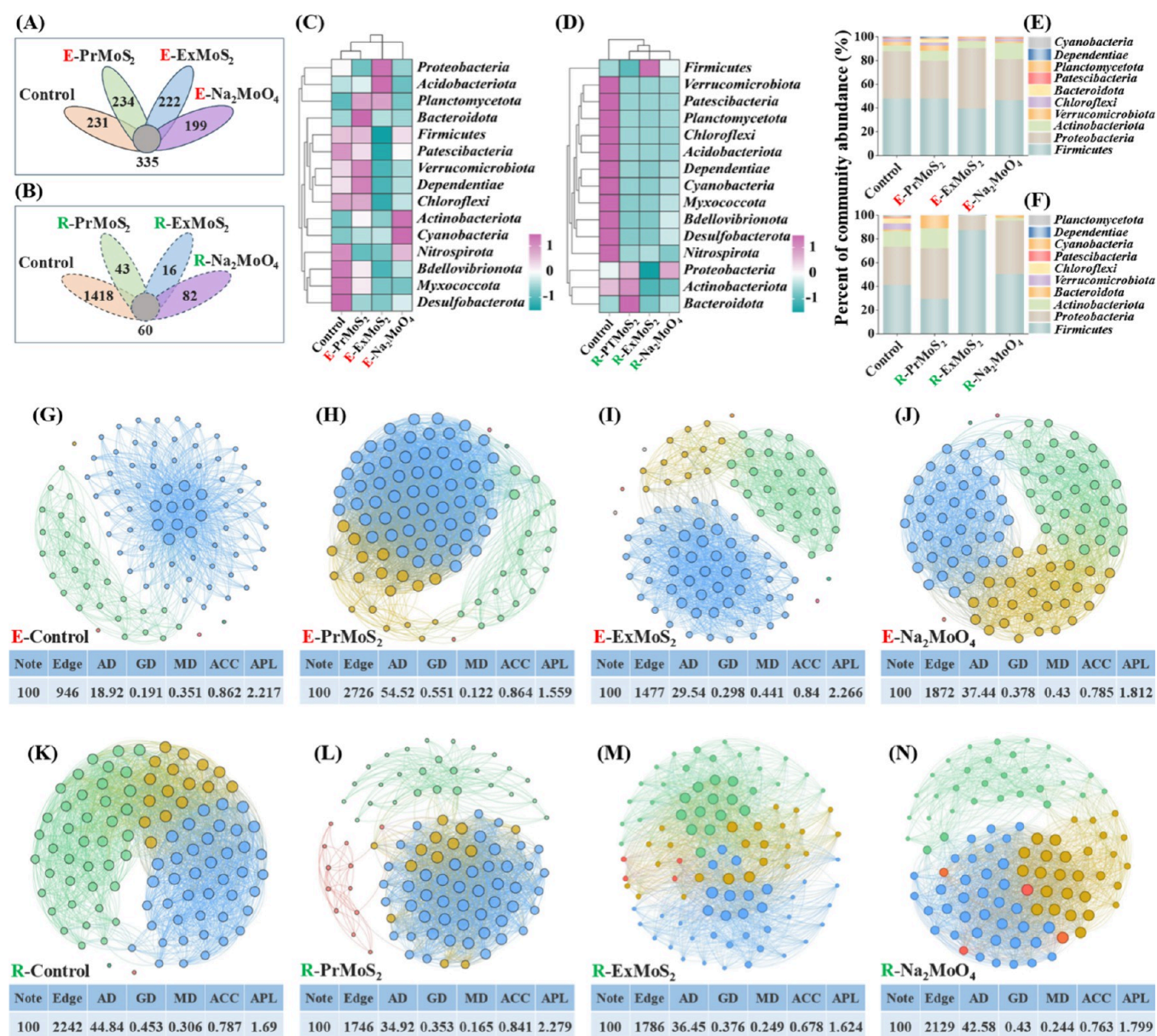


Figure 6. Endosymbiotic microbiota profile of earthworms (*Eisenia fetida*) exposed to PrMoS₂, ExMoS₂, or Na₂MoO₄ at a concentration of 50 mg Mo/kg soil (d.w.) for 7 days (marked as E for exposure) followed by another 7 days of recovery in clean soils (marked as R for recovery). (A, B) Venn maps of microbes involving the OTU numbers. (C, D) Hierarchical clustering analysis and (E, F) composition calculation of the endosymbiotic community at the phylum level. (G–N) Co-occurrence networks of the microbial consortium for different treatments were conducted by calculating pairwise associations between OTUs using the Spearman rank correlation method and visualized via Gephi (version 0.10.1). Each node represents an OTU, and the size of each node is proportional to its degree in the network. The node colors signify different microbial phylum levels. The structure and stability of each network were characterized by parameters including the number of nodes and edges, average degree (AD), graph density (GD), modularity index (MD), average clustering coefficient (ACC), and average path length (APL).

while this phenomenon was not observed with ExMoS₂ or Na₂MoO₄ treatments (Figure S5). This indicates that both macromolecules adsorbed onto PrMoS₂ via a peripheral binding pattern, forming larger nanobio aggregates with increased hydration diameter.⁵² Both MoS₂ variants reduced the synchronous fluorescence spectral intensity ($\Delta\lambda = 60$ nm) of lumbricinase and polyphenol oxidase from 2.23 to 10.77% of the control (Figure S6), which is associated with fluorescence quenching of Trp residues.⁵³ Additionally, compared to PrMoS₂, ExMoS₂ specifically quenched the Tyr fluorescence inside the lumbricinase molecule, as reflected by the decreased intensity (2.25%) of synchronous fluorescence spectra at $\Delta\lambda = 15$ nm (Figure S7). This results in amino acid

residue microenvironment changes such as tighter encapsulation, decreased local refractive indices, and enhanced polarity.⁵³ These insights are summarized in Figure 4G–J, establishing a molecular-level connection between the functional regulation and conformational changes of earthworm immune macromolecules upon exposure to different molybdenum forms.

