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Photosynthetic, antioxidative, and metabolic adjustments of a crop plant to elevated levels of La and Ce exposure

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ABSTRACT

Rare earth elements (REEs) have been widely applied as fertilizers in farmland of China for decades to improve the yield and quality of crops. Unfortunately, adverse effects on plants have been observed due to overdosing with REEs. Until now, the toxicology of REEs was mainly evaluated based on phenotypic responses, but knowledge gaps still exist concerning their metabolic effects. Here, the physiological responses and nontargeted metabolomics studies were combined to systematically explore the potential effects of La and Ce on a crop plant, wheat *Triticum aestivum*. It was observed that REEs accumulated in the shoots of wheat, with significant reduction of the shoot biomass at higher exposure doses. The disturbance of photosynthesis and induced oxidative stress were identified by analyzing indicators of the photosynthetic (chlorophyll a/b, carotenoid and rubisco) and antioxidant systems (POD, CAT, SOD, GSH and MDA). Furthermore, the global metabolic profiles of REEs treatment groups and the non-exposed control group were screened and compared, and the metabolomic disturbance of REEs was dose-dependent. A high overlap of significantly changed metabolites and matched disturbed biological pathways was found between La and Ce treatments, indicating similarity of their toxicity mechanism in wheat shoots. Generally, the perturbed metabolomic pathways were mainly related to carbohydrate, amino acid and nucleotide/side metabolism, suggesting a disturbance of carbon and nitrogen metabolism, which finally affected the growth of wheat. We thus proved the potential adverse effect of inappropriate application of REEs in crop plants and postulated metabolomics as a feasible tool to identify the underlying toxicological mechanisms.

1. Introduction

Rare earth elements (REEs), including strontium (Sc), yttrium (Y) and 15 lanthanides, have become a highly valuable commodity in view of their increased use in industrial applications, like magnets, carbon-arc lighting, and electrodes (Gwenzi et al., 2018). Besides, REEs also have been widely used in the development of agriculture, like as REE fertilizers (Sun et al., 2019). Given the similar ionic radius of REEs and calcium (Ca) ion, the chemical characteristics of REEs conform to those of Ca, and REEs thus share the same binding sites in organisms with Ca. Consequently, REEs show a similar physiological effect as Ca and the deficiency symptoms of Ca in plant could especially be mitigated by addition of lanthanum (La) (Hu et al., 2004). It has been reported that the addition of appropriate amounts of REEs can promote the germination of seed, stimulate the growth of roots, increase the content of chlorophyll, enhance the resistance, and subsequently increase the output of crops (Tommasi et al., 2021). For example, both La and cerium (Ce) exert stimulatory effects on *Crocus sativus*, with enhanced cell growth and crocin production (Chen et al., 2004). Addition of Ce could alleviate the Ca-deficient symptoms in spinach plants by improving the photosynthesis and nitrogen metabolism (Chao et al., 2008). Therefore, commercial REE-based fertilizers have been widely used in farmland to increase the yield and quality of crops in China since the 1990's. The application of REEs for agricultural purposes is becoming one of the leading demanding branches of REEs in China (Hu et al., 2004; Pang et al., 2002).

