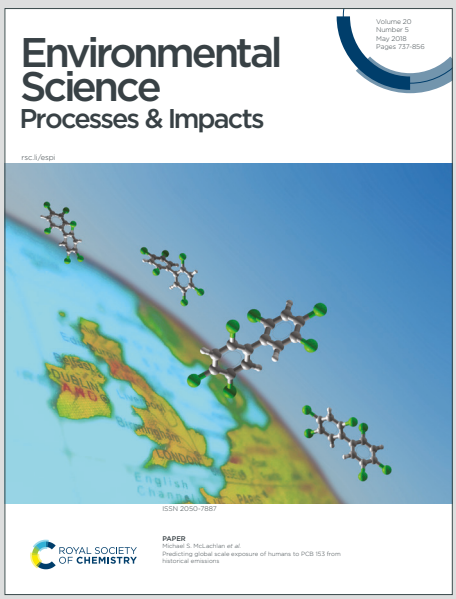
Environmental Science
Processes & Impacts

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: C. He, S. Zhang, J. Hou, Y. Chi, J. Wang, J. Xu and D. Lin, *Environ. Sci.: Processes Impacts*, 2025, DOI: 10.1039/D5EM00371G.



ROYAL SOCIETY OF CHEMISTRY

rsc.li/espi

View Article Online
DOI: 10.1039/D5EM00371G

Nanoplastics Enhance Florfenicol Toxicity through Disturbing Detoxification and Metabolic Processes in Nematodes

Caijiao He[a], Shuang Zhang[a], Jie Hou[a,b], Yi Chi[c], Jing Wang[d], Jiang Xu[a,b], Daohui Lin[a,b,*]

[a] Zhejiang Provincial Key Laboratory of Organic Pollution Process and Control, Department of Environmental Science, Zhejiang University, Hangzhou 310058, China

[b] State Key Laboratory of Soil Pollution Control and Safety, Zhejiang University, Hangzhou 310058, China

[c] Hangzhou Ecological and Environmental Monitoring Center of Zhejiang Province, Hangzhou 310007, China

[d] Ecological and Environmental Monitoring Center of Zhejiang Province, Hangzhou 310012, China

*Corresponding author: Daohui Lin (lindaohui@zju.edu.cn)

View Article Online
DOI: 10.1039/D5EM00371G

Abstract

Nanoplastics (NPs) and antibiotics are ubiquitous contaminants that frequently coexist and undergo interactions in various environments. While their combined toxicity is known to depend on NPs physicochemical properties, the mechanistic basis of their toxicological interactions, particularly how surface charge and particle size modulate combined effects, remains unclear. Using *Caenorhabditis elegans* as a model, we investigated the combined toxicity of florfenicol (FF) with four polystyrene nanoplastics (PS-NPs) differing in size (100 nm (PS-100) and 500 nm (PS-500)) and surface modification (-NH_2 (PS-NH_2) and -COOH (PS-COOH)), and the mechanisms were explained through integrated analyses of bioaccumulation, detoxification gene expression, and metabolic homeostasis. The results revealed that while the NP coexposures did not significantly alter analyte bioaccumulation in *C. elegans*, they suppressed detoxification genes, with PS-100 and PS-NH_2 causing more severe dysfunction than PS-500 or PS-COOH. Metabolomics perturbations in the combined exposures were 2.12- to 4.86-fold greater than the FF exposure alone, with different NPs exacerbating oxidative stress and toxicity *via* divergent metabolic pathway disruptions. Building upon these transcriptomic and metabolomic mechanisms, the positively-charged PS-NH_2 and smaller-sized PS-100 amplified FF toxicity, as quantified through both survival rate and body length reductions, more than their negatively-charged (PS-COOH) and larger-sized (PS-500) counterparts. The findings advance mechanistic understanding of NP-antibiotic interactions, supporting evidence-based environmental risk assessment of co-occurring pollutants.

1. Introduction

Nanoplastics (NPs) are plastic fragments with sizes between 1-1000 nm,[1] which originate from two different sources, including direct leaching (primary source) or decomposition, fragmentation, and degradation of larger plastics (secondary source).[2] NPs are widely detected at trace levels (0-1.59 mg/L) in the environment[3] and are easily ingested by organisms, posing potential toxicities because of their small size, large specific surface area, and biological permeability.[4] Moreover, NPs usually

View Article Online
DOI: 10.1039/D5EM00371G

induce combined pollution with a wide range of environmental contaminants, such as heavy metals, antibiotics, organic halogens, and other emerging contaminants.[5] Compared to single contaminants, the interaction between NPs and environmental contaminants may induce significant alterations in the surface properties of NPs, altering the bioaccumulation of NPs and environmental contaminants in exposed organisms.[6] The combined toxicity of such compounds may exhibit synergistic, antagonistic, potentiating or additive effects, owing to the differentially affected bioaccumulation and metabolism patterns.[5,7] Thus, the combined toxicity and potential mechanisms of NPs and other environmental contaminants still need to be studied, for accurately assessing the ecological risk of plastic pollution.

Antibiotics are widely used to prevent and treat bacterial infections. It is estimated that unrestricted use will increase by 200% to 126 billion defined daily doses from 2015 to 2030.[8] However, 30-90% of antibiotics are released into the environment either unchanged or as metabolic byproducts, triggering ecological risks.[9] Antibiotics have been frequently identified in water, soil, and sediment, sharing similar sources and migration pathways with microplastics (MPs) and NPs. Consequently, the coexistence of these pollutants in natural environments is inevitable.[9] In areas co-polluted by antibiotics and NPs, antibiotic concentrations can reach as high as 1.11 mg/L.[10] NPs exhibit a strong affinity for antibiotic compounds, with the adsorption capacity ranging from ng/g to mg/g dependent on the characteristics of NPs (*e.g.*, particle size and surface charge) and antibiotics.[9,11,12] Therefore, NPs can act as carriers for antibiotics, promoting their internalization and accumulation in organisms, and further altering the toxic effects of antibiotics.[9] Among antibiotics, florfenicol (FF) is one of the most predominantly utilized veterinary drugs in terrestrial and aquatic edible animals,[13,14] having been detected in various environments due to its high solubility and persistence, with concentrations as high as 11 mg/L in aquatic systems.[15] Consequently, the combined effects of NPs and FF deserve further research.

Nematodes constitute the most abundant metazoans on Earth, comprising approximately 80% of all individual multicellular animals, occupying the main trophic level of all soil food webs, and

View Article Online
DOI: 10.1039/D5EM00371G

playing an important role in maintaining ecosystem functions.[16] *Caenoehabditis elegans* (*C. elegans*) is a free-living nematode found in almost all habitats (marine, freshwater, and soil) and occupies a critical position in the benthic food web, linking low and high trophic levels.[16,17] Given the ubiquitous and highly coexisting NPs and antibiotics in various environmental media,[9] *C. elegans* inevitably exposed to co-contaminants, inducing complex interactive toxicity. Our previous study has revealed the charge- and size-dependent uptake preference and toxicity of NPs along toward *C. elegans*.[18] Meanwhile antibiotics may undergo detoxification in organisms *via* three consecutive metabolic phases (Phases I, II, and III). Generally, antibiotics are firstly oxidized by the cytochrome P450 enzymes (CYPs, Phase I), then transformed and transported by enzymes such as glutathione-S-transferase (GSTs) and UDP-glucuronosyltransferases (UGTs, Phase II), and finally excreted by ATP-binding cassette (ABC) transporters such as P-glycoprotein (PGPs, Phase III).[19,20] Nevertheless, emerging evidence suggests that plastics may interfere with antibiotic detoxification processes.[21] This interaction could exacerbate the toxicity of antibiotics by disrupting metabolic homeostasis in exposed organisms, highlighting the need for more targeted investigations into these combined effects.

Current evidence indicates that the combined toxicity of NPs and antibiotics primarily arises from alterations in antibiotic bioavailability or impaired detoxification processes in organisms. However, these interactions highly depend on NPs' characteristics, particularly particle size and surface charge, and the underlying toxicological mechanisms remain unclear. To reveal the effects of NPs' surface charge and particle size on FF toxicity during coexposure in nematodes, we examined four commercially available PS-NPs with different sizes (100 nm and 500 nm, named PS-100 and PS-500, respectively) and surface modifications (-NH_2 and -COOH, named PS-NH_2 and PS-COOH, respectively, of 100 nm in size). The combined toxicities of FF and different PS-NPs were measured and the underlying mechanisms were explained by measuring the FF bioaccumulation, FF adsorption on PS-NPs, FF uptake rates, and avoidance of nematodes, as well as the alterations in detoxification genes and metabolic processes. The findings are expected to demonstrate the role and mechanism of NPs in FF toxicity, enhancing our understanding of environmental risks of NP-antibiotic coexposures.