Immune-Specific Gene Transcriptional Initiation Mechanism

Transcriptomic analysis was employed to elucidate the gene regulatory mechanisms of differential immune responses as a function of exposure to different molybdenum forms. Partial

least-squares discriminant analysis (PLS-DA) for all immune-specific unigenes indicates significant separation between ExMoS₂ and Na₂MoO₄ groups versus the control and PrMoS₂ group (Figure 5A). After the 7 day recovery period, the gene expression patterns of PrMoS₂, ExMoS₂, and molybdate treatments were well separated from those of the control (Figure 5B). Furthermore, the overt distinction of different molybdenum forms between exposure and recovery periods, along with the specific regulation of a number of differentially expressed genes (DEGs), underscores the irreversibility of overall gene expression reprogramming (Figure 5C,D, Figures S8 and S9). Volcano plot visualization analysis counted a greater number of downregulated DEGs during the exposure period, regardless of the molybdenum form (Figure 5E). However, compared to the PrMoS₂ group, downregulated gene expression in the ExMoS₂ and Na₂MoO₄ groups persisted after 7 days of recovery (Figure 5F). Hierarchical clustering (HCL) analysis indicates that the shared DEGs in earthworms exposed to ExMoS₂ and molybdenum ions (Na₂MoO₄) were clustered together based on expression levels (Figure S10A), but that the two types of MoS₂ exhibited distinct expression regulation patterns compared to their ionic counterpart upon cessation of exposure (Figure S10B).

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses (Figure 5G,H and Figure S11) show that Na₂MoO₄ exposure significantly reprogrammed the expression of genes encoding metalloendopeptidases, potentially affecting pathogen-associated protein degradation and immune cell recognition processes.⁵⁴ During recovery, genes responsible for NOD-like receptors (NLRs) and transforming growth factor-beta (TGF- β) signaling pathways were activated (Figure S11B) to compensate for Na₂MoO₄-induced immunosuppression and to enhance immune tolerance.⁵⁵ Unlike those under Na₂MoO₄ exposure, DEGs were significantly enriched in Chitinase activity in the PrMoS₂ group during exposure. Chitinases are known to participate in biodegradation and nutrient cycling by hydrolyzing chitin and regulating the Toll pathway to engage in innate immune responses.⁵⁶ The synchronous enrichment of polysaccharide degradation supported the potential digestive dysfunction induced by PrMoS₂ exposure. Compared with PrMoS₂, ExMoS₂ exposure significantly impacted energy supply processes, including oxidoreductase activity and NADPH oxidase activity, persistently activating compensatory genes related to redox homeostasis. Initiation of fat digestion and absorption in KEGG mapping validated energy homeostasis instability, which can expedite substance utilization to compensate for energy deprivation (Figure S11A).¹⁴

Gene set enrichment analysis (GSEA) shows that earthworms exhibiting significantly changed leading pathways of synaptic vesicles (Figure S12A) and enzyme-linked receptor protein signaling pathways (Figure S12B) during exposure and recovery periods of the PrMoS₂ group, potentially responsible for signaling transduction imbalance and postexposure immune memory formation. Conversely, genes related to exocytosis were significantly enriched with exposure to ExMoS₂ (Figure S12C), phenotypically mediating the persistent secretion of filamentous extracellular DNA (Figure 3). The activated glutathione enzyme activity pathway in response to ExMoS₂ during the recovery period (Figure S12D) indicates an active initiation of detoxification and antioxidant compensation in earthworms upon cessation of exposure, aligning with the GO

and KEGG functional enrichment analyses. Leading genes in the Na₂MoO₄ exposure group were involved in impaired lipid transport in the exposure period and enhanced mitochondrial function in the recovery period (Figure S12E,F); this may disturb the energy supply processes. Given the similar energy pathway enrichment and significant ion release observed for the ExMoS₂ and Na₂MoO₄ groups, the additional regulation of soluble ionic species in ExMoS₂-induced transcriptional reprogramming warrants further study.

Cooperative Immune Profile from the Innate and Synergistic Viewpoints

Host endosymbiotic microbiota play a crucial role in maintaining host health and immune function, as well as in mediating responses to xenobiotic invasion through self-reassembling community structures.⁵⁷ PLS-DA clearly distinguishes ExMoS₂ and Na₂MoO₄ groups from the control along the first and second axes, both in the exposure and recovery periods, at the operational taxonomic unit (OTU) level, while the PrMoS₂ group shows only slight separation from the control (Figure S13). The decline in unique OTU counts (Figure 6A,B) and the abundance-based coverage estimation (ACE) index (Figure S14) in earthworms during the 7 day recovery period demonstrate the sensitive response of microbial community diversity as a function of exposure to different molybdenum forms. The analysis of the average degree of variation confirms the loss of microbial community stability in earthworms during the exposure period (Figure S15). These observations, as derived from the control of adequate sample size, appropriate grouping, and satisfying species uniformity (Figures S16–18), are consistent with previous studies reporting that the microbial community in earthworms is highly sensitive to exogenous substance exposure, including metals, pesticides, and nanoparticles.^{58–60}