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Meanwhile, large amounts of REEs are released into the environment during the long-term application of REE-enriched fertilizers and mining activities connected with their extraction (Dinali et al., 2019; Gonzalez et al., 2014). It has been reported that REE-based phosphate fertilizers may increase the probability of environmental contamination (Carpenter et al., 2015). Accumulation of REEs in soil, water, and biota is being associated with these agricultural and industrial activities. Elevated contents of REEs in the environment, plants and animals were demonstrated by various researchers (Li et al., 2013; Liang et al., 2005; Zhang et al., 2022). As reported, Ce was the most accumulated REE in soils around the mining areas in Baotou with concentrations ranging from 93 to 12,170 mg/kg, followed by La (40 −6905 mg/ kg) (Li et al., 2008). Another study also found the same order of REEs accumulated in surface soils near the Bayan Obo mine region (Wang et al., 2015). These results were consistent with the fact that Ce was the most abundant REEs in earth's crust, followed by La. Despite some research efforts have been devoted to evaluate the effect of REEs in terrestrial system, there is still no consensus on the safe levels of exposure to REEs. In soils, La adversely affected soil organisms, including invertebrates, bacteria, and plants, at levels slightly higher than its natural background contents (7−50 mg/kg) (Li et al., 2018). Differently, no toxicity symptoms in onion bulbs were observed at concentrations up to 200 mg/kg of La and Ce in soil. The dose-response relationships of REEs often exhibit biphasic trends, showing beneficial effects at low concentrations (hormesis), while toxic effects at high concentrations (Pagano et al., 2015). Previous studies have proven the adverse effects induced by REEs in aquatic and terrestrial organisms. The growth of duckweed, *Lemna minor* L. was increased under the treatment of relatively low Ce concentrations, but was reduced at higher exposure levels (Zicari et al., 2018). Addition of La, Ce, and their mixtures inhibited primary root elongation, dry weight of roots and shoots of wheat, *T. aestivum*, and the damage increased with increasing concentrations of REEs in culture medium (Hu et al., 2002). An obvious dose-dependent response was observed for the relative root elongation of *T. aestivum* exposed to Y, La, and Ce in individual and in mixture scenarios (Gong et al., 2019). Generally, it has been widely accepted that exposure to excess concentration of REEs may lead to toxic effects on organisms, while the underlying mechanisms to interpret these biological effects have not been universally accepted (Herrmann et al., 2016). It has been detected that the chlorophyll and carotenoid levels were reduced and the activity of antioxidants was increased in *Lemna minor* L. treated with a high concentration of REEs (Zicari et al., 2018). A higher concentration of La was associated with an increase of oxidative stress, with the content of malondialdehyde (MDA) and hydrogen peroxide elevated in the seeds of rice (Liu et al., 2012). However, these targeted physiological indicators, e.g., antioxidant system, and photosynthesis could neither provide a holistic detection of plant responses to REEs stress, nor reveal the underlying toxicity mechanisms.

The emergence of nontargeted metabolomic approaches could give a broader detection of the disturbance of metabolites and biological pathways in organisms under stress at the molecular level (Fiehn, 2002; Viant et al., 2019). Hence, metabolomics could serve as a tool to elucidate the mechanisms behind the phenotypic changes induced by employing chemicals to enhance agricultural production (Chen et al., 2019; Majumdar and Keller, 2021). In recent years, metabolomics has become a mature technology in academic research, particularly within the biomedical and plant sciences (Viant et al., 2019). The detected dysregulated metabolites and biological pathways explained the reduced root growth of wheat and corn exposed to excessive level of molybdenum trioxide nanoparticles, which were applied in agriculture as micronutrients (Huang et al., 2021). By applying metabolomics, the disturbance of energy, antioxidant and defense related pathways were found in cucumber leaf exposure to copper-based pesticides (Zhao et al., 2018). Inappropriate application of pesticides trigged molecular alterations in rice, which were involved in the pathways of carbohydrate metabolism, amino acid metabolism, and fatty acid metabolism, subsequently affecting crop quality (Lin et al., 2020; Liu and Zhu, 2020). These studies suggest that metabolomics profiling provides a comprehensive view of plant responses to the application of fertilizers or pesticides and supports the optimization of their applied dose. Still, the metabolomics approach has not been applied for exploring the mechanisms of the simulated effects of REEs on crops. Moreover, the molecular level studies to elucidate the mechanisms of action of lanthanides are mostly limited to La (Gonzalez et al., 2014). As far as is known, REEs-induced metabolic changes in plant shoots have rarely been studied. The question remains open whether two rare earth elements with similar chemical properties can be treated uniformly when assessing their phytotoxicity. Hence, further research is needed to identify if common mechanisms of toxic action are shared across lanthanides.