View Article Online
DOI: 10.1039/D5EM00371G

2. Materials and methods

2.1. Test materials and nematodes' exposure

Nematode (N2 strains) and *Escherichia coli* (*E. coli*, OP50 strain) were from our own laboratory.[20] Standardized commercial PS-NPs with controlled properties enable systematic comparative investigations that are crucial for assessing their environmental risks and for elucidating the toxicological mechanisms and their interactions with environmental contaminants. Consequently, commercial PS-NPs have been widely employed in studies examining environmental behaviors and fates of NPs.[5,12,18] Two fluorescence labelled PS-NPs were specifically used to visualize their distributions in nematodes. The green PS-NPs were used in most experiments, while the red PS-NPs were used for tracking the distribution of PS-NPs in nematodes. The green PS-100 and PS-500 were obtained from Tianjin Baseline Chromtech Research Center (Tianjin, China). The green PS-COOH and PS-NH_2 and the red PS-100 were obtained from Huge Biotechnology Co. (Shanghai, China). The red PS-COOH, PS-NH_2, and PS-500 were purchased from Tianjin Big Goose Scientific Co. (Tianjin, China). FF was purchased from Shanghai Macklin Biochemical Co. (Shanghai, China), and chloramphenicol-D5 (CAP-D5) were purchased from ANPEL Laboratory Technologies (Shanghai) Co. (shanghai, China). All PS-NPs and FF stock solutions (100 mg/L) were diluted with deionized water and stored at 4 ℃. CAP-D5 stock solutions (100 mg/L) were diluted with methanol and stored at -20 ℃.

Age-synchronized L1 larvae N2 (approximately 8000 L1 larvae/mL) were grown in K-medium in conical flasks with *E. coli* (OD600 = 0.4) as food. To simulate potential environmental hotspots or extreme contamination conditions and assess potential risks, the exposure concentrations of PS-NPs and FF were both set at 1 mg/L, according to their reported levels in polluted environments.[3,10,15] FF (1 mg/L) and four PS-NPs (1 mg/L) were set alone as single treatment groups. All exposure treatments were maintained on an orbital shaker (180 rpm, 20 °C) and incubated in the dark for 72 h.

2.2. Observation and measurements of PS-NPs in nematodes

Nematodes were exposed to K-medium containing FF (1 mg/L) and red PS-NPs (1 mg/L) for 72

View Article Online
DOI: 10.1039/D5EM00371G

h, then they were washed twice with K-medium and killed. The distribution of PS-NPs in nematodes was observed using confocal laser scanning microscopy (CLSM, LSM 880, Zeiss).[22] Specifically, nematodes were digested with tetramethylammonium hydroxide (15%, w/w), then were ultrasonically extracted twice and centrifuged to obtain the supernatants. Triton X-45 (10%, w/w) were added into the supernatants and bathed in water for 30 min (45 ℃). The mixtures were centrifuged (3000*g*, 20 min). The PS-NPs remained in the supernatants were labeled by gold (III) ethylenediamine complex ($Au(en)_2Cl_3$, 100 μL, 0.75 g/L) and were measured by single particle inductively coupled plasma mass spectrometry (SP-ICP-MS, Fisher Scientific, USA).[18,23]

2.3. Measurements of FF accumulations in nematodes

FF in nematodes was measured based on previous study.[24] Specifically, 100 μL of 50 μg/L internal standard (CAP-D5) was added into 50 mg of nematodes, and then was ground to obtain the homogenate. Then, 50 μL of ammonia water and 1 mL of ethyl acetate were added. After being vortexed (10 min), samples were centrifuged (8000*g*, 5 min) to achieve phase separation. The supernatant was transferred to an 8 mL glass bottle and the precipitate was extracted again. The extracted supernatants were combined and dried with nitrogen at room temperature. About 1 mL of methanol: water (1:9, v/v) mixture was added into the residue, and then was ultrasonically extracted for 1 h. About 2 mL of n-hexane was added and vortexed for 30 sec, and centrifuged at 1500*g* for 10 min. The upper layer of n-hexane was discarded. Another 2 mL of n-hexane was added and removed again. Then, the lower aqueous phase was filtered through a 0.22 μm filter membrane, and the contained FF was analyzed with liquid chromatography tandem mass spectrometry (LC-MS/MS, US Waters). The recoveries of 50 μg/kg FF and CAP-D5 were 118 ± 1% and 107 ± 1%, respectively.

2.4. Adsorption experiments of FF on PS-NPs

PS-NPs (10 mg/L) were mixed with FF (10 mg/L) in 250 mL glass conical flasks containing 150 mL of K-medium (pH 6) and maintained in an orbital shaker (180 rpm, 20 °C). About 5 mL of suspension was sampled and filtered through 50 nm nylon membranes (Haining Yibo, Zhejiang) to remove PS-NPs at a given time interval, then the concentration of FF was measured by LC-MS/MS.

View Article Online
DOI: 10.1039/D5EM00371G

The initial ratio of NPs to contaminants can significantly affect the adsorption efficiency, as previously demonstrated by the substantial adsorption of antibiotics (*e.g.*, ciprofloxacin and tetracycline) at a ratio of NPs to contaminants up to 500.[25,26] We further investigated the potential FF (1 mg/L) adsorption by extreme high concentrations (200, 500, and 1000 mg/L) of NPs. This experimental design aimed to specifically address the potential concentration-dependent adsorption of FF on PS-NPs. FF solutions without PS-NPs or organisms were used as blank controls.

2.5. Pharyngeal pumping and avoidance behavior assays

Pharyngeal pumping rates were recorded based on a previous method.[27] At least 10 nematodes were recorded for 30 sec to obtain the average pumping rates. Avoidance behavior of nematodes was evaluated by counting the number of nematodes on both sides of the nematode growth medium (NGM) plate.[18] Specifically, a NGM plate was separated into two equal parts, and one side was covered with test material and another added nothing. One hundreds of L4 nematodes were dropped in the center of a plate and maintained for 1 h, then avoidance was calculated through the following formula.[28]

$$\text{Avoidance} = \frac{\text{Nematodes' number on the test area} - \text{Nematodes' number on the blank area}}{\text{Total number of nematodes}}$$

2.6. Toxicity assays of PS-NPs and FF

To evaluate the toxicity of PS-NPs and FF, the body length and survival rate were measured.[18] Besides, the related indicators of oxidative stress, including the activities of superoxide dismutase (SOD) and the contents of malondialdehyde (MDA), glutathione (GSH), and oxidized glutathione (GSSG) were also determined. More than 30 nematodes were measured to obtain the body length. Survival rate was counted through optical microscopy (Nikon H600L, Japan) and was further quantified as the percentage of surviving nematodes relative to the initial population. The related indicators of oxidative stress were measured using the corresponding reagent kits from Nanjing Jiancheng Biological Co., Ltd. (China).

2.7. Transcriptomics and metabolomic analyses

Nematodes were maintained with FF (1 mg/L) alone and in coexposure with PS-NPs (1 mg/L) in an orbital shaker (180 rpm, 20 °C). After 72 h of exposure, transcriptomic and metabolomic

analyses were carried out by Majorbio Bioinformatics Technology Co., Ltd. (Shanghai, China). Specifically, approximately 50 mg of nematodes was collected for each treatment after the 72-h exposure, then total RNA was extracted using the TRIzol Reagent (Invitrogen, USA) and was separated by magnetic frame to obtain mRNA. The extracted mRNA was then analyzed using a NovaSeq X Plus (Illumina, USA).

For metabolomic analysis, nematodes were mixed with 400 μL of extraction solution (methanol: water=4: 1, v/v), with L-2-chloroalanine (20 mg/L) as an internal standard. The mixture was ground to obtain homogenate, then was ultrasonically extracted (30 min, 5 ℃) and centrifuged (13000*g*, 15 min) to obtain supernatant. The supernatant was analyzed by LC-MS/MS (Thermo Fisher, USA).

2.8. Statistical analyses

Experimental data are presented as mean ± standard deviation (SD), and error bars indicate the SD. Data normality was confirmed using the Shapiro-Wilk test and Q-Q plots, and homogeneity of variances was verified using Levene's test. Since assumptions for analysis of variation (ANOVA) were met, group comparisons were conducted using a one-way ANOVA followed by Tukey's HSD post hoc test. Statistical analyses were performed using SPSS 21.0 with $p < 0.05$ taken as statistically significant. Signals *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For each treatment, three independent biological replicates were performed by exposing approximately 8000 synchronized L1-stage nematodes larvae/mL in separate conical flasks (n = 3), and ten independent replicates (n = 10) were performed for the avoidance test.