Differences in the community composition of earthworm endosymbiotic microbes in response to particulate and sheetlike MoS₂ were assessed at the phylum level. Compared to the PrMoS₂ and Na₂MoO₄ exposure groups, symbiotic microbes in the ExMoS₂ group were categorized into a distinct cluster based on abundance in both the exposure and recovery periods (Figure 6C,D). In addition, *Actinobacteria* and *Bacteroidota* abundance were significantly higher in earthworms that underwent 7 days of recovery after PrMoS₂ exposure compared to those from the exposure period (Figure 6E,F). This could be attributed to PrMoS₂-induced abnormalities in earthworm intestinal tissue permeability, as well as increased susceptibility of the overall immune response to exogenous pollutants.⁶¹ Compared to the PrMoS₂ group (80%), earthworm symbiotic microbes upon ExMoS₂ exposure exhibited higher abundances of *Proteobacteria* and *Firmicutes* (90%), and these further dominated (99%) during the recovery period. *Proteobacteria* and *Firmicutes* have been documented to actively participate in metal resistance and reduction, as well as sulfide oxidation, as a means to reduce the bioavailability of metals and their derived nanoparticles, thereby maintaining redox homeostasis.⁶² This aligns with the sustained activation of stress-compensatory genes induced by ExMoS₂ through the recovery period (Figure 5G,H). Co-occurrence network analysis was employed to provide insight into the earthworm's intrinsic microbial community structure in terms of interspecies interactions. Under PrMoS₂ exposure, impacts on microbial network connectivity and community structure were most pronounced (as explained by the highest

graph density (GD) and smallest average path length (APL) values), representing a highly synergistic pattern among earthworm endosymbiotic microbiota (Figure 6G–J). Once exposure ceased, microbial module clustering and community connectivity reverted to levels below those of the control group (Figure 6K–N).

Mechanistically, the relative abundance-frequency relationships of earthworm microbial taxa adhered more closely to the neutral community model (NCM) in the exposure period ($R^2 = 0.4348$) compared to those in the recovery period ($R^2 = 0.0272$) (Figure S19). This result suggests a stronger diffusion potential driven by niche differences between microbial communities under different molybdenum form stress, whereas host-mediated processes (e.g., immune responses and gene regulation) exerted a more central role in shaping the community structure during the recovery period.⁶³ The higher migration rate parameters (m) observed in the exposure period (0.022) than in the recovery period (0.0052) further support this interpretation. Furthermore, using iCAMP analysis based on the null model algorithm with phylogenetic binning, we found that, unlike PrMoS_2 and Na_2MoO_4 exposures, the clustering of microbial communities during the ExMoS_2 exposure period was driven by deterministic processes (Figure S20A). However, the relative contribution of the randomized processes was enhanced after recovery (Figure S20B). This may be attributed to the retention of ingested MoS_2 particles in the earthworm gut, offering a temporary and additional opportunity for microbial colonization.⁵⁷ These results mechanistically highlight the key processes that shape the deterministic nature of earthworm endosymbiotic microbiota under MoS_2 exposure, particularly ExMoS_2 , by influencing niche selection and dispersal limitation.

Orthogonal Analyses of Phenotypic Immune Function, Transcriptome, and Endosymbiotic Microbial Communities

Transcriptome, microbiome, and phenotypic data were further integrated for orthogonal analysis to explore the cascading regulatory pathways from the earthworm upstream molecular response to the phenotype as a function of exposure to different molybdenum forms. Weighted gene coexpression network analysis (WGCNA) visually linked gene expression to immune function. All unigenes were clustered into 16 or 17 modules (clusters of genes with similar expression patterns) after screening during the exposure and recovery periods, respectively (Figure S21). Nine significant modules expressed during exposure and three during recovery showed significant correlations with phenotype ($p < 0.05$). These modules were associated with the shared extracellular secretion, metal binding, and immune defense function, suggesting sustained top-down transcription-phenotype coregulation in earthworms (Figure S22). In addition to the transcriptional involvement in immune regulation, Mantel analyses confirmed the microbial synergism and highlighted a positive *in vivo* link between microbes and phenotypic immune function after recovery (Figure S23). To quantitatively evaluate and compare the differences in earthworm immune regulatory pathways during the exposure and recovery periods, a conceptual framework of the partial least-squares pathway model (PLS-PM) was established (Figure S24). During the exposure period, molybdenum stress directly activated earthworm immune resistance by significantly affecting *in vivo* accumulation (path coefficient = 0.94, $p < 0.001$) and immune cell behavior

(extrusion of extracellular DNA; path coefficient = 0.64, $p < 0.05$). After recovery, the residual MoS_2 in earthworms stimulated sustained immunity mediated through the transcriptional activation of immune resistance genes. However, this was accompanied by impaired immune cell behavior, which ultimately had a negative impact on phenotypic immune function tolerance (path coefficient = -0.6 , $p < 0.05$). The integration of these multidimensional insights underscores the top-down cascade immunomodulatory profile centered on *in vivo* accumulation of molybdenum-based nanomaterials, to rapidly respond and enhance immune tolerance during exposure and actively sustain organismal health after exposure.

