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Mitigating combined internalized toxicity of nanoplastics and cadmium in rice through metabolic and biochemical regulations under supply of biochar biofilters derived from *Mikania Micrantha*

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Abstract

Nanoplastics and cadmium are common pollutants in agricultural systems, posing significant risks to rice. This study explored the effectiveness of biochar biofilters derived from invasive plant *Mikania micrantha* in mitigating the combined toxicity of polystyrene nanoplastics and cadmium on rice. The combined toxic effects of cadmium and polystyrene nanoplastics were more severe than their individual impacts. Polystyrene nanoplastics under cadmium stress adversely affected rice growth, reducing biomass by 16.46%, whereas the invasive plant *Mikania micrantha* biochar biofilters significantly improved biomass by 84.60% and 52.59% when applied alone or together with polystyrene nanoplastics under cadmium stress, respectively. Additionally, total chlorophyll content improved by 82.09% in the MBC treatment and by 36.66% in the MBC + PS NPs treatment compared to sole cadmium stress conditions. The invasive plant *Mikania micrantha* biochar biofilters alleviated these stress effects by reducing the cadmium translocation to roots and shoots, restoring chlorophyll and carotenoid levels, proteins, carbohydrates, preserving cellular structures, and enhancing oxidative defence through gene modulation. Scanning electron microscopy revealed the polystyrene nanoplastics internalization in root cells, but biochar biofilters acted as a physical barrier, limiting their translocation. Furthermore, the invasive plant *Mikania micrantha* biochar biofilters improved rice performance under dual stress by regulating metabolic pathways, nutrients cycle, TCA cycles and nitrogen transport. In contrast, polystyrene nanoplastics disrupted ATP-binding transporters and hormone signalling, increasing cadmium absorption and intensifying toxicity, thereby impairing growth, root development, and photosynthesis. These findings underscore the potential of invasive plant *Mikania micrantha* biochar biofilters in mitigating the environmental impacts of cadmium and nanoplastics in agricultural systems.

Highlights

- Polystyrene nanoplastics were observed to internalize within rice root.
- Polystyrene nanoplastics acted as carriers for cadmium, facilitating the translocation of heavy metals through a synergistic interaction.

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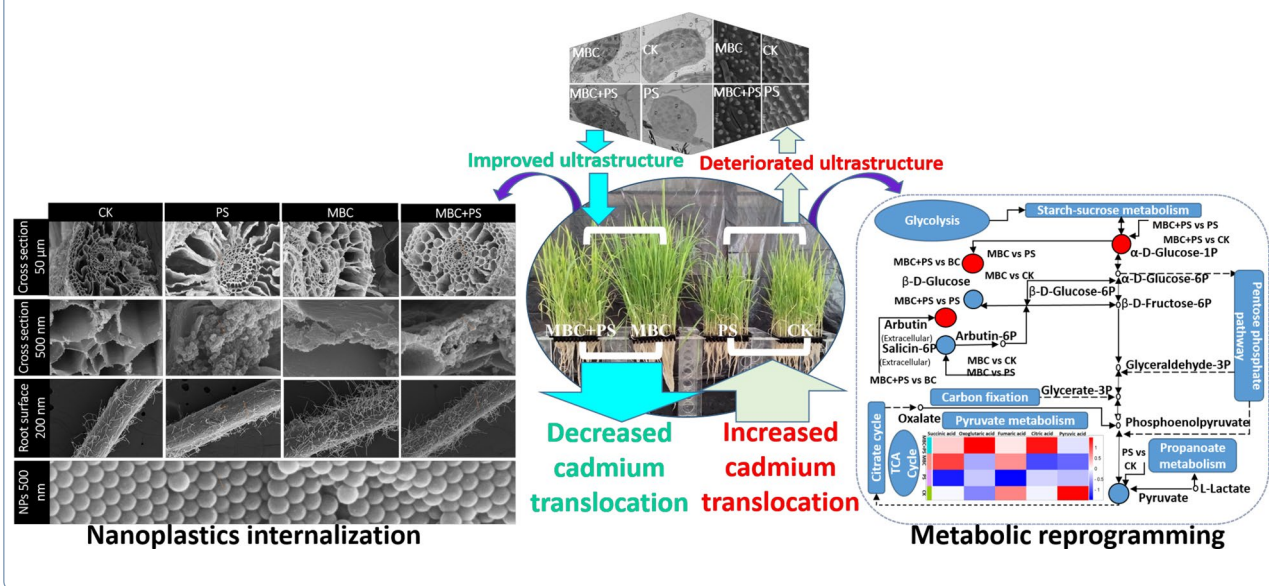


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- The application of invasive plant *Mikania micrantha* biochar biofilters alleviated the phytotoxic effects of polystyrene nanoplastics under cadmium stress in rice.
- Invasive plant *Mikania micrantha* biochar biofilters functioned as containers for both heavy metals and polystyrene nanoplastics, reducing their mobility and toxicity in rice.
- Invasive plant *Mikania micrantha* biochar biofilters modulated the metabolic variations and strengthened oxidative defence.

Keywords Nanoplastic, Cadmium, Stress mitigation, Biochar biofilters, Metabolites, Biochemical regulations

Graphical Abstract



1 Introduction

Microplastics (MPs < 5 mm) and NPs (1–100 nm) have emerged as global environmental pollutants due to their harmful impacts on plants, living organisms, and ecosystem functions (Li et al. 2024a). These particles primarily originate from common plastic items such as bags, fishing nets, bottles, and agricultural tunnel plastics, breaking down through mechanical abrasion, photochemical reactions, and biological degradation (Lian et al. 2020; Nohara et al. 2024). MPs and NPs are particularly widespread in terrestrial ecosystems, where their estimated abundance is 4 to 23 times higher compared to marine environments (Dong et al. 2020; Lian et al. 2022). Globally, in some cases, plastic particles can account for approximately 7% of soil weight, with concentrations ranging from 10 to 12,560 items per kilogram in farmland (Gong et al. 2021; Horton et al. 2017; Rillig 2018).

The biological and toxic effects of NPs are more complex and challenging to manage compared to MPs (Mitrano et al. 2021). NPs possess a higher surface area

and an enhanced capability to cross biological obstacles, which intensifies their biochemical interactions with living organisms and other pollutants (Frias and Nash 2019; Wang et al. 2023b). Recent studies have highlighted the phytotoxicity of NPs (Maity et al. 2022; Maity and Pramanick 2020), which primarily leads to reduced seed germination and plant biomass, inhibited root function and elongation, increased oxidative stress, impaired photosynthesis, and suppressed gene expression (Yu and Li 2024). Studies have shown that nanoparticles can enter epidermal cells through endocytosis (Bandmann et al. 2012) and may even traverse the vessels of the root xylem via apoplastic spaces, which facilitates their rapid movement within the root system through the vascular tissues (Su et al. 2019; Yin et al. 2021). Li et al. (2022) have demonstrated that plants like lettuce and wheat can absorb nanoparticles as small as 0.2 µm, indicating that the absorption of nanoparticles by plants is not random, and their presence within plant tissues is possible. Additionally, NPs have been found to reduce plant disease resistance (Cao

et al. 2024). For instance, Jiang et al. (2019) have indicated that PS NPs can cause severe oxidative stress and genetic damage in *Vicia faba*. Therefore, it is crucial to understand the impacts of NPs on plant health and physiology.

Globally, the presence of heavy metals (HMs) in agroecosystems is a major concern (Rana et al. 2020a), as they plant photosynthetic, physiological, and biochemical processes, potentially leading to plant death (Li et al. 2024a; Rana et al. 2025b). Specifically, Cd is known as one of the most toxic and persistent pollutants in soil, raising global concerns due to its contribution to soil contamination and its accumulation in plants (Shanmugaraj et al. 2019). When NPs coexist with HMs, they can act as efficient carriers, introducing a range of toxic effects in plants (Naziri et al. 2023; Song et al. 2023). This interaction may facilitate the rapid spread of Cd within plant tissues. Research has shown that MPs can exacerbate arsenic toxicity in rice seedlings (Dong et al. 2020). However, MPs have also been observed to have no impacts on copper toxicity in the *Tetraselmis chuii* (Davaranah and Guilhermino 2015). Conversely, PS NPs have been found to increase Cd translocation in the roots of *Arabidopsis thaliana*, resulting in a triggered oxidative stress responses (Li et al. 2024a). Therefore, compared to MPs, NPs are generally considered more harmful to plants (Pelegrini et al. 2023).

In addition to the environmental toxicity caused by HMs and NPs, invasive plants pose a significant threat to global ecosystems due to their adaptability and rapid growth, which can disrupt local ecosystems (Li et al. 2023b). One such invasive species, *Mikania micrantha* Kunth, poses a major threat to native ecosystems in South China. Chemical control methods for managing invasive plants often rely on compounds containing trace metals such as copper (Cu), Cd, arsenic (As), and lead (Pb). These metals can leach into water bodies, posing risks to aquatic ecosystems and human health (Abdellah et al. 2024). Therefore, there is an urgent need to explore cost-effective and sustainable strategies for controlling invasive plant species (Osunkoya et al. 2019; Westbrook et al. 2022).

Studies suggest that BC derived from invasive plant species can effectively alleviate the ecological damage caused by biological invasions (Feng et al. 2021; Wang et al. 2023a). It also has the potential to reduce Cd toxicity and adsorb environmental pollutants (Wang et al. 2024). Biochar has been proven highly effective in removing NPs from water bodies (Ganie et al. 2021; Yu and Li 2024). Similarly, Li et al. (2023a) reported that optimal concentrations of corncob-derived BC can effectively mitigate the inhibiting effects of NPs on lettuce growth. This strategy provides a dual benefit by addressing both

the proliferation of invasive plant species and environmental contamination caused by heavy metals and nanoparticles.

Rice is widely cultivated in China, serving as a key food source and a significant raw material for the chemical industry. Metabolomics, a high-throughput analytical method, is emerging as an influential tool for detecting changes in metabolic programming and understanding mechanisms of metabolic variations (Rana et al. 2025a). Recent studies have leveraged metabolomics analyses to explore the toxic effects of HMs and nanomaterials on various organisms (Kim et al. 2019; Zhang et al. 2021). Drawing from these studies, we proposed that the co-exposure of NPs and HMs, in the presence of IPMM BC biofilters, may influence plant growth and development. To explore this, we investigated the individual and combined effects of PS NPs and Cd on rice. Our study evaluated how PS-NPs and Cd, along with IPMM BC biofilters, impact the plant physiological and biochemical processes, elemental metabolism, and molecular responses. Additionally, we examined how PS NPs, combined with IPMM BC biofilters, affect the translocation and phytotoxicity of Cd and NPs within the plant. These insights will deepen our understanding of the biological risks and toxicological implications arising from the co-existence of NPs, HMs, and IPMM BC biofilters.

2 Materials and methods

2.1 Experimental treatments, plant material, growth conditions, and preparation of engineered IPMM BC biofilters

The invasive plant *Mikania micrantha*, sourced from South China Agricultural University in Guangzhou, China, was used as the raw material. The plants were washed with tap water and allowed to air-dry before carbonization. After drying, the material was crushed and sieved through a 2-mm sieve to ensure uniformity. Carbonization was performed at 500 °C for 2 h in a limited-oxygen environment. The physicochemical properties of the resulting IPMM BC were assessed using methods established in previous research (Zhang and Luo 2014). The engineered IPMM BC biofilters were assembled by filling a perforated plastic tube (5 cm long and 3 cm in diameter) with 1.0 g of IPMM BC. To minimize BC loss, a 0.5-cm layer of sand was placed both above and below the BC. Additionally, to promote even flow, 0.5-cm-thick layers of gravel were added above and below the sand layers. Cheesecloth was used to separate and secure the layers of IPMM BC, sand, and gravel. The characterization of BC was carried out by the previously described methods (Qu et al. 2024a, 2024b). The microstructure of the BC was investigated via field emission scanning electron microscope (SEM) (Hitachi, SU8010, Tokyo, Japan). Fig. S1A shows a SEM image of

the BC derived from IPMM, revealing its rough, irregular, and porous texture. The surface elemental compositions and distribution were determined by an energy dispersive X-ray spectroscopy (EDS, HORIBA EMAX mics2, Hitachi, Tokyo, Japan) detector attached to an SEM. Furthermore, abundant C, O, K and Cl elements were uniformly distributed on the BC surface (Fig. S1B, C, D, E, G). A diagram illustrating the design of the IPMM BC biofilter is shown in Fig. S1F.

Rice seeds were germinated on cheesecloth in a growth chamber maintained at 28 ± 2 °C. Following germination, uniform plants were shifted to pots containing a nutrient solution diluted to a quarter of the standard Hoagland's concentration. The growth chamber conditions were maintained at 28 ± 2 °C with a photosynthetic photon flux density of $820 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a relative humidity between 65% and 70%. Each pot, measuring 13 cm long, 9 cm wide, and 12 cm high, had 96 holes in its lid and was filled with 1 L of the nutrient solution. Each experimental treatment was performed in triplicate. The PS NPs (100 nm) were obtained from Jiangsu Zhichuan Technology Co., Ltd. in China. The rice seedlings were placed in the holes of the pot lids and initially grown in a nutrient solution diluted to one-fourth the standard Hoagland's concentration for the first 48 h. Then, the plants were transferred to a solution diluted to half the strength of Hoagland's solution for the following 48 h. The experimental treatments were applied in various combinations, including: CK (100 μM CdCl_2), PS NPs (50 mg L^{-1} + 100 μM CdCl_2), BC-BioF (IPMM BC biofilter + 100 μM CdCl_2), and BC-BioF + PS NPs (IPMM BC biofilter + 50 mg L^{-1} PS NPs + 100 μM CdCl_2). The treatment with only CdCl_2 was labelled as CK. Seedlings were provided with a Hoagland nutrient solution for 30 days. The Hoagland nutrient solution included: 9.1 μM MnSO_4 , 1 mM $(\text{NH}_4)_2\text{SO}_4$, 20 μM Fe-EDTA, 1 mM KH_2PO_4 , 1 mM $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 1 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2 mM $\text{Na}_2\text{SiO}_3 \cdot 9 \text{H}_2\text{O}$, 1 μM $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.1 μM $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, 1 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 10 μM H_3BO_3 . The pH of the solution was consistently maintained at 6.5 ± 0.05 daily by adding either 0.1 mM hydrochloric acid (HCl) or sodium hydroxide (NaOH). The solution was replaced every three days throughout the experiment. At the end of the 30-day growth period, the plants were harvested, and their physiological traits were recorded. Approximately half of the plants from each pot were dried in an oven, while the other half were stored at -80 °C for chemical and molecular.

2.2 Determination of root architecture, growth and development characteristics, osmoregulation, gas exchange and photosynthetic pigments

At the harvest time (30 days), 25 rice plants from each experimental group were carefully selected to measure plant height. The plants were then separated into shoot

and root components and were gently washed with distilled water to remove any remaining media. The root architecture and various morphological traits, including root length, surface area, volume, diameter, and the number of root tips, were analysed through WinRhizo system version 4.0b (Yue et al. 2019). Then, the samples were oven oven-dried 80 °C to record the dry weights of shoots and roots. For gas exchange measurements, fully expanded mature leaves from each plant were used, and the measurements were recorded according to the previously described methods described (Austin 1990; Saleem et al. 2020). Chlorophyll and carotenoids were measured using a spectrophotometer (UV-VIS 2550, Shimadzu, Japan), following the previously described method (Imran et al. 2019a). Leaf protein concentration was determined using the G-250 assay technique. Approximately 0.2 g of fresh leaf tissue was ground in sodium phosphate buffer (50 mM, pH 7.0) containing 2% polyvinylpyrrolidone-40 and EDTA- Na_2 (1 mM). The homogenate was then centrifuged at 10,000 g (15 min, 4 °C), and the absorbance of the supernatant was measured in triplicate at 595 nm using a spectrophotometer. For proline quantification, the ninhydrin method was used. The reaction mixture was extracted with 5 mL of toluene, and absorbance was measured at 520 nm. Proline levels were determined by comparing the absorbance to a standard curve.

2.3 Lipid peroxidation and reactive oxygen species determination

Fresh leaf samples (0.2 g) were finely ground in liquid nitrogen to form a homogeneous powder. This powder was mixed with 1 mL of trichloroacetic acid (TCA-0.1%) to normalize the samples. The mixture was then centrifuged at 12,000 g (20 min, 4 °C) to precipitate proteins and other cellular debris, yielding a clear supernatant for hydrogen peroxide (H_2O_2) quantification. The assay mixture for H_2O_2 measurement included 1 mL of potassium iodide solution (1 M), 0.5 mL of potassium phosphate buffer (100 mM, pH 6.8), and 0.5 mL of the supernatant. H_2O_2 concentration was determined by measuring the absorbance at 390 nm using a UV-VIS spectrophotometer (Valentovic et al. 2006). For malondialdehyde (MDA) content assessment, fresh leaves were homogenized in cold TCA (0.1% w/v) and centrifuged at 12,000 g (20 min, 4 °C). The reaction mixture comprised 2.5 mL of thiobarbituric acid (TBA) solution (0.5% in 20% TCA) and 0.5 mL of the supernatant. The mixture was boiled (30 min), cooled, and centrifuged at 12,000 g (5 min). MDA contents were measured by the difference in absorbance at 532 nm and 600 nm, using an extinction coefficient of 155 mM cm^{-1} . Electrolyte leakage (EL) in leaf tissues was assessed according to the previously

described methods (Imran et al. 2020; Valentovic et al. 2006).