In this study, as one of the primary producers in the contaminated agricultural soils, wheat (*T. aestivum*) was selected to investigate its response to various exposure doses of REEs. Because of their relatively high crustal abundance, La and Ce were chosen as the representatives of REEs to explore the existence of similar or dissimilar toxicology mechanisms of different lanthanides. High-throughput nontargeted metabolomics was applied to detect the disturbed metabolites and the relevant biological functions induced by REEs at the molecular level. Furthermore, indicators of growth, and the antioxidant and photosynthetic systems of the shoot were evaluated to determine the physiological responses of wheat to REEs exposure. This work will contribute to a deeper understanding of the adverse effects caused by excessive application of REEs from a molecular-scale perspective.

2. Material and methods

2.1. Wheat cultivation and plant toxicity assays

Winter wheat (*T. aestivum* L. cv. Xiaoyan 22) seeds were purchased from Nanjing Agricultural University (China) and chosen as test species in the current study as a representative crop plant. The wheat seeds were sterilized and pre-germinated following the method of Wang et al. (2013). After germination, seedlings with root lengths of approximately 1 cm were chosen for the experiments.

The toxicity test of REEs was performed in hydroponic cultures. The nutrient solution was used as the basic solution for preparing test solutions of various concentrations of La and Ce. The composition of the nutrient solution can be found elsewhere (Li et al., 2020). The pH values of all test solutions were adjusted to 6.0 ± 0.1 by diluted NaOH or HCl and buffered with 4-morpholineethanesulfonic acid (MES). In total, five treatments were established including control (nutrient solution without La and Ce), 0.5 μM La, 1.0 μM La, 0.5 μM Ce and 1.0 μM Ce. The measured concentrations of REEs were always within 10% of the nominal concentrations. Six replicates were performed for each treatment. For each replicate, four wheat seedlings were cultured in a glass beaker filled with 250 mL test solutions. The plant toxicity experiments were performed in a climate chamber which was maintained at 20 ℃, 75% relative humidity and 16 h light/8 h dark cycle. After 4 days of exposure, the shoots of wheat were collected, rinsed with deionized water, weighted, and stored for further physiological and metabolomic analyses. The impact of external stressors on wheat plants is often studied by analysis of shoot and root responses (Lipiec et al., 2016; Severini et al., 2020). In a companion paper, we have investigated the metabolomic alterations in wheat roots exposed to REEs (He et al., 2022). In the present study, we choose to only focus on the responses of plant shoots, mainly because plant shoots can serve as a direct indicator for the effects of rare earth elements on crop growth. Besides, the metabolic changes of plant shoots under REEs exposures can also be interpreted together with the phenotypic measurements of the photosynthetic responses and this kind of analysis is only applicable for plant shoots.

2.2. REEs contents analysis

The collected wheat shoots of each replicate were dried, weighed, cut into small sections and placed into glass digestion tubes. For each tube, 2 mL of purified HNO_3 (65 %–68 %, GR) was added, sealed with sealing film and kept for 24 h. Then, the digestion was performed with a graphite digestion instrument (DigiPREP MS, SCP Science) following a standard procedure: 85 ℃ for 2 h until no obvious tissue residue was left in the solution, and 140 ℃ until almost no residual liquid was left in the tube. Finally, the digests in the glass tubes were diluted to 5 mL with deionized water and the concentration of La and Ce was determined by inductively coupled plasma optical emission spectrometer (ICP-OES, iCAP7600, Thermo Fisher) to calculate the content of REEs in the wheat shoots.

2.3. Photosynthesis and oxidative stress analysis

Fresh leaves (300 mg) were cut into pieces and transferred into a mortar. A small amount of quartz sand and calcium carbonate powder and 10 mL of 95 % ethanol were added to extract the pigment of the leaves. The liquid in the mortar was filtered, transferred to a 25 mL volumetric flask, and mixed with 95% ethanol to a constant volume. The absorbance of the extracted solution was measured by UV-Visible spectrophotometer at 665 nm, 649 nm and 470 nm to determine the contents of chlorophyll a, chlorophyll b and carotenoid, respectively.