ENVIRONMENTAL IMPLICATIONS

Nowadays, a significant and rapidly growing literature is focused on the development, use, and agricultural benefit assessment of novel nanofertilizers.^{64–67} However, in addition to considering crop-enhancing effects, potential ecological risks must always be considered, including impacts on soil quality and associated biota. This study mechanistically demonstrates the irreversible immune stress on model soil biota of a representative two-dimensional agricultural nanomaterial, MoS_2 nanosheets (ExMoS_2), that has shown promise for enhancing crop growth in laboratory-scale studies. Specifically, in a realistic exposure-recovery exposure scenario, MoS_2 nanosheets can persistently reprogram earthworm gene expression related to energy homeostasis and extracellular secretion, phenotypically mediating brown body encapsulation and filamentous extracellular DNA assembly. Beyond that, ExMoS_2 can alter the biomolecular skeletal structure and inner amino acid residue microenvironment upon incorporation into immune macromolecular agglomerates, thereby irreversibly dysregulating the phenotypic immune receptor function. Compared to their precursor bulk particles (PrMoS_2), this specific immune stress is unique to MoS_2 nanosheet exposure, and partially stems from the persistent *in vivo* accumulation and enhanced ionic species solubilization associated with the nanosized two-dimensional structure. Furthermore, we found that deterministic processes and niche selection drove the weakening of dispersal limitation in earthworm endosymbiotic microbial communities under MoS_2 nanosheet exposure and revealed a top-down immune regulation cascade to resist MoS_2 nanosheet exposure and accumulation and actively maintain *in vivo* homeostasis upon the cessation of exposure. This study highlights the necessity of thorough consideration of the trade-offs between advancing nanoenhanced agriculture and holistic ecological health.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.5c14710>.

Supplementary methods; elemental distribution of MoS_2 ; extracellular DNA content and metabolic activity of immune cells; multispectral analyses of MoS_2 interactions with immune macromolecules; PLS-DA, Venn, hierarchical clustering, KEGG enrichment, and GSEA results of transcriptomics analyses; dimensionality reduction, diversity index, stability index, Pan/Core curves, dilution curves, similarity analysis, NCM model, and ecological processes analysis of earthworm microbiome; module clustering and functional analysis in

WGCNA results; correlation analysis between phenotype and microbiome; PLS–PM analysis in earthworm immune regulatory pathways; detection of actual exposure concentrations; quantification of ionic Mo release from MoS₂ (PDF)

AUTHOR INFORMATION

Corresponding Authors

Peng Zhang — School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom; Department of Environmental Science and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China; Email: zhangpeng1987@ustc.edu.cn

Erkai He — School of Geographic Sciences, East China Normal University, Shanghai 200241, China; orcid.org/0000-0002-4866-3001; Email: ekhe@geo.ecnu.edu.cn

Hao Qiu — School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China; orcid.org/0000-0002-4743-9702; Email: haoqiu@sjtu.edu.cn

Authors

Kailun Sun — School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

Jason C. White — The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504, United States; orcid.org/0000-0001-5001-8143

Iseult Lynch — School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom; orcid.org/0000-0003-4250-4584

Willie J.G.M. Peijnenburg — Center for the Safety of Substances and Products, National Institute of Public Health and the Environment, Bilthoven 3720BA, The Netherlands; Institute of Environmental Sciences, Leiden University, Leiden 2300RA, The Netherlands; orcid.org/0000-0003-2958-9149

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.est.5c14710>

Author Contributions

K.S.: Investigation, Methodology, Writing — original draft; J.C.W.: Writing — review and editing; I.L.: Writing — review and editing; P.Z.: Methodology, Visualization, Writing — review and editing; E.H.: Resources, Supervision, Writing — review and editing; W.J.G.M.P.: Writing — review and editing; H.Q.: Conceptualization, Supervision, Writing — review and editing, Funding acquisition

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (no. 42377268 and no. 423B2706), the National Key Research and Development Program of China (No. 2023YFC3711500), the Postdoctoral Fellowship Program and China Postdoctoral Science Foundation (BX20250011 and 2025M782496), and the Open Research Fund of State Key Laboratory of Estuarine and Coastal

Research (SKLEC-KF202408). We also gratefully acknowledge funding from the Horizon 2020 Marie Skłodowska-Curie Actions project CompSafeNano (Grant agreement ID: 101008099) and from the Horizon Europe project INSIGHT (Grant agreement ID: 101137742) via the UKRI Innovate UK Horizon Guarantee Funding (Grant number 10097888).

