Environmental Pollution 266 (2020) 115159

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Metabolomics revealing the response of rice (*Oryza sativa* L.) exposed to polystyrene microplastics☆

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ARTICLE INFO

Article history:
Received 9 March 2020
Received in revised form
25 May 2020
Accepted 30 June 2020
Available online 7 July 2020

Keywords:
Microplastics
Polystyrene
Rice
Metabolites disturbance

ABSTRACT

Large amounts of microplastics accumulate in the agricultural soil. Microplastics would stress the crops but the underlying mechanism remains unclear. Herein, a laboratory exposure and field trials were carried out to investigate the response of rice (*Oryza sativa* L. II You. 900) to stress induced by polystyrene microplastics (PS-MPs) using a metabolomic approach. After laboratory exposure for 21 days, the decreases in shoot biomass of rice exposed to low, medium and high doses of PS-MPs were 13.1% (CV = 4.1%), 18.8% (CV = 3.7%), and 40.3% (CV = 9.2%), respectively, while the antioxidant enzymes showed an inverted upper-U shape when exposed to PS-MPs. A total of 24 samples from three exposure dose levels were included in the metabolic analysis. The metabolites of 12 amino acids, 16 saccharides, 26 organic acids and 17 others (lipids and polyols) in leaves decreased after the exposure to both 50 mg L^{-1} and 250 mg L^{-1} PS-MPs doses with hydroponically-cultured. The inhibition of perturbed biological pathway causes the biosynthesis of amino acids, nucleic acids, fatty acids and some secondary metabolites decreased which indicate that the energy expenditure exceeded the substance accumulation. In order to further validate the effects of PS-MPs on rice leaves obtained from the laboratory-scale experiments, a field-trial experiment was conducted. After 142 days of cultivation in farmland, the results with a maximum of 25.9% lower biomass in the crops exposed with PS-MPs. As such, the presence of PS-MPs may affect rice production by altering the metabolic systems of rice. Long-term exposure of PS-MPs to rice might be a potential risk to rice safety and quality.

1. Introduction

Since the first report of microplastics (MPs) particles in 1972, MPs have been ubiquitously detected around the world, especially in those areas with intensive human activities (Carpenter et al., 1972; Van Cauwenberghe et al., 2015). Environmental concentrations of microplastics are from almost zero to several thousand particles per m^3 with different particles size (Rezania et al., 2018). e.g. 3.4–25.8 items L^{-1} in surface water and 11.0–234.6 items kg^{-1} dw in sediments with the size of 100–1000 μm in Taihu Lake, China (Su et al., 2016), 1400–4900 particles L^{-1} with the size of 10–5000 μm in Amsterdam canals, the Netherlands (Leslie et al., 2017) or up to 67.5 g kg^{-1} in industrial soil in Sydney, Australia (Fuller and Gautam, 2016). Currently, the studies of MPs mainly focus on the source, migration, distribution and biological effects of MPs in marine animals (Cozar et al., 2014; Rillig et al., 2019; Thompson et al., 2004). However, information on the effects of microplastics on terrestrial fauna is still lacking. At present, there are only studies on how microplastics affects *L. terrestris* biomass and reproduction (Huerta Lwanga et al., 2016). The total amount of MPs in the soil is reported to be 4 to 23-fold than that in seawater (Nizzetto et al., 2016), indicating that soil can be also a main reservoir for the MPs. It is reported that the abundance of MPs ranges from 7100 up to 42,960 items kg^{-1} (18,760 items kg^{-1} on average, >250 μm) in the farmland soil in southwestern China

☆ This paper has been recommended for acceptance by Jörg Rinklebe.

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https://doi.org/10.1016/j.envpol.2020.115159
0269-7491/

(Zhang and Liu, 2018). Larger plastic fragments in the environment can break into micron-sized or even nano-sized plastic particles by physicochemical and biological processes (da Costa et al., 2016; do Sul and Costa, 2014). Long-term and large-area application of plastic film covering, wastewater reuse irrigation, and sludge recycling technologies in agricultural production lead to the accumulation of large amounts of MPs in the soil of agricultural areas (Nizzetto et al., 2016).

Once deposited at the farmland soil via various contamination pathways (Blasing and Amelung, 2018), microplastics may adversely affect soil biota (Huerta Lwanga et al., 2017) and also can alter the physicochemical properties of the soil, e.g., soil aggregation, bulk density, and water holding capacity. Those changes have been reported to have adverse effects on root traits, growth and nutrient uptake by plants (Machado et al., 2018; Wan et al., 2019).

However, information on the effects of MP on plants was quite limited till now. There is an urgent need to elucidate the effects of PS-MPs on plants growth. MPs sourced from the atmosphere can be deposited and adsorbed on the shoots of the plants, while MPs in groundwater and soil can be adsorbed on the surface of the roots. MPs adsorbed on the surface of plants may inhibit photosynthesis, possibly causing an adverse effect on crop growth (Rillig et al., 2019). Qi et al. (2018) reported that 10 g kg^{-1} (1% (w/w)) MPs had adverse effects on both root and shoot systems of wheat, causing negative effects on both vegetative and reproductive growth. Jiang et al. (2019) reported that 5 μm PS-MPs significantly inhibited growth of V. faba at the concentration of 10–100 mg L^{-1} PS-MPs. The ideas and approaches such as using germination, biomass and omics-based techniques from these studies could shed light on the possible mechanism of microplastics interaction with plants (Ng et al., 2018; Wang et al., 2016).

Metabolomics provides information about metabolites and metabolic pathway changes in the plants during the response to toxicants and environmental stressors (Fiehn et al., 2000). It was proved to be an effective tool in understanding the changes in the chemical compositions of crops and further revealing the mechanisms of the process (Matich et al., 2019; Soria et al., 2017). Mass spectrometry has been widely used for metabolite profiling, which could offer acceptable sensitivity and reliability in separating and identifying the metabolites and intermediates in plant tissue (Lisec et al., 2006). Metabolic changes on the roots and shoots of crops were identified under nanoparticle exposure, indicating an important detoxification mechanism that could defend the plant from stress (Wu et al., 2017; Zhang et al., 2019). However, few studies have been investigated the feasibility of using metabolomics to estimate the effects of MPs on the crops.

In this study, rice (*Oryza sativa* L.) is chosen as the model crop to be investigated. Rice is a major food source, which comprises up to 25% of the total caloric intake of the population of the world (Kusano et al., 2015). Polystyrene (PS) was chosen as the model MP. For the laboratory-scale experiments, different doses of commercial PS-MPs were exposed to rice for 3 weeks to investigate: 1) the effect on physiological and biochemical parameters of the rice plant, including biomass and lipid peroxidation, and 2) using Gas Chromatography-Mass Spectrometry (GC-MS)-based metabolomics, the changes of metabolites in the plant under different exposure doses. In addition, In order to further validate the effects of PS-MPs on rice leaves obtained from the laboratory-scale experiments, a field-trial experiment was conducted.We would like to offer a deeper insight to the potential metabolic response of rice to MPs exposure. Collectively, the results of this research aim to elucidate the potential influence of MP on the yield and quality of crops, thus providing deeper insights into the interaction between PS-MPs and rice.