In addition, parts of the collected leaf tissues (80–120 mg) were immediately frozen in liquid nitrogen and grounded into fine powder in a mortar. The frozen leaves were weighed and mixed with phosphate buffer solution (0.1 M, pH 7–7.4) with a ratio of 1.0 g to 9.0 mL. The mixture was homogenized in an ice water bath and then centrifuged at 3500 rpm for 10 min. The supernatant was extracted for measuring the oxidative stress related parameters, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione (GSH) and MDA in wheat shoots. These parameters were determined using the corresponding commercial assay kits (Nanjing Jiancheng Bioengineer Institute, Nanjing, China), following the instructions of the manufacturer.

2.4. Metabolomics extraction and analysis

For the extraction and analysis of metabolites in wheat shoots, first of all, the fresh leaf tissues (80–120 mg) were frozen and ground in liquid nitrogen following the method described above. Next, 1.0 mL methanol/acetonitrile/water (2:2:1, v/v/v) was added to the homogenized solution, the mixture was centrifuged for 20 min (14,000 g, 4 °C), and the supernatant was dried in a vacuum centrifuge to obtain the extracts containing the metabolites. The samples were stored at − 80 ℃ and redissolved in 100 μL acetonitrile/water (1:1, v/v) solvent for analysis of the metabolites. In order to monitor the stability and repeatability of the instrumental analysis, quality control (QC) samples were prepared by pooling 10 μL of each sample and regularly inserted and analyzed during the analysis of the other samples. In this study, metabolites were analyzed by UHPLC-Q-TOF MS, the ultrahigh performance liquid chromatography (UHPLC) (1290 Infinity LC, Agilent Technologies) coupled with quadrupole time-of-flight (Q-TOF) (AB Sciex TripleTOF 6600). LC-MS/MS data were acquired in both positive and negative electrospray ionization (ESI) modes. Quantification was reported as peak height using the unique ion as default. Metabolites were unambiguously assigned by the BinBase identifier numbers using retention index and mass spectrum as the two most important identification criteria. The obtained raw MS data were normalized and transformed for further statistical analysis. More details regarding the instrumental settings, running parameters and data processing are described in our previous study (He et al., 2020).

2.5. Statistical analysis

For the weight and accumulation of REEs as well as the photosynthesis and oxidative stress related parameters in shoot of wheat, one-way analysis of variance (ANOVA) and Tukey's test were performed to see if there is a significant difference among different treatments. A value of $p < 0.05$ was considered statistically significant. The results are presented as mean ± standard errors ($n = 3$).

For the metabolic analysis, the processed data were subjected to SIMCA 14.1 software for multivariate data analysis, including Pareto-scaled principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) to obtain a global screen of the sample separation in the control and the treatments of different exposure doses of La/Ce. The 7-fold cross-validation and response permutation testing were used to evaluate the robustness of the model. The variable importance in the projection (VIP) value of each metabolite in the PLS-DA model was calculated to indicate its contribution to the overall classification. Metabolites with VIP value > 1.0 were further applied to Student's t-test at univariate level to measure the significance of variation of each metabolite between the REE treatment group and the control group. Values of *p* values less than 0.05 were considered as statistically significant. The significantly changed metabolites (SCMs) were further used for the hierarchical clustering (HCL) analysis and relevant biological pathways identification. HCL clustering of SCMs in positive and negative modes were performed by the Multiple Experiment Viewer (MeV 4.9) and the relative abundance of SCMs was presented in the form of a heatmap to show the trend of change of metabolic profiles. Pathway enrichment analysis was performed with MetaboAnalyst 4.0 and pathways with p value < 0.05 and fold enrichment $>$ 2.0 were determined as significantly altered by La and Ce exposure. The relative changes of the abundance of SCMs involved in the perturbed pathways are illustrated in the boxplots using Origin 9.2.

3. Results and discussion

3.1. Physiological indicators of REEs stress

3.1.1. Weight and accumulation of REEs

Compared to the control, the weight of the shoots was significantly ($p < 0.05$) reduced by 10.5% and 20.2% after exposure to a high dose (1.0 μM) of La and Ce, respectively (Fig. 1A). This adverse effect was not obvious for the low exposure dose (0.5 μM) of REEs. The accumulation of REEs in the shoots of *T. aestivum* was observed, with an obvious dose-dependent trend from 1.81 to 2.14 mg/kg and from 2.12 to 2.79 mg/kg under low and high exposure dose of La and Ce, respectively (Fig. 1**B**). This indicates that the enrichment and adverse effects of REEs could be induced in wheat shoots when their environmental abundance exceeds certain threshold.