REFERENCES

- (1) Li, M.; Gao, L.; White, J. C.; Haynes, C. L.; O'Keefe, T. L.; Rui, Y.; Ullah, S.; Guo, Z.; Lynch, I.; Zhang, P. Nano-Enabled Strategies to Enhance Biological Nitrogen Fixation. *Nat. Nanotechnol.* **2023**, *18* (7), 688–691.
- (2) Guo, S.; Hu, X.; Wang, Z.; Yu, F.; Hou, X.; Xing, B. Zinc Oxide Nanoparticles Cooperate with the Phyllosphere to Promote Grain Yield and Nutritional Quality of Rice under Heatwave Stress. *Proc. Natl. Acad. Sci. U.S.A.* **2024**, *121* (46), No. e2414822121.
- (3) Lombi, E.; Donner, E.; Dusinska, M.; Wickson, F. A One Health Approach to Managing the Applications and Implications of Nanotechnologies in Agriculture. *Nat. Nanotechnol.* **2019**, *14* (6), 523–531.
- (4) Zhao, L.; Bai, T.; Wei, H.; Gardea-Torresdey, J. L.; Keller, A.; White, J. C. Nanobiotechnology-Based Strategies for Enhanced Crop Stress Resilience. *Nat. Food* **2022**, *3* (10), 829–836.
- (5) Li, M.; Zhang, P.; Guo, Z.; Cao, W.; Gao, L.; Li, Y.; Tian, C. F.; Chen, Q.; Shen, Y.; Ren, F.; Rui, Y.; White, J. C.; Lynch, I. Molybdenum Nanofertilizer Boosts Biological Nitrogen Fixation and Yield of Soybean through Delaying Nodule Senescence and Nutrition Enhancement. *ACS Nano* **2023**, *17* (15), 14761–14774.
- (6) Li, M.; Zhang, P.; Guo, Z.; Zhao, W.; Li, Y.; Yi, T.; Cao, W.; Gao, L.; Tian, C. F.; Chen, Q.; Ren, F.; Rui, Y.; White, J. C.; Lynch, I. Dynamic Transformation of Nano-MoS₂ in a Soil–Plant System Empowers Its Multifunctionality on Soybean Growth. *Environ. Sci. Technol.* **2024**, *58* (2), 1211–1222.
- (7) Wei, L.; Liu, J.; Jiang, G. Nanoparticle-Specific Transformations Dictate Nanoparticle Effects Associated with Plants and Implications for Nanotechnology Use in Agriculture. *Nat. Commun.* **2024**, *15* (1), 7389.
- (8) Xu, Z.; Lu, J.; Zheng, X.; Chen, B.; Luo, Y.; Tahir, M. N.; Huang, B.; Xia, X.; Pan, X. A Critical Review on the Applications and Potential Risks of Emerging MoS₂ Nanomaterials. *J. Hazard. Mater.* **2020**, *399*, No. 123057.
- (9) Sun, K.; van Gestel, C. A. M.; Qiu, H. Two-Dimensional Layered Nano-MoS₂ Induces Earthworm Immune Cell Apoptosis by Regulating Lysosomal Maintenance and Function: Toward Unbiased Screening and Validation of Suspicious Pathways. *Environ. Sci. Technol.* **2024**, *58* (45), 19948–19959.
- (10) Liebeke, M.; Strittmatter, N.; Fearn, S.; Morgan, A. J.; Kille, P.; Fuchser, J.; Wallis, D.; Palchykov, V.; Robertson, J.; Lahive, E.; Spurgeon, D. J.; McPhail, D.; Takáts, Z.; Bundy, J. G. Unique Metabolites Protect Earthworms against Plant Polyphenols. *Nat. Commun.* **2015**, *6* (1), 7869.
- (11) Organization for Economic Co-operation and Development (OECD) *Guidelines for the Testing of Chemicals*, No. 222: *Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)*, 2006, Vol. 1, pp 1–18.
- (12) International Organization for Standards (ISO) *Soil Quality Effects of Pollutants on Earthworms-Determination of Effects on Reproduction of Eisenia fetida/Eisenia andrei (Endorsed by AENOR in September of 2015)*, 2015, Part 2, 11268–2.
- (13) Wang, H.; Guo, S.; He, F.; Li, X.; Wang, Y.; Wang, T.; Tian, G.; Liu, R. The Combined Effects of Polystyrene Nanoplastics with Nickel on Oxidative Stress and Related Toxic Effects to Earthworms from Individual and Cellular Perspectives. *Sci. Total Environ.* **2024**, *912*, No. 168819.
- (14) Sun, K.; White, J. C.; He, E.; Van Gestel, C. A. M.; Qiu, H. Surface Defects Regulate the in Vivo Bioenergetic Response of Earthworm *Eisenia fetida* Coelomocytes to Molybdenum Disulfide Nanosheets. *ACS Nano* **2023**, *17* (3), 2639–2652.