2. Materials and methods

2.1. Preparation of PS-MPs suspensions

The PS-MPs (<50 μm) used in this study were provided by Zhongcheng Plastic Co. (Dongguan, China) used for scrub materials. PS-MPs have a mean hydrodynamic diameter measured by dynamic light scattering (BT-9300ST, Bettersize 118 Instruments, China) of 19.6 μm in deionized water and particle size range of 8.5–30.7 μm. Particles were thoroughly suspended in the Hoagland nutrient solution(Wu and Zhu, 2019) till a dispersion was achieved. A magnetic stirrer (about 20 r min^{-1}) was placed on the bottom of the PS-MP solution container to keep it even and stable. Three different doses of the PS-MPs were chosen in the experiments. The doses of PS-MPs were environment-relevant content. Control group was prepared according to the same procedure that PVP suspension solution without the addition of PS-MPs. The morphology of PS-MPs in the nutrient solution was characterized by a high-resolution transmission electron microscope (FTEM, TECNAI G2 F30, FEI Ltd., Netherlands). Details regarding PS-MPs used in this study can be seen in the supporting information (SI, Fig. S1 and Text S1).

2.2. Plant growth and PS-MPs exposure

Rice seeds (*O. sativa* L. II You. 900) were obtained from BioRice Co., Ltd (Hunan Province, China). Seeds were sterilized for 12 h in a 3 wt% H_2O_2 solution before germination. Plants under similar growth conditions after germinating for 14 days (e.g., size and root length) were selected and transferred to 2 L glass pots with 1.8 L PS-MPs stable dispersed suspensions. The seedlings were fixed on the perforated metal sheet with only roots submerged into the suspensions. Each pot (3 exposure pots and 1 control pot) had 4 bundles of seedlings with 9 seedlings in each bundle. And then the seedlings grew in a greenhouse under natural lighting (about 14 h a day) and ambient temperature (25 °C) for 21 days. After exposed, each pot with 36 rice seedlings was collected, and grounded into one mixture and subsequentially used in biological and biochemical experiments. And, for the metabolic analysis, eight paralleled samples were randomly selected from each mixture, and a total of 24 samples were included in the analysis.

2.3. PS-MPs exposed field trials

Field trials were conducted in agricultural land in Shaoyang County (Fig. S2), Hunan Province, China. Each experimental field contained a gridded and isolated farmland by wood slides with a width of 75 cm, a length of 182 cm, and a depth of 35 cm. The soil texture is paddy soil with sand/silt/clay percentage of 2.5%, 86.1% and 11.4%. Soil pH is 7.10 ± 0.18. Loss-onignition organic matter is 3.36 mg g^{-1}. Three soil samples were collected by the diagonal method on each test field and were then merged into one sample. The samples were stored in an aluminum sealed bag and transported back to the laboratory immediately after collection under ambient conditions. Using a quarter method, 200 g soil was taken from each sample and quantified for the doses of the microplastic before use (Han et al., 2019). All the soil in the test field (depth of 35 cm) was then dug out, air-dried, sieved through a 5 mm sieve, and then spiked with PS-MPs powder at selected exposure doses and then put back into the tillage layer (0–30 cm) field. Rice plant for field trials was germinated in Hoagland's nutrient solution for 21 days, transplanted to fields, and harvested after 142 days of transplantation. Samples of roots and shoots were taken after the harvest and evaluated *in-situ*. Details of the field study are provided in SI (Fig. S2).

2.4. Physiological and biochemical analysis of rice (O. sativa L.) leaves

Catalase (CAT) and peroxidase (POD) activities were measured following Zhang's protocol (Zhang and Kirkham, 1994). The activities of superoxide dismutase (SOD) and reactive oxygen species (ROS) were determined using Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (Meimian Technology Ins., China). Malondialdehyde (MDA) was measured by the thiobarbituric acid reactive substances (TBARS) method (Aravind and Prasad, 2003). Details regarding these assays can be seen in SI (Text S2 and S3).

2.5. Metabolite analysis in rice (O. sativa L.) leaves

The targeted metabolites in rice leaf samples are low molecular weight metabolites including primary (saccharides and amino acids) and secondary (organic acids and fatty acids) metabolites according to the previous reports (Wu et al., 2017). The related sample preparation protocol followed the methods described by Chen et al. (2018), by using a GC-MS, (Agilent® 7890B-5977B) for OMIC-based non-target screening techniques. The metabolites were identified in the GC-MS by the MassHunter Qualitative Analysis Software B.07.00 (Agilent, USA), then annotated and confirmed by comparison with the NIST 14 library. Details about sample preparation and GC-MS instrumental parameters can be found in SI (Text S4).

2.6. Statistical analysis

In this study, the exposure experiment was performed at least triplicates (n > 3) while the metabolite assay was performed in eight samples from the same plant. Data distribution in the results was tested by Kolmogorov–Smirnov (K–S test). Percentiles of measurements were presented with mean and CV (coefficient of variation). One-way analysis of variance, least-significant difference (ANOVA, LSD) was applied in the study for comparisons among different groups. All data was tested for normal distribution using K–S test prior to the Anova description. Statistical analysis in this study was carried out using SPSS Statistics 18.0 (IBM, New York), and statistical significance was accepted at $p < 0.05$.

Peak area for identified metabolites was then normalized (by the peak area of ribitol) and logarithm transformed to achieve pseudo-quantification. For identifying the altered metabolites in the leaves, a partial least squares discriminant analysis (PLS-DA) clustering was used, while a heat map was prepared using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/) (Chong et al., 2018). The variable with a Variable importance in projection (VIP) > 1 is regarded as responsible for the separation, defined as a biomarker of metabolite in this study (Chong et al., 2018). Metabolic pathway analysis was performed based on GC-MS data using MetaboAnalyst. The impact value threshold calculated for pathway identification was set at 0.1 (Chen et al., 2018).

3. Results

3.1. Biomass change of rice leaves tissues after the exposure to PS-MPs

After 21 days of exposure to PS-MPs, discernible differences can be found between the shoots of rice seedlings in biomass and lengths when compared with the control group without the addition of PS-MPs (Fig. 1), while the biomass and length of rice root showed indiscernible differences ($p > 0.1$) compared to control group. As shown in Fig. 1b, at lower doses of PS-MPs (50 and 250 mg L^{-1}), the shoot lengths in rice were reduced by 12%