2.4 Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

For SEM analysis, fresh leaves were randomly selected from various plants. Small leaf sections, approximately 1 mm², were first cleaned with distilled water and then fixed in a 4% glutaraldehyde and 0.2 M sodium phosphate buffer (pH 6.8) solution at 4 °C for 6 h. After fixation, the samples underwent a series of ethanol dehydration steps, and were treated twice with isoamyl acetate following four rinses with a 0.1 M sodium phosphate buffer (pH 6.8), and then dried using a critical-point drying apparatus (Emitech K850, Quorum Technologies, UK). The dried leaf samples were coated with a conductive metal layer and examined using a field emission scanning electron microscope (model S4800, Hitachi, Ltd., Tokyo, Japan). For transmission electron microscopy (TEM), fresh leaf samples measuring 1–3 mm in length were fixed in a glutaraldehyde solution (4%) mixed with 0.2 M sodium phosphate buffer (PBS, pH 7.2) for 8 h. The samples were then post-fixed with 1% osmium tetroxide (OsO₄) solution for 1 h, rinsed in 0.2 M PBS (pH 7.2) for 2 h to remove excess OsO₄, and dehydrated through a graded ethanol series (50%, 60%, 70%, 80%, 90%, 95%, and 100%) followed by acetone. After dehydration, the samples were infiltrated and embedded in Spurr's resin. Ultrathin sections of the embedded samples were prepared and mounted onto copper grids for examination using a transmission electron microscope (Roland and Vian 1991).

2.5 Oxidative defence system attributes determination

Fresh leaf tissue was ground into a fine powder using liquid nitrogen and then mixed with a cold sodium phosphate buffer (pH 7.8) to prepare a homogenate. The mixture was centrifuged at 4000 rpm for 10 min at 4 °C, and the supernatant was separated from the crude fiber. Superoxide dismutase (SOD; EC 1.15.1.1) activity was analysed using a reaction mixture containing the enzyme extract, sodium phosphate buffer (pH 7.8), nitro blue tetrazolium (NBT), riboflavin, methionine, EDTA-Na₂, and distilled water. SOD activity was assessed by measuring the absorbance at 560 nm with a UV–VIS spectrophotometer (Rana et al. 2025b). Peroxidase (POD; EC 1.11.1.7) activity was measured by grinding fresh samples in a cold extraction buffer containing sodium phosphate (50 mM, pH 5.5). The mixture was centrifuged at 3000 rpm for 10 min, and the resulting supernatant was used in the reaction mixture, which included enzyme extract, sodium phosphate buffer (50 mM, pH 5.5), H₂O₂ (0.3%), and guaiacol (0.2%). Absorbance was recorded at

470 nm using a spectrophotometer. Catalase (CAT; EC 1.11.1.6) activity was determined by grinding fresh samples in a sodium phosphate buffer (50 mM, pH 7.8). After centrifugation at 4000 rpm for 15 min at 4 °C, the supernatant was used in the assay, which contained distilled water, sodium phosphate buffer (50 mM, pH 7.8), H₂O₂ (0.1 M), and the enzyme extract. Absorbance (240 nm) was measured using a spectrophotometer. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured by grinding leaves in sodium phosphate buffer (50 mM, pH 5.5) containing EDTA-Na₂ (0.2 mM). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C, and the supernatant was used for APX activity measurement. The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined using commercial kits 'A006-1' and 'A061-2', respectively (www.njcbio.com).

2.6 Total RNA extraction and qRT-PCR analysis

Freshly collected rice leaves were used to isolate total RNA with TRIzol reagent (Invitrogen, Carlsbad, California, USA). After extraction, the quality and quantity of the total RNA were assessed according to standard protocols. cDNA synthesis was performed, and Real-Time PCR experiments were conducted as previously described (Imran et al. 2019b). Detailed information, including nucleotide sequences (Imran et al. 2020) are provided in Table S1. Three biological replicates were prepared for each sample, and Ct (cycle threshold) values were normalized against ACTIN Ct values. Gene expression levels were quantified using the 2^{−ΔΔCt} method for relative normalization (Pfaffl 2001).

2.7 Primary, secondary and micro-elemental assays

Plant samples were initially dried and ground. Then these were digested in H₂SO₄ (180 °C; 3 h). The concentration of nitrogen (N) in the plant samples was determined following the previously described method (Barbano and Clark 1990; Rana et al. 2020c, 2018). Potassium (K) concentration was assessed using a flame photometer (Model 410, USA) while phosphorus (P) content was measured colorimetrically using the previously described Mo-blue method (Lu et al. 1999; Rana et al. 2020b, 2020d, 2020e, 2025c). For calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), and copper (Cu) analysis, dried plant samples were processed using a microwave oven with a mixture of nitric acid and perchloric acid (5:1 v/v). This method dissolves plant tissues, releasing the metals for subsequent analysis using Inductively Coupled Plasma (ICP) spectroscopy or other suitable techniques. Cd and other elements, including Ca, Mg, Zn, Fe, Mn, and Cu, were measured in the digested samples using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with a Vista-PRO

instrument (Varian, Inc., Palo Alto, CA, USA) and ICP-mass spectrometry (ICP-MS) (ELAN DRC-e, Perkin-Elmer Sciex, Wilmington, DE, USA).

2.8 Metabolomics analysis

Samples were retrieved from storage at -80°C , and 60 mg was transferred to a 1.5-mL centrifuge tube pre-loaded with two small steel balls and 400 μL of a methanol–water solution (7:3), which contained internal standards at a concentration of $4\text{ }\mu\text{g mL}^{-1}$. The mixture was ground using a grinder at 60 Hz for 2 min, followed by ultrasonic extraction in an ice-water bath for 30 min. After extraction, the samples were stored at -40°C overnight. The next day, the extracts were centrifuged at 12,000 rpm and 4°C for 10 min. From the supernatant, 100 μL was transferred to a glass vial and evaporated using a centrifugal evaporator. Once dried, 80 μL of methoxyamine hydrochloride ($15\text{ }\mu\text{g mL}^{-1}$ in pyridine) was added, vortexed for 2 min, and incubated at 37°C for 60 min to initiate oximation. Following this, 50 μL of BSTFA derivatization reagent and 20 μL of *n*-hexane were added, vortexed for 2 min, and derivatized at 70°C for 60 min. The samples were then cooled to room temperature for 30 min before being subjected to GC–MS metabolomics analysis. Quality control (QC) samples were prepared by pooling equal amounts of extracts from all individual samples. After derivatization, the samples were analysed using an Agilent 7890B gas chromatograph coupled with an Agilent 5977A mass selective detector (MSD), both from Agilent Technologies Inc., CA, USA. Separation of the derivatives was accomplished using a DB-5MS fused-silica capillary column (30 m length, 0.25 mm diameter, 0.25 μm film thickness) from Agilent J & W Scientific, Folsom, CA, USA. Helium, with a purity exceeding 99.999%, was employed as the carrier gas at a constant flow rate of 1 mL min^{-1} . A 1 μL sample was injected in splitless mode at an injector temperature of 260°C . The oven temperature program began at 60°C for 0.5 min, followed by an increase to 125°C at $8^{\circ}\text{C min}^{-1}$, then to 210°C at $5^{\circ}\text{C min}^{-1}$, to 270°C at $10^{\circ}\text{C min}^{-1}$, and finally to 305°C at $20^{\circ}\text{C min}^{-1}$, where it was held for 5 min. The mass spectrometer was set with the quadrupole and ion source temperatures at 150°C and 230°C , respectively, and operated with an electron impact energy of 70 eV. Data acquisition occurred in full-scan mode, with a mass-to-charge ratio (*m/z*) range of 50–500 and a solvent delay of 5 min.

2.9 Data processing and statistical analysis

The raw GC–MS data files in .D format were converted to .abf format using analysis base file converter software to enable easier data access. These converted files were then imported into MS-DIAL software for a range

of analytical tasks, including peak detection, identification, deconvolution with MS2Dec, characterization, peak alignment, wave filtering, and interpolation of missing values. Metabolite characterization was performed using the LUG database as a reference. A detailed three-dimensional data matrix was created, containing information on the samples, peak names, retention times, retention indices, mass-to-charge ratios, and signal intensities for each detected compound. For each sample, peak signal intensities were segmented and normalized against the internal standards, applying a relative standard deviation (RSD) threshold of greater than 0.1 for filtering. After normalization, the data underwent redundancy elimination and peak merging to complete the final data matrix. The data matrix was imported into R software to perform Principal Component Analysis (PCA), allowing for visualization of sample distribution and evaluation of the analytical process consistency. To identify metabolic differences specific to groups, Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) and Partial Least-Squares Discriminant Analysis (PLS-DA) were performed. To prevent model overfitting, a sevenfold cross-validation and 200 iterations of Response Permutation Testing (RPT) were executed to assess the robustness of the models. The Variable Importance of Projection (VIP) scores derived from the OPLS-DA model were used to determine the significance of each variable in differentiating between groups. Additionally, a two-tailed Student's T-test was performed to evaluate the statistical significance of the identified differential metabolites. Metabolites were classified as differential if their VIP scores exceeded 1.0 and their *P*-values were below 0.05. Furthermore, the elemental and physiological data were analyzed statistically using a Completely Randomized Design, facilitated by Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA). Means and variances were compared using the least significant difference (LSD) test at a significance level of $P \leq 0.05$.

3 Results

3.1 The impact of IPMM BC biofilters on phenotypic traits and plant physiological characteristics under combined toxicity from Cd and PS NPs

The incorporation of an IPMM BC biofilter significantly mitigated the detrimental effects of Cd and PS NPs on rice plant growth compared to treatments with Cd alone (100 μM) or in combination with PS NPs (Table 1). The application of the IPMM BC biofilter, whether used alone or in the presence of PS NPs, notably enhanced root and shoot lengths, as well as dry biomass ($P \leq 0.05$). As detailed in Table 1, the IPMM BC biofilter resulted in substantial increases in stem diameter (6.62%), biomass dry weight (81.26%-shoot and 101.76%-root), and height

Table 1 The effect of IPMM BC biofilters on plant physiological characteristics and plant phenotype under combined toxicity of Cd and PS NPs

Treatments	Plant growth indicators					Plant biomass production			
	Root length (cm)	Shoot length (cm)	Root:shoot length	Plant height (cm)	Stem diameter (mm)	Root (mg plant ⁻¹)	Shoot (mg plant ⁻¹)	Root:shoot biomass	Total (mg plant ⁻¹)
CK	11.15 ± 0.02a	19.78 ± 0.56c	0.56 ± 0.017a	30.93 ± 0.54c	2.06 ± 0.09a	25.66 ± 1.73c	131.67 ± 4.14c	0.19 ± 0.016c	157.33 ± 4.07c
PS	8.44 ± 0.20a	16.53 ± 1.22d	0.51 ± 0.038a	24.98 ± 1.27d	2.03 ± 0.13a	19.42 ± 1.94c	112.00 ± 3.07d	0.17 ± 0.022d	131.43 ± 2.07d
MBC	15.43 ± 0.98c	25.48 ± 0.32a	0.60 ± 0.031a	40.91 ± 1.30a	2.20 ± 0.11a	51.77 ± 2.16a	238.67 ± 5.53a	0.22 ± 0.013a	290.44 ± 4.54a
MBC + PS	13.59 ± 0.76b	22.18 ± 0.19b	0.61 ± 0.038a	35.78 ± 0.65b	2.10 ± 0.05a	41.74 ± 2.19b	198.33 ± 4.39b	0.21 ± 0.016b	240.08 ± 2.39b

The data are the average of three replicates ± SE from different independent treatments. Different lowercase (a, b) represents significant differences by the LSD test ($P \leq 0.05$, $n = 3$) among different treatment

(root-38.40% and shoot-34.19%) compared to conditions without the IPMM BC biofilter and PS NPs under Cd toxicity. However, combining the IPMM BC biofilter with PS NPs resulted in only a minimal reduction in the negative effects of Cd and PS NPs on rice growth. Notably, PS NPs alone adversely affected rice growth, reducing biomass by 16.46%, whereas the IPMM BC biofilter either alone or with PS NPs under Cd stress increased biomass by 84.60% and 52.59%, respectively. Furthermore, the root-to-shoot ratio increased by 1.15% with IPMM BC biofilter + PS NPs treatment compared to the control. The IPMM BC biofilter demonstrated greater effectiveness in reducing Cd and PS NPs toxicity than PS NPs alone, suggesting its significant role in alleviating physiological toxicity caused by these contaminants in plants.

3.2 Co-exposure influence of IPMM BC biofilters and PS NPs supply on Cd translocation, primary, secondary and micro-elemental assays

Exogenous application of Cd and PS NPs significantly altered the concentrations of primary, secondary, and micro-elements in rice. Specifically, in the shoots, the treatment with IPMM BC biofilter led to notable increases in the contents of N (92.40%), P (200%), K (95.78%), Ca (9.71%), and Mg (55.55%) compared to plants under Cd stress. Similar patterns for these macro-nutrients were observed in the roots with MBC + PS NPs treatments under Cd stress conditions (Table 2). Plants treated with PS NPs showed the highest copper (Cu) concentration in the roots (11.55 $\mu\text{g g}^{-1}$), followed by the CK (10.86 $\mu\text{g g}^{-1}$) and MBC + PS NPs (8.42 $\mu\text{g g}^{-1}$), while the MBC plants had the lowest Cu concentration (7.05 $\mu\text{g g}^{-1}$). Furthermore, the PS NPs treatment reduced Zn content in the roots by 19.62% compared to the sole Cd-contaminated treatment (CK). In contrast, the MBC and MBC + PS NPs treatments increased Zn concentration by 56.41% and 24.20%, respectively. The highest levels of Fe were found in plants treated with the IPMM BC biofilter, with Fe concentrations of 121.89 $\mu\text{g g}^{-1}$ in

roots and 56.54 $\mu\text{g g}^{-1}$ in shoots, followed by MBC + PS NPs (92.56 $\mu\text{g g}^{-1}$ and 46.42 $\mu\text{g g}^{-1}$), CK (83.03 $\mu\text{g g}^{-1}$ and 39.42 $\mu\text{g g}^{-1}$), and PS NPs (69.68 $\mu\text{g g}^{-1}$ and 30.55 $\mu\text{g g}^{-1}$). Mn contents were higher in the roots (21.16%) of the MBC compared to other treatments. The IPMM BC biofilter treatment was particularly effective in enhancing the translocation of Mo in both root and shoot tissues compared to other treatments involving Cd and PS NPs. Cadmium levels varied among treatments, with the highest levels found in plants subjected to PS NPs stress (root-18.74% and shoot-35.62%). The IPMM BC biofilter reduced Cd levels in roots by approximately 39.46% and 24.20% in MBC and MBC + PS NPs treatments, respectively.

3.3 IPMM BC biofilter and PS NPs induced effect on osmoregulation, photosynthetic efficiency, chlorophyll and carotenoids concentration in Cd contaminated environment

Rice plants exhibited a significant reduction in gas exchange parameters under Cd and PS NPs stress compared to other treatments (Fig. 1). The transpiration rate was lowest in the CK treatment (2.83 $\text{mol m}^{-2} \text{s}^{-1}$) compared to PS NPs (3.12 $\text{mol m}^{-2} \text{s}^{-1}$), MBC + PS NPs (3.96 $\text{mol m}^{-2} \text{s}^{-1}$), and MBC (4.87 $\text{mol m}^{-2} \text{s}^{-1}$). Whereas, the supply of IPMM BC biofilters led to notable ($P \leq 0.05$) increases in photosynthetic rate (Pn), stomatal conductance (Gs), and CO_2 concentration compared to treatments exposed to Cd and PS NPs (Fig. 1A, B, C, D). The PS NPs treatment under Cd stress caused the most substantial adverse effects on photosynthesis. The Pn, Gs, and CO_2 content decreased by 36.51%, 40.75%, and 2.95%, respectively, in PS NPs compared to CK. Moreover, PS NPs significantly reduced the stomatal conductance up to 40.75% as compared to other treatments. Conversely, gas exchange results for CK, PS NPs, MBC, and MBC + PS NPs treatments were not significantly different. Chlorophyll pigments and carotenoid contents were also significantly reduced under Cd and PS NPs stress compared

Table 2 The influence of Cd and PS NPs contaminated environment on the primary, secondary and micro nutrients of rice in the presence and absence of IPMM BC biofilters