- (15) Zhuo, Y.; Yang, Y.; Zhang, H.; Wang, X.; Cao, M.; Wang, Y. Toxicological Evaluation and Metabolic Profiling of Earthworms (*Eisenia fetida*) after Exposure to Microplastics and Acetochlor. *Environ. Res.* **2025**, 276, No. 121546.
- (16) Sun, K.; He, E.; Van Gestel, C. A. M.; Cao, X.; Zhao, L.; Xu, X.; Peijnenburg, W. J. G. M.; Qiu, H. Biosafety of Two-Dimensional Molybdenum Disulfide Nanomaterials: Natural and Engineered Transformation Pathways and Their Role in the Toxicity Profile. *Crit. Rev. Environ. Sci. Technol.* **2024**, 54 (10), 840–863.
- (17) Keller, A. A.; Zheng, Y.; Praetorius, A.; Quik, J. T. K.; Nowack, B. Predicting Environmental Concentrations of Nanomaterials for Exposure Assessment - a Review. *NanoImpact* **2024**, 33, No. 100496.
- (18) Adam, V.; Wu, Q.; Nowack, B. Integrated Dynamic Probabilistic Material Flow Analysis of Engineered Materials in All European Countries. *NanoImpact* **2021**, 22, No. 100312.
- (19) Gottschalk, F.; Sun, T.; Nowack, B. Environmental Concentrations of Engineered Nanomaterials: Review of Modeling and Analytical Studies. *Environ. Pollut.* **2013**, 181, 287–300.
- (20) Hong, H.; Part, F.; Nowack, B. Prospective Dynamic and Probabilistic Material Flow Analysis of Graphene-Based Materials in Europe from 2004 to 2030. *Environ. Sci. Technol.* **2022**, 56 (19), 13798–13809.
- (21) Lotufo, G. R.; Boyd, R. E.; Harmon, A. R.; Bednar, A. J.; Smith, J. C.; Simini, M.; Sunahara, G. I.; Hawari, J.; Kuperman, R. G. Accumulation of Insensitive Munition Compounds in the Earthworm *Eisenia andrei* from Amended Soil: Methodological Considerations for Determination of Bioaccumulation Factors. *Environ. Toxicol. Chem.* **2021**, 40 (6), 1713–1725.
- (22) Sun, K.; White, J. C.; Qiu, H.; van Gestel, C. A. M.; Peijnenburg, W. J. G. M.; He, E. Coupled Lipidomics and Digital Pathology as an Effective Strategy to Identify Novel Adverse Outcome Pathways in *Eisenia fetida* Exposed to MoS₂ Nanosheets and Ionic Mo. *Environ. Sci. Technol.* **2023**, 57 (30), 11009–11021.
- (23) Sun, K.; Li, M.; Song, Y.; Tang, J.; Liu, R. Organism and Molecular-Level Responses of Superoxide Dismutase Interaction with 2-Pentanone. *Chemosphere* **2022**, 286, No. 131707.
- (24) Shi, T.; Hou, X.; Guo, S.; Zhang, L.; Wei, C.; Peng, T.; Hu, X. Nanohole-Boosted Electron Transport between Nanomaterials and Bacteria as a Concept for Nano–Bio Interactions. *Nat. Commun.* **2021**, 12 (1), 493.
- (25) Wang, Z.; Mi, B. Environmental Applications of 2D Molybdenum Disulfide (MoS₂) Nanosheets. *Environ. Sci. Technol.* **2017**, 51 (15), 8229–8244.
- (26) Chen, K.; Xu, X.; Li, X.; Gui, X.; Zhao, L.; Qiu, H.; Cao, X. The Colloidal Stability of Molybdenum Disulfide Nanosheets in Different Natural Surface Waters: Combined Effects of Water Chemistry and Light Irradiation. *Water Res.* **2024**, 261, No. 121973.
- (27) Pradhan, G.; Sharma, A. K. Anomalous Raman and Photoluminescence Blue Shift in Mono- and a Few Layered Pulsed Laser Deposited MoS₂ Thin Films. *Mater. Res. Bull.* **2018**, 102, 406–411.
- (28) Cançado, L. G.; Jorio, A.; Ferreira, E. H. M.; Stavale, F.; Achete, C. A.; Capaz, R. B.; Moutinho, M. V. O.; Lombardo, A.; Kulmala, T. S.; Ferrari, A. C. Quantifying Defects in Graphene via Raman Spectroscopy at Different Excitation Energies. *Nano Lett.* **2011**, 11 (8), 3190–3196.
- (29) Lee, C.; Yan, H.; Brus, L. E.; Heinz, T. F.; Hone, J.; Ryu, S. Anomalous Lattice Vibrations of Single- and Few-Layer MoS₂. *ACS Nano* **2010**, 4 (5), 2695–2700.
- (30) Helmlinger, J.; Sengstock, C.; Groß-Heitfeld, C.; Mayer, C.; Schildhauer, T. A.; Köller, M.; Eppler, M. Silver Nanoparticles with Different Size and Shape: Equal Cytotoxicity, but Different Antibacterial Effects. *RSC Adv.* **2016**, 6 (22), 18490–18501.
- (31) Szalaj, U.; Chodara, A.; Gierlotka, S.; Wojnarowicz, J.; Łojkowski, W. Enhanced Release of Calcium Ions from Hydroxypatite Nanoparticles with an Increase in Their Specific Surface Area. *Materials* **2023**, 16 (19), 6397.