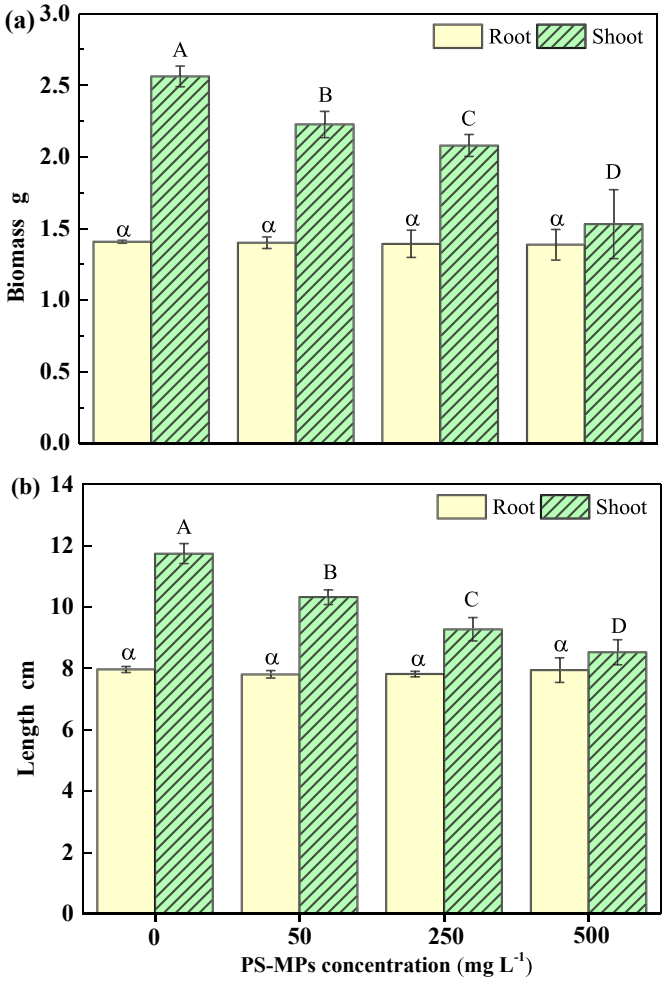

Fig. 1. Biomass and lengths of rice roots and shoots after 21-day exposure to different concentrations of PS-MPs: (a) Biomass of roots and shoots. (b) Length of roots and shoots. Values are means ± SE (n = 8). Means with the same letter (e.g. α or A) are not significantly different ($p < 0.05$).

(CV = 2.3%) and 21% (CV = 4.1%) (% of the control), respectively, which were lower than those in rice when exposed to high dose (500 mg L^{-1}, 27%, CV = 4.8% for shoot lengths). Similar impacts on shoot biomass can be noted after exposing the rice samples to PS-MPs (Fig. 1a). To exemplify, the decreases in shoot biomass of rice exposed to low, medium and high doses (50, 250 and 500 mg L^{-1}) of PS-MPs were 13.1% (CV = 4.1%), 18.8% (CV = 3.7%), and 40.3% (CV = 9.2%), respectively, which were significantly ($p < 0.05$) lower than control.

3.2. Antioxidative enzyme activities and MDA contents of rice leaves

As the biomass and length of the shoot of the rice were significantly different between PS-MPs exposure and the control groups, antioxidative enzyme activities and MDA content of the rice leaves were also investigated. Results showed that PS-MPs caused oxidative damage to principal macromolecules as lipids at the molecular level in rice seedlings decreased significantly (Fig. 2).

All three levels of exposure of rice to PS-MPs inhibit the activities of SOD (Fig. 2A), POD (Fig. 2B) and MDA (Fig. 2C) in rice leaves, while the degree of severity increased with the increasing doses of PS-MPs. In rice leaves tissues, the responses of SOD and POD were

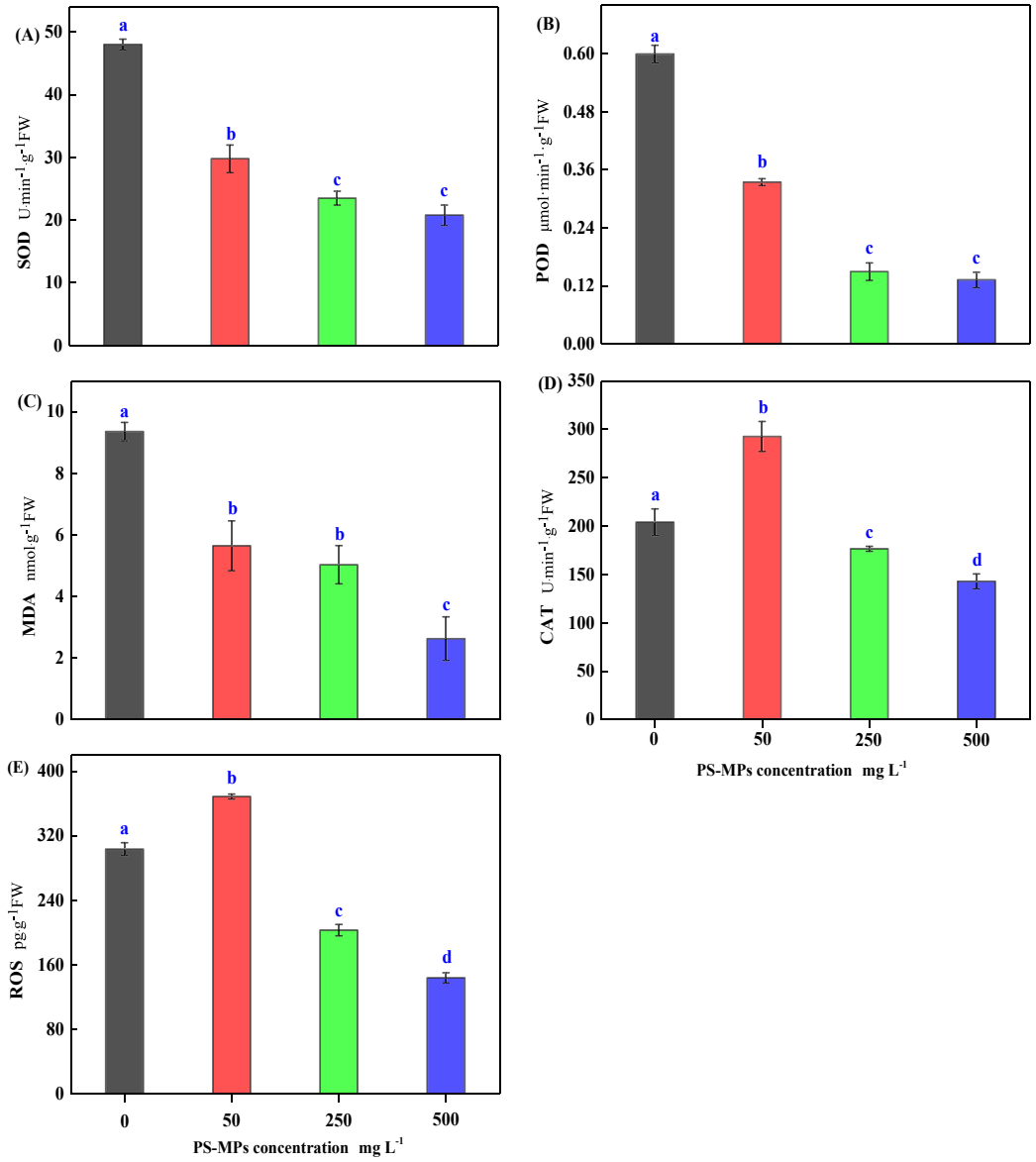

Fig. 2. Parameters of the antioxidant defense system in rice leaves after 21-day exposure to different concentrations of PS-MPs. (A) Superoxide dismutase (SOD) activity, (B) Peroxidase (POD) contents, (C) Malonaldehyde (MDA) contents, (D) Catalase (CAT) activity, and (E) Reactive oxygen species (ROS). One unit of CAT is the amount of enzyme necessary to decompose 1 μmol of H_2O_2 per minute. One unit of SOD activity is the amount of enzyme that causes a 50% decrease in the rate of nitro-blue tetrazolium reduction. Values are means ± SE (n = 8). Means with the same letter (a, b, c, d) are not significantly different ($p < 0.05$).

stimulated over the entire treatment range compared with each control. In tissues of rice leaves, the responses of the activities SOD and POD to PS-MPs exposure were dose-dependent: The activities decreased ($p < 0.05$) over the entire treatment compared with the control groups. MDA contents in leaves decreased compared with those in controls for all treatments after exposure to different doses of PS-MPs, especially at higher doses, showing a decreased degree of lipid peroxidation.