Treatment	Primary nutrients (%)				Secondary nutrients (mg g ⁻¹)				Micro-nutrients (µg g ⁻¹)				Heavy metal (µg g ⁻¹)	
	N	P	K	Mg	Ca	Mn	Zn	Cu	Fe	Mo	Cd			
CK	Root	0.83 ± 0.051c	1.04 ± 0.026b	0.57 ± 0.035c	1.72 ± 0.099c	6.52 ± 0.035b	50.69 ± 1.10b	37.19 ± 2.31c	10.86 ± 1.48ab	83.03 ± 4.17b	2.13 ± 0.046b	382.45 ± 6.45b		
PS	con-	0.35 ± 0.006d	0.78 ± 0.088c	0.45 ± 0.023d	1.17 ± 0.025d	5.94 ± 0.198b	48.03 ± 2.99b	29.89 ± 0.667d	11.55 ± 1.52a	69.68 ± 2.36c	1.28 ± 0.048a	454.13 ± 6.36a		
MBC	centra-	1.62 ± 0.020a	1.44 ± 0.059a	0.86 ± 0.031a	2.49 ± 0.087a	7.46 ± 0.336a	61.42 ± 1.99a	58.17 ± 1.33a	7.05 ± 0.307b	121.89 ± 4.10a	4.76 ± 0.077d	231.52 ± 2.62d		
MBC + PS	tion	1.24 ± 0.030b	1.18 ± 0.038b	0.72 ± 0.011b	2.05 ± 0.050b	6.79 ± 0.230ab	54.92 ± 3.71ab	46.19 ± 1.84b	8.42 ± 1.50ab	92.56 ± 3.05b	4.49 ± 0.122c	289.89 ± 3.84c		
CK	Shoot	0.26 ± 0.032b	0.17 ± 0.012c	0.32 ± 0.003c	1.08 ± 0.023b	3.26 ± 0.032b	22.79 ± 0.960b	18.9 ± 1.06c	4.47 ± 1.86ab	39.42 ± 1.10c	1.34 ± 0.058b	86.39 ± 1.66c		
PS	con-	0.16 ± 0.015c	0.13 ± 0.015c	0.19 ± 0.020d	0.8 ± 0.074c	3.18 ± 0.087b	22.98 ± 2.32b	12.45 ± 0.492d	4.71 ± 2.42a	30.55 ± 0.173d	1.12 ± 0.042a	117.89 ± 3.71c		
MBC	centra-	0.51 ± 0.043a	0.51 ± 0.026a	0.62 ± 0.031a	1.68 ± 0.057a	3.58 ± 0.156a	34.02 ± 1.53a	50.19 ± 1.22a	3.78 ± 0.088c	56.54 ± 1.13a	2.81 ± 0.096d	39.19 ± 2.02b		
MBC + PS	tion	0.32 ± 0.018b	0.43 ± 0.019b	0.44 ± 0.022b	1.24 ± 0.041b	3.45 ± 0.061ab	28.01 ± 2.25ab	37.79 ± 2.38b	4.16 ± 0.035bc	46.42 ± 0.90b	2.27 ± 0.161c	64.23 ± 3.84a		
CK	Total	1.09 ± 0.041c	1.21 ± 0.025c	0.89 ± 0.033c	2.8 ± 0.0115c	9.78 ± 0.324bc	73.49 ± 0.203c	56.09 ± 1.36c	15.32 ± 1.66ab	122.45 ± 4.98c	3.47 ± 0.095c	469.38 ± 7.38b		
PS	con-	0.51 ± 0.015d	0.91 ± 0.084d	0.64 ± 0.044d	1.97 ± 0.100d	9.12 ± 0.179c	71.01 ± 1.86c	42.34 ± 0.79d	16.26 ± 1.58a	100.24 ± 2.53d	2.4 ± 0.008a	572.02 ± 3.19a		
MBC	centra-	2.13 ± 0.049a	1.95 ± 0.075a	1.48 ± 0.020a	4.17 ± 0.123a	11.04 ± 0.389a	95.45 ± 2.91a	108.36 ± 2.38a	10.84 ± 0.369b	178.43 ± 3.23a	7.57 ± 20.024d	270.71 ± 0.848d		
MBC + PS	tion	1.56 ± 0.012b	1.61 ± 0.025b	1.16 ± 0.032b	3.29 ± 0.085b	10.24 ± 0.284ab	82.93 ± 3.65b	83.98 ± 3.43b	12.59 ± 1.52ab	138.98 ± 3.96b	6.77 ± 0.277b	354.12 ± 3.05c		

The data are the average of three replicates ± SE from different independent treatments. Different lowercase (a, b) represents significant differences by the LSD test ($P \leq 0.05$, $n = 3$) among different treatments

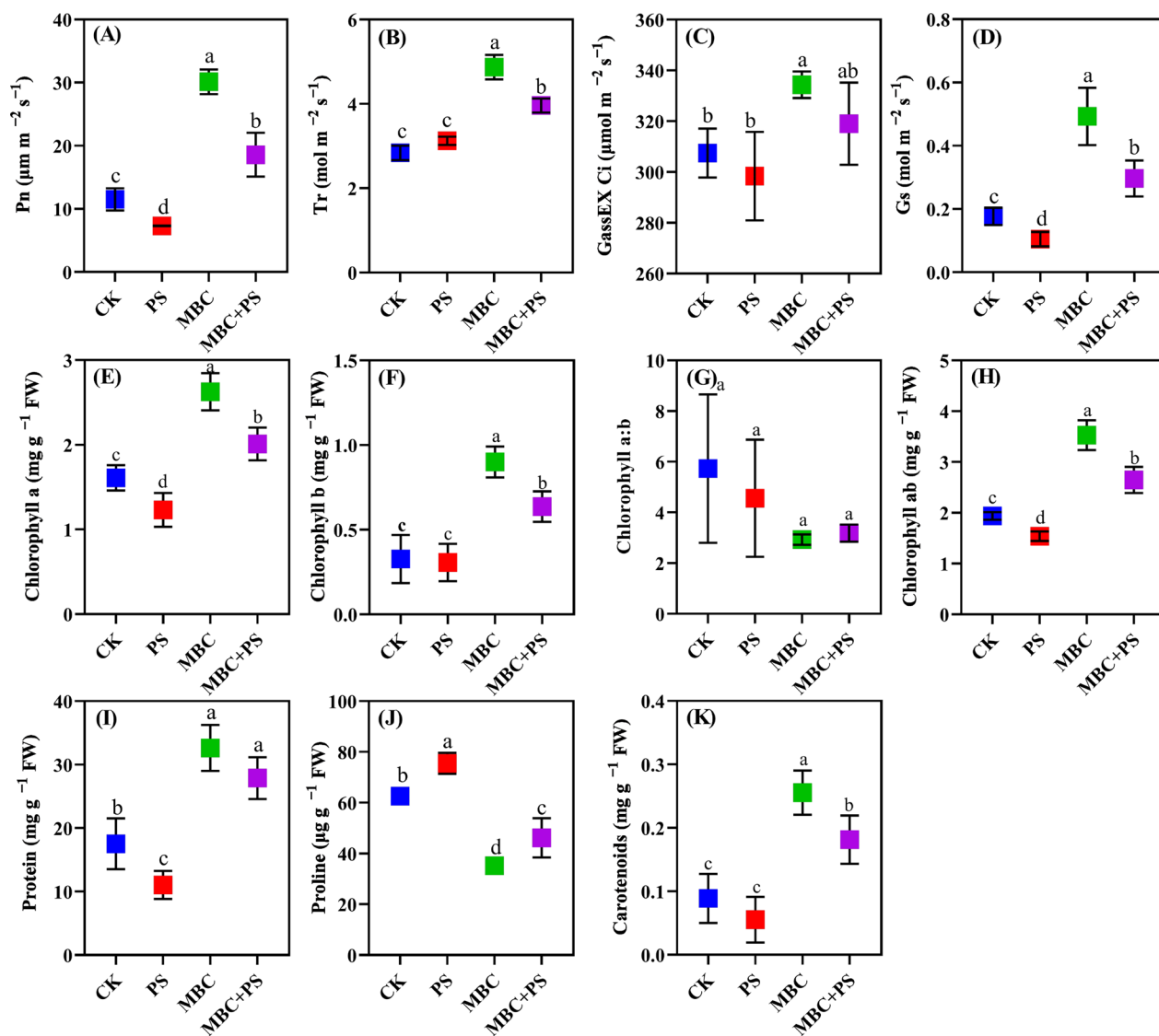


Fig. 1 The effect of IPMM BC biofilters on photosynthetic rate (A), transpiration rate (B), intercellular leaf CO_2 (C), stomatal conductance (D), chlorophyll a (E), chlorophyll b (F), chlorophyll a:b (G), total chlorophyll (H), protein (I), proline (J) and carotenoids (K) in Cd and NPs contaminated environment. The bar above the mean shows the S.E. ($n=3$) and different lowercase letters indicate the statistically significant differences among different treatments through LSD test at $P \leq 0.05$

to other treatments (Fig. 1E, F, G, H, K). The combined toxic effect of Cd and PS NPs was especially pronounced in carotenoid concentrations. The highest decrease of 37.91% in carotenoids was observed with PS NPs treatment. Whereas, the application of IPMM BC biofilter in a Cd-contaminated environment led to a substantial increase of 187.87% in carotenoid contents. Total chlorophyll content improved by 82.09% in the MBC treatment and 36.66% in the MBC+PS NPs treatment compared to sole Cd stress conditions. However, chlorophyll contents significantly reduced to $1.93 \text{ mg g}^{-1} \text{FW}$ in CK and $1.53 \text{ mg g}^{-1} \text{FW}$ in PS NPs treatments. Additionally,

the chlorophyll a:b ratio declined from 5.73 in the CK to 4.55, 2.92, and 3.18 in PS NPs, MBC, and MBC+PS NPs treatments, respectively. The exposure to PS NPs and Cd significantly ($P \leq 0.05$) altered soluble protein and proline concentration in rice leaf tissues. The IPMM BC biofilter alleviated the negative effects of these toxic elements and notably increased soluble protein concentration under Cd toxicity compared to PS NPs treatment (Fig. 1I). Specifically, the MBC and MBC+PS NPs treatments increased soluble protein content by 86.22% and 59.10%, respectively, whereas PS NPs reduced protein content by 37.01% compared to sole Cd stress treatment.

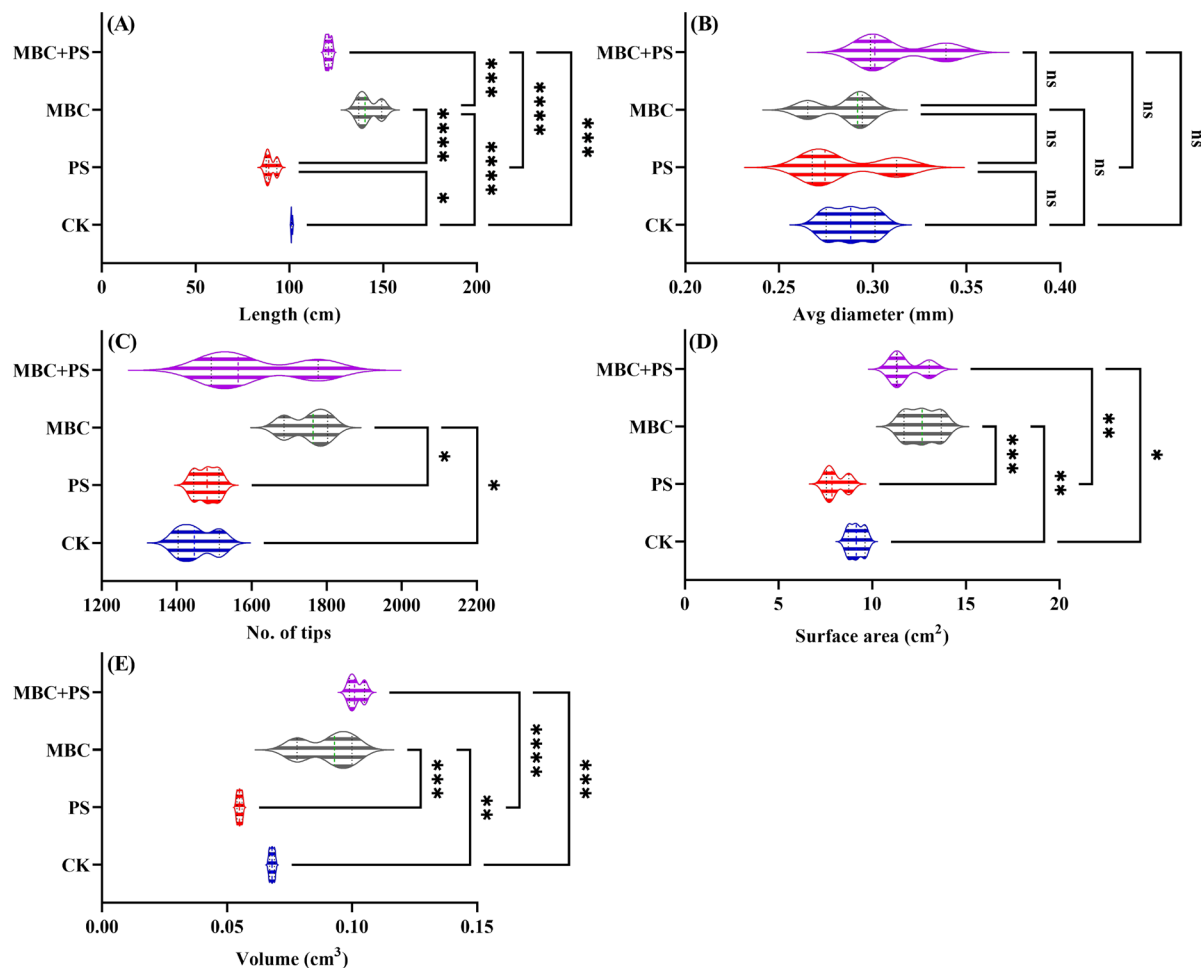


Fig. 2 Effect of IPMM BC biofilters under Cd and PS NPs toxicity on root growth and development. The stars above the mean show the statistically significant differences among different treatments through the LSD test at $P \leq 0.05$

Interestingly, IPMM BC biofilters reduced the proline concentration (Fig. 1j). Under Cd contamination, proline levels were higher in the following order: PS NPs ($72.52 \mu\text{g g}^{-1}$ FW) > CK ($62.64 \mu\text{g g}^{-1}$ FW) > MBC+PS NPs ($46.19 \mu\text{g g}^{-1}$ FW) > MBC ($35.19 \mu\text{g g}^{-1}$ FW). These findings suggest that the IPMM BC biofilter plays a crucial role in osmoregulation and chlorophyll formation, thereby mitigating the negative effects of Cd and PS NPs on rice.

3.4 Root growth and developmental alterations in response to IPMM BC biofilter and PS NPs under Cd contaminated environment

The beneficial impact of the IPMM BC biofilter under Cd and PS NPs toxicity is evident in its effects on root morphological characteristics such as total root length, root tip count, surface area, average diameter, and root volume (Fig. 2). The IPMM BC biofilter improved root architecture in the following order: MBC > MBC+PS

NPs > CK > PS NPs (Fig. 2A, B, C, D, E). Specifically, the maximum increases in cumulative root length, surface area, and volume were 40.46%, 38.39%, and 32.84%, respectively, in the MBC treatment compared to the control, while decrease of 11.09%, 12.14%, and 19.11% was observed with PS NPs treatment. Interestingly, no significant effect was observed on the average root diameter across treatments. The root system structure is crucial for water and nutrient absorption, as well as overall plant health and longevity. The IPMM BC biofilter significantly increased total root length, tip count, and surface area under combined Cd and PS NPs stress ($P \leq 0.05$). Conversely, PS NPs under Cd toxicity had a detrimental effect on root architecture. As shown in Fig. 2, the combined exposure to Cd and PS NPs had a more pronounced negative impact on root architecture compared to the IPMM BC biofilter treatment in a Cd-contaminated environment.