- (32) Chen, S.; Shi, N.; Huang, M.; Tan, X.; Yan, X.; Wang, A.; Huang, Y.; Ji, R.; Zhou, D.; Zhu, Y.-G.; Keller, A. A.; Gardea-Torresdey, J. L.; White, J. C.; Zhao, L. MoS₂ Nanosheets–Cyanobacteria Interaction: Reprogrammed Carbon and Nitrogen Metabolism. *ACS Nano* **2021**, 15 (10), 16344–16356.
- (33) Zeng, H.; Hu, X.; Zhou, Q.; Luo, J.; Hou, X. Extracellular Polymeric Substances Mediate Defect Generation and Phytotoxicity of Single-Layer MoS₂. *J. Hazard. Mater.* **2022**, 429, No. 128361.
- (34) Zhao, L.; Chen, S.; Tan, X.; Yan, X.; Zhang, W.; Huang, Y.; Ji, R.; White, J. C. Environmental Implications of MoS₂ Nanosheets on Rice and Associated Soil Microbial Communities. *Chemosphere* **2022**, 291, No. 133004.
- (35) Zhang, W.; Liu, K.; Li, J.; Liang, J.; Lin, K. Impacts of BDE209 Addition on Pb Uptake, Subcellular Partitioning and Gene Toxicity in Earthworm (*Eisenia fetida*). *J. Hazard. Mater.* **2015**, 300, 737–744.
- (36) Briones, M. J. I.; Alvarez-Otero, R. Body Wall Thickness as a Potential Functional Trait for Assigning Earthworm Species to Ecological Categories. *Pedobiologia* **2018**, 67, 26–34.
- (37) Zhang, H.; Wang, K.; Yue, S.; Qiao, Y. The Impact of Soil Cadmium on the Ultrastructural Cuticle of Two Earthworms Species *Eisenia fetida* and *Metaphire californica*. *Ecol. Environ. Sci.* **2017**, 26 (10), 1807–1813.
- (38) Lei, L.; Liu, M.; Song, Y.; Lu, S.; Hu, J.; Cao, C.; Xie, B.; Shi, H.; He, D. Polystyrene (Nano)Microplastics Cause Size-Dependent Neurotoxicity, Oxidative Damage and Other Adverse Effects in *Caenorhabditis elegans*. *Environ. Sci.: Nano* **2018**, 5 (8), 2009–2020.
- (39) Mateos-Cárdenas, A.; van Pelt, F. N. A. M.; O'Halloran, J.; Jansen, M. A. K. Adsorption, Uptake and Toxicity of Micro- and Nanoplastics: Effects on Terrestrial Plants and Aquatic Macrophytes. *Environ. Pollut.* **2021**, 284, No. 117183.
- (40) Fortin, C.; Campbell, P. G. Silver Uptake by the Green Alga *Chlamydomonas Reinhardtii* in Relation to Chemical Speciation: Influence of Chloride. *Environ. Toxicol. Chem.* **2000**, 19 (11), 2769–2778.
- (41) Valembois, P.; Seymour, J.; Lassegues, M. Evidence of Lipofuscin and Melanin in the Brown Body of the Earthworm *Eisenia-fetida-andrei*. *Cell Tissue Res.* **1994**, 277 (1), 183–188.
- (42) Valembois, P.; Lassegues, M.; Roch, P. Formation of Brown Bodies in the Coelomic Cavity of the Earthworm *Eisenia fetida andrei* and Attendant Changes in Shape and Adhesive Capacity of Constitutive Cells. *Dev. Comp. Immunol.* **1992**, 16 (2), 95–101.
- (43) Tong, Y.; Feng, A.; Hou, X.; Zhou, Q.; Hu, X. Nanoholes Regulate the Phytotoxicity of Single-Layer Molybdenum Disulfide. *Environ. Sci. Technol.* **2019**, 53 (23), 13938–13948.
- (44) Homa, J. Earthworm Coelomocyte Extracellular Traps: Structural and Functional Similarities with Neutrophil NETs. *Cell Tissue Res.* **2018**, 371 (3), 407–414.
- (45) Homa, J.; Ortmann, W.; Kolaczowska, E. Conservative Mechanisms of Extracellular Trap Formation by Annelida *Eisenia andrei*: Serine Protease Activity Requirement. *PLoS One* **2016**, 11 (7), No. e0159031.
- (46) Tumminello, R. A.; Fuller-Espie, S. L. Heat Stress Induces ROS Production and Histone Phosphorylation in Coelomocytes of *Eisenia hortensis*. *Invert. Surviv. J.* **2013**, 10, 50–57.
- (47) Zhu, Z.; Deng, X.; Xie, W.; Li, H.; Li, Y.; Deng, Z. Pharmacological Effects of Bioactive Agents in Earthworm Extract: A Comprehensive Review. *Animal Model. Exp. Med.* **2024**, 7, 653–672.
- (48) Homa, J.; Stalmach, M.; Wilczek, G.; Kolaczowska, E. Effective Activation of Antioxidant System by Immune-Relevant Factors Reversely Correlates with Apoptosis of *Eisenia andrei* Coelomocytes. *J. Comp. Physiol. B* **2016**, 186 (4), 417–430.
- (49) Ghanem, M. M. E.; Abd-Elaziz, A. M.; Mohamed, M. A. Biochemical and Toxicological Characteristics of Polyphenol Oxidase from Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Comp. Biochem. Phys. C* **2025**, 287, No. 110044.
- (50) Patel, R.; Maurya, N.; Paray, M. U. d.; Farooq, N.; Siddique, A.; Verma, K. L.; Dohare, N. Esterase Activity and Conformational Changes of Bovine Serum Albumin toward Interaction with Mephedrone: Spectroscopic and Computational Studies. *J. Mol. Recognit.* **2018**, 31 (11), No. e2734.