Compared with the other two antioxidative enzymes, the activity of CAT (Fig. 2D) showed a different trend in rice leaves: An inverted U-shape was noted. To be specific, the activity of CAT was highest in leaves when exposed to 50 mg L^{-1} of PS-MPs, while it showed a significant decrease in leaves when exposed to 250 mg L^{-1} and 500 mg L^{-1} of PS-MPs.

Similar to the CAT, the activity of ROS (Fig. 2E) in rice leaf tissues also presented an inverted U-shape: With the maximum activity of ROS was achieved when exposed to 50 mg L^{-1} of PS-MPs, while a significant decline was observed when exposed to 250 mg L^{-1} and 500 mg L^{-1} of PS-MPs compared with the control ($p < 0.05$).

3.3. Metabolic changes in rice leaves

GC-MS based metabolomics identified and pseudo-quantified 249 metabolites in rice leaves after exposure to 50 mg L^{-1} and 250 mg L^{-1} of PS-MPs. It was observed that the rice leaves exposed to 500 mg L^{-1} of PS-MPs showed significantly lowered biomass and poorer growth condition than those that were exposed to 50 mg L^{-1} and 250 mg L^{-1} of PS-MPs. The result indicated that the

exposure to 500 mg L^{-1} of PS-MPs could induce irreversible damage to the rice leaves. In order to gain a better understanding of the metabolic changes induced by PS-MPs in the rice plant, the metabolomic analysis was only conducted for exposure doses of 0, 50 and 250 mg L^{-1} of PS-MPs.

Over 70% of the metabolites significantly changed in the exposed rice leaves, as the contents of most amino acids, organic acids and saccharides decreased with the increasing of PS-MPs doses. Among all PS-MP exposed samples, 83 of the metabolites changed significantly compared to control ($p < 0.05$). The identified metabolomics of amino acids, saccharides, organic acids, and other secondary metabolites with significant differences were presented in Table S1−S4 of the supporting information, respectively.

For most of secondary metabolites, the contents of lipid (such as Glycerol and Gluconic acid lactone), polyols (*i.e.,* Citronellol, Phytol, 2,3-Butanediol, and 1-Phenylethanol), glycosides (*i.e.,* Methyl β-D-glucopyranoside), cell nucleus process(*i.e.,*2′-Deoxy-D-ribose and D-Ribose) and amine compounds (*i.e.,* ethanolamine and Oleamide) also decreased by 62.4%, 36.2%, 29.6%, 80.5% and 90.9% after exposure to 50 mg L^{-1} of PS-MPs, and 90.5%, 95.7%, 80.9%, 99.6% and 95.4% for 250 mg L^{-1} of PS-MPs, respectively.

Not all metabolites in rice leaves were inhibited after the exposure experiment to microplastics. The doses of several amino acids, e.g., cycloserine and D-asparagine, increased 1.4 and 5.1 folds (at low and medium dose levels, respectively) after exposure to PS-MPs. The doses of two types of organic acids (*i.e.,* 2-hydroxyundecanoate and oxalic acid), two types of lipid (*i.e.,* cystathionine and phosphatidylcholine) and urea increased up to about 2−8 folds after exposure to low and medium doses of PS-MPs compared with the control. Similarly, the concentrations of isoborneol, β-D-lactose, 5,6-dihydrouracil, L-proline and L-aspartic acid in leaves also showed an inverted U-shape when exposed to different doses of PS-MPs, which increased initially after exposure to 50 mg L^{-1} but decreased gradually after exposure to 250 mg L^{-1}dose.

Heat Map and the PLS-DA model. The relative abundances of fluctuated metabolites in all experimental groups were presented on the heat map (Fig. S3). The 24 samples in all three groups were clearly separated from each other when exposed to different doses of PS-MPs. From the results shown in the heatmap, it was noted that the normalized concentrations of most metabolites in rice leaves decreased after exposure to PS-MPs compared with the control.

To develop a visual plot for metabolites profiles in different samples in terms of a nonbiased evaluation of changes, a PLS-DA model was developed based on the data set of experimental and control groups. The PLS-DA score plots showed that the group of control was represented by the group exposed to 50 mg L^{-1} of PS-MPs and the group exposed to 250 mg L^{-1} of PS-MPs, as these groups collectively explained 41.6% of the total changes (Fig. 3A). Moreover, the variable importance in projection (VIP) score of metabolites (>1) was defined as the potential biomarkers (Fig. 3B) (Zhang et al., 2013). In this study, the VIP scores of about 15% of the metabolites were higher than 1, revealing the metabolic changes in rice leaves. The normalized concentration of these 12 biomarkers, including saccharides, organic acids, and polyols, were shown in the box-whisker plot (Fig. 4). As the exposure doses of PS-MPs increased, all biomarkers decreased dose, indicating adverse impacts on the metabolisms of rice leaves. These results of the one-way ANOVA analysis (Fig. 4) further confirmed that these metabolic changes in rice leaves were statistical, indicating that the exposure to PS-MPs can change the metabolisms of rice leaves.

3.4. Perturbed biological pathway in rice leaves

The results from biological pathway analysis showed evidence of ten major (>0.1) metabolic pathways have been involved in damage to rive leaves after exposure to different doses of PS-MPs. As shown in Fig. S4, the metabolic pathways of fatty acid, saccharide, amino acid and organic acid in rice leaves were significantly perturbed after exposure to different doses of PS-MPs. Fig. 5 showed the relationships among the metabolic pathways, such as pentose phosphate pathway, glycerolipid metabolism, TCA cycle, amino acid metabolism, and the primary metabolic process glycolysis.

As shown in Fig. 5, the energy metabolisms of glycolysis, the pentose phosphate pathway, and the TCA cycle were significantly declined after exposure to PS-MPs. The metabolic pathway of glycolysis converts glucose to pyruvate by a series of intermediate metabolites. While the energy anabolic process was lowered, the pentose phosphate metabolic pathway showed reduced activity.

In the TCA cycle, the concentration of citric acid was decreased by 56.8% and 94.2% after exposure to 50 and 250 mg L^{-1} of PS-MPs, respectively. Similar trends were found in the levels of malic, succinic and fumaric acid that was decreased by 42.8% and 67.9%, 71.9% and 98.7%, 64.2%, and 89.7%, after exposure to 50 and 250 mg L^{-1} of PS-MPs, respectively.

3.5. Field test verification

The length and biomass of root showed an indiscernible difference ($p > 0.05$) compared with the control (Table S5). However, the biomass of stem exposed with 50 and 250 mg kg^{-1} (environment-relevant concentration) of dose PS-MPs decreased by 12.8% (CV = 5.6%) and 25.9% (CV = 6.8%), respectively, compared with the control group.

4. Discussion

4.1. Effects of physiological and biochemical

The biomass of rice leaves was significantly decreased under the exposure of PS-MPs.The results reported by Qi and Jiang (Jiang et al., 2019; Qi et al., 2018) can support the findings in this study that the exposure to microplastics can also inhibit the growth of crop leaves. Under exposure to PS-MPs, the cell connections in V. faba roots probably be blocked and disrupted the nutrients transport to leaves (Jiang et al., 2019).