3. Results and discussion

3.1. Nanoplastics enhanced the toxicity of FF to nematodes

After the 72-h exposure, the survival rates of nematodes were significantly reduced by both the PS-100 and the PS-NH_2 treatments and the body length were affected by the four PS-NPs treatments (Figure 1a), which were consistent with our previous study.[18] The survival rates of nematodes in the FF, PS-100 + FF, PS-COOH + FF, PS-NH_2 + FF, and PS-500 + FF groups were 87.2%, 79.9%,

View Article Online
DOI: 10.1039/D5EM00371G

81.3%, 69.6%, and 85.8%, respectively (Figure 1a). Compared to the FF alone group, the survival rates of nematodes in PS-COOH + FF, PS-100 + FF, and PS-NH_2 + FF groups were decreased by 5.9%, 7.3%, and 17.6%, respectively ($p < 0.05$), and the body lengths were shortened by 2.3%, 3.1%, 4.6% , and 6.2% in PS-500 + FF, PS-COOH + FF, PS-100 + FF, and PS-NH_2 + FF groups, respectively ($p < 0.05$). Indicators of oxidative stress showed that the PS-NH_2 + FF group had a significant decrease in SOD activity in comparison with the FF group ($p < 0.001$), and the PS-100 + FF, PS-COOH + FF, and PS-500 + FF groups also exhibited a decreasing trend in SOD level (Figure 1c), indicating that the combined exposure to PS-NPs and FF impaired the nematodes' ability to counteract oxidative stress. No significant change in GSH content was observed between the PS-NPs + FF groups and the FF alone group (Figure 1d, $p > 0.05$). In contrast, the MDA levels in the PS-NPs + FF groups increased by 1.20-1.31 folds (Figure 1e). MDA is a biomarker of oxidative stress. The increased MDA content suggested that the PS-NPs + FF treatments resulted in more serious oxidation stress in nematodes. GSH functions as a critical endogenous antioxidant system that plays a vital role in neutralizing toxic oxidants.[29] While GSH depletion typically leads to elevated MDA levels as an indicator of oxidative damage, emerging evidence suggests that oxidative stress can intensify without concomitant GSH reduction. This phenomenon occurs through compensatory regulation by other antioxidant enzymes.[30] The increased MDA levels and unchanged GSH content have been reported in *Mytilus coruscus*, mice, and *Eriocheir sinensis*,[30-32] which was primarily mediated by decreased activity of key antioxidant enzymes, including glutathione peroxidase 4, SOD, and catalase. Consequently, the coexposures of PS-NPs and FF caused an exacerbation of oxidative damage in nematodes, which could impact their growth and development (in four PS-NPs + FF groups) and even result in mortality (in PS-100 + FF, PS-COOH + FF, and PS-NH_2 + FF groups). According to the results, we can conclude that PS-NPs worsened the toxicity induced by FF, and the exacerbation caused by the positively-charged (PS-NH_2) and smaller-sized (PS-100) PS-NPs was severer than that caused by the negatively-charged (PS-COOH) and larger-sized (PS-500) PS-NPs. The enhancement effect of positively-charged or smaller-sized NPs has been

View Article Online
DOI: 10.1039/D5EM00371G

reported during the single and combined exposures,[12,18,33] indicating that the characteristics of PS-NPs may dominate the toxic effects during combined exposures.

3.2. Detoxification rather than bioaccumulation were modulated by nanoplastics

The phenomenon of toxicity enhancement is typically associated with an increase in the concentration of contaminants accumulated by organisms.[12] Subsequent results demonstrated that the bioaccumulation of FF in nematodes after being exposed to FF, PS-100 + FF, PS-COOH + FF, PS-NH_2 + FF, and PS-500 + FF were 2.35 ± 0.22, 2.74 ± 0.03, 2.81 ± 0.49, 2.26 ± 0.05, 2.06 ± 0.12 mg/kg (dry weight), respectively (Figure 2a). Obviously, the bioaccumulation of FF in nematodes was not significantly affected by the coexposures to PS-NPs. The bioaccumulation of PS-NPs in nematodes revealed that the coexposure with FF did not significantly affect the bioaccumulation of the four PS-NPs in nematodes either. The accumulations of PS-100, PS-COOH, PS-NH_2, and PS-500 were 112 ± 30, 136 ± 44, 99.3 ± 7.2, and 1247 ± 184 mg/kg in the single-exposure groups, and were 122 ± 5, 128 ± 31, 94.5 ± 7.2, and 1117 ± 78 mg/kg in the combined-exposure groups, respectively (Figure 2b). In addition, the distribution of PS-NPs in nematodes was predominantly confined to the intestine, both in the single-exposure groups and the combined-exposure groups (Figure S1 in the Supporting information), which was consistent with our previous finding.[18] The results indicate that the increased toxicity of FF to nematodes could not be related to changes in the overall bioaccumulations of FF or PS-NPs.

The adsorption of antibiotics by plastics is considered an important factor affecting the bioaccumulation of antibiotics.[21] Adsorption results of FF on PS-NPs showed that the removal rates of FF by different PS-NPs in 72 h were all less than 3.3% in the given experimental system (PS-NPs: FF = 1:1) and extreme concentration ratios (PS-NPs: FF = 1000:1), indicating that the adsorption effect of PS-NPs on FF was negligible (Figure 3a, b). The nematodes were observed to absorb FF and PS-NPs at the same pumping rate with control (about 127 min^{-1}) in both the single-exposure and combined-exposure groups (Figure S2a), indicating that the ingestion of PS-NPs and FF relied on instinctively mechanical motion of pharyngeal muscles. Hence, the nematodes could accumulate

View Article Online
DOI: 10.1039/D5EM00371G

considerable amounts of FF and PS-NPs. Considering the defense response of nematodes to toxic materials,[18,34] nematodes exhibited certain avoidance behavior towards the PS-NPs and FF (Figure S2b). Although nematodes in the PS-NPs + FF groups exhibited stronger avoidance than those in the FF group, the accumulation of FF in nematodes remained constant due to nematodes exhibiting avoidance behavior regardless of encountering FF or PS-NPs during the test. According to above results, the physicochemical interactions between PS-NPs and FF were relatively weak *in vitro* and their contributions to FF toxicity were insignificant. Therefore, it was speculated that biological processes, might dominate the enhanced toxicity of FF under the coexposures of PS-NPs.

The detoxification ability of organisms (such as metabolic enzyme activity and antioxidant defense system) directly affects the apparent toxicity of contaminants.[29,35] According to previous study,[21] the increase in toxicity of antibiotics by MPs might be attributed to both the Trojan horse effect and the disruption of detoxification. The disruption of detoxification might dominate the FF toxicity to nematodes after coexposures with PS-NPs, since there was no significant increase in FF bioaccumulation after the addition of PS-NPs. To investigate the effect of PS-NPs + FF coexposure on the expression of detoxification genes in nematodes, the transcriptomic sequencing was conducted. Principal component analysis (PCA) demonstrated partial but not complete overlap between the treatment groups and the control group (Figure 4a), suggesting subtle but discernible transcriptomic differences. To further elucidate these variations, downstream analyses were focused on differentially expressed genes (DEGs) identified in each group. Comparative transcriptomics identified 377, 270, 589, 632, and 302 DEGs in response to FF, PS-100 + FF, PS-COOH + FF, PS-NH_2 + FF, and PS-500 + FF exposures, respectively (Figure 4b), with most DEGs showing downregulation among all treatment groups (Figure S3). Functional classification demonstrated that 59.7-63.9% of DEGs were associated with membrane-related processes (Figure 4c). Gene Ontology (GO) enrichment analysis further revealed that all PS-NPs + FF groups exhibited significant enrichment in extracellular region-related terms compared to the FF group (Figure 4d). Previous studies suggested that alterations in extracellular regions might reflect structural and compositional changes in cells, where extracellular

View Article Online
DOI: 10.1039/D5EM00371G

space expansion resulted from cellular damage and subsequent leakage of intracellular components.[36,37] These findings imply that PS-NPs exposure may induce membrane disruption and protein degradation in nematode cells. Notably, the PS-100 + FF and PS-NH_2 + FF groups showed specific enrichment in glucuronosyltransferase and UDP-glycosyltransferase activities. UGTs play a critical role in phase II detoxification by catalyzing the conjugation of lipophilic compounds (*e.g.*, environmental contaminants, pharmaceuticals, and endogenous substances) with sugar moieties, thereby enhancing their polarity and excretion.[38] In nematodes, UGTs function as key detoxification enzymes that are widely found in somatic cells, and their impairment can lead to diverse toxicological phenotypes due to compromised xenobiotic elimination.[39,40]

Notably, exposure to PS-NPs + FF significantly disrupted protein homeostasis in nematodes (Figure 4e), as evidenced by pronounced downregulation of key genes involved in protein folding (*e.g.*, *abu*, *grl*, *grd*), protein synthesis (*e.g.*, *scl*, *wrt*), and proteolysis (*e.g.*, *pcp*, *asp*, *nas*). This comprehensive dysregulation suggests a substantial impairment of normal protein balance mechanisms. The detoxification capacity was particularly compromised, with significant suppression of critical detoxification genes,[41] such as *abf*, *pgp*, *gcy*, *gst*, *oac*, *clec*, *cyp*, and *ugt*. Wherein CYPs carries out the majority of oxidative Phase I reactions and reduces toxicity from accumulation of chemicals. UGTs are important phase II enzymes and catalyze the conjugation of pollutants to sugar groups, and PGPs are vital for the export of xenobiotics and metabolites out of the cell in Phase III.[38,42] The Phases I, II, and III detoxification genes regulate the xenobiotic metabolism and stress responses of nematodes to contaminants, and the suppression of these genes indicted that detoxification functions were affected by PS-NPs. According previous research,[21] MPs significantly inhibited GST activity and the expression of detoxification genes in *Tegillarca granosa*, leading to an increased food safety risk of antibiotic contamination through aggravating the bioaccumulation. Although the bioaccumulation of FF in nematodes was not significantly affected in our study, the significant downregulation of these genes suggested that the detoxification process was disrupted by the exposure of nematodes to PS-NPs (Figure 4e), which might partially explain the increase in the

toxicity of FF *via* weak resistance. Because the *col* genes are considered important genes in the composition of nematode epidermis.[43] All *col* genes were significantly downregulated in the PS-NPs + FF groups and upregulated in the FF group, indicating that the coexposures of PS-NPs might have disrupted the barrier function of nematodes' epidermis, thereby decreasing the resistance and enhancing the susceptibility to FF. Meanwhile, the more enrichment of detoxification and resistance in PS-100 + FF and PS-NH_2 + FF groups than that of PS-COOH + FF and PS-500 + FF groups were able to explain the stronger enhancement of FF toxicity in the former groups.