A previous study showed that the application of rare earth-based fertilizers induced an increased concentration of 15 REEs in roots, stem, and leaves of plants, including wheat, rice, and vegetables (Wen et al., 2001). The accumulation of praseodymium (Pr), neodymium (Nd), samarium (Sm), terbium (Tb), dysprosium (Dy) and erbium (Er) was observed both in the belowground and aboveground parts of plants grown in contaminated soils around REE-mining areas (Carpenter et al., 2015). The translocation and partitioning of La and Ce between shoots and roots were dependent on the exposure routes, e.g., the foliar application or soil exposure (Gwenzi et al., 2018). Adverse effects of metals could be induced when the residual of metals in the plant exceeds the Critical Body Residue (CBR), which is the threshold concentration of a substance in an organism that marks the transition between no effect and adverse effect (Vijver et al., 2004). Significant inhibition of root elongation was determined for wheat exposed to individual and binary mixture of La, Ce and Y, showing an obvious dose-response relationship (Gong et al., 2019). The direct linkage between bioaccumulation and toxicity of La and Ce was determined by expressing the survival rate of

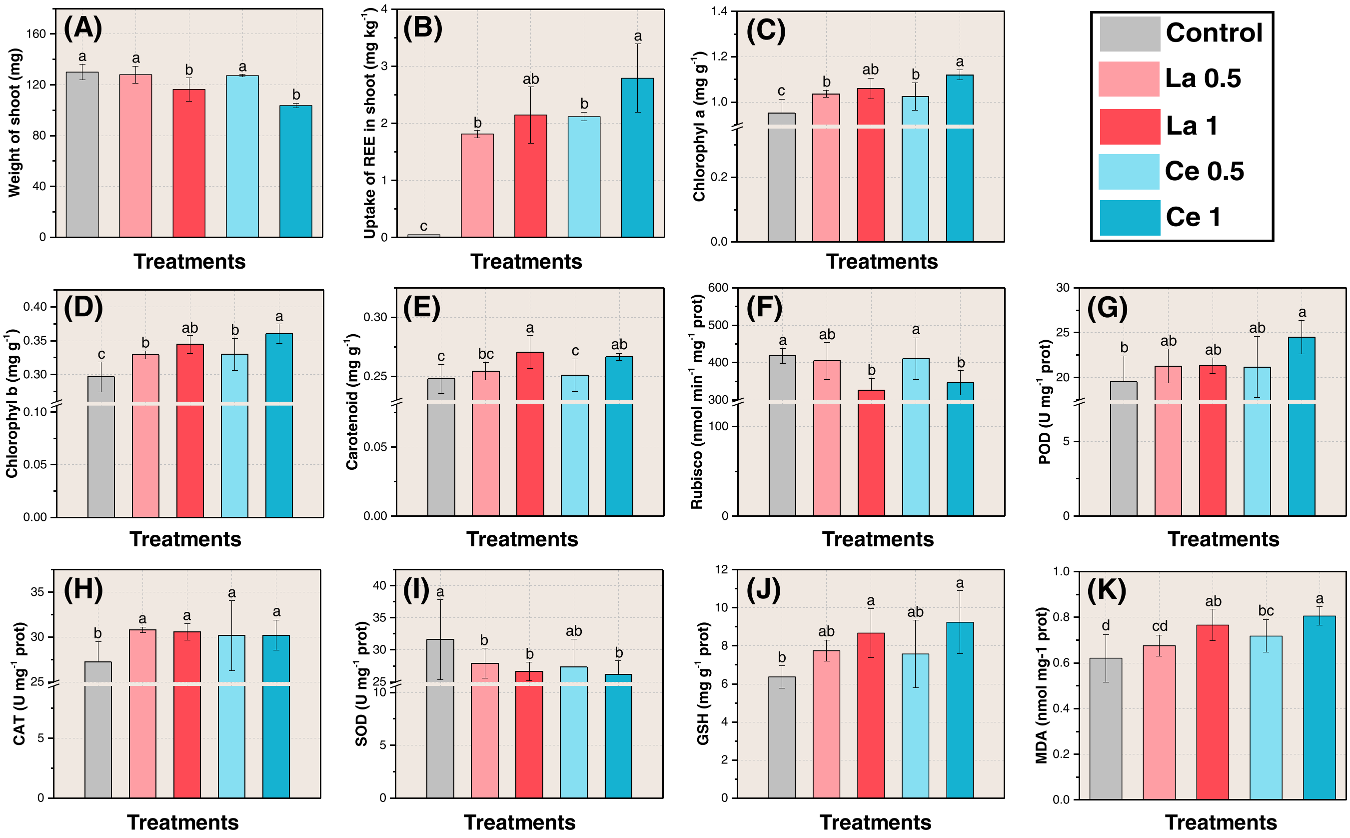

Fig. 1. The physiological responses of wheat shoots (*Triticum aestivum*) exposed to low (0.5 μM) and high level (1.0 μM) of La and Ce, respectively. (A) Weight of shoots (mg); (B) Uptake of La/Ce in shoots (mg kg^{-1}); (C-F) Indicators of photosynthetic systems, including chlorophyll a, chlorophyll b, carotenoids (mg g^{-1}), and rubisco (nmol min^{-1}mg^{-1} prot); (G-K) Indicators of the antioxidant systems, including POD, CAT, SOD (U mg^{-1} prot), GSH (mg g^{-1}), and MDA (nmol mg^{-1} prot). Here, prot stands for protein.

organisms to their body concentration under varied exposure levels of La and Ce (Huang et al., 2020b). A deep analysis of REEs-induced effects is therefore needed.

3.1.2. Photosynthetic system