3.5 Physiochemical attributes connectivity and biochemical responses under the combined exposure to Cd and PS NPs in the presence of IPMM BC biofilters

The evaluation of antioxidant enzyme activities provided insights into the role of the IPMM BC biofilter in enhancing oxidative defence mechanisms in rice exposed to Cd and PS NPs toxicity. The results show that exposure to Cd and PS NPs significantly reduced the activities of POD, SOD, CAT, and APX in rice compared to the MBC treatment (Fig. 3A, B, C, D). Notably, the IPMM BC biofilter mitigated these effects by enhancing the activities of these antioxidant enzymes. Specifically, under Cd contamination, enzyme activities in the IPMM BC biofilter treatment increased as follows: SOD activity increased from $132.22 \text{ U g}^{-1} \text{ FW}$ (CK) to $302.89 \text{ U g}^{-1} \text{ FW}$, POD from 37.34 to $62.23 \text{ U g}^{-1} \text{ FW}$, CAT from 19.78 to $50.91 \text{ U min}^{-1} \text{ g}^{-1} \text{ FW}$, and APX from 2.05 to $4.90 \text{ U min}^{-1} \text{ g}^{-1} \text{ FW}$ under the Cd contaminated environment. Conversely, the PS NPs treatment led to a decrease in the activity of SOD, POD, CAT, and APX by 67.51% , 19.95% , 5.16% , and 14.28% , respectively, compared to CK. Furthermore, the levels of glutathione were notably diminished under the influence of Cd and PS NPs contamination alone (Fig. 3E). In contrast, oxidized glutathione levels were substantially higher compared to other treatments (Fig. 3F). Under Cd stress, PS NPs caused a 30.35% decrease in reduced glutathione

(GSH) levels, while MBC and MBC + PS NPs increased GSH content by 108.57% and 46.09% , respectively. On the other hand, GSSG levels were reduced by 47.62% and 26.65% in the MBC and MBC + PS NPs treatments, respectively, whereas they were increased by 79.92% in the PS NPs treatment. Moreover, IPMM BC biofilters enhanced the GSH:GSSG ratio in rice leaves under Cd stress compared to the CK. However, these results were not significant for the CK and PS NPs treatments (Fig. 3G). Figure 3 (I, J, K, L) demonstrates the effect of the IPMM BC biofilter on the expression of genes associated with the oxidative defence system in rice under PS NPs and Cd toxicity. The genetic expression levels of antioxidant enzyme genes (SOD, POD, CAT, APX) were reduced in the presence of Cd and PS NPs. However, the addition of MBC led to an upregulation in the expression of genes for CAT, SOD, APX, and POD under the combined toxicity of Cd and PS NPs. The fold changes in gene expression ranged from -50% to 37.05% for SOD, -39.47% to 203.94% for POD, -22.86% to 351.16% for CAT, and -8.19% to 160.10% for APX across the different treatments, with the most pronounced changes observed in the MBC treatment. Conversely, PS NPs treatment significantly downregulated the genetic expression of SOD, POD, CAT, and APX genes by 50% , 39.47% , 22.86% , and 8.19% , respectively, compared to the CK. The integrity of the cellular membrane in rice, which is linked to the accumulation of H_2O_2 , EL, and MDA, was significantly

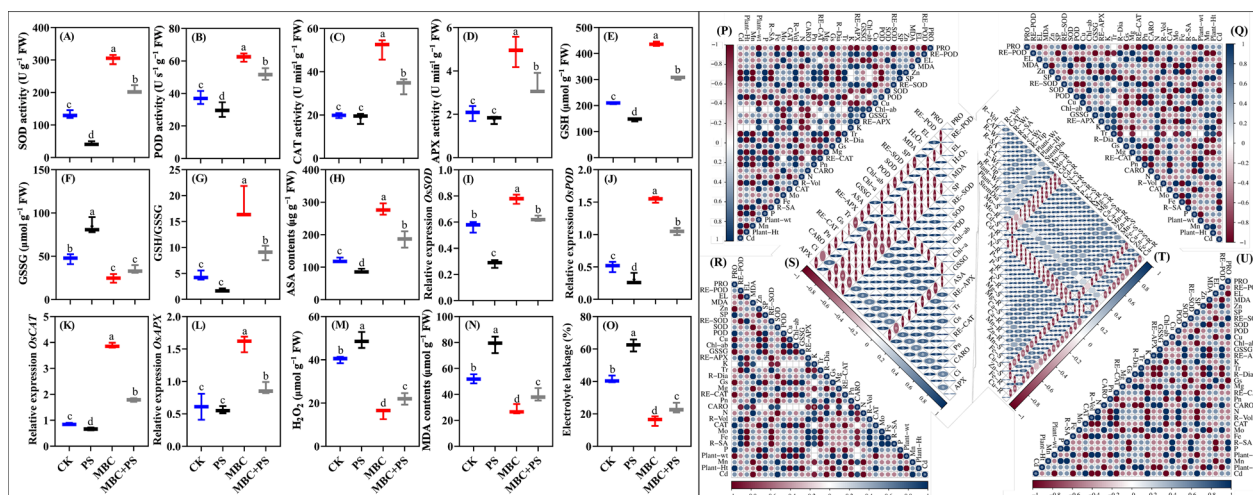


Fig. 3 Oxidative stress, genetic expression and membrane permeability response of rice plant under combined toxicity of Cd and NPs in the presence of IPMM BC biofilters. Figure (A), (B), (C), and (D) represent (Enzymatic oxidative stress) superoxidase, peroxidase, catalase and ascorbate peroxidase activities while figure (E), (F), (G), and (H) represent (Non-enzymatic oxidative stress) reduced glutathione, oxidized glutathione, reduced glutathione to oxidized glutathione ratio (GSH/GSSG) and ascorbic acid, respectively, in rice plants. Figures (I), (J), (K), (L) and (M), (N), (O) represent the genetic expression and biochemical responses of rice under Cd, NPs and IPMM BC biofilter supply. Figures (P), (Q), (R), (U) indicate the CK, PS, MBC, MBC + PS and (S), (T) represent the overall correlations respectively among biochemical and molecular indicators affected by different treatments. Chl, chlorophyll; EL, electrolyte leakage; CARO, carotenoids; RE, Relative expressions; R, root; S, shoot; SA, surface area; Dia, diameter; Vol, volume; Wt, weight; Ht, height. RE, S and R before and after different indices indicate the relative expression, shoot and root respectively

compromised by Cd and PS NPs stress, as evidenced by increased levels of H_2O_2 , MDA, and EL.

Specifically, Cd and PS NPs treatments significantly ($P \leq 0.05$) increased the levels of H_2O_2 , MDA, and EL by $40.16 \mu\text{mol g}^{-1}$ FW and $48.99 \mu\text{mol g}^{-1}$ FW, $52.0 \mu\text{mol g}^{-1}$ FW and $78.67 \mu\text{mol g}^{-1}$ FW, $41.13 \mu\text{mol g}^{-1}$ FW and $62.3 \mu\text{mol g}^{-1}$ FW, respectively (Fig. 3 M, N, O). In contrast, the provision of MBC led to the most substantial reductions in H_2O_2 , MDA, and EL by 61.81%, 45.77%, and 61.44%, whereas the PS NPs treatment under Cd toxicity resulted in the highest increases of 21.97%, 51.27%, and 51.46%, respectively. These findings highlight that the IPMM BC biofilter effectively preserved membrane integrity by regulating oxidative defence mechanisms and maintaining redox homeostasis in rice under the combined toxicity of Cd and Ps NPs, demonstrating its significant role in mitigating toxicity. A Pearson's correlation analysis was performed to examine how IPMM BC biofilters and PS NPs affect rice growth and biochemistry under Cd toxicity (Fig. 3P, Q, R, S, T, U). Cd levels were found positively correlated with oxidative stress but negatively with plant growth metrics like biomass, height, photosynthesis, and soluble proteins. Antioxidant enzyme activity showed only a weak correlation with enzyme transcript abundance. The elemental profile in roots and shoots was positively correlated with plant height, biomass, root volume, diameter, length, and root tips. Conversely, transpiration rate, stomatal conductance, photosynthetic rate, intercellular CO_2 concentration, and soluble protein content were negatively correlated with ROS and proline. Photosynthetic pigments and oxidative stress markers were strongly correlated with chlorophyll content, highlighting beneficial interactions between plant biological indices and physiological mechanisms in the presence of IPMM BC biofilters, while revealing negative effects under Cd and PS NPs toxicity.

3.6 Combined toxic effect of PS NPs and Cd

on ultrastructural characterization of rice leaf cells

Under Cd toxicity, both Cd alone and Cd combined with PS NPs significantly reduced stomatal widths, lengths, and their opening degree (Fig. 4A, B, C, D). However, the IPMM BC biofilter, whether used alone or with PS NPs under Cd stress, effectively restored these characteristics, showing the most positive impact on stomatal structure compared to other treatments. Figure 4 (E, F, G, H) illustrates the ultrastructural changes in rice mesophyll cells under various treatments. Plants treated with the IPMM BC biofilter under Cd stress displayed well-organized thylakoid grana and stroma, with clear chloroplast features such as a defined shape, intact

envelope, and well-formed plastoglobuli. In both MBC and MBC+PS NPs treatments, thylakoids showed minimal cracks and connected seamlessly with stroma thylakoids, while maintaining an intact mitochondrial structure (Fig. 4G, H). In contrast, plants treated with PS NPs alone under Cd stress exhibited poorly developed structures. The chloroplasts exhibited irregularities such as disorganized grana stacking, distorted membranes, and disrupted thylakoid systems (Fig. 4F). Severe stress caused chloroplasts to swell and adopt a spherical shape, deviating from the typical spindle-like form seen in MBC treatment. The thylakoid lamellae were loosely packed, grana thylakoids appeared wavy, and stroma lamellae were disorganized. Some chloroplast envelopes ruptured, releasing internal contents into the cytoplasm. The IPMM BC biofilter treatments showed varying levels of detoxification effects under the toxicity of Cd and PS NPs.

3.7 IPMM BC biofilters mitigate PS NPs accumulation in rice root

To gain structural insights into how IPMM BC biofilters reduce the PS NPs bioaccumulation and rescue rice root growth, the roots exposed to CK, PS NPs, MBC, and MBC+PS NPs were examined using SEM. The SEM micrographs highlighted significant differences between the CK, PS NPs, MBC, and MBC+PS NPs groups (Fig. 5). The results showed that PS NPs were present on the surfaces of rice roots and penetrated internal structures, such as the xylem and the cell walls of the cortex tissue, indicating bioaccumulation of PS NPs in rice. Additionally, cross-sections of rice roots exposed to PS NPs exhibited structural defects relative to the control group. In the MBC group, root tissues displayed the most extensive distribution of root hairs on their surfaces, followed by the MBC+PS NPs group (Fig. 5K, L). Whereas, the PS NPs under Cd stress showed a noticeable reduction in root hairs along with significant disruption to intercellular tissues (Fig. 5B, I). The root cross-section diagrams further revealed that stress from Cd and PS NPs led to increased channels within the cortical regions, cortical layer, and vascular bundle of the root. Furthermore, the dimensions and quantity of sieve tubes, periductal phloem, and companion cells were decreased under the stress of Cd and PS NPs (Fig. 5A, B, C, D). Interestingly, the application of IPMM BC biofilters alleviated the structural damage caused by PS NPs and limited their entry into the root cells (Fig. 5F, G, H). This reduction in PS NPs bioaccumulation and toxicity in roots under Cd stress contributed to the recovery of root architecture.

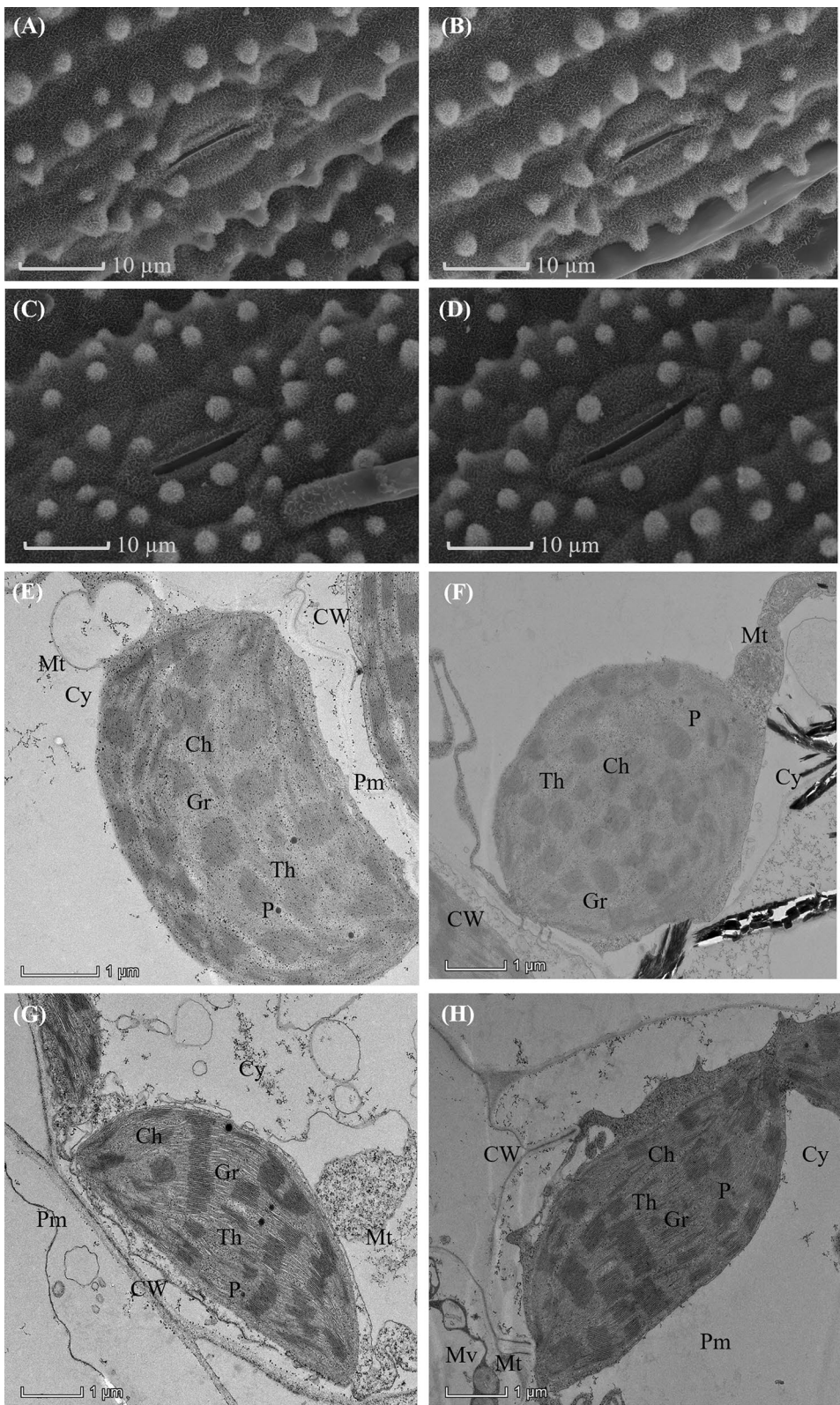


Fig. 4 Effect of IPMM BC biofilters, Cd and PS NPs on leaf ultra-structure. Figures (A), (B), (C), (D) and (E), (F), (G), (H) represent the variation of stomata and chloroplast in CK, PS, MBC and MBC + PS treatment respectively. Ch, chloroplasts; P, plastoglobuli; CW, cell wall; Gr, grana; Th, thylakoid; Scale bars (Chloroplast) = 1 µm Stomata = 10 µm)

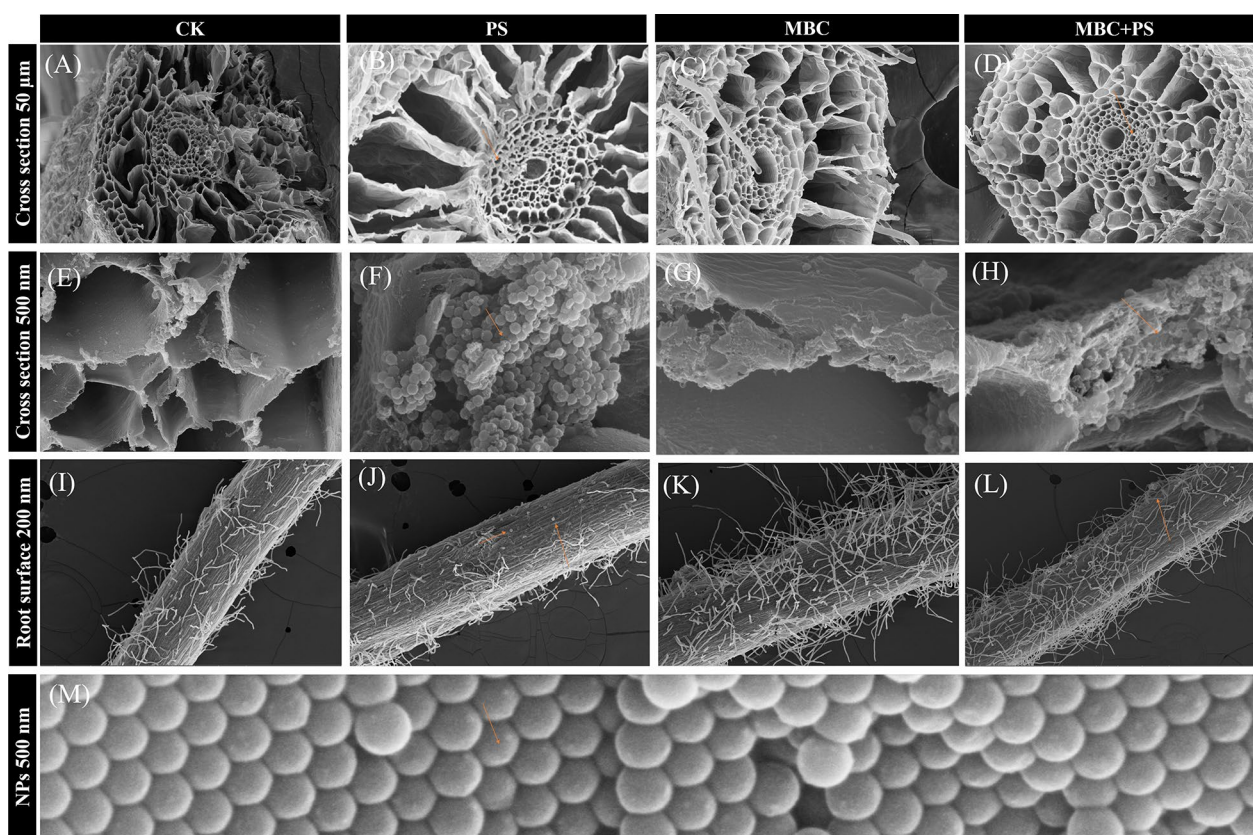


Fig. 5 SEM micrographs of rice root in response to PS NPs (100 nm), Cd, MBC and their combined application (PS + MBC). PS NPs aggregated were detected on the root surface and inside the root. The red arrow indicates the presence of PS NPs