(51) Xu, M.; Wan, J.; Niu, Q.; Liu, R. PFOA and PFOS Interact with Superoxide Dismutase and Induce Cytotoxicity in Mouse Primary Hepatocytes: A Combined Cellular and Molecular Methods. *Environ. Res.* **2019**, *175*, 63–70.

(52) Pan, Z.; Peng, J.; Chen, Y.; Zang, X.; Peng, H.; Bu, L.; Xiao, H.; He, Y.; Chen, F.; Chen, Y. Simultaneous Determination of Five Fluoroquinolones by the Selective High Performance Liquid Chromatography Associating with Sensitive Resonance Light Scattering and Mechanism Study. *Microchem. J.* **2018**, *136*, 71–79.

(53) Zhang, H.; Wang, Y.; Zhou, Q. Fluorimetric Study of Interaction of Benzidine with Trypsin. *J. Lumin.* **2010**, *130* (5), 781–786.

(54) McMahon, M.; Ye, S.; Pedrina, J.; Dlugolenski, D.; Stambas, J. Extracellular Matrix Enzymes and Immune Cell Biology. *Front. Mol. Biosci.* **2021**, *8*, No. 703868.

(55) Tsankov, B. K.; Luchak, A.; Carr, C.; Philpott, D. J. The Effects of NOD-like Receptors on Adaptive Immune Responses. *Biomed. J.* **2024**, *47* (1), No. 100637.

(56) Liu, M.; Chen, C.; Wu, Q.-C.; Chen, J.-L.; Dai, L.-S.; Hui Chu, S.; Liu, Q.-N. Chitinase Involved in Immune Regulation by Mediated the Toll Pathway of Crustacea *Procambarus clarkii*. *Fish Shellfish Immun.* **2021**, *110*, 67–74.

(57) Wang, Z.; Fan, N.; Li, X.; Yue, L.; Wang, X.; Liao, H.; Xiao, Z. Trophic Transfer of Metal Oxide Nanoparticles in the Tomato-*Helicoverpa armigera* Food Chain: Effects on Phyllosphere Microbiota, Insect Oxidative Stress, and Gut Microbiome. *ACS Nano* **2024**, *18* (39), 26631–26642.

(58) Li, W.; He, E.; Zhang, P.; Li, Y.; Qiu, H. Multiomics Analyses Uncover Nanoceria Triggered Oxidative Injury and Nutrient Imbalance in Earthworm *Eisenia fetida*. *J. Hazard. Mater.* **2022**, *437*, No. 129354.

(59) Zhu, D.; Zheng, F.; Chen, Q.-L.; Yang, X.-R.; Christie, P.; Ke, X.; Zhu, Y.-G. Exposure of a Soil Collembolan to Ag Nanoparticles and AgNO₃ Disturbs Its Associated Microbiota and Lowers the Incidence of Antibiotic Resistance Genes in the Gut. *Environ. Sci. Technol.* **2018**, *52* (21), 12748–12756.

(60) Wang, H.-T.; Gan, Q.-Y.; Li, G.; Zhu, D. Effects of Zinc Thiazole and Oxytetracycline on the Microbial Metabolism, Antibiotic Resistance, and Virulence Factor Genes of Soil, Earthworm Gut, and Phyllosphere. *Environ. Sci. Technol.* **2024**, *58* (1), 160–170.

(61) Zhang, J.; Xia, X.; Ma, C.; Zhang, S.; Li, K.; Yang, Y.; Yang, Z. Nanoplastics Affect the Bioaccumulation and Gut Toxicity of Emerging Perfluoroalkyl Acid Alternatives to Aquatic Insects (*Chironomus kiinensis*): Importance of Plastic Surface Charge. *ACS Nano* **2024**, *18* (7), 5752–5765.

(62) Xu, H.; Zhang, P.; He, E.; Peijnenburg, W. J. G. M.; Cao, X.; Zhao, L.; Xu, X.; Qiu, H. Natural Formation of Copper Sulfide Nanoparticles via Microbially Mediated Organic Sulfur Mineralization in Soil: Processes and Mechanisms. *Geoderma* **2023**, *430*, No. 116300.

(63) Yan, X.; White, J. C.; He, E.; Peijnenburg, W. J. G. M.; Zhang, P.; Qiu, H. Temporal Dynamics of Copper-Based Nanopesticide Transfer and Subsequent Modulation of the Interplay Between Host and Microbiota Across Trophic Levels. *ACS Nano* **2024**, *18* (37), 25552–25564.

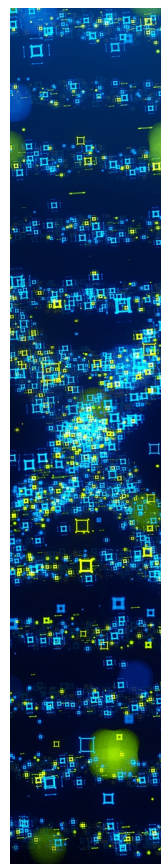
(64) Mullen, A. Expectations from Nano in Agriculture. *Nat. Nanotechnol.* **2019**, *14* (6), 515–516.

(65) Lowry, G. V.; Giraldo, J. P.; Steinmetz, N. F.; Avellan, A.; Demirer, G. S.; Ristorph, K. D.; Wang, G. J.; Hendren, C. O.; Alabi, C. A.; Caparco, A.; da Silva, W.; González-Gamboa, I.; Grieger, K. D.; Jeon, S.-J.; Khodakovskaya, M. V.; Kohay, H.; Kumar, V.; Muthuramalingam, R.; Poffenbarger, H.; Santra, S.; Tilton, R. D.; White, J. C. Towards Realizing Nano-Enabled Precision Delivery in Plants. *Nat. Nanotechnol.* **2024**, *19* (9), 1255–1269.

(66) Kah, M.; Tufenkji, N.; White, J. C. Nano-Enabled Strategies to Enhance Crop Nutrition and Protection. *Nat. Nanotechnol.* **2019**, *14* (6), 532–540.

(67) Wang, D.; Saleh, N. B.; Byro, A.; Zepp, R.; Sahle-Demessie, E.; Luxton, T. P.; Ho, K. T.; Burgess, R. M.; Flury, M.; White, J. C.; Su,

C. Nano-Enabled Pesticides for Sustainable Agriculture and Global Food Security. *Nat. Nanotechnol.* **2022**, *17* (4), 347–360.



CAS BIOFINDER DISCOVERY PLATFORM™

STOP DIGGING THROUGH DATA —START MAKING DISCOVERIES

CAS BioFinder helps you find the
right biological insights in seconds

Start your search



A Division of the
American Chemical Society