Photosynthesis is the main energy source for the maintenance of green plants growth, metals could exert their toxic action by damaging chloroplasts and disturbing the photosynthesis (Aggarwal et al., 2011). The content of light-absorbing pigments (chlorophyll a, chlorophyll b and carotenoid) and the activity of key enzymes of the Calvin cycle (rubisco) in shoots of *T. aestivum* were analyzed to indicate the influence of REEs exposure on the photosynthetic system. The content of chlorophyll a/b was significantly increased in the REEs treatment relative to that of the control treatment (Fig. 1C, D). For carotenoids, the change was not significant for low dose treatment of REEs but increased by 12.4 % and 10.7 % for high dose treatment of La and Ce, respectively, as compared with the control (Fig. 1E). Evident inhibition of the activity of rubisco was only observed under high level exposure of REEs (Fig. 1F).

These findings indicate that the photosynthesis of plants is sensitive to abiotic stress, which is consistent with previous studies. For the exposure of silver nanoparticles and sliver ions, the chlorophyll content of cyanobacteria was significantly changed upon exposure to high concentrations, but not for the low and medium concentration treatments (Huang et al., 2020a). The content of chlorophyll a/b in rice seeds was highly dependent on the exposure level of CeO$_2$ nanoparticles (Rico et al., 2013). These findings indicate that the photosynthetic system of *T. aestivum* was possibly changed under REEs exposure stress, especially at relatively high exposure dose, but there is no obvious discrepancy between La and Ce treatments. Further hard evidence is still needed to elucidate the relationship between changes in photosynthetic pigments and the disturbance in photosynthetic pathway. Carbohydrates are the most-important direct organic product of photosynthesis in the majority of green plants (Aggarwal et al., 2011). Hence, the disturbance of photosynthetic system will subsequently lead to growth inhibition of *T. aestivum*. This is supported by the reduced weight of wheat shoots under a high exposure concentration of REEs.