3.3. Mechanisms of combined toxicity between different nanoplastics and florfenicol

Metabolomics can reflect the composition, dynamics, and functional roles of small-molecule metabolites in organisms after being exposed to environmental contaminants, which has been widely used in toxicological mechanism studies.[44,45] PCA of metabolomics displayed incomplete overlap between the treatment groups and the control group, with the PS-NH_2 + FF group showing particularly pronounced separation from PS-100 + FF, PS-COOH + FF, and PS-500 + FF (Figure 5a), indicating that the positively-charged PS-NH_2 induced different toxic mechanisms compared to the other three PS-NPs. This metabolomics analysis successfully identified 159, 536, 662, 773, and 337 metabolites that exhibited significant changes in FF, PS-100 + FF, PS-COOH + FF, PS-NH_2 + FF, and PS-500 + FF groups, respectively (Figures 5b, S4). The number of metabolites showing significant changes in PS-NPs + FF groups was 2.12- to 4.86- fold higher than in the FF alone group. Notably, PS-NH_2 + FF affected more metabolites than the other three PS-NPs + FF groups. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the metabolic responses in nematodes varied with both particle size and surface charge of the PS-NPs (Figure 5c). The PS-100 + FF primarily affected nucleotide metabolism, biotin metabolism, as well as purine and pyrimidine metabolism; PS-COOH + FF showed strong enrichment in nucleotide metabolism, pyrimidine metabolism, and glutathione metabolism; PS-NH_2 + FF mainly enriched in the one carbon pool by folate and the neuroactive ligand receptor interaction; and PS-500 + FF mainly impacted glutathione metabolism (Figure 5c). These metabolic perturbations have critical biological

View Article Online
DOI: 10.1039/D5EM00371G

implications. Nucleotide-related metabolism (including purine and pyrimidine metabolism) disruption compromises genetic material synthesis, cellular proliferation, and energy homeostasis, potentially leading to developmental defects and redox imbalance.[46-48] It has been reported that exposure to foreign toxicants could trigger dysregulation of nucleotide-related metabolism, mediating developmental defects in zebrafish embryos.[47,49] GSH metabolism impairment reduces detoxification capacity and antioxidant defenses, increasing susceptibility to toxins.[50] Previous researches suggest that dysfunction of these metabolisms may induce oxidative stress and inflammation, and even death.[47,51] The observed toxicity to nematodes is presumably attributable to the disruption of these pathways, with oxidative stress and metabolic imbalances serving as key contributing factors.

The metabolites in main metabolism pathways of nematodes exposed to PS-NPs + FF were further analyzed to elucidate the metabolic homeostasis (Table S1 in the Supporting information). In nucleotide-related metabolism pathways (PS-100 + FF and PS-COOH + FF), the levels of adenine and cytidine were significantly upregulated, where uridine 5'-monophosphate, 5'-guanylic acid, deoxyadenosine monophosphate, and deoxycytidine monophosphate were inhibited. In GSH metabolism pathways, the levels of GSSG were upregulated in the PS-COOH + FF group; the levels of glutamic acid were upregulated while GSH were downregulated in the PS-500 + FF group. These alterations demonstrated the disruption of nucleotide-related metabolism and GSH metabolism in nematodes following exposures to PS-100 + FF, PS-COOH + FF or PS-500 + FF, which may contribute to the observed antioxidant system damage, development inhibition, and survival rate reduction. Compared to the other three PS-NPs coexposure groups (PS-100, PS-COOH, and PS-500), PS-NH_2 + FF interfered with the one carbon pool by folate in nematodes. The one carbon pool by folate can serve as a carbon source for the synthesis of various substances and has been shown to affect the lifespan of nematodes.[52,53] Abnormal folate metabolism pathways are associated with many diseases, including impaired DNA synthesis, restricted cell proliferation, increased risk of developmental abnormalities, and reduced lifespan.[54] Metabolites detected in the PS-NH_2 + FF group showed the decrease in 5,10-methylenetetrahydrofolate and the increase in homocysteine (Table S1),

View Article Online
DOI: 10.1039/D5EM00371G

indicating the obstruction of the folate cycle and methionine cycle pathways (Figure 5d). Elevated homocysteine has been demonstrated to impair methylation, a process crucial to DNA synthesis and repair, which can induce DNA strand breakage, oxidative stress, and apoptosis.[55,56] The heatmap of the top 30 metabolites exhibited that GSSG was a collectively enhanced metabolite in PS-100, PS-COOH, and PS-500 (Figure 5e), revealing the disruption of the balance between oxidation and antioxidation. Previous study has proved that homocysteine enters the sulfur-containing amino acid metabolism pathways, and cystathionine participates in methionine sulfur-transferring and serves as the primary route for L-cysteine production in animals.[44,57] L-cysteine functions as both a direct reactive oxygen species scavenger and a precursor of reduced GSH, controlling the redox status in organisms. Although GSSG in the PS-NH_2 + FF group was lower than that in the FF group, homocysteine may induce death of nematodes through increased oxidative stress.

Integration of transcriptomics and metabolomics analyses can help to reveal distinct toxic mechanisms associated with different NPs and antibiotic combinations.[58,59] Specifically, the downregulation of detoxification genes could block metabolic synthesis pathways, leading to systemic biological dysfunction. A key example is the *oac* gene, which can play a critical role in sulfur-containing amino acid metabolism by facilitating the conversion of free cysteine to GSH, thereby enhancing stress response in nematodes.[60] The consistent downregulation of *oac* genes in PS-COOH + FF, PS-NH_2 + FF, and PS-500 + FF groups correlated with their respective dysregulated defense mechanisms involved in glutathione and folate metabolism (Figures 4e, 5c). In addition, PS-100 + FF and PS-COOH + FF directly inhibited DNA replication by interfering with nucleotide-related metabolism pathways, leading to global downregulation of gene expression.[47] In contrast, PS-COOH + FF and PS-500 + FF induced severe oxidative stress, inducing a fundamental shift in defensive responses through GSH metabolism. The altered GSH levels could modulate detoxification gene transcription.[58,60] PS-NH_2 + FF caused DNA synthesis impairment and elevated oxidative stress possibly through dysregulation of folate metabolism pathway.[55] Notably, both direct (through DNA synthesis and repair interference) and indirect (*via* oxidative and antioxidative imbalance)

mechanisms of genetic damage were observed in PS-COOH + FF and PS-NH_2 + FF groups.

Based on the results above, the detailed mechanisms enhancing the toxicity of FF upon the coexposures to PS-NPs are illustrated schematically in Figure 6. Although the four PS-NPs all enhanced the FF toxicity, the toxic mechanisms induced by the differently sized or charged PS-NPs were distinct. Specifically, the vectors of PS-NPs on FF bioaccumulation and their contributions to the toxicity of FF were insignificant. However, all PS-NPs disturbed the biological processes of nematodes through disrupting differently metabolic pathways. For example, PS-100 caused disorder in nucleotide-related and biotin metabolism; PS-COOH disturbed the pathways of nucleotide-related and GSH metabolism; PS-NH_2 disturbed the pathways of one carbon pool by folate; and PS-500 disturbed the pathways of GSH metabolism. According to our previous study,[18] the 72-h exposure to four PS-NPs (0.1 and 1 mg/L) exhibited toxicity to nematodes in the order of PS-NH_2 > PS-100 > PS-COOH > PS-500. This study further revealed that the coexposure to PS-NH_2 or PS-100 with FF enhanced growth inhibition and reduced survival rate of nematodes, likely due to metabolic disruption. These findings collectively suggest that PS-NH_2 and PS-100 induce stronger physiological dysfunction compared to PS-COOH and PS-500. Taken together, these metabolic perturbations by PS-NPs might cause cell structural defects, cellular energy imbalance, and nutrient synthesis obstruction, collectively impairing the nematodes' detoxification capacity and FF resistance; the enhanced toxicity was particularly pronounced with positively-charged (PS-NH_2 > PS-COOH) and smaller (PS-100 > PS-500) PS-NPs, correlating with their greater cell interaction potential.

4. Conclusion

This study found that the coexposures to PS-NPs enhanced the toxicity of FF to nematodes, with positively-charged and smaller-sized PS-NPs exhibiting pronounced effects compared to their negatively-charged and larger-sized counterparts. It was further revealed that the disruption of detoxification and metabolic processes could play major roles in the enhancement of FF toxicity by PS-NPs, while the carrier effect of PS-NPs on FF was negligible under. Specifically, our findings reveal plastic-specific metabolic perturbations: (1) PS-100 primarily disrupted both nucleotide-

View Article Online
DOI: 10.1039/D5EM00371G

related and biotin metabolisms; (2) PS-COOH affected both nucleotide-related and GSH metabolic pathways; (3) PS-NH_2 particularly impaired the one carbon pool by folate pathway; while (4) PS-500 induced dysfunction in GSH metabolic pathway. The dysfunctions of detoxification and metabolism could weaken the resistance of nematodes to FF, thereby exacerbating growth and survival reductions. The positively-charged and smaller-sized PS-NPs exhibited stronger dysfunction of detoxification, and thus induced higher toxicity of FF to nematodes. By decoding how NP size and charge modulate antibiotic toxicity, this work enables targeted risk evaluation of NP-antibiotic combinations in contaminated ecosystems, supporting establishment of environmental management strategies for emerging co-pollutants.