3.8 Metabolomics response in rice under Cd and PS NPs stress

The observed physiological changes are likely the results of alterations in intracellular metabolic processes. To explore these modifications, a metabolomics analysis and quantitative profiling of metabolites in rice plants were carried out through gas GC–MS. In total, 165 metabolites ($VIP > 1$, $P < 0.05$) were identified. The detected compounds primarily consisted of alkaloids, benzoids, lipids, organic acids, nucleosides, and various other substances. Among the detected metabolites, organooxygen compounds, carbohydrates, and organic molecules accounted for the largest proportions, comprising 32.73%, 24.24%, and 32.73%, respectively (Fig. S2). Multivariate analyses including PLS-DA and OPLS-DA revealed distinct separations between the Cd, PS NPs, and MBC treatments in rice, indicating substantial metabolic reprogramming in response to Cd and PS NPs stress. The component scores from these analyses are displayed in a three-dimensional plot, illustrating the distinct metabolic responses of rice plants to the application of IPMM BC biofilters and PS NPs under Cd toxicity. Differences along the PC2 axis highlighted the variations in metabolic profiles (Fig. 6). PLS-DA and OPLS-DA

served as the classification model and the evaluation was based on the predictive ability metrics (Q^2) and goodness of fit (R^2). In all cases, the R^2 values for all samples were found greater than corresponding Q^2 values, which confirmed the consistency of the model. The PLS-DA 3D plot clearly demonstrates the overall impact of the treatments on metabolite variation (Fig. 6A). The OPLS-DA 3D plot analysis revealed minimal variation between the IPMM BC biofilter and MBC+NPs treatments, while the impact of CK and PS NPs was more pronounced compared to the other treatments (Fig. 6B, C, D, E, F, G). The OPLS-DA loading diagram indicated that under Cd toxicity, PS NPs altered the distribution of root metabolites in rice compared to the IPMM BC biofilters (Fig. 6I, J, K, L, M, N). The MBC and MBC+PS NPs treatments clustered closer together, whereas CK and PS NPs groups formed distinct clusters, far from those exposed to Cd and PS NPs alone. This shows that IPMM BC biofilters had the most significant impact on the rice plant metabolic profiles. The S-plot further confirmed that the influence of metabolites in the PS NPs vs CK comparison group was lower compared to MBC vs PS NPs (Fig. 6Q), MBC+PS NPs vs BC (Fig. 6R), and MBC+PS NPs vs PS (Fig. 6T). Permutation testing

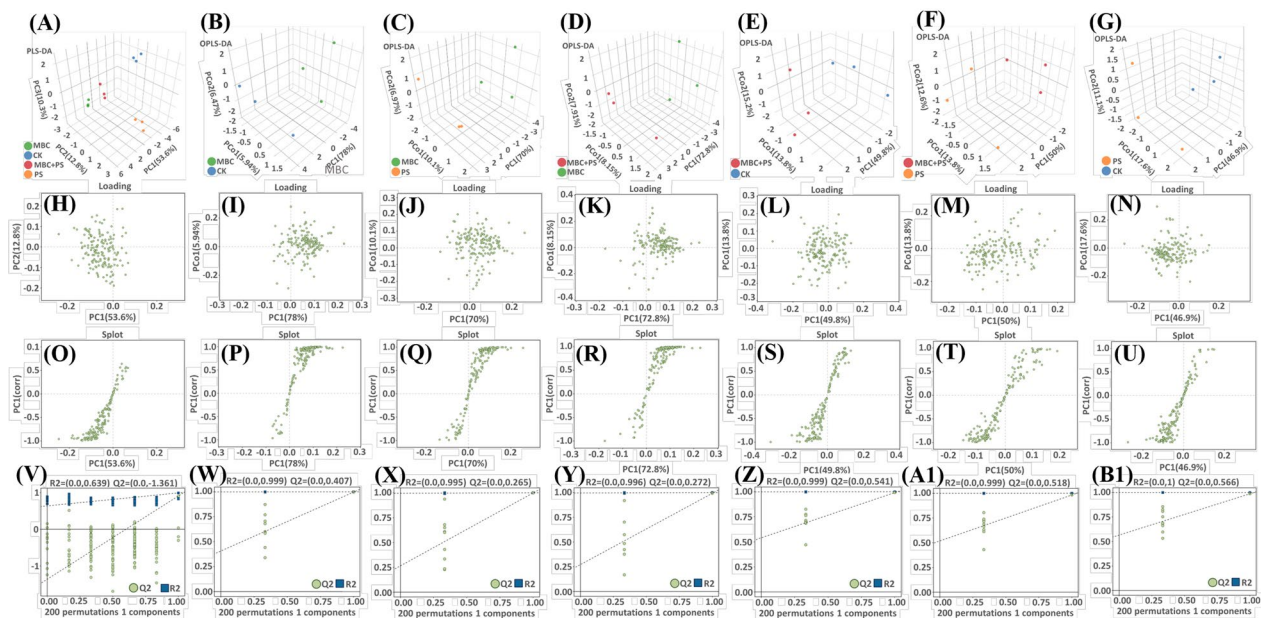


Fig. 6 Overall partial least-squares discriminant analysis (PLS-DA) score plot (A), PLS-DA loading score (H), PLS-DA-Splot (O) and model effectiveness evaluations through permutation plot (V) of metabolic alterations in the rice plant. The Fig. (B, C, D, E, F, G, (I, J, K, L, M, N), (P, Q, R, S, T, U), (W, X, Y, Z, A1, B1) represent the pairwise alterations of metabolites through orthogonal partial least squares discriminant analysis (OPLS-DA), OPLS-DA loading score, OPLS-DA-splot and OPLS-DA model effectiveness evaluations through Permutation plot respectively in MBC vs CK (Fig. B, I, P, W), MBC vs PS (Fig. C, J, Q, X), MBC + PS vs BC (Fig. D, K, R, Y), MBC + PS vs CK (Fig. E, L, S, Z), MBC + PS vs PS (Fig. F, M, T, A1), PS vs CK (Fig. G, N, U, B1)

(Fig. 6V, W, X, Y, Z, A1, B1) validated the consistency of the OPLS-DA and PLS-DA model results, confirming that PS NPs and IPMM BC biofilters significantly influenced the metabolite changes. The permutation testing showed that all the green Q2 values on the left side were lower than the original points on the right, and the green regression line (Q2 points) intersected the vertical axis at or below zero. This confirmed both the effectiveness of the model and the accuracy of its evaluation of treatment impacts. The four treatments were clearly distinguished by the first two principal components (Fig. 6), demonstrating that the application of the IPMM BC biofilter under Cd and PS NPs stress had a significant ameliorative effect on rice metabolites. Additionally, correlation analysis revealed a significant strong correlation among identified metabolites across different treatment groups (Fig. S4). The CK, PS, MBC, and MBC+PS treatments under Cd stress were significantly separated from the control and PS NPs treatments, highlighting their detrimental impacts on rice and emphasizing the protective role of the IPMM BC biofilters.

3.9 Identification of differentially accumulated metabolites (DAMs) and Kyoto encyclopedia of genes and genomes (KEGG) pathways enrichment analysis.

A heat map analysis was performed to further categorize the various metabolites. This revealed that the

normalized levels of most metabolites in the PS NPs, MBC+PS NPs, and MBC treatment groups showed significant changes compared to the CK treatment. Across all treatments, 50 differentially expressed metabolites (DEMs) (P value < 0.05) in rice are represented in the heat map (Fig. 7A, B, C, D, I, J). The distribution of these metabolites was further illustrated using a Venn diagram (Fig. 7M). Specifically, significant alterations in metabolite expression were observed in response to Cd and PS NPs exposure. Comparisons between different treatment groups, CK vs MBC, MBC vs MBC+PS, PS vs MBC+PS, PS + MBC, CK vs MBC+PS, and CK vs PS, revealed the number of downregulated metabolites as 55, 2, 13, 45, 20, and 36, respectively, while the number of upregulated metabolites was 0, 57, 29, 3, 15, and 6, respectively (Fig. 7E, F, G, H, K, L). The addition of IPMM BC biofilter imparted a significant positive effect on the identified metabolites and effectively counteracted the adverse effects of Cd and PS NPs exposure. The treatments with IPMM BC biofilter led to the upregulation of D-threonic acid, N-acetylmannosamine, and indoleacrylic acid compared to PS NPs. However, in the MBC+PS treatment, indoleacrylic acid and linoleic acid were downregulated relative to MBC under Cd toxicity (Fig. S3 A, B, C, D, E, F). Additionally, PS NPs showed a pronounced effect on indoleacrylic acid, linoelaidic acid,

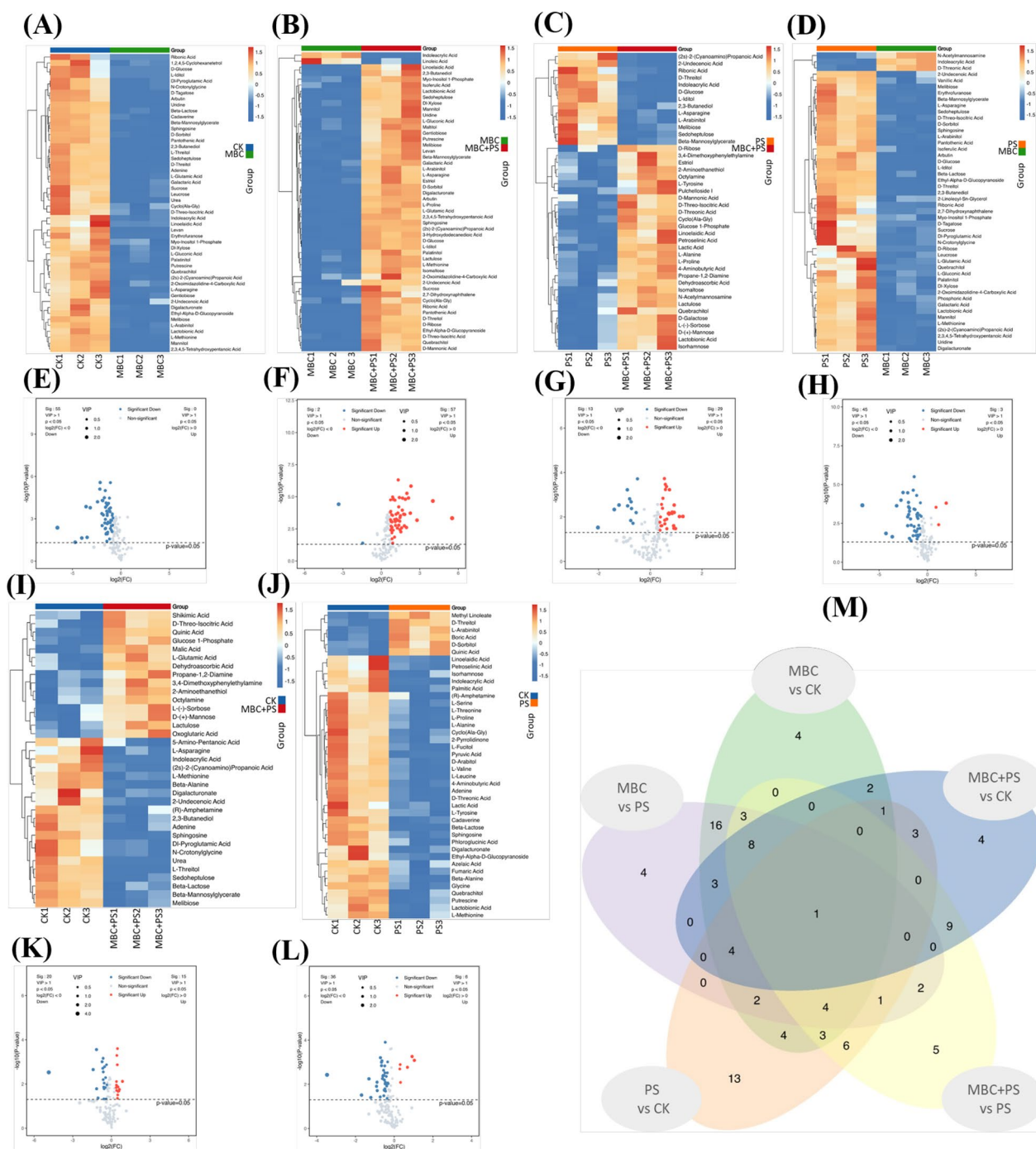


Fig. 7 Comparative heat map of top 50 detected metabolites, volcano plot, and ven diagram of differentially expressed metabolites in MBC vs CK (Fig. A, E), MBC vs PS (Fig. B, F), MBC + PS vs CK (Fig. C, G), MBC + PS vs PS (Fig. D, H), MBC + PS vs PS (Fig. I, K), PS vs CK (Fig. J, L). The horizontal axis in the heat map represents the sample name, and the vertical axis represents the differential metabolites. The colours from blue to red represent the expression abundance of metabolites from low to high. The red dots in the volcano plot analysis represent significantly upregulated differential metabolites in the experimental group, blue dots represent significantly downregulated differential metabolites, and grey dots represent metabolites with no significant difference. Each dot in the figure represents a metabolite, the horizontal axis is the log₂(FC) value of the two groups, and the vertical axis is the -log₁₀(P-value). Red dots are significantly upregulated differential metabolites ($P < 0.05$, VIP > 1 and FC > 1), and blue dots are significantly downregulated differential metabolites ($P < 0.05$, VIP > 1 and FC < 1)

quebrachitol, petroselinic acid, cyclo (Ala-Gly), palmitic acid, digalacturonate, 4-aminobutyric acid, adenine, and D-threonic acid, which were the major components of DEM in the PS NPs group under Cd toxicity (Fig. S3F). Notably, L-proline was downregulated with the addition of IPMM BC biofilters compared to the MBC+PS NPs treatment under Cd stress. Similarly, after PS NPs stress, methionine and valine were downregulated.

The metabolic pathway enrichment analysis, based on data from the KEGG database for various treatments, revealed a significant enhancement ($P < 0.05$) in specific metabolic pathways in rice, including purine and nitrogen metabolism, as well as the citric acid cycle (TCA cycle) (Fig. 8). The metabolic pathways included fructose and mannose metabolism, starch and sucrose metabolism, inositol phosphate metabolism, galactose metabolism, ascorbate and aldarate metabolism, and, pentose and glucuronate interconversions associated with carbohydrate metabolism. Additionally, fatty acid biosynthesis

and elongation in mitochondria were part of these pathways, as well as the tricarboxylic acid (TCA) cycle and glyoxylate and dicarboxylate metabolism related to organic acid metabolism. For amino acid metabolism, pathways involved valine, leucine, and isoleucine biosynthesis, as well as the metabolism of arginine and proline, cysteine and methionine, and glycine, serine, and threonine. Furthermore, the metabolism of pyrimidines and purines, especially concerning uridine and guanosine, was also affected. The metabolic pathways resulting from the investigation of different groups exposed to Cd, PS NPs and IPMM BC biofilters are shown in Fig. 8. In a Cd-contaminated environment, significant enrichment was observed in several metabolic pathways in rice, including the metabolisms of arginine and proline, sphingolipid, fructose and mannose, glyoxylate and dicarboxylate, alanine, aspartate, and glutamate biosynthesis, when compared to the MBC treatment (Fig. 8A). The addition of IPMM BC biofilters further enhanced the enrichment

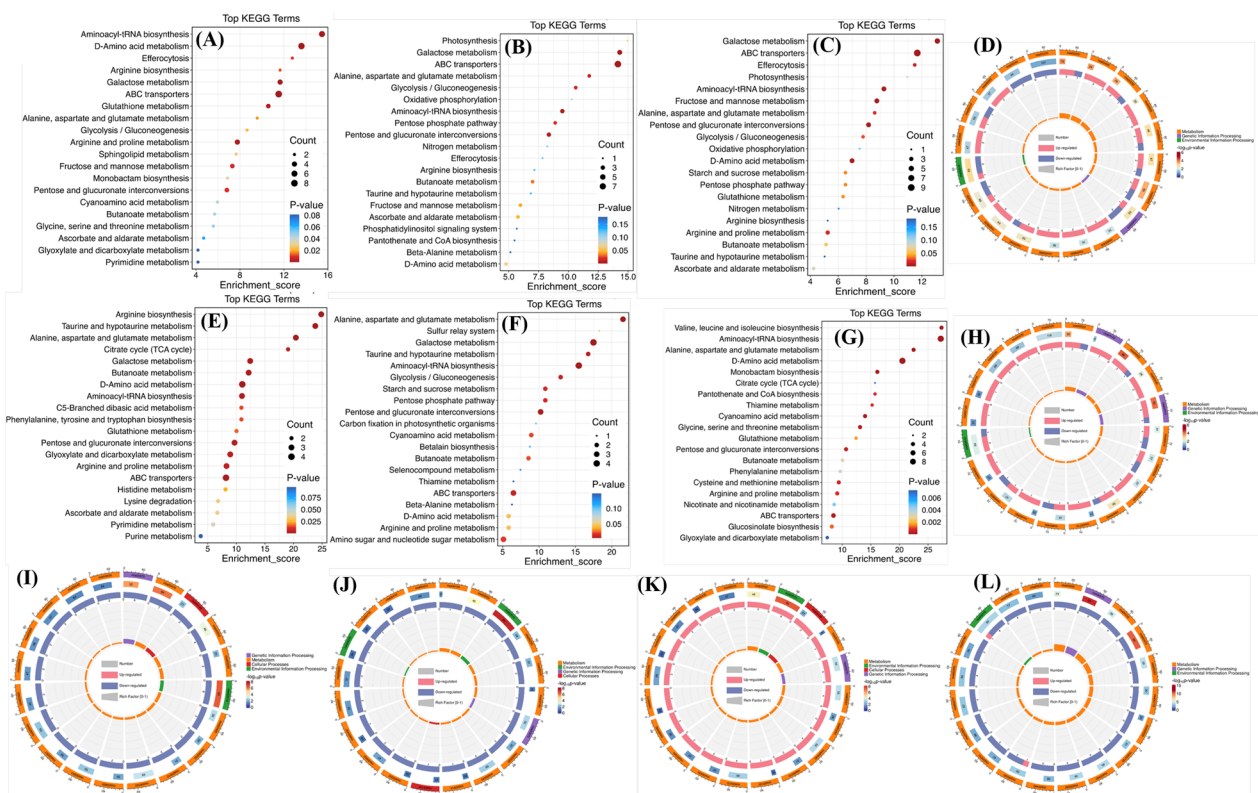


Fig. 8 Differential metabolic pathways through KEGG bubble analysis and KEGG enrichment analysis through circle plot with the smallest P -value in MBC vs CK (Fig. A, I), MBC vs PS (Fig. B, J), MBC + PS vs BC (Fig. C, K), MBC + PS vs CK (Fig. D, E), MBC + PS vs PS (Fig. F, H), PS vs CK (Fig. G, L). In KEGG bubble analysis, the horizontal axis enrichment score in the figure is the enrichment score, and the vertical axis is the top 20 pathway information. The bubble colour changes from blue to red, and smaller the enrichment P -value is, the greater the significance is. The first circle in the KEGG circle analysis indicates the enriched classification, outside the circle is the coordinate scale of the number of metabolites, and different colours represent different classifications. The second circle: the number of this category in the background metabolism and the P -value. The more metabolites there are, the longer the bar is, smaller the value, redder the color, and larger the value, the bluer the color. The third circle: Up-and down-regulated metabolic ratio bar chart, light red represents the up-regulated metabolite ratio, and light blue represents the down-regulated metabolite ratio. The fourth circle: Rich Factor value of each category, each small grid of the background auxiliary line represents 0.2