In this study, the activities of SOD, POD, and CAT from the rice leaves tissues after exposure to PS-MPs were lower than those of the antioxidant enzymes, which is in consistence with the less accumulated ROS contents (Fig. 2E). ROS are considered as one of the most reliable indexes reflecting oxidative stress in plants (Choudhury et al., 2017). The reduced activities of antioxidant enzymes (or ROS, to be more specific) confirmed that the oxidative stress had exceeded their scavenging abilities, thus causing damage in the rice growth (Koca et al., 2007). ROS showed a stimulatory effect on the activities of antioxidant enzymes that are responsible for scavenging different free radicals in rice. The results of There may exist a dynamic balance between oxidative stress and anti-oxidation in plants (Jiang et al., 2017). The exposure to PS-MPs may inhibit the activities of POD and SOD, particularly when the enhanced ROS level was beyond the scavenging ability of the enzymes. Excessive ROS may be formed beyond the scavenging capacity of the antioxidant system, thus leading to decreased membrane activity as indicated by the change in MDA content. As the nutrients adsorption was disrupted in roots, these results could act as indirect evidence that PS-MPs exposure could deteriorate the

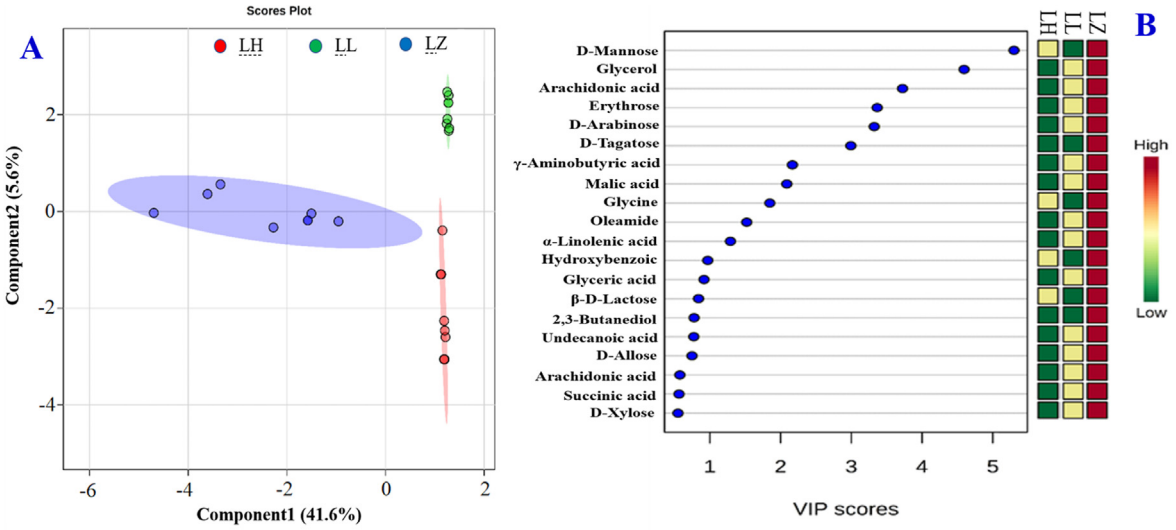

Fig. 3. PLS-DA results obtained from GC-MS data of metabolites extracted from rice leaves exposed to 0, 50, and 250 mg L^{-1} of PS-MPs (represented by LZ, LL, LH). (A) Scores plot (PC1 vs PC2) of partial least-squares discriminant analysis (PLS-DA). (B) Variable importance in projection (VIP) scores of the top 20 metabolites in rice exposed to 0, 50, and 250 mg L^{-1} of PS-MPs.

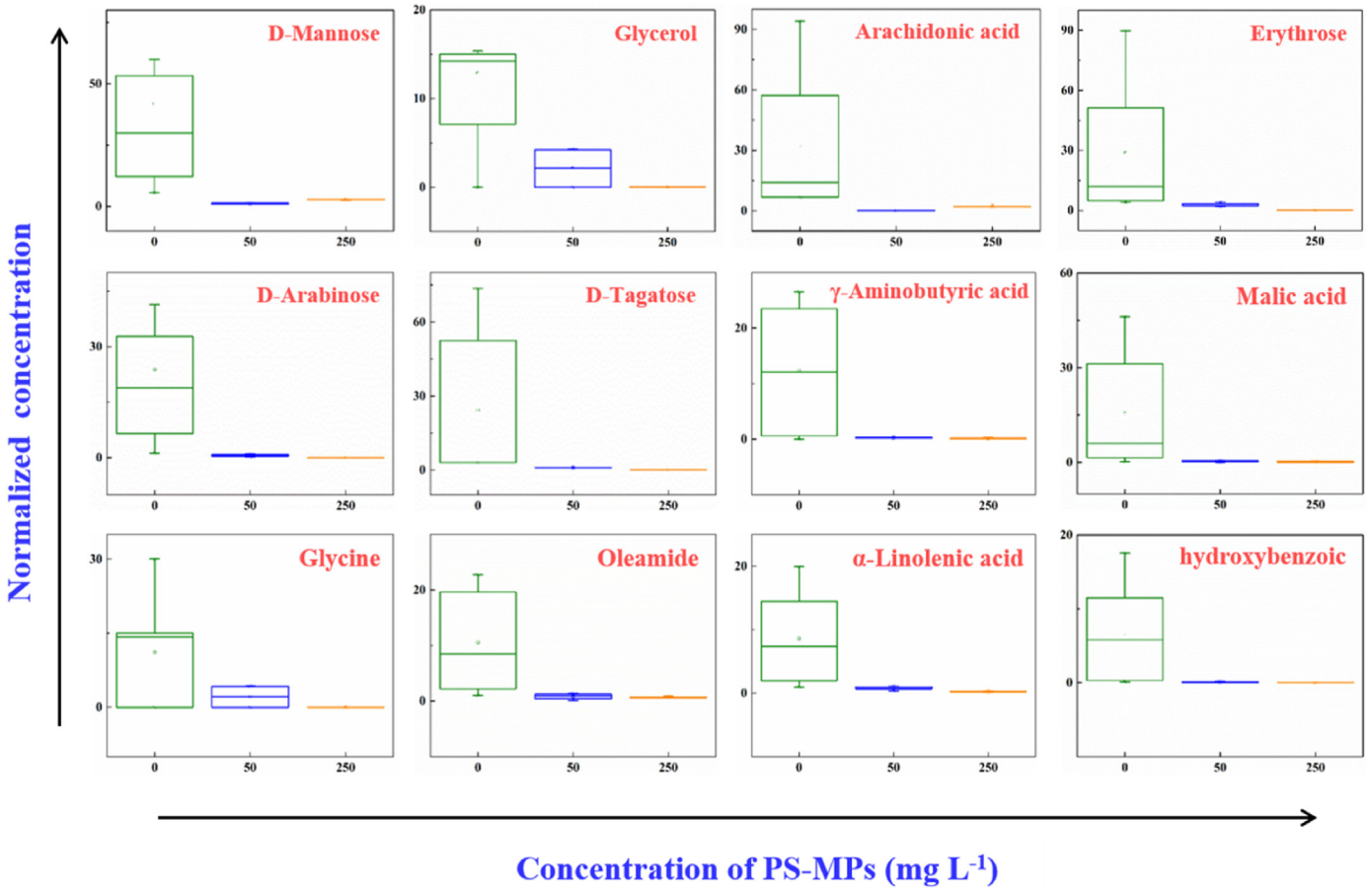

Fig. 4. The box plot of the top 12 significantly different metabolites among the four groups of rice through the PLS-DA analysis. Different colors are used to distinguish different groups that are consistent with the PLS-DA results. Every blue point represents a kind of metabolite in the VIP score. The relative contents of metabolites were displayed by the color boxes, with red indicating high and green indicating low contents. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