According to the results obtained in this study, NPs-associated FF toxicity in nematodes was predominantly mediated through metabolic disruption rather than the carrier effect, with the affected metabolic pathways showing distinct dependence on both particle size and surface charge characteristics. The results highlight the critical role of organismal metabolism and detoxification processes in determining the bioaccumulation and toxicity of organic pollutants, underscoring the necessity to incorporate metabolic considerations when assessing the combined effects of plastics and pollutants. While adsorption exhibited a negligible role in this study, it remains a vital interaction mechanism between NPs and pollutants that warrants careful consideration. Under real environmental conditions where factors such as NP aging and biofilm formation occur, adsorption may emerge as a predominant factor influencing combined toxicity.[9,11,61] Hence, future research should systematically investigate both metabolic and adsorptive mechanisms to fully understand the complex interactions between NPs and co-existing pollutants in environmental systems.

Data availability

Data will be made available on request.

Author contributions

Caijiao He: Conceptualization, Data curation, Formal analysis, Methodology, Writing-original

View Article Online
DOI: 10.1039/D5EM00371G

draft. **Shuang Zhang**: Data curation. **Jie Hou**: Methodology. **Yi Chi**: Resources. **Jing Wang**: Resources. **Jiang Xu:** Methodology. **Daohui Lin**: Conceptualization, Writing-review & editing, Supervision, Funding acquisition.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42192573), the Key Research and Development Program of Zhejiang Province (2024C03228), Zhejiang Society for Environmental Sciences (2022HT0003), and Hangzhou Science and Technology Bureau (20220919Y165).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XX.XXX.XX.

References

1 J. Gigault, A. ter Halle, M. Baudrimont, P. Y. Pascal, F. Gauffre, P. Thuy-Linh, H. El Hadri, B. Grassl, S. Reynaud, Current opinion: What is a nanoplastic? *Environ. Pollut.*, 2018, **235**, 1030-1034.

2 B. Pandey, J. Pathak, P. Singh, R. Kumar, A. Kumar, S. Kaushik, T. K. Thakur, Microplastics in the ecosystem: An overview on detection, removal, toxicity assessment, and control release, *Water*, 2023, **15**, 51.

3 D. Materić, M. Peacock, J. Dean, M. Futter, T. Maximov, F. Moldan, T. Röckmann, R. Holzinger, Presence of nanoplastics in rural and remote surface waters, *Environ. Res. Lett.*, 2022, **17**, 054036.

4 T. Atugoda, H. Piyumali, H. Wijesekara, C. Sonne, S. S. Lam, K. Mahatantila, M. Vithanage, Nanoplastic occurrence, transformation and toxicity: A review, *Environ. Chem. Lett.*, 2023, **21**,

363-381.

5 J. Bhagat, N. Nishimura, Y. Shimada, Toxicological interactions of microplastics/nanoplastics and environmental contaminants: Current knowledge and future perspectives. *J. Hazard. Mater.*, 2021, **405**, 123913.

6 Y. Zhang, Z. Zhang, M. Zhang, C. Zhang, H. Chen, F. Wang, L. Xie, J. Mu, Surface functional groups on nanoplastics delay the recovery of gut microbiota after combined exposure to sulfamethazine in marine medaka (*Oryzias melastigma*), *Aquat. Toxicol.*, 2024, **267**, 106813.

7 G. Magara, F. R. Khan, M. Pinti, K. Syberg, A. Inzirillo, A. C. Elia, Effects of combined exposures of fluoranthene and polyethylene or polyhydroxybutyrate microplastics on oxidative stress biomarkers in the blue mussel (*Mytilus edulis*). *J. Toxicol. Env. Health Part A*, 2019, **82**, 616-625.

8 E. Y. Klein, T. P. Van Boeckel, E. M. Martinez, S. Pant, S. Gandra, S. A. Levin, H. Goossens, R. Laxminarayan, Global increase and geographic convergence in antibiotic consumption between 2000 and 2015, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E3463-E3470.

9 Y. Wang, Y. Yang, X. Liu, J. Zhao, R. Liu, B. Xing, Interaction of microplastics with antibiotics in aquatic environment: Distribution, adsorption, and toxicity, *Environ. Sci. Technol.*, 2021, **55**, 15579-15595.

10 Y. Guo, W. Shao, W. Zhao, H. Zhu, The occurrence, distribution, environmental effects, and interactions of microplastics and antibiotics in the aquatic environment of China. *Water*, 2024, 16, 1435.

11 Z. Zheng, Y. Huang, L. Liu, Wang, L. Tang, J., Interaction between microplastic biofilm formation and antibiotics: Effect of microplastic biofilm and its driving mechanisms on antibiotic resistance gene. *J. Hazard. Mater.*, 2023, **459**, 132099.

12 J. Zhang, X. Xia, C. Ma, S. Zhang, K. Li, Y. Yang, Z. Yang, 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**, 5752-5765.

13 I. M. Assane, K. S. Gozi, G. M. Ramos Valladao, F. Pilarski, Combination of antimicrobials as an approach to reduce their application in aquaculture: Emphasis on the use of thiamphenicol/florfenicol against *Aeromonas hydrophila*, *Aquaculture*, 2019, **507**, 238-245.

14 S. Zou, W. Xu, R. Zhang, J. Tang, Y. Chen, G. Zhang, Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: Impacts of river discharge and aquaculture activities, *Environ. Pollut.*, 2011, **159**, 2913-2920.

15 H. Zong, D. Ma, J. Wang, J. Hu, Research on florfenicol residue in coastal area of dalian (Northern China) and analysis of functional diversity of the microbial community in marine sediment, *Bull. Environ. Contam. Toxicol.*, 2009, **84**, 245-249.

16 J. van den Hoogen, S. Geisen, D. Routh, H. Ferris , W. Traunspurger, D. A. Wardle, R. G. M. de Goede, B. J. Adams, W. Ahmad, W. S. Andriuzzi, R. D. Bardgett, M. Bonkowski, R. Campos-Herrera, J. E. Cares, T. Caruso, L. de Brito Caixeta, X. Chen, S. R. Costa, R. Creamer, J. Mauro da Cunha Castro *et al.*, Soil nematode abundance and functional group composition at a global scale, *Nature*, 2019, **572**, 194-198.

17 M. Schratzberger, M. Holterman, D. van Oevelen, J. Helder, A worm's world: Ecological flexibility pays off for free-living nematodes in sediments and soils, *Bioscience*, 2019, **69**, 867-876.

18 C. He, X. Lin, P. Li, J. Hou, M. Yang, Z. Sun, S. Zhang, K. Yang, D. Lin, Nematode uptake preference toward different nanoplastics through avoidance behavior regulation, *ACS Nano*, 2024, **18**, 11323-11334.

19 E. M. Leslie, R. G. Deeley, S. P. C. Cole, Multidrug resistance proteins: Role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense, *Toxicol. Appl. Pharmacol.*, 2005, **204**, 216-237.

20 N. T. Popovic, R. S. Klobucar, I. Strunjak-Perovic, M. Jadan, J. Barisic, R. Coz-Rakovac, Piscine cytochromes P450 (CYP) and their response to antimicrobial drugs, *Aquac. Res.*, 2015, **46**, 257-271.

21 W. Zhou, Y. Han, Y. Tang, W. Shi, X. Du, S. Sun, G. Liu, Microplastics aggravate the bioaccumulation of two waterborne veterinary antibiotics in an edible bivalve species: Potential mechanisms and implications for human health, *Environ. Sci. Technol.*, 2020, **54**, 8115-8122.

22 S. Shaham, Methods in cell biology, *In WormBook*, 2006, 1-75.

23 P. Li, C. He, D. Lin, Extraction and quantification of polystyrene nanoplastics from biological samples. *Environ. Pollut.*, 2022, **314**, 120267.

24 J. Hou, Y. Lu, Q. Chen, X. Liao, X. Wu, K. Sang, J. C. White, J. L. Gardea-Torresdey, J. Xu, J. Zhang, K. Yang, L. Zhu, D. Lin, Multifunctional biomolecular corona- inspired nanoremediation of antibiotic residues, *Proc. Natl. Acad. Sci. U. S. A.*, 2024, **121**, e2409955121.

25 M. Yilimulati, L. Wang, X. Ma, C. Yang, N. Habibul, Adsorption of ciprofloxacin to functionalized nano-sized polystyrene plastic: Kinetics, thermochemistry and toxicity. *Sci. Total Environ.*, 2021, **750**, 142370.

26 L. Feng, Y. Shi, X. Li, X. Sun, F. Xiao, J. Sun, Y. Wang, X. Liu, S. Wang, X. Yuan, Behavior of tetracycline and polystyrene nanoparticles in estuaries and their joint toxicity on marine microalgae *Skeletonema costatum*. *Environ. Pollution.*, 2020, **263**, 114453.

27 D. Raizen, B. m. Song, N. Trojanowski, Y. J. You, Methods for measuring pharyngeal behaviors. *In WormBook*, 2012, 1-13.