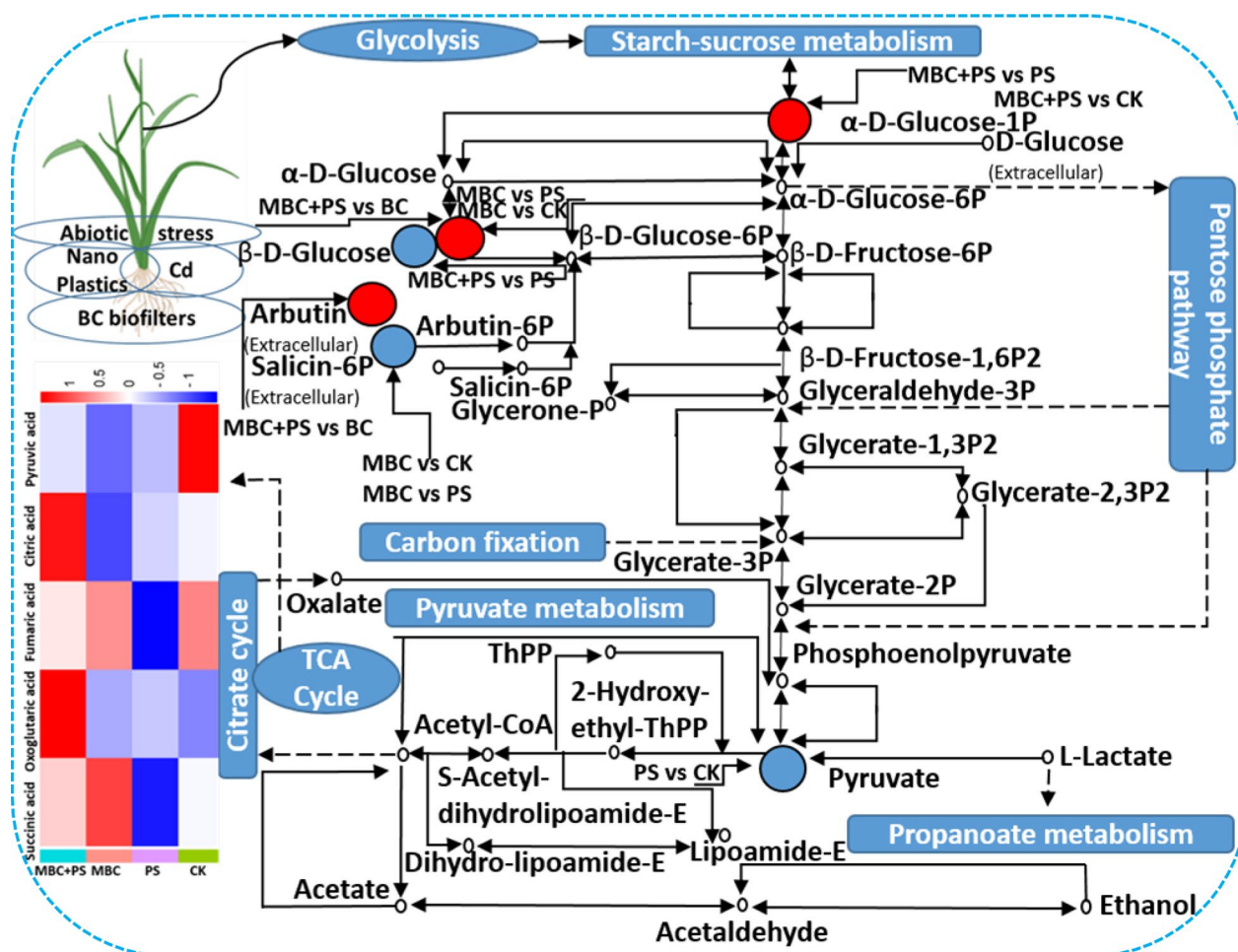


Fig. 9 Schematic diagram of proposed metabolic pathways in rice plant. The differential metabolic pathways are displayed through the KEGG pathway mapper function, and the differential metabolites are colored according to the up- and down-regulation information. The small circles in the metabolic pathway diagram represent metabolites. The metabolites marked in red in the pathway diagram are up-regulated metabolites detected in the experiment, and the metabolites marked in blue are down-regulated metabolites. The relative content of metabolites in citrate cycle (TCA cycle) is shown in the form of a heat map

of photosynthesis pathways in rice plants compared to the PS NPs treatment under Cd toxicity (Fig. 8B). Furthermore, the MBC+PS NPs treatment significantly enriched the TCA cycle, histidine and galactose metabolism, and phenylalanine biosynthesis pathways compared to CK. The ten pathways with the highest list-hits and the lowest *P*-values from the KEGG enrichment analysis are presented in the chord analysis (Fig. S5), demonstrating the pronounced impact of IPMM BC biofilters on metabolic pathways under Cd and PS NPs contamination. These findings indicate that the combination of PS NPs and Cd stress exerted a more substantial effect on rice metabolic pathways than other treatments, suggesting that the combined stress from PS NPs and Cd had a more severe impact on rice than Cd alone. The KEGG pathway database, which tracks molecular interaction networks within cells and monitors specific changes

in organisms, classifies biological metabolic pathways into seven groups: organismal systems, cellular processes, diseases, environmental information processing, metabolism, genetic information processing, and drug development. The analysis of these molecular interaction networks, as shown in the circos plot, underscores the effectiveness of IPMM BC biofilters in maintaining metabolism, environmental and genetic information processing, as well as cellular processes. In contrast, PS NPs significantly disrupted these networks under Cd toxicity (Fig. 8D, H, I, J, K, L).

Additionally, the schematic diagram of proposed metabolic pathways in rice (Fig. 9) shows that β -D-glucose was upregulated in the MBC treatment compared to CK and PS NPs, but downregulated in the MBC+PS NPs treatment. In pyruvate metabolism, PS NPs negatively affected pyruvate synthesis compared to CK under Cd

stress conditions. These findings demonstrate that Cd and PS NPs significantly impacted rice metabolic pathways. Notably, the pathways influenced by Cd and PS NPs appeared more complex, suggesting that plants were more susceptible to stress induced by Cd and PS NPs than by other treatments. This implies that the differentially DAMs and the associated pathways may play a crucial role in conferring tolerance to Cd and PS NPs in rice when supplied with IPMM BC biofilters.

4 Discussion

The impact of multiple stress factors on plants is more complex than that of single stress, and the consequences of PS NPs and Cd combined internalization in the context of IPMM BC biofilters on plant health remain unclear. Therefore, this study aims to explore the ecological risks and food safety concerns posed by the combined influence of PS NPs and Cd in the presence of IPMM BC biofilters. The results revealed that simultaneous exposure to PS NPs and Cd negatively affected root structure and physiology, photosynthetic activities, and both enzymatic and non-enzymatic oxidative stress markers in rice. It also altered genetic expression, root and leaf ultrastructure, as well as metabolic and biochemical processes, resulting in increased Cd accumulation in rice. The findings suggest that using IPMM BC biofilters could serve as an innovative strategy to reduce the toxicity of PS NPs and Cd by triggering the biochemical and molecular variations in rice.

4.1 Rice growth, NPs internalization and root developmental alterations in response to IPMM BC biofilter under PS NPs and Cd contaminated environment

This investigation revealed that the combined stress of PS NPs and Cd had more severe phenotypic effects on plants than either stressor alone and IPMM BC biofilters effectively mitigated this combined stress. Prior research has shown that the PS NPs in Cd-contaminated environments can negatively impact plant growth and development. For instance, PS NPs can hinder water absorption, intensify oxidative stress, and increase Cd toxicity, leading to reduced dry weight of maize stems and roots in Cd-polluted conditions (Wang et al. 2020). In this study, rice plants exposed to both PS NPs and Cd showed a significant decrease in dry biomass, as well as reduced root and shoot length, compared to those exposed to Cd alone or treated with IPMM BC biofilters (Table 1). This suggests that PS NPs augmented the toxic effects of Cd by enhancing its translocation within the plant. The entrance of Cd ions into the plant can lead to reduced chlorophyll content, decreased antioxidant enzyme activity, and altered cell membrane permeability, which may inhibit plant

growth and development (Li et al. 2023c). It is supposed that Cd may have replaced Mg in the chlorophyll synthesis, contributing to the observed reduction in chlorophyll content. Similar observations have been reported in the spinach (Rydzynski et al. 2019). Whereas, the diameter of rice plants was not significantly influenced by the PS NPs in the presence of Cd (Fig. 2). The use of IPMM BC biofilters alleviated the phytotoxic effects of both Cd and PS NPs in rice, likely due to reduced uptake of these toxic substances (Table 2).

Roots serve as the primary organs for nutrients absorption that act as the initial interface with the external environment. In this study, PS NPs were internalized into rice roots, facilitating increased uptake of Cd. This observation aligns with previous studies indicating that nanoparticles can penetrate in plants through root tips and cell walls (Li et al. 2024a). Under Cd stress, PS NPs reduced root length, total number of tips and root surface area while also disrupting root structure morphology. Similar disruptions under HMs stress have been reported by Redjala et al. (2011) and Meng et al. (2021). The SEM micrograph revealed the presence of PS NPs dispersed across the root tissues and within the vascular bundles. The possible route for PS NPs entry involves root absorption, followed by upward translocation through the vascular bundles, driven by transpiration flow (Yu et al. 2024). A parallel mechanism of uptake has been observed for copper oxide nanoparticles into cabbage and lettuce (Xiong et al. 2017). However, the application of IPMM BC biofilters significantly reduced the internalization of both PS NPs and Cd in rice roots and shoot, thereby mitigating their toxic effects and preserving root structure (Fig. 2). One potential mechanism behind this alleviation is the adsorption capacity of IPMM BC biofilters, which may act as reservoirs, sequestering PS NPs and Cd. Notably, the internalization of Cd was more pronounced in PS NP treatments than in Cd-alone treatments (Table 2), suggesting that PS NPs enhance Cd accumulation. Similar findings have been reported by Wang et al. (2022b) who observed that nanoparticles can form stable associations with HMs through both surface and internal adsorption. This interaction may contribute to increased Cd accumulation in rice plants, thereby impairing their growth and development.

4.2 Combined toxic effect of PS NPs and Cd on the ultrastructure of rice leaf

The negative impacts of PS NPs on cellular organization under Cd contamination are crucial for understanding the adverse effects on rice physiological systems. The combined phytotoxicity of Cd and PS NPs on chloroplast ultrastructure is associated with various biochemical abnormalities in proteins, lipids, and cellular

connections. TEM micrographs of rice exposed to both stressors revealed significant structural changes in chloroplasts, such as amoeboid shapes and extensive damage to the thylakoid membranes. In contrast, rice treated with IPMM BC biofilters exhibited a well-organized internal structure with properly aligned thylakoids, compared to controls or other treatments (Fig. 4). Cadmium stress can also disrupt glucose transport, leading to distorted mesophyll cells and chloroplast structures (Adil et al. 2020; Grato et al. 2009; Mosa et al. 2016). This damage was more severe in plants exposed to both PS NPs and Cd (Fig. 4F) than in those treated with IPMM BC filters or MBC + PS NPs. Moreover, the phytotoxic effects of PS NPs in the presence of Cd also resulted in significant alterations in mitochondrial morphology, suggesting a detoxification response to mitigate the adverse effects of PS NPs. The alterations in mitochondrial dimensions and morphology may result from the production of ROS, which can lead to enlargement and damage to the outer mitochondrial membrane (Li and Xing 2011; Ott et al. 2007). Additionally, Cd-induced phytotoxicity may disrupt thylakoid molecular structure through biochemical impairments, such as protein denaturation and imbalances in protein-lipid interactions that affect membrane protein complexes (El-Banna et al. 2018; Maleki et al. 2017). The protective impact of IPMM BC biofilters alleviated these negative impacts on mesophyll leaf cells caused by PS NPs and Cd (Fig. 4G, H). These findings underscore the potential of IPMM BC biofilters to counteract the structural and functional damage in plant cells caused by Cd and PS NPs, emphasizing their importance in enhancing plant resilience under HMs stress.

4.3 Co-exposure influence of PS NPs and Cd on primary, secondary and micro-elemental composition and Cd translocation under the supply of IPMM BC biofilters

The surface charge of nanoparticles plays a significant role in their absorption and translocation within plants (Azeem et al. 2021; Li et al. 2024a). NPs readily internalized and progressively accumulated in the root xylem (Fig. 5). PS NPs adsorb Cd via electrostatic interactions, leading to extensive translocation within the roots. These electrostatic interactions cause PS NPs to quickly aggregate and bind Cd within the polymer matrix (Wang et al. 2022b). In plant growth mediums, plants typically absorb and retain HMs through their root systems. However, the presence of plastic materials can decrease the sorption capacity for metals (Jędruchiewicz et al. 2023), thereby increasing the Cd bioavailability to rice plants. Additionally, plastic particles can obstruct water uptake by clogging the pores in plant cell walls (Wang et al. 2022a). This blockage leads to reduced stomatal conductance which is associated with lower transpiration rates. In turn, this

blockage limits the absorption of essential nutrients, such as Ca, Fe, Mn, and Mg (Table 2). As a result, plants showed decreased biomass and lower productivity. The presence of PS NPs also elevated Cd levels in both the roots and shoots, while IPMM BC biofilters reduced Cd content. This reduction in Cd translocation by IPMM BC biofilters is likely due to their role as a repository for these contaminants. Research in agricultural systems has shown that MPs and nanoparticles can increase the bioavailability of HMs. For instance, PE-MPs have been found to raise Cd levels in plants (Li et al. 2024b); PMMA-NPs have been shown to facilitate higher arsenic uptake in rapeseed (Dong et al. 2022); and a high concentration of PE-MPs has been shown to decrease the metal adsorption capability of soil (Li et al. 2021). In line with these findings, our study suggests that the NPs enhance metal bioavailability to plants. Understanding the interactions between NPs, HMs, and plants is therefore crucial for assessing their combined effects.

4.4 Role of IPMM biochar biofilters in mitigating Cd and PS NPs-induced adverse effects on photosynthetic efficiency and related attributes

In plants, chlorophyll contents are crucial for photosynthesis and directly impact plant productivity (Simkin et al. 2022; Xu et al. 2022). Significant differences in photosynthetic attributes were observed among the CK, PS, MBC, and MBC + PS treatments. The presence of PS NPs under Cd exposure significantly reduced the GasEx Ci, Pn, Gs, Tr, Chlorophyll a and b, and soluble protein levels compared to CK, MBC and MBC + PS exposures. The higher production of ROS under the exposure of Cd and NPs likely contributed to the inhibition of photosynthesis and plant growth. The multiple stressors may disrupt chlorophyll structure and hinder its synthesis by triggering excessive ROS production within the plant (Bi et al. 2020; Wang et al. 2023b). This reduction in chlorophyll could be linked to a decreased carbon assimilation capacity in plants under HMs stress (Sardar et al. 2022). A decline in chlorophyll levels can induce adverse impacts on plant physiology and limit the energy available for seedling growth (Kalaji et al. 2016). Research has shown that nanoparticles can damage the structure of cellular organelles, while Cd can disrupt chlorophyll production by replacing Mg in the chlorophyll molecule (Grajek et al. 2020; Hu et al. 2021). A previous study on combined stress has observed that pollutant like PE-As can alter the morphology of leaf cells (Sun et al. 2020). The primary reason for the reduced chlorophyll content under PS NPs and Cd stress may be the inhibition of enzyme synthesis, which in turn lowers photosynthesis rates. The SP is also interlinked with photosynthetic activity

and considered as a significant indicator for assessing the nutritional value of plants. Both PS NPs and Cd impaired nutrient absorption, potentially decreasing SP levels in rice seedlings. PS NPs have also been shown to interfere with amino acid metabolism, resulting in reduced SP content (Zhang et al. 2022b). However, the presence of IPMM BC biofilters improved SP levels in rice, even in the presence of Cd or PS NPs. This underscores the increased sensitivity of these processes to Cd and PS NPs stress while demonstrating that the IPMM BC biofilter effectively reduced the detrimental impact of these contaminants.