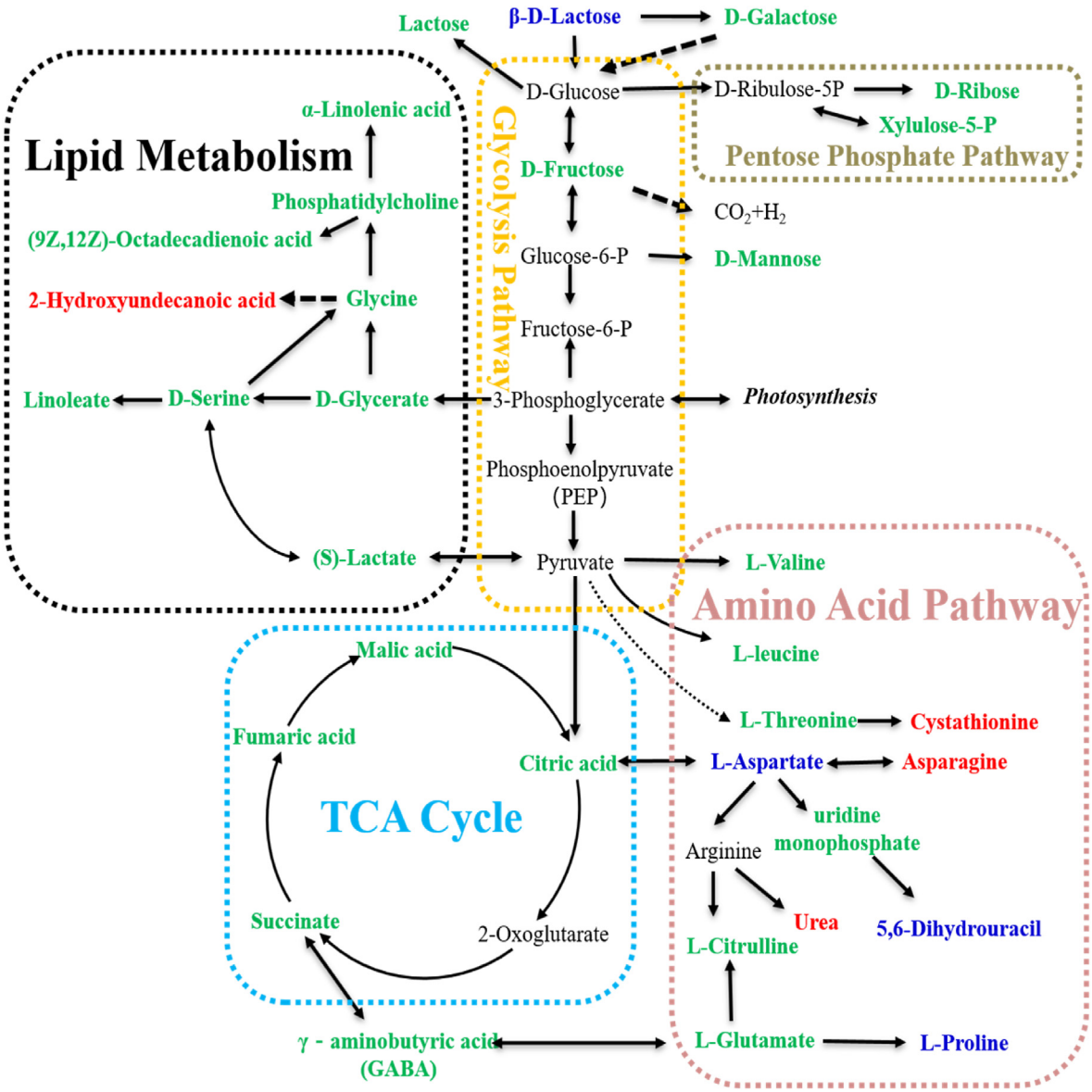

Fig. 5. Changes of metabolites mapped to the metabolic pathways in leaves of rice exposed to PS-MPs. The FC represents the relative contents of metabolites. The points were colored according to the relative quantity ($\log_2^{FC}$) of control. The red means up-regulation, the green means down-regulation, and the blue means increased under 50 mg L^{-1} PS MPs then decreased under 250 mg L^{-1} PS MPs. The dotted and solid lines represent the indirect and direct connections between two metabolites, respectively, and the arrows present the transformation direction. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

activity of antioxidants in rice leaves (Gill and Tuteja, 2010; Wu et al., 2017).

It is also confirmed in the field experiment, that after exposure to PS-MPs, the biomass of the stem were lowered. Though the difference was not significant, it still indicate that the effect of exposure exists in the agricultural application scenario.

4.2. Metabolomics

Alteration in Amino acids. Amino acids typically play an important role in modulating plant biological functioning by acting as osmolytes, regulating ion transport, modulating stomatal opening, serving as precursors for the synthesis of defense-related metabolites, and signaling metabolites (Chen et al., 2018; Zhang et al., 2013).

Changes in L-proline content is often closely related to environmental stress, as it is beneficial in clearing ROS (Pidatala et al., 2016). Similarly, phenylalanine plays an important role in plant defenses, as it is the precursor for many intermediary and secondary metabolites (Tzin and Galili, 2010). The down-regulation of phenylalanine may reflect the abnormal stress response of rice leaves to MPs.

Amino acids are the primary substances for the synthesis of proteins in rice leaves (Chen et al., 2019). Moreover, the descended levels of most amino acids resulted in the reduction of the contribution of amino acid-related metabolic pathways in protein synthesis, including glycine, serine, and threonine metabolism, and alanine, aspartate and glutamate metabolism. These two biosynthesis pathways may be responsible for the decrease in the biomass of rice leaves. Due to the roles of amino acids in impairing protein biosynthesis and promoting plant growth (Zhang et al., 2019), it can be deduced that the decrease of amino acids in rice leaves might result in the incapability of rice leaves to sustain the stress imposed by PS-MPs through scavenging ROS from the leaves, thus inhibiting

the growth of rice leaves.

Alteration in Saccharides. The down-regulation saccharides in response to the exposure of PS-MPs might suggest the perturbation of the glycolytic pathway. To be specific, saccharides play an important role in protecting and stabilizing cell membranes, which were functioned as ROS scavengers and signals in stress-induced pathways (Ribeiro et al., 2016).

In this study, most saccharides, including various hexoses and disaccharides, were down-regulated in rice leaves significantly during all the exposure periods to PS-MPs.