28 Y. Zhou, M. Loeza-Cabrera, Z. Liu, B. Aleman-Meza, J. K. Nguyen, S. K. Jung, Y. Choi, Q.

Shou, R. A. Butcher, W. Zhong, Potential nematode alarm pheromone induces acute avoidance in *Caenorhabditis elegans*, *Genetics*, 2017, **206**, 1469-1478.

29 C. Umansky, A. E. Morellato, M. Rieckher, M. A. Scheidegger, M. R. Martinefski, G. A. Fernandez, O. Pak, K. Kolesnikova, H. Reingruber, M. Bollini, G. P. Crossan, N. Sommer, M. E. Monge, B. Schumacher, L. B. Pontel, Endogenous formaldehyde scavenges cellular glutathione resulting in redox disruption and cytotoxicity, *Nat. Commun.*, 2022, **13**, 745.

30 P. Yang, Y. Shang, B. Qu, J. Zhang, M. F. Khalil, M. Hu, Y. Wang, Effects of functionalized nanoplastics on oxidative stress in the mussel *Mytilus coruscus*. *Mar. Pollut. Bull.*, 2025, **211**, 117437.

31 J. Tang, W. Bu, W. Hu, Z. Zhao, L. Liu, C. Luo, R. Wang, S. Fan, S. Yu, Q. Wu, X. Wang, X. Zhao, Ferroptosis is involved in sex-specific small intestinal toxicity in the offspring of adult mice exposed to polystyrene nanoplastics during pregnancy. *ACS Nano*, 2023, **17**, 2440-2449.

32 P. Huang, L. Cao, J. Du, Y. Guo, Q. Li, Y. Sun, H. Zhu, G. Xu, J. Gao, Polystyrene nanoplastics amplify the toxic effects of PFOA on the Chinese mitten crab (*Eriocheir sinensis*). *J. Hazard. Mater.*, 2025, **488**, 137488.

33 J. Chen, Q. Liang, Y. Zheng, Y. Lei, X. Gan, H. Mei, C. Bai, H. Wang, J. Ju, Q. Dong, Y. Song, Polystyrene nanoplastics induced size-dependent developmental and neurobehavioral toxicities in embryonic and juvenile zebrafish. *Aquat. Toxicol.*, 2024, **267**, 106842.

34 H. Schulenburg, C. L. Kurz, J. J. Ewbank, Evolution of the innate immune system: the worm perspective, *Immunol. Rev.*, 2004, **198**, 36-58.

35 J. Hou, C. Hu, J. C. White, K. Yang, L. Zhu, D. Lin, Nano-Zoo interfacial interaction as a design principle for hybrid soil remediation technology, *ACS Nano*, 2021, **15**, 14954-14964.

36 J. L. Dziki, G. Hussey, S. F. Badylak, Alarmins of the extracellular space, *Semin. Immunol.*, 2018, **38**, 33-39.

37 H. Wang, A. Inoue; Y. Lei, H. Wu, L. Hong, X. Cheng, Cathepsins in the extracellular space: Focusing on non-lysosomal proteolytic functions with clinical implications, *Cell. Signal.*, 2023, **103**, 110531.

38 J. H. Hartman, S. J. Widmayer, C. M. Bergemann, D. E. King, K. S. Morton, R. F. Romersi, L. E. Jameson, M. C. K. Leung, E. C. Andersen, S. Taubert, J. N. Meyer, Xenobiotic metabolism and transport in *Caenorhabditis elegans*, *J. Toxicol. Env. Health-Pt b-Crit. Rev.*, 2021, **24**, 51-94.

39 Y. Cui, S. J. McBride, W. A. Boyd, S. Alper, J. H. Freedman, Toxicogenomic analysis of *Caenorhabditis elegans* reveals novel genes and pathways involved in the resistance to cadmium toxicity, *Genome Biol.*, 2007, **8**, R122.

40 P. Fontaine, K. Choe, The transcription factor SKN-1 and detoxification gene *ugt-22* alter

albendazole efficacy in *Caenorhabditis elegans*, *Int. J. Parasitol.-Drugs Drug Resist.*, 2018, **8**, 312-319.

41 L. Larigot, D. Mansuy, I. Borowski, X. Coumoul, J. Dairou, Cytochromes P450 of *Caenorhabditis elegans*: Implication in biological functions and metabolism of xenobiotics, *biomolecules*, 2022, **12**, 342.

42 S. Y. M. Lim, M. Alshagga, C. Kong, M. A. Alshawsh, S. A. Alshehade, Y. Pan, CYP35 family in *Caenorhabditis elegans* biological processes: Fatty acid synthesis, xenobiotic metabolism, and stress responses, *Arch. Toxicol.*, 2022, **96**, 3163-3174.

43 J. M. Kramer, G. N. Cox, D. Hirsh, Comparisons of the complete sequences of 2 collagen genes from *Caenorhabditis elegans*, *Cell*, 1982, **30**, 599-606.

44 S. L. Hughes, J. G. Bundy, E. J. Want, P. Kille, S.R. Stürzenbaum, The Metabolomic responses of *Caenorhabditis elegans* to cadmium are largely independent of metallothionein status, but dominated by changes in cystathionine and phytochelatins, *J. Proteome Res.*, 2009, **8**, 3512-3519.

45 R. Mkuye, C. Yang, F. Masanja, S. Ibrahim, X. Yang, H. Mwemi, P. Mrope, M. Salman, A. Alfatat, Y. Deng, Omics insights in responses of bivalves exposed to plastic pollution. *Aquat. Toxicol.*, 2025, **279**, 107224.

46 D. Ting, S. Ge, L. Xinran, X. Meihong, L. Yong, Nucleotides as optimal candidates for essential nutrients in living organisms: A review, *J. Funct. Food.*, 2021, **82**, 104498-104498.

47 Y. Lv, Z. Lin, J. Chen, K. Jiang, A. Wang, B. Wang, Y. Wu, Z. Xu, J. Wang, W. Yao, Hyoscyamine induces developmental toxicity by disrupting metabolism in zebrafish embryo (*Danio rerio*), *Food Chem. Toxicol.*, 2023, **177**, 113860.

48 S. L. Baldin, K. d. P. Pickler, A. C. Salvador de Farias, H. T. Bernardo, R. Scussel, B. d. C. Pereira, S. D. Pacheco, E. R. Dondossola, R. A. Machado-de-Avila, A. G. Wanderley, E. P. Rico, Gallic acid modulates purine metabolism and oxidative stress induced by ethanol exposure in zebrafish brain, *Purinergic Signal.*, 2022, **18**, 307-315.

49 C. Merino, M. Casado, B. Pina, M. Vinaixa, N. Ramirez, Toxicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in early development: A wide-scope metabolomics assay in zebrafish embryos, *J. Hazard. Mater.*, 2022, **429**, 127746.

50 E. J. Martinez-Finley, M. Aschner, Revelations from the nematode *Caenorhabditis eleganson* the complex interplay of metal toxicological mechanisms. *J. Toxicol.*, 2011, **2011**, 1-10.

51 S. Lin, H. Zhang, C. Wang, X. Su, Y. Song, P. Wu, Z. Yang, M. Wong, Z. Cai, C. Zheng, Metabolomics reveal nanoplastic-induced mitochondrial damage in human liver and lung cells. *Environ. Sci. Technol.*, 2022, **56**, 12483-12493.

52 A. Annibal, R. G. Tharyan, M. F. Schonewolff, H. Tam, C. Latza, M. M. K. Auler, S. Grönke,

L. Partridge, A. Antebi, Regulation of the one carbon folate cycle as a shared metabolic signature of longevity, *Nat. Commun.,* 2021, **12**, 3486.

53 F. Cabreiro, C. Au, K. Y. Leung, N. Vergara-Irigaray, H. M. Cochemé, T. Noori, D. Weinkove, E. Schuster, N. D. E. Greene, D. Gems, Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism, *Cell.*, 2013, **153**, 228-239.

54 Y. Zheng, L. C. Cantley, Toward a better understanding of folate metabolism in health and disease, *J. Exp. Med.*, 2019, **216**, 253-266.

55 M. P. Mattson, T. B. Shea, Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders, *Trends Neurosci.*, 2003, **26**, 137-146.

56 G. Gokul, J. Singh, Dithiothreitol causes toxicity in *C. elegans* by modulating the methionine-homocysteine cycle, *eLife*, 2022, **11**, e76021.

57 D. Mendoza-Cózatl, H. Loza-Tavera, A. Hernández-Navarro, R. Moreno-Sánchez, Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants, *Fems Microbiol. Rev.*, 2005, **29**, 653-671.

58 M. Jiang, M. Peng, A. Meng, W. Zhang, H. Shi, Q. Han, R. Peng, X. Jiang, Multi-omics analysis reveals the toxic mechanism of tributyltin exposure causing digestive gland oxidative stress in cuttlefish (*Sepia pharaonis*). *J. Hazard. Mater.*, 2025, **489**, 137547.

59 S. Fang, T. Li, P. Zhang, C. Liu, B. Cong, S. Liu, Integrated transcriptome and metabolome analyses reveal the adaptation of Antarctic moss Pohlia nutans to drought stress. *Front. Plant Sci.*, 2022, **13**, 924162.

60 C. He, S. Zhang, Y. Chi, J. Wang, J. Xu, J. Hou, D. Lin, Nanoplastics-Induced hormesis enhances cadmium bioaccumulation in nematodes by stimulating glutathione synthesis. *Sci. Total Environ.*, 2025, **993**, 179983.