4.5 Rice defence system regulations and membrane integrity under combined PS NPs and Cd toxicity

Plants have highly proficient oxidative defence systems that resist to ROS toxicity (Dumanović et al. 2021). The improved oxidative defence system—including enzymes such as APX, SOD, CAT and POD—plays a critical role in eliminating excessive ROS (Lai and Luo 2019). MDA, H₂O₂ and EL are the key indicators of membrane lipid peroxidation, reflecting the intensity of injury to the plasma membrane (Li et al. 2013). The co-exposure of PS NPs and Cd significantly increased the concentration of MDA, H₂O₂ and EL resulted in severe injury to the plant cell membrane. Excessive ROS can affect the transcription of DNA in plant cells, resulting in the suppression of antioxidant enzyme synthesis (Ge et al. 2021; You and Chan 2015). PS NPs and Cd treatments reduced the expression of antioxidant enzyme genes, weakening the oxidative defence system and impairing rice performance. Similar findings have been reported by Zhang et al. (2022a), indicating that disruptions in oxidative gene expression can impact the functionality of antioxidant systems. The reduced activity of oxidative enzymes in rice treated with PS NPs under Cd contamination is likely due to the suppressed expression of CAT, SOD, APX, and POD genes. This suppression may result from the ROS (Xu et al. 2023) induced by PS NPs and Cd. Elevated levels of ROS, coupled with decreased activity of SOD, APX, CAT, and POD, and their corresponding genetic expression, highlight the toxicity of PS NPs and Cd in rice, indicating compromised cell membrane integrity. This damage to cell membrane facilitated the passive absorption of Cd and PS NPs through the damaged roots, leading to increased Cd uptake in rice plants. This reduction in antioxidant enzymatic activity may be linked to a reduction in essential elements in rice when PS NPs are present under Cd contamination. In contrast, IPMM BC biofilter groups produced opposite results. Consistent with previous observations (Lu et al. 2018; Maria

et al. 2014), oxidative stress induced by PS NPs in a Cd-contaminated environment was alleviated by the presence of IPMM BC biofilters, which helped in reducing the oxidative damage from Cd and PS NPs toxicity.

4.6 Metabolomics responses in rice against Cd and PS NPs stress under the presence of IPMM BC biofilters

Metabolomics analysis of rice under PS NP treatment and Cd contamination revealed significant changes in metabolites including nucleotides, organic acids, and sugars. Metabolic processes serve as key indicators of plant performance and provide valuable insights into the various assimilation processes occurring within the plant. The stress from PS NPs disrupted normal metabolic processes in rice, leading to variations in plant metabolites (Fig. 6). The heat map displays a total of 50 differentially expressed metabolites (DEMs) ($P < 0.05$) in rice across all treatments (Fig. 7A, B, C, D, I, J). Notably, the addition of IPMM BC biofilters showed a significant positive impact on metabolite levels, mitigating the negative impacts of Cd and PS NPs by regulating key metabolites. For example, the IPMM BC biofilter reduced sorbitol levels compared to the PS NPs treatment under Cd stress. Sorbitol is a primary form of carbon transported from photosynthetic activity and it can significantly impair plant photosynthesis when its concentration increases (Gao et al. 2014). Additionally, the application of IPMM BC biofilters also led to a notable increase in the enrichment score for amino acid metabolism compared to the PS NPs treatment under Cd exposure (Fig. 8). Amino acids are integral part of key metabolic pathways in plants, influencing various physiological processes (Hildebrandt et al. 2015), and serving as essential sources of nitrogen and carbon for plant growth (Jones et al. 2005; Miller et al. 2008). These results indicate that amino acid metabolism is significantly affected by the treatments, including pathways such as β -Alanine metabolism, Arginine biosynthesis, and the biosynthesis of Tryptophan, Phenylalanine and Tyrosine. This suggests an interconnection among these effects with the upregulation of amino acid metabolism potentially representing another metabolic strategy employed by plants to mitigate oxidative stress. The biosynthesis and metabolic pathways of amino acids can impact plant growth and development both directly and indirectly. This is attributed to the critical role of amino acid metabolism in nitrogen metabolism and the production of secondary metabolites, in addition to their fundamental function in protein synthesis (Scheible et al. 2004). However, the low enrichment scores for these metabolites indicate that PS NPs and Cd have disturbed this important process and distressed the glycine, serine, and threonine metabolism associated to N metabolism (Zhang et al. 2019). Consequently, disturbances in

nitrogen-related metabolic pathways might have led to reduced chlorophyll content and a decline in the net photosynthetic rate, eventually disturbing the plant growth. But further investigation is needed to clarify the specific mechanisms behind the changes in amino acid levels in plant leaves.

KEGG pathway enrichment analysis revealed disruptions in rice immune response and key metabolic pathways essential for energy production, including the TCA cycle, glutathione metabolism, and arginine biosynthesis (Fig. 8). These metabolic disturbances led to reduced nutrient uptake and growth limitations in rice under stress conditions. This provides a clear explanation for the observed declines in chlorophyll content, photosynthesis, nutrient uptake, and biomass due to exposure to Cd and PS NPs. In this study, the significant disturbances in metabolites may have led to increased oxidative stress in rice plants. A comparison between PS NPs and CK treatments revealed that several carbohydrates were disturbed in response to PS NPs. Carbohydrates are considered a potential energy source and function as signalling molecules for optimum plant growth and development (Eveland and Jackson 2012). Interestingly, CK showed higher level of L-glutamic acid compared to the MBC treatment. Glutamate is a key constituent of glutathione, which serves as a precursor for phytochelatins and has been previously reported to aid in the sequestration of HMs (Tomaszewska 2002). Previous studies have also reported elevated levels of glutamic acid under Cd toxicity (Xie et al. 2014). The enhanced activity of photosynthetic product glucose suggests that rice leaves may have tried to fix more carbon in response to PS NPs and Cd exposure. Additionally, changes in cell wall components suggest that rice plants might have adapted their cell wall composition to enhance tolerance to Cd, possibly by binding Cd ions or reducing cell wall permeability. Furthermore, dicarboxylate and glyoxylate metabolism, which are important in carbohydrate biosynthesis (Chen et al. 2019), play a crucial role as energy sources and signalling molecules in response to various stresses (Krasavina et al. 2014). The disruption of these metabolic pathways specifies that the combined stress of PS NPs and Cd can lead to significant metabolic reprogramming in rice. The concentration of bioavailable elements directly influences the uptake of HMs by plants. Under pollutant stress, plants secrete a substantial amount of organic acids, which can help in mitigating the toxic effects of pollutants (Mei et al. 2022). These organic acids can form complexes with HMs, facilitating their translocation to the above-ground parts of the plant. Initially, HMs are transported to the vacuoles in the roots via complex peptides, and then they are conveyed to the leaves through specific transport proteins. However, high

concentrations of cations in cells can significantly hinder the transport of metal ions. At the same time, organic acids within the plant bind with HMs to form complexes that facilitate their movement to the leaves (Parveen et al. 2020). This mechanism explains the increased accumulation of Cd in rice when exposed to PS NPs under Cd contamination. The results from metabolomics profiling revealed that PS NPs and Cd interfered with the accumulation of plant nutrients by impacting carbohydrate, amino acid, and lipid metabolism, whereas IPMM BC biofilters enhanced plant resistance to environmental stress by modulating the synthesis and metabolism of these metabolites.

5 Conclusion

Cd and NPs pollution remains a significant environmental challenge, posing serious risks to agricultural productivity and human health. This study demonstrates that the strategic use of indigenous resources such as BC biofilters derived from IPMM can ensure vigorous rice growth in Cd-NPs contaminated environments while simultaneously mitigating the adverse effects of these contaminants. IPMM BC biofilters improved physiological, biochemical, and molecular responses of rice by regulating gene expression and root development, which were inhibited by PS NPs and HMs. This was accomplished by modulating metabolic processes related to nutrient cycles, the TCA cycle, nitrogen transport, and mechanistic pathways of metabolites, while also reducing Cd bioavailability and limiting PS NP translocation into root cells. The simultaneous stress of Cd and PS NPs significantly transformed the uptake and translocation of nutrients in the rice, leading to an imbalance in the metabolic equilibrium of essential nutrients in rice seedlings. Our research provides a theoretical foundation for a comprehensive understanding of the co-toxicity of Cd and NPs in crops under the influence of IPMM BC biofilters. Thus, biofilters derived from invasive plants hold the potential to address the challenges posed by NPs, HMs, and the invasion of invasive plants in contaminated environments through a multifaceted manner.

Supplementary Information

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Supplementary file 1.

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Author contributions

Muhammad Shoaib Rana: Conceptualization, Writing an original draft, Methodology, Data curation, Software, Writing review & editing, Formal analysis, Investigation. Muhammad Imran: Visualization, Data curation. Yousif Abdelrahman Yousif Abdellah: Validation, Investigation. Hongyu Chen: Conceptualization, Data curation, Software. Shiwen Deng: Data curation, Visualization, Rongjie Ren: Conceptualization, Software. Jiayu Lin: Visualization, Investigation. Jiaxin Li: Data curation, Software. Ruilong Wang: Supervision, Visualization, Methodology, Writing, review & editing, Project administration, Funding acquisition.

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Data availability

The authors declare that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All listed authors have approved the manuscript before submission.

Competing interests

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.

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References

- Abdellah YAY, Chen H-Y, Deng S-W, Li W-T, Ren R-J, Yang X, Rana MS, Sun S-S, Liu J-J, Wang R-L (2024) *Mikania micrantha* Kunth and its derived biochar impacts on heavy metal bioavailability and siderophore-related genes during chicken manure composting. *Biochar* 6:56
- Adil MF, Sehar S, Chen G, Chen Z-H, Jilani G, Chaudhry AN, Shamsi IH (2020) Cadmium-zinc cross-talk delineates toxicity tolerance in rice via differential genes expression and physiological/ultrastructural adjustments. *Ecotoxicol Environ Saf* 190:110076
- Austin RB (1990) Prospects for genetically increasing the photosynthetic capacity of crops. *Plant Biol* 10:395
- Azeem I, Adeel M, Ahmad MA, Shakoor N, Jiangcuo GD, Azeem K, Ishfaq M, Shakoor A, Ayaz M, Xu M (2021) Uptake and accumulation of nano/microplastics in plants: a critical review. *Nanomaterials* 11:2935
- Bandmann V, Müller JD, Köhler T, Homann U (2012) Uptake of fluorescent nano beads into BY2-cells involves clathrin-dependent and clathrin-independent endocytosis. *FEBS Lett* 586:3626–3632
- Barbano D, Clark J (1990) Total nitrogen content of milk: a rapid microwave Kjeldahl digestion.
- Bi M, He Q, Chen Y (2020) What roles are terrestrial plants playing in global microplastic cycling? ACS Publications, Washington
- Cao X, Wang C, Luo X, Yue L, White JC, Wang Z, Xing B (2024) Nano-and microplastics increase the occurrence of bacterial wilt in tomato (*Solanum lycopersicum* L.). *ACS Nano* 18:18071–18084
- Chen J, Le XC, Zhu L (2019) Metabolomics and transcriptomics reveal defense mechanism of rice (*Oryza sativa*) grains under stress of 2, 2', 4, 4'-tetrabromodiphenyl ether. *Environ Int* 133:105154
- Davarpanah E, Guilhermino L (2015) Single and combined effects of microplastics and copper on the population growth of the marine microalgae *Tetraselmis chuii*. *Estuar Coast Shelf Sci* 167:269–275
- Dong Y, Gao M, Song Z, Qiu W (2020) Microplastic particles increase arsenic toxicity to rice seedlings. *Environ Pollut* 259:113892
- Dong R, Liu R, Xu Y, Liu W, Wang L, Liang X, Huang Q, Sun Y (2022) Single and joint toxicity of polymethyl methacrylate microplastics and As (V) on rapeseed (*Brassica campestris* L.). *Chemosphere* 291:133066
- Dumanović J, Nepovimova E, Natić M, Kuča K, Jačević V (2021) The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. *Front Plant Sci* 11:552969
- El-Banna MF, Mosa A, Gao B, Yin X, Ahmad Z, Wang H (2018) Sorption of lead ions onto oxidized bagasse-biochar mitigates Pb-induced oxidative stress on hydroponically grown chicory: Experimental observations and mechanisms. *Chemosphere* 208:887–898
- Eveland AL, Jackson DP (2012) Sugars, signalling, and plant development. *J Exp Bot* 63:3367–3377
- Feng Q, Wang B, Chen M, Wu P, Lee X, Xing Y (2021) Invasive plants as potential sustainable feedstocks for biochar production and multiple applications: a review. *Resour Conserv Recycl* 164:105204
- Frias JP, Nash R (2019) Microplastics: finding a consensus on the definition. *Mar Pollut Bull* 138:145–147
- Ganie ZA, Khandelwal N, Tiwari E, Singh N, Darbha GK (2021) Biochar-facilitated remediation of nanoplastic contaminated water: effect of pyrolysis temperature induced surface modifications. *J Hazard Mater* 417:126096
- Gao S, Zheng Z, Gu W, Xie X, Huan L, Pan G, Wang G (2014) Photosystem I shows a higher tolerance to sorbitol-induced osmotic stress than photosystem II in the intertidal macro-algae *Ulva prolifera* (*Chlorophyta*). *Physiol Plant* 152:380–388
- Ge J, Li H, Liu P, Zhang Z, Ouyang Z, Guo X (2021) Review of the toxic effect of microplastics on terrestrial and aquatic plants. *Sci Total Environ* 791:148333
- Gong W, Zhang W, Jiang M, Li S, Liang G, Bu Q, Xu L, Zhu H, Lu A (2021) Species-dependent response of food crops to polystyrene nanoplastics and microplastics. *Sci Total Environ* 796:148750
- Grajek H, Rydzynski D, Piotrowicz-Cieślak A, Herman A, Maciejczyk M, Wiczorek Z (2020) Cadmium ion-chlorophyll interaction—examination of spectral properties and structure of the cadmium-chlorophyll complex and their relevance to photosynthesis inhibition. *Chemosphere* 261:127434
- Gratão PL, Monteiro CC, Rossi ML, Martinelli AP, Peres LE, Medici LO, Lea PJ, Azevedo RA (2009) Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium. *Environ Exp Bot* 67:387–394
- Hildebrandt TM, Nesi AN, Araújo WL, Braun H-P (2015) Amino acid catabolism in plants. *Mol Plant* 8:1563–1579
- Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C (2017) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci Total Environ* 586:127–141
- Hu T, Wang T, Wang G, Bi A, Wassie M, Xie Y, Xu H, Chen L (2021) Overexpression of FaHSP17. 8-ClI improves cadmium accumulation and tolerance in tall fescue shoots by promoting chloroplast stability and photosynthetic electron transfer of PSII. *J Hazard Mater* 417:125932
- Imran M, Hu C, Hussain S, Rana MS, Riaz M, Afzal J, Aziz O, Elyamine AM, Ismael MAF, Sun X (2019a) Molybdenum-induced effects on photosynthetic efficacy of winter wheat (*Triticum aestivum* L.) under different nitrogen