The content of β-D-Lactose and D-galactose increased when exposed to a low dose (50 mg L^{-1}) of PS-MPs, possibly indicating that the decomposition pathway of lactose was inhibited. As the exposure dose of PS-MPs increased, the produced glucose in rice leaves would perturb lactose synthesis. These saccharides (i.e., β- D-lactose and D-galactose) may perturb galactose metabolism, thus affecting the formation of some glycoproteins in rice (Chen et al., 2018; Wu et al., 2017). Moreover, they could also act as primary substances to synthesize starch in rice leaves, and contribute to the transformation of proteins and lipids as signaling molecules, thus providing energy for plant growth and development.

Alteration in Organic Acid. The changes in organic acids showed a similar trend: Their relative abundance significantly decreased after exposure to PS-MPs. Particularly, the content of hydroxybenzoic acid decreased with the increasing of the doses of PS-MPs, which indicated that the cell wall composition might be changed as hydroxybenzoic acid is an integral component of cell walls (Zhang et al., 2019). In addition, the increased concentration of oxalic acid might act as signaling and defense molecules (Zhao et al., 2016a).

For rice leaves, organic acids in the exposure group with the doses in down-regulated could not work as non-enzymatic antioxidants to alleviate oxidative stress induced by PS-MPs. Organic acids are intermediates of major carbon metabolism (photosynthesis and respiration) in plant cells (Drincovich et al., 2016). The decreased organic acid content in rice leaves exposed to PS-MPs induces the interference with photosynthesis and respiration in rice.

From the aforementioned discussion, it can be concluded that the decreased levels of amino acids from rice leaves might damage the amino acids metabolic pathways of the rice leaves after exposure to different doses of PS-MPs. The down-regulation of amino acids and saccharides indicated that the exposure to PS-MPs affected the nitrogen and carbon profiles in rice leaves. The mechanism of organic acid profile changes under xenobiotic exposure is a result of the reprogramming of multiple metabolic pathways (Zhao et al., 2016b). Also, organic acids are considered as an important media for plant material exchange with the outside world, playing an important role in plant nutrient activation and uptake (Igamberdiev and Eprintsev, 2016).

4.3. Metabolic pathways

Changes in metabolic pathways may interfere with crop antioxidant defense systems, including energy metabolism and anabolism. The stress of ROS may affect energy metabolism by declining the degree of anabolism, thus resulting in a decreased content of biomass. The changes in the composition of amino acids and saccharides might also affect the metabolic pathways of galactose and amino acids. And the rice biomass might decline as a result of the down-regulation of the metabolites of galactose and amino acids.

As is known, malic acid can protect living organisms from external stress and defend oxidative stress (Du et al., 2017). Succinic acid was important in the respiratory system in mitochondria (Ratnasekhar et al., 2015). Fumaric acid was used by cells to produce energy in the form of adenosine triphosphate (Gold et al., 2012). And these organic acids were also important intermediates responsible for the TCA cycle. The decreases in concentrations of malic, succinic and fumaric acid indicate that the defense and respiratory have been affected, and the TCA cycle and related energy production might be interfered with.

5. Conclusions

Currently, the knowledge of the effects of microplastics on plants is still lacking. There is an urgent need to elucidate the effects of PS-MPs on rice growth. This study, for the first time, evaluated the disturbance of metabolic systems of rice plants by PS-MPs using omics-based non-target screening techniques. The concentrations of intermediates (citric acid, 56.7%, and 94.2%) decreased after exposure to different doses of PS-MPs, indicating that the antioxidant defense system of rice was affected after exposure to different doses of PS-MPs. And the biomass of rice decreased as proved by the results of field-trial experiments after a cultivation time of 142 days when exposed to PS-MPs. Collectively, it can be found that the disturbance in the metabolic pathways by PS-MPs could significantly reduce rice production. However, there are some limitations to this study. Firstly, there are numerous types of MPs that can be detected in the soil environment, but only one type of PS-MPs was considered as a representative contaminant in this study. The effects of the particle sizes of microplastics on rice were also ignored in this study. The scale of a field test in this study is small, thus leading to less reliability. Therefore, studies concerning a wider range of microplastics and their effects on plant growth will be further investing in the future.

CRediT authorship contribution statement

Xiang Wu: Methodology, Software, Formal analysis, Investigation, Writing - original draft. **Yao Liu:** Investigation. **Shanshan Yin:** Conceptualization, Writing - review & editing. **Keke Xiao:** Writing - review & editing. **Qiao Xiong:** Investigation. **Shijie Bian:** Supervision. **Sha Liang:** Software, Validation. **Huijie Hou:** Data curation. **Jingping Hu:** Writing - review & editing. **Jiakuan Yang:** Writing - review & editing.

Declaration of competing interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgement

This work was jointly supported by the National Natural Science Foundation of China (21906060), the National Key Research and Development Program of China (2018YFD1100604) and China Postdoctoral Science Foundation (2019M662631). Additionally, we would like to thank the Analytical and Testing Center of Huazhong University of Science and Technology for providing experimental measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115159.

References

Aravind, P., Prasad, M.N., 2003. Zinc alleviates cadmium-induced oxidative stress in