61 X. Xi, D. Ding, H. Zhou, B. Baihetiyaer, H. Sun, Y. Cai, N. Wang, X. Yin, Interactions of pristine and aged nanoplastics with heavy metals: Enhanced adsorption and transport in saturated porous media. *J. Hazard. Mater.*, 2022, **437**, 129311

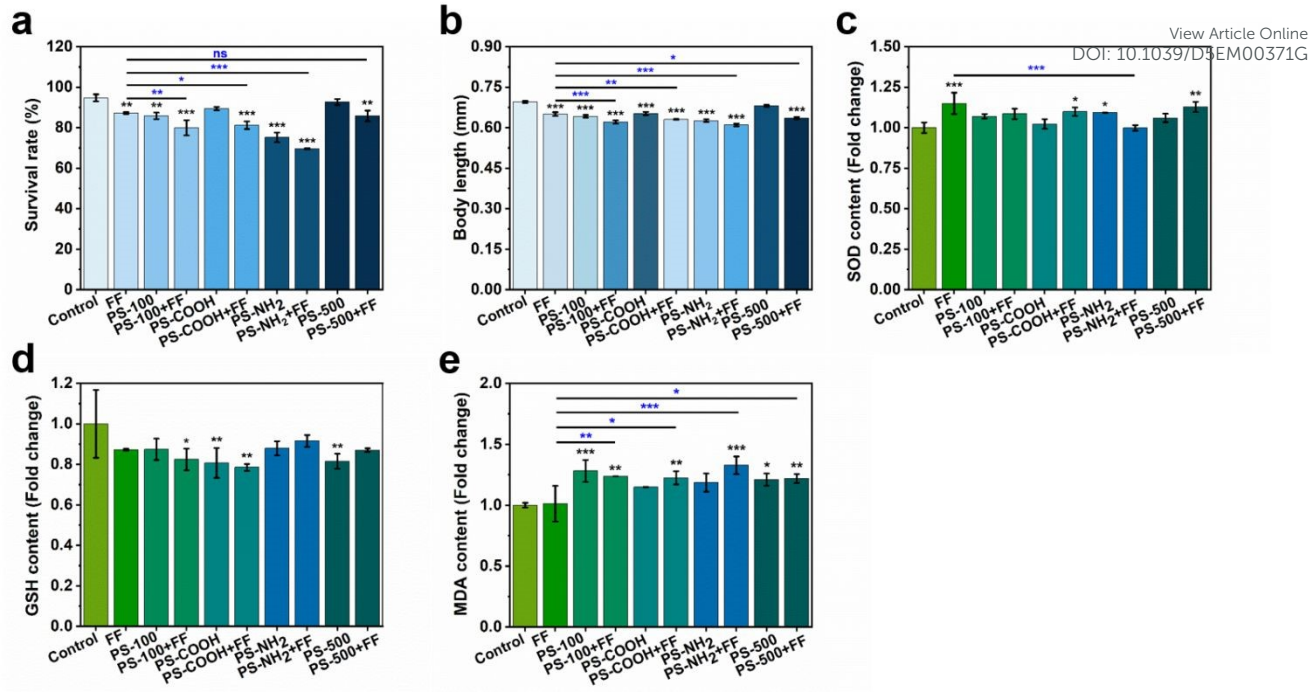


Figure 1. Toxicity effects of PS-NPs and FF on nematodes. (a) Survival rate. (b) Body length (n > 30). (c) SOD content. (d) GSH content. (e) MDA content. Nematodes in the control group were fed with *E. coli* only. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ns represents no significance (n = 3). The black * represents comparison with control, and the blue * represents comparison with FF treatment.

View Article Online
DOI: 10.1039/D5EM00371G

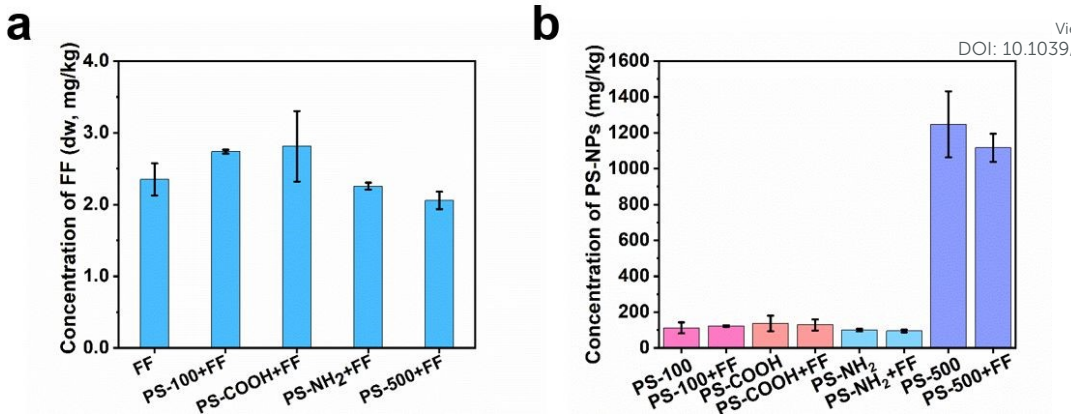


Figure 2. The accumulated (a) FF and (b) PS-NPs in nematodes after the 72-h exposures.

View Article Online
DOI: 10.1039/D5EM00371G

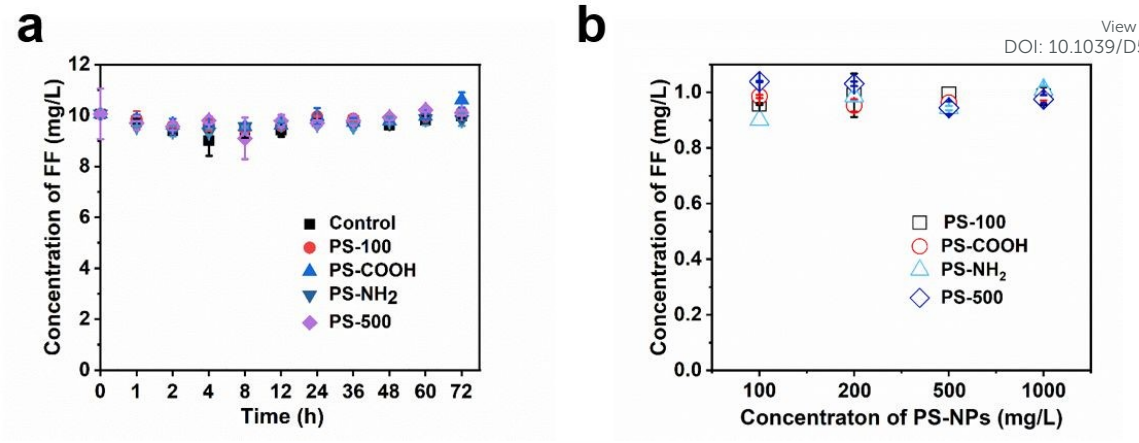


Figure 3. Adsorption of FF on different PS-NPs. (a) Variation of aqueous FF (10 mg/L) with and without PS-NPs (10 mg/L) in 72 h. (b) Variation of aqueous FF (1 mg/L) with increasing concentration of PS-NPs after equilibration for 72 h.