- sources are associated with nitrogen assimilation. *Plant Physiol Biochem* 141:154–163
- Imran M, Sun X, Hussain S, Ali U, Rana MS, Rasul F, Saleem MH, Moussa MG, Bhantana P, Afzal J (2019b) Molybdenum-induced effects on nitrogen metabolism enzymes and elemental profile of winter wheat (*Triticum aestivum* L.) under different nitrogen sources. *Int J Mol Sci* 20:3009
- Imran M, Hussain S, El-Esawi MA, Rana MS, Saleem MH, Riaz M, Ashraf U, Potcho MP, Duan M, Rajput IA (2020) Molybdenum supply alleviates the cadmium toxicity in fragrant rice by modulating oxidative stress and antioxidant gene expression. *Biomolecules* 10:1582
- Jędruchiewicz K, Bogusz A, Chańko M, Bank MS, Alessi DS, Ok YS, Oleszczuk P (2023) Extractability and phytotoxicity of heavy metals and essential elements from plastics in soil solutions and root exudates. *Sci Total Environ* 905:166100
- Jiang X, Chen H, Liao Y, Ye Z, Li M, Klobučar G (2019) Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant *Vicia faba*. *Environ Pollut* 250:831–838
- Jones DL, Shannon D, Junvee-Fortune T, Farrar JF (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations. *Soil Biol Biochem* 37:179–181
- Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Cetner MD, Łukasik I, Goltsev V, Ladle RJ (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiol Plant* 38:1–11
- Kim HM, Lee D-K, Long NP, Kwon SW, Park JH (2019) Uptake of nanoplastyrene particles induces distinct metabolic profiles and toxic effects in *Caenorhabditis elegans*. *Environ Pollut* 246:578–586
- Krasavina MS, Burmistrova NA, Raldušina GN (2014) The role of carbohydrates in plant resistance to abiotic stresses. Emerging technologies and management of crop stress tolerance. Elsevier, Amsterdam
- Lai JL, Luo XG (2019) High-efficiency antioxidant system, chelating system and stress-responsive genes enhance tolerance to cesium ionotoxicity in Indian mustard (*Brassica juncea* L.). *Ecotoxicol Environ Saf* 181:491–498
- Li Z, Xing D (2011) Mechanistic study of mitochondria-dependent programmed cell death induced by aluminium phytotoxicity using fluorescence techniques. *J Exp Bot* 62:331–343
- Li FT, Qi JM, Zhang GY, Lin LH, Fang PP, Tao AF, Xu JT (2013) Effect of cadmium stress on the growth, antioxidative enzymes and lipid peroxidation in two kenaf (*Hibiscus cannabinus* L.) plant seedlings. *J Integr Agric* 12:610–620
- Li M, Wu D, Wu D, Guo H, Han S (2021) Influence of polyethylene-microplastic on environmental behaviors of metals in soil. *Environ Sci Pollut Res* 28:28329–28336
- Li X, Du J, Song B, Zhang X, Riaz M (2022) Fomesafen drift affects morphology of sugar beet. *Chemosphere* 287:132073
- Li J, Yu Y, Chen X, Yu S, Cui M, Wang S, Song F (2023a) Effects of biochar on the phytotoxicity of polyvinyl chloride microplastics. *Plant Physiol Biochem* 195:228–237
- Li Y, Abdo AI, Shi Z, Merwad A-RM, Zhang J (2023b) Biochar derived from invasive plants improved the pH, macronutrient availability and biological properties better than liming for acid rain-affected soil. *Biochar* 5:59
- Li Y, Rahman SU, Qiu Z, Shahzad SM, Nawaz MF, Huang J, Naveed S, Li L, Wang X, Cheng H (2023c) Toxic effects of cadmium on the physiological and biochemical attributes of plants, and phytoremediation strategies: a review. *Environ Pollut* 325:121433
- Li J, Zhang Y, Zhou Y, Liu W, Maryam B, Cui J, Liu M, Liu X (2024a) Polystyrene nanoplastics distinctly impact cadmium uptake and toxicity in *Arabidopsis thaliana*. *Environ Pollut* 356:124373
- Li Y, Shi X, Qin P, Zeng M, Fu M, Chen Y, Qin Z, Wu Y, Liang J, Chen S (2024b) Effects of polyethylene microplastics and heavy metals on soil-plant microbial dynamics. *Environ Pollut* 341:123000
- Lian J, Wu J, Zeb A, Zheng S, Ma T, Peng F, Tang J, Liu W (2020) Do polystyrene nanoplastics affect the toxicity of cadmium to wheat (*Triticum aestivum* L.)? *Environ Pollut* 263:114498
- Lian Y, Liu W, Shi R, Zeb A, Wang Q, Li J, Zheng Z, Tang J (2022) Effects of polyethylene and polylactic acid microplastics on plant growth and bacterial community in the soil. *J Hazard Mater* 435:129057
- Lu Y, Wassmann R, Neue H, Huang C (1999) Impact of phosphorus supply on root exudation, aerenchyma formation and methane emission of rice plants. *Biogeochemistry* 47:203–218
- Lu K, Qiao R, An H, Zhang Y (2018) Influence of microplastics on the accumulation and chronic toxic effects of cadmium in zebrafish (*Danio rerio*). *Chemosphere* 202:514–520
- Maity S, Pramanick K (2020) Perspectives and challenges of micro/nanoplastics-induced toxicity with special reference to phytotoxicity. *Glob Change Biol* 26:3241–3250
- Maity S, Guchhait R, Sarkar MB, Pramanick K (2022) Occurrence and distribution of micro/nanoplastics in soils and their phytotoxic effects: a review. *Plant, Cell Environ* 45:1011–1028
- Maleki M, Ghorbanpour M, Kariman K (2017) Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. *Plant Gene* 11:247–254
- Maria VL, Ribeiro MJ, Amorim MJ (2014) Oxidative stress biomarkers and metallothionein in *Folsomia candida*-responses to Cu and Cd. *Environ Res* 133:164–169
- Mei K, Wu G, Liu J, Hong H, Lu H, Yan C (2022) Dynamics of low-molecular-weight organic acids for the extraction and sequestration of arsenic species and heavy metals using mangrove sediments. *Chemosphere* 286:131820
- Meng F, Yang X, Riksen M, Xu M, Geissen V (2021) Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. *Sci Total Environ* 755:142516
- Miller AJ, Fan X, Shen Q, Smith SJ (2008) Amino acids and nitrate as signals for the regulation of nitrogen acquisition. *J Exp Bot* 59:111–119
- Mitrano DM, Wick P, Nowack B (2021) Placing nanoplastics in the context of global plastic pollution. *Nat Nanotechnol* 16:491–500
- Mosa A, El-Banna MF, Gao B (2016) Biochar filters reduced the toxic effects of nickel on tomato (*Lycopersicon esculentum* L.) grown in nutrient film technique hydroponic system. *Chemosphere* 149:254–262
- Naziri A, Mina T, Manoli K, Beretsou VG, Christou A, Michael C, Agathokleous E, Fatta-Kassinos D (2023) Looking into the effects of co-contamination by micro (nano) plastics in the presence of other pollutants on irrigated edible plants. *Sci Total Environ* 892:164618
- Nohara NML, Ariza-Tarazona MC, Triboni ER, Nohara EL, Villarreal-Chiu JF, Cedillo-González EI (2024) Are you drowned in microplastic pollution? A brief insight on the current knowledge for early career researchers developing novel remediation strategies. *Sci Total Environ* 918:170382
- Osunkoya OO, Froese JG, Nicol S (2019) Management feasibility of established invasive plant species in Queensland, Australia: a stakeholders' perspective. *J Environ Manage* 246:484–495
- Ott M, Gogvadze V, Orrenius S, Zhivotovskiy B (2007) Mitochondria, oxidative stress and cell death. *Apoptosis* 12:913–922
- Parveen A, Saleem MH, Kamran M, Haider MZ, Chen J-T, Malik Z, Rana MS, Hassan A, Hur G, Javed MT (2020) Effect of citric acid on growth, ecophysiology, chloroplast ultrastructure, and phytoremediation potential of jute (*Corchorus capsularis* L.) seedlings exposed to copper stress. *Biomolecules* 10:592
- Pelegrini K, Pereira TCB, Maraschin TG, Teodoro LDS, Basso NRDS, De Galland GLB, Ligabue RA, Bogo MR (2023) Micro-and nanoplastic toxicity: a review on size, type, source, and test-organism implications. *Sci Total Environ* 878:162954
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45–e45
- Qu J, Li Y, Bi F, Liu X, Dong Z, Fan H, Yin M, Fu L, Cao W, Zhang Y (2024a) Smooth vetch (*Vicia villosa* var.) coupled with ball-milled composite mineral derived from shell powder and Phosphate rock for remediation of Cadmium-polluted farmland: Insights into synergetic mechanisms. *ACS ES&T Engineering* 4:2054–2067
- Qu J, Li Z, Wang S, Lin Q, Zhang Z, Wu Z, Hu Q, Jiang Z, Tao Y, Zhang Y (2024b) Enhanced degradation of atrazine from soil with recyclable magnetic carbon-based bacterial pellets: Performance and mechanism. *Chem Eng J* 490:151662
- Rana MS, Murtaza G, Sun X-c, Imran M, Afzal J, Elyamine AM, Farag MA, Riaz M, Waris MI, Hu C-x (2018) Nitrate leaching losses reduction, N-use efficiency optimization in *Triticum aestivum* L. and *Oryza sativa* L. rotation to improve production and soil reclamation rate with gypsum in salt-affected soil. *Indian J Agric Res* 52:604
- Rana M, Bhantana IM, Moussa M, Khan Z (2020a) A novel strategy: microbes in relation to heavy metals toxicity and future prospects. *J Soil Plant Biol* 1:113–118

- Rana MS, Hu CX, Shaaban M, Imran M, Afzal J, Moussa MG, Elyamine AM, Bhan-tana P, Saleem MH, Syaifudin M (2020b) Soil phosphorus transformation characteristics in response to molybdenum supply in leguminous crops. *J Environ Manage* 268:110610
- Rana MS, Murtaza G, Hu C, Sun X, Imran M, Afzal J, Elyamine AM, Khan Z, Riaz M, Shaaban M (2020c) Nitrate leaching losses reduction and optimization of N use efficiency in *Triticum aestivum* L. and *Oryza sativa* L. crop rotation for enhancing crop productivity. *Pak J Agric Sci* 57:3
- Rana MS, Sun X, Imran M, Ali S, Shaaban M, Moussa MG, Khan Z, Afzal J, Binyamin R, Bhan-tana P (2020d) Molybdenum-induced effects on leaf ultra-structure and rhizosphere phosphorus transformation in *Triticum aestivum* L. *Plant Physiol Biochem* 153:20–29
- Rana MS, Sun X, Imran M, Khan Z, Moussa MG, Abbas M, Bhan-tana P, Syaifudin M, Din IU, Younas M (2020e) Mo-inefficient wheat response toward molybdenum supply in terms of soil phosphorus availability. *J Soil Sci Plant Nutr* 20:1560–1573
- Rana MS, Alshehri D, Wang R-L, Imran M, Abdellah YAY, Rahman FU, Alatawy M, Ghabban H, Abeer AH, Hu C-x (2025a) Effect of molybdenum supply on crop performance through rhizosphere soil microbial diversity and metabolite variation. *Front Plant Sci* 15:1519540
- Rana MS, Chen H, Deng S, Imran M, Abdellah YAY, Li W, Lin J, Li J, Wang R (2025b) Biological insights into alleviating heavy metal toxicity through the simultaneous supply of biochar biofilters derived from the *Mikania micrantha* and molybdenum nanoparticles. *Biochar* 7:23
- Rana MS, Wang R-L, Imran M, Abdellah YAY, Riaz M, Hu C-x (2025c) Insights into the rhizosphere and non-rhizosphere phosphorus bioavailability and plant responses under molybdenum supply. *J Soil Sci Plant Nutr* 21:1–18
- Redjala T, Zelko I, Sterckeman T, Legué V, Lux A (2011) Relationship between root structure and root cadmium uptake in maize. *Environ Exp Bot* 71:241–248
- Rillig MC (2018) Microplastic disguising as soil carbon storage. *ACS Publications*, Washington
- Roland J, Vian B (1991) General preparation and staining of thin sections. *Electr Microsc Plant Cells* 1:67
- Rydzynski D, Piotrowicz-Cieslak A, Grajek H, Michalczyk D (2019) Chlorophyll degradation by tetracycline and cadmium in spinach (*Spinacia oleracea* L.) leaves. *Int J Environ Sci Technol* 16:6301–6314
- Saleem MH, Fahad S, Adnan M, Ali M, Rana MS, Kamran M, Ali Q, Hashem IA, Bhan-tana P, Ali M (2020) Foliar application of gibberellic acid endorsed phytoextraction of copper and alleviates oxidative stress in jute (*Corchorus capsularis* L.) plant grown in highly copper-contaminated soil of China. *Environ Sci Pollut Res* 27:37121–37133
- Sardar R, Ahmed S, Yasin NA (2022) Titanium dioxide nanoparticles mitigate cadmium toxicity in *Coriandrum sativum* L. through modulating antioxidant system, stress markers and reducing cadmium uptake. *Environ Pollut* 292:118373
- Scheible W-R, Morcuende R, Czechowski T, Fritz C, Osuna D, Palacios-Rojas N, Schindelasch D, Thimm O, Udvardi MK, Stitt M (2004) Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of *Arabidopsis* in response to nitrogen. *Plant Physiol* 136:2483–2499
- Shanmugaraj BM, Malla A, Ramalingam S (2019) Cadmium stress and toxicity in plants: an overview. *Cadmium Tox Toler Plants* 1:1–17
- Simkin AJ, Kapoor L, Doss CGP, Hofmann TA, Lawson T, Ramamoorthy S (2022) The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. *Photosynth Res* 152:23–42
- Song Z, Zhao X, Dong Y, Bai L, Wang S, Gao M (2023) Effects of polystyrene nanoplastics with different functional groups on the accumulation and toxicity of Pb on dandelion. *Chemosphere* 310:136874
- Su Y, Ashworth V, Kim C, Adeleye AS, Rolshausen P, Roper C, White J, Jassby D (2019) Delivery, uptake, fate, and transport of engineered nanoparticles in plants: a critical review and data analysis. *Environ Sci Nano* 6:2311–2331
- Sun X-D, Yuan X-Z, Jia Y, Feng L-J, Zhu F-P, Dong S-S, Liu J, Kong X, Tian H, Duan J-L (2020) Differentially charged nanoplastics demonstrate distinct accumulation in *Arabidopsis thaliana*. *Nat Nanotechnol* 15:755–760
- Tomaszewska B (2002) Glutathione and thiol metabolism in metal exposed plants. *Physiology and biochemistry of metal toxicity and tolerance in plants*. Springer, Cham
- Valentovic P, Luxova M, Kolarovic L, Gasparikova O (2006) Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant Soil Environ* 52:184
- Wang F, Zhang X, Zhang S, Zhang S, Adams CA, Sun Y (2020) Effects of co-contamination of microplastics and Cd on plant growth and Cd accumulation. *Toxics* 8:36
- Wang F, Feng X, Liu Y, Adams CA, Sun Y, Zhang S (2022a) Micro (nano) plastics and terrestrial plants: Up-to-date knowledge on uptake, translocation, and phytotoxicity. *Resour Conserv Recycl* 185:106503
- Wang L, Lin B, Wu L, Pan P, Liu B, Li R (2022b) Antagonistic effect of polystyrene nanoplastics on cadmium toxicity to maize (*Zea mays* L.). *Chemosphere* 307:135714
- Wang J, Lu X, Jing Q, Zhang B, Ye J, Zhang H, Xiao Z, Zhang J (2023a) Spatiotemporal characterization of heavy metal and antibiotics in the Pearl River Basin and pollutants removal assessment using invasive species-derived biochars. *J Hazard Mater* 454:131409
- Wang Q, Meng L, Liu W, Zeb A, Shi R, Lian Y, Su C (2023b) Single and combined effects of polystyrene nanoplastics and Cd on submerged plants *Ceratophyllum demersum* L. *Sci Total Environ* 872:162291
- Wang X, Zheng W-L, Yuan H-M, van Kleunen M, Yu F-H, Li M-H (2024) Biochar produced from diverse invasive species improves remediation of cadmium-contaminated soils. *Biol Invasions* 26:1–12
- Westbrooks RG, Manning ST, Waugh JD (2022) Early detection and rapid response: a cost-effective strategy for minimizing the establishment and spread of new and emerging invasive plants by global trade, travel and climate change. *Invasive species and global climate change*. CABI GB.
- Xie Y, Hu L, Du Z, Sun X, Amombo E, Fan J, Fu J (2014) Effects of cadmium exposure on growth and metabolic profile of bermudagrass [*Cynodon dactylon* (L.) Pers.]. *PLoS one* 9: e115279.
- Xiong T, Dumat C, Dappe V, Vezin H, Schreck E, Shahid M, Pierart A, Sobanska S (2017) Copper oxide nanoparticle foliar uptake, phytotoxicity, and consequences for sustainable urban agriculture. *Environ Sci Technol* 51:5242–5251
- Xu X, Shen R, Mo L, Yang X, Chen X, Wang H, Li Y, Hu C, Lei B, Zhang X (2022) Improving plant photosynthesis through light-harvesting upconversion nanoparticles. *ACS Nano* 16:18027–18037
- Xu G, Lin X, Yu Y (2023) Different effects and mechanisms of polystyrene micro- and nano-plastics on the uptake of heavy metals (Cu, Zn, Pb and Cd) by lettuce (*Lactuca sativa* L.). *Environmental Pollution* 316: 120656.
- Yin L, Wen X, Huang D, Du C, Deng R, Zhou Z, Tao J, Li R, Zhou W, Wang Z (2021) Interactions between microplastics/nanoplastics and vascular plants. *Environ Pollut* 290:117999
- You J, Chan Z (2015) ROS regulation during abiotic stress responses in crop plants. *Front Plant Sci* 6:1092
- Yu Y, Li J (2024) Biochar-derived dissolved and particulate matter effects on the phytotoxicity of polyvinyl chloride nanoplastics. *Sci Total Environ* 906:167258
- Yu Z, Xu X, Guo L, Jin R, Lu Y (2024) Uptake and transport of micro/nanoplastics in terrestrial plants: Detection, mechanisms, and influencing factors. *Sci Total Environ* 907:168155
- Yue L, Lian F, Han Y, Bao Q, Wang Z, Xing B (2019) The effect of biochar nanoparticles on rice plant growth and the uptake of heavy metals: Implications for agronomic benefits and potential risk. *Sci Total Environ* 656:9–18
- Zhang Y, Luo W (2014) Adsorptive removal of heavy metal from acidic wastewater with biochar produced from anaerobically digested residues: kinetics and surface complexation modeling. *BioResources* 9:2
- Zhang H, Lu L, Zhao X, Zhao S, Gu X, Du W, Wei H, Ji R, Zhao L (2019) Metabolomics reveals the “invisible” responses of spinach plants exposed to CeO₂ nanoparticles. *Environ Sci Technol* 53:6007–6017
- Zhang L-J, Qian L, Ding L-Y, Wang L, Wong MH, Tao H-C (2021) Ecological and toxicological assessments of anthropogenic contaminants based on environmental metabolomics. *Environ Sci Ecotechnol* 5:100081
- Zhang J, Huang D, Deng H, Zhang J (2022a) Responses of submerged plant *Vallisneria spiralis* growth and leaf biofilms to water contaminated with microplastics. *Sci Total Environ* 818:151750
- Zhang Y, Yang X, Luo Z-x, Lai J-I, Li C, Luo X-g (2022b) Effects of polystyrene nanoplastics (PSNPs) on the physiology and molecular metabolism of corn (*Zea mays* L.) seedlings. *Science Total Environ* 806:150895