Ceratophyllum demersum L.: a free floating freshwater macrophyte. Plant Physiol. Biochem. 41, 391–397.
Blasing, M., Amelung, W., 2018. Plastics in soil: analytical methods and possible sources. Sci. Total Environ. 612, 422–435.
Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. Science 178, 749–750.
Chen, J., Le, X.C., Zhu, L.Z., 2019. Metabolomics and transcriptomics reveal defense mechanism of rice (Oryza sativa) grains under stress of 2,2',4,4'-tetrabromodiphenyl ether. Environ. Int. 133, 12.
Chen, J., Li, K.L., Le, X.C., Zhu, L.Z., 2018. Metabolomic analysis of two rice (Oryza sativa) varieties exposed to 2,2',4,4'-tetrabromodiphenyl ether. Environ. Pollut. 237, 308–317.
Chong, J., Soufan, O., Li, C., Caraus, I., Li, S.Z., Bourque, G., Wishart, D.S., Xia, J.G., 2018. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 46, W486–W494.
Choudhury, F.K., Rivero, R.M., Blumwald, E., Mittler, R., 2017. Reactive oxygen species, abiotic stress and stress combination. Plant J. 90, 856–867.
Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez-Leon, S., Palma, A.T., Navarro, S., Garcia-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Nat. Acad. Sci. USA 111, 10239–10244.
da Costa, J.P., Santos, P.S., Duarte, A.C., Rocha-Santos, T., 2016. (Nano)plastics in the environment - sources, fates and effects. Sci. Total Environ. 566, 15–26.
do Sul, J.A.I., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364.
Drincovich, M.F., Voll, L.M., Maurino, V.G., 2016. On the diversity of roles of organic acids. Front. Plant Sci. 7, 2.
Du, C.L., Zhang, B., He, Y.L., Hu, C.Y., Ng, Q.X., Zhang, H., Ong, C.N., ZhifenLin, 2017. Biological effect of aqueous C_{60} aggregates on Scenedesmus obliquus revealed by transcriptomics and non-targeted metabolomics. J. Hazard Mater. 324, 221–229.
Fiehn, O., Kopka, J., Trethewey, R.N., Willmitzer, L., 2000. Identification of uncommon plant metabolites based on calculation of elemental compositions using gas chromatography and quadrupole mass spectrometry. Anal. Chem. 72, 3573–3580.
Fuller, S., Gautam, A., 2016. A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 50, 5774–5780.
Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Gold, R., Linker, R.A., Stangel, M., 2012. Fumaric acid and its esters: an emerging treatment for multiple sclerosis with antioxidative mechanism of action. Clin. Immunol. 142, 44–48.
Han, X.X., Lu, X., Vogt, R., 2019. An optimized density-based approach for extracting microplastics from soil and sediment samples. Environ. Pollut. 254, 7.
Huerta Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). Environ. Sci. Technol. 50, 2685–2691.
Huerta Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2017. Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environ. Pollut. 220, 523–531.
Igamberdiev, A.U., Eprintsev, A.T., 2016. Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. Front. Plant Sci. 7, 15.
Jiang, X.F., Chen, H., Liao, Y.C., Ye, Z.Q., Li, M., Klobucar, G., 2019. Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environ. Pollut. 250, 831–838.
Jiang, Y.S., Tang, X.X., Zhou, B., Sun, T.L., Chen, H.M., Zhao, X.Y., Wang, Y., 2017. The ROS-mediated pathway coupled with the MAPK-p38 signalling pathway and antioxidant system plays roles in the responses of Mytilus edulis haemocytes induced by BDE-47. Aquat. Toxicol. 187, 55–63.
Koca, H., Bor, M., Ozdemir, F., Turkan, I., 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp. Bot. 60, 344–351.
Kusano, M., Yang, Z.G., Okazaki, Y., Nakabayashi, R., Fukushima, A., Saito, K., 2015. Using metabolomic approaches to explore chemical diversity in rice. Mol. Plant 8, 58–67.
Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142.
Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., Fernie, A.R., 2006. Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat. Protoc. 1, 387–396.
Machado, A.A.D., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of microplastics on the soil biophysical environment. Environ. Sci. Technol. 52, 9656–9665.
Matich, E.K., Soria, N.G.C., Aga, D.S., Atilla-Gokcumen, G.E., 2019. Applications of metabolomics in assessing ecological effects of emerging contaminants and pollutants on plants. J. Hazard Mater. 373, 527–535.
Ng, E.L., Lwanga, E.H., Eldridge, S.M., Johnston, P., Hu, H.W., Geissen, V., Chen, D.L., 2018. An overview of microplastic and nanoplastic pollution in agroecosystems. Sci. Total Environ. 627, 1377–1388.
Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50, 10777–10779.
Pidatala, V.R., Li, K.F., Sarkar, D., Ramakrishna, W., Datta, R., 2016. Identification of biochemical pathways associated with lead tolerance and detoxification in Chrysopogon zizanioides L. Nash (Vetiver) by metabolic profiling. Environ. Sci. Technol. 50, 2530–2537.
Qi, Y.L., Yang, X.M., Pelaez, A.M., Lwanga, E.H., Beriot, N., Gertsen, H., Garbeva, P., Geissen, V., 2018. Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Sci. Total Environ. 645, 1048–1056.
Ratnasekhar, C., Sonane, M., Satish, A., Mudiam, M.K.R., 2015. Metabolomics reveals the perturbations in the metabolome of Caenorhabditis elegans exposed to titanium dioxide nanoparticles. Nanotoxicology 9, 994–1004.
Rezania, S., Park, J., Din, M.F.M., Taib, S.M., Talaiekhozani, A., Yadav, K.K., Kamyab, H., 2018. Microplastics pollution in different aquatic environments and biota: a review of recent studies. Mar. Pollut. Bull. 133, 191–208.
Ribeiro, D.M., Silva, D.D., Cardoso, F.B., Martins, A.O., Silva, W.A., Nascimento, V.L., Araujo, W.L., 2016. Growth inhibition by selenium is associated with changes in primary metabolism and nutrient levels in Arabidopsis thaliana. Plant Cell Environ. 39, 2235–2246.
Rillig, M.C., Lehmann, A., Machado, A.A.D., Yang, G., 2019. Microplastic effects on plants. New Phytol. 223, 1066–1070.
Soria, N.G.C., Montes, A., Bisson, M.A., Atilla-Gokcumen, G.E., Aga, D.S., 2017. Mass spectrometry-based metabolomics to assess uptake of silver nanoparticles by Arabidopsis thaliana. Environ. Sci. Nano 4, 1944–1953.
Su, L., Xue, Y.G., Li, L.Y., Yang, D.Q., Kolandhasamy, P., Li, D.J., Shi, H.H., 2016. Microplastics in Taihu Lake, China. Environ. Pollut. 216, 711–719.
Thompson, R., Olsen, Y., Mitchell, R., Davis, A., Rowland, S., John, A.W., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838.
Tzin, V., Galili, G., 2010. The biosynthetic pathways for shikimate and aromatic amino acids in Arabidopsis thaliana. Arabidopsis Book 8, e0132.
Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environ. Pollut. 199, 10–17.
Wan, Y., Wu, C.X., Xue, Q., Hui, X.M.N., 2019. Effects of plastic contamination on water evaporation and desiccation cracking in soil. Sci. Total Environ. 654, 576–582.
Wang, J., Lv, S.H., Zhang, M.Y., Chen, G.C., Zhu, T.B., Zhang, S., Teng, Y., Christie, P., Luo, Y.M., 2016. Effects of plastic film residues on occurrence of phthalates and microbial activity in soils. Chemosphere 151, 171–177.
Wu, B.Y., Zhu, L.Z., Le, X.C., 2017. Metabolomics analysis of TiO_2 nanoparticles induced toxicological effects on rice (Oryza sativa L.). Environ. Pollut. 230, 302–310.
Wu, X., Zhu, L.Z., 2019. Prediction of organic contaminant uptake by plants: modified partition-limited model based on a sequential ultrasonic extraction procedure. Environ. Pollut. 246, 124–130.
Zhang, G.S., Liu, Y.F., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. Sci. Total Environ. 642, 12–20.
Zhang, H.L., Lu, L., Zhao, X.P., Zhao, S., Gu, X.Y., Du, W.C., Wei, H., Ji, R., Zhao, L.J., 2019. Metabolomics reveals the "invisible" responses of spinach plants exposed to CeO2 nanoparticles. Environ. Sci. Technol. 53, 6007–6017.
Zhang, J.X., Kirkham, M.B., 1994. Drought-stress-induced changes in activities of superoxide dismutase, Catalase, and peroxidase in wheat species. Plant Cell Physiol. 35, 785–791.
Zhang, L., Wang, X.Y., Guo, J.Z., Xia, Q.L., Zhao, G., Zhou, H.N., Xie, F.W., 2013. Metabolic profiling of Chinese tobacco leaf of different geographical origins by GC-MS. J. Agric. Food Chem. 61, 2597–2605.
Zhao, L.J., Huang, Y.X., Hu, J., Zhou, H.J., Adeleye, A.S., Keller, A.A., 2016a. H-1 NMR and GC-MS based metabolomics reveal defense and detoxification mechanism of cucumber plant under nano-Cu stress. Environ. Sci. Technol. 50, 2000–2010.
Zhao, L.J., Ortiz, C., Adeleye, A.S., Hu, Q.R., Zhou, H.J., Huang, Y.X., Keller, A.A., 2016b. Metabolomics to detect response of lettuce (Lactuca sativa) to $Cu(OH)_2$ nanopesticides: oxidative stress response and detoxification mechanisms. Environ. Sci. Technol. 50, 9697–9707.