View Article Online
DOI: 10.1039/D5EM00371G

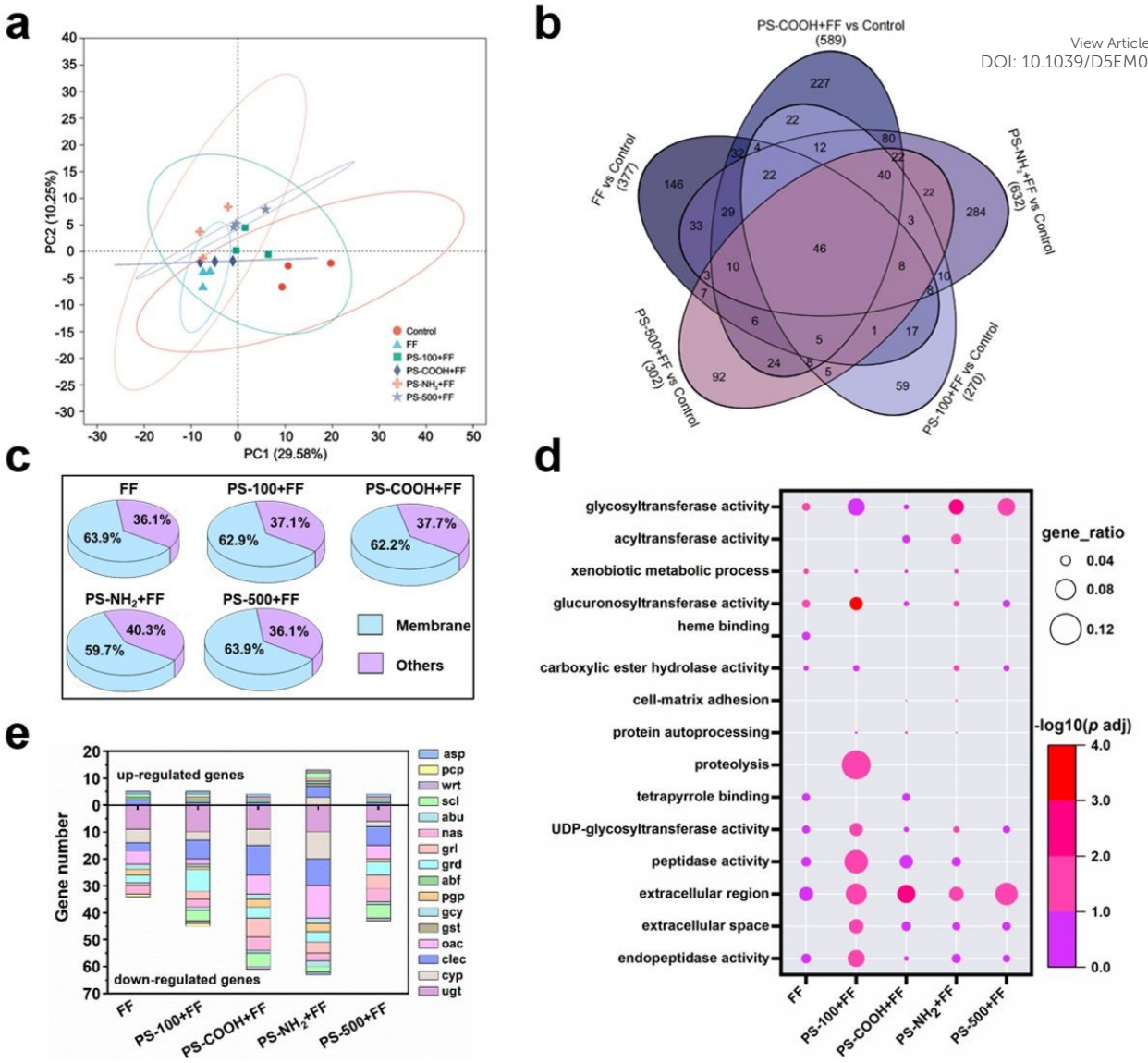


Figure 4. Transcriptomic analyses of nematodes after the 72-h exposures to PS-NPs and FF. (a) Principal component analysis (PCA). (b) Venn diagram illustration of differentially expressed genes (DEGs). (c) Proportion of membrane related genes. (d) Top 15 Gene Ontology (GO) terms with significant (*p* adjusted < 0.05) enrichment of DEGs. (e) Number of genes involved in proteolysis and detoxification.

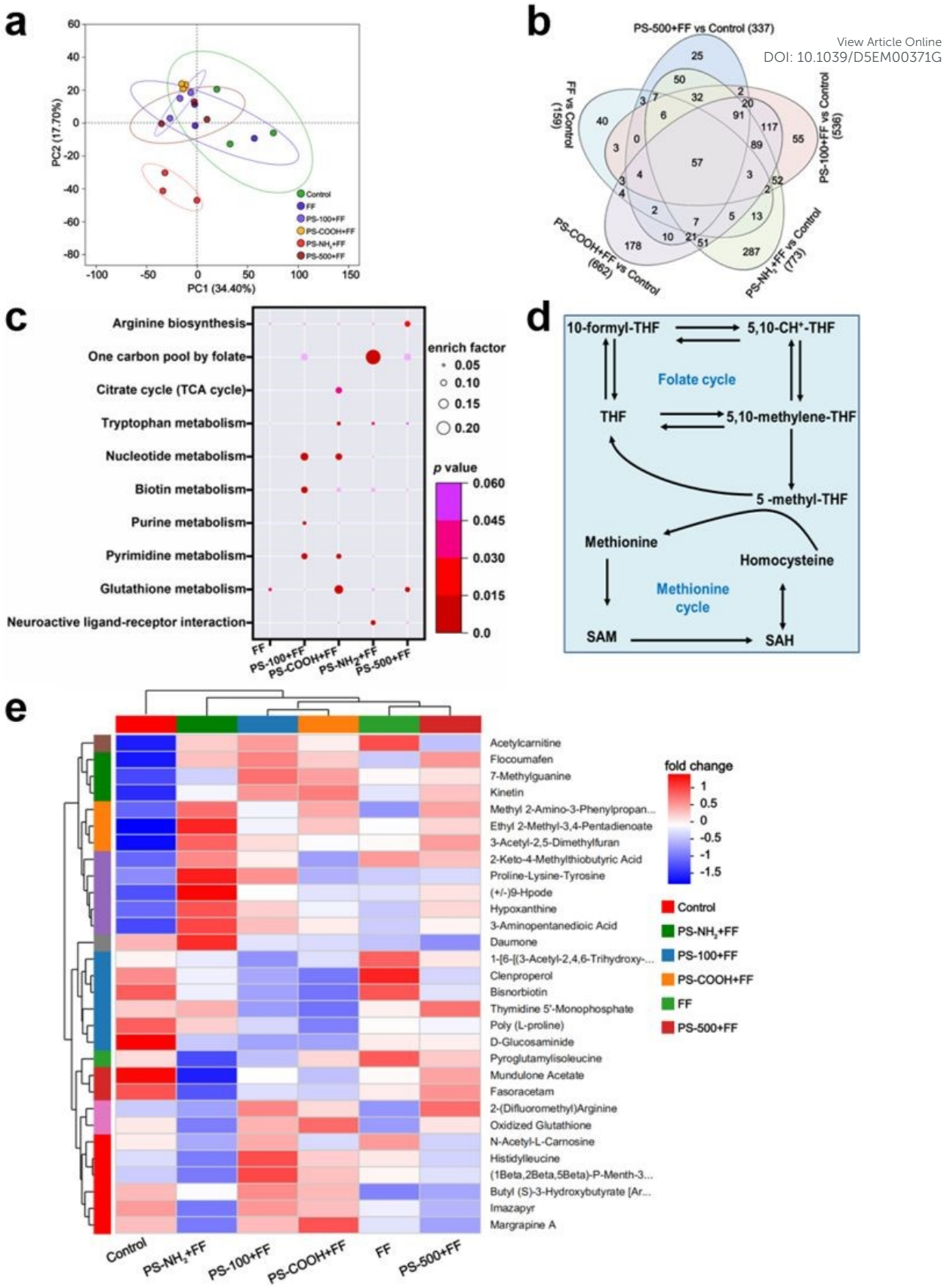


Figure 5. Metabolomic analyses of nematodes exposed to FF and PS-NPs. (a) PCA. (b) Venn diagram of the number of changed metabolites. (c) Top 10 KEGG pathways with significant (*p* adjusted < 0.05) enrichment of changed metabolites. (d) Proposed folate and methionine cycling pathways. (e) Heatmap of changed metabolites.

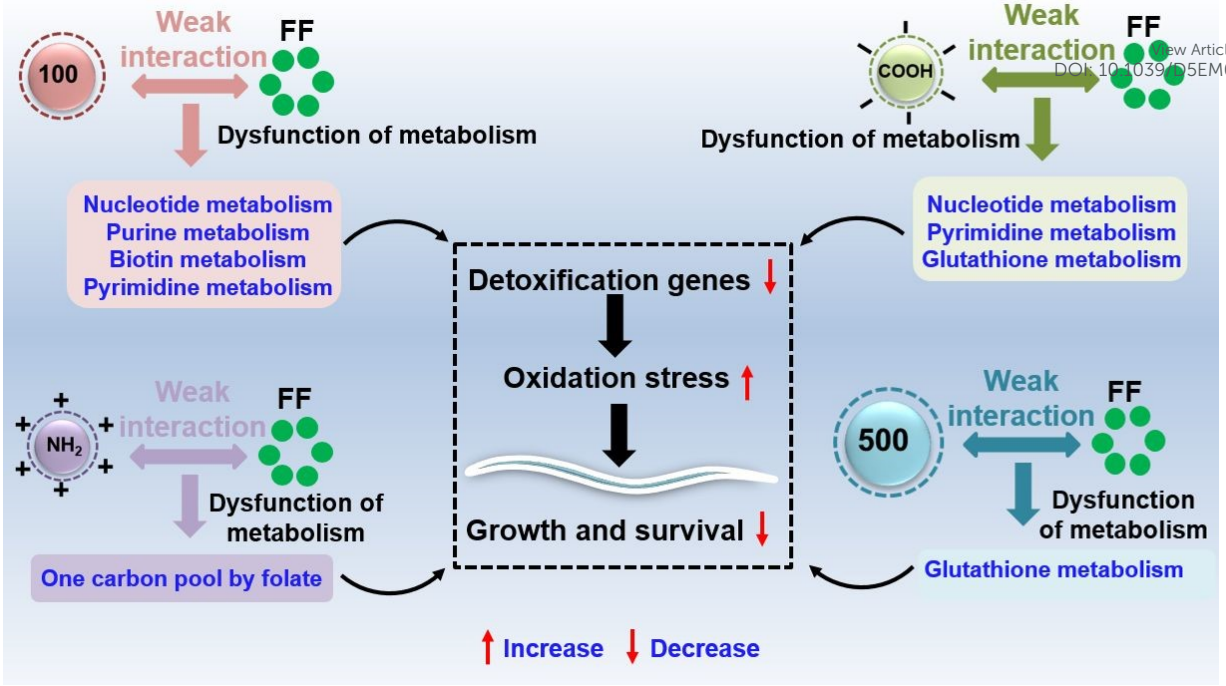


Figure 6. Mechanism illustration for the effects of four PS-NPs on the toxicity of FF in nematodes.

View Article Online
DOI: 10.1039/D5EM00371G

Data availability

Data will be made available on request.