3.1.3. Antioxidant system

The induced oxidative stress in plants could be evaluated by the change of activity of antioxidant enzymes such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), which can scavenge and control the level of reactive oxygen species (ROS) generated in plants as byproduct of abiotic stress (Foyer and Noctor, 2011; Rico et al., 2013). In this study, the activities of POD and CAT were increased by 8.9 and 12.4, and 25.3 % and 10.9 % for La and Ce, respectively. The activity of SOD was reduced by 18.5% and 20.7% for the high dose treatments of La and Ce separately (Fig. 1G-I). GSH is central to redox control, and it is an abundant and stable antioxidant with an appropriate redox potential for protection of cells against ROS (Foyer and Noctor, 2011). The exposure of La and Ce caused significant elevation of reduced glutathione (GSH), exhibiting an increasing trend with exposure dose (Fig. 1J).

The induced oxidative stress in organisms by REEs exposure has been reported previously. Antioxidant system analysis of algae, *Chlorella vulgaris* and *Phaeodactylum tricornutum* under La exposure confirmed increase in the activities of antioxidants such as SOD and GSH (Sun et al., 2019). The treatments of single and mixture of REEs induced an increase in total GSH in the roots of wheat, *Triticum durum* (d'Aquino et al., 2009). When the balance between production and removal of ROS could not be maintained by biota, the overproduction of ROS could subsequently lead to cell membrane damage (Foyer and Shigeoka, 2011). As the lipid peroxidation product, the level of MDA reflects the cell damage under oxidative stress (Oukarroum et al., 2012). It can be seen from Fig. 1K that the MDA content increased with increasing levels of La and Ce, indicating damage of cell membranes as caused by REEs exposure. Overall, the disturbed photosynthesis and induced oxidative stress could explain the observed inhibition of shoot growth of *T. aestivum*.

3.2. Metabolomic response to REEs exposure

3.2.1. Overview of metabolites profiles in T. aestivum exposed to REEs

The above examination of physiological indicators suggests that the adverse effects were induced in the shoots of wheat. Further exploration of the metabolite changes at the molecular level would be helpful to understand the underlying mechanisms of the involved toxicology process and defense response in wheat to REEs exposure (Zhao et al., 2017). Using nontargeted UPLC-QTOF-MS-based metabolomics, a total of 165 and 295 up/downregulated metabolites were identified and semi-quantified under positive mode and negative mode, respectively. A general overview of the clustering information among different treatments was obtained by unsupervised principal component analysis (PCA) (Fig. 2A, B) and supervised partial least squares-discriminant analysis (PLS-DA) (Fig. 2C, D). From the score plots, it can be seen that the La/Ce treatment groups were clearly distinguished from the non-exposed control group and that they showed a dose-dependent trend. Compared to the low exposure dose (0.5 μM) treated samples, the samples under high exposure dose (1.0 μM) of La/Ce were separated more from the samples in the control group. The separation was not so obvious between the groups of La and Ce treatments of the same exposure dose. Furthermore, to obtain a global view of the trend of metabolites change for all treatments, significantly changed metabolites (SCMs) (VIP>1.0, $p < 0.05$) were determined by PLS-DA and ANOVA analysis, subjected to hierarchical clustering analysis and presented in a heatmap. As shown in Fig. S1-S2, more extensive metabolite profile alteration was observed with increasing exposure dose of REEs, but the variation between La and Ce treatments was relatively small. Based on these data, it can be concluded that exposure to REEs induced molecular changes in *T. aestivum*, which was mainly dependent on the exposure dose.

Usually, lanthanides are considered as a chemically uniform group of elements due to their similar physicochemical behavior. Subsequently, a similar behavior and similar biological effects of individual lanthanides could be expected (Khan et al., 2017). The long-term LC50 values for La, Ce, praseodymium (Pr), neodymium (Nd) and gadolinium (Gd) to freshwater crustaceans were evaluated and it turned out that the differences between individual lanthanides were not significant (Blinova et al., 2018). On the other hand, Gonzalez et al. (2015) assessed the toxicity of a light REE (Ce) and two heavy REEs (Gd and Lu) for six aquatic species, and showed that the toxicity of REEs increased with atomic number. When exposing *Hydra attenuate* to eleven REEs, toxicity was found to decrease with increasing atomic number, except for the two heaviest elements (Blaise et al., 2018). Not in accordance with the atomic number order, the toxicity of REEs to *Enchytraeus crypticus* showed a decreasing trend for Ce, Gd and La (Huang et al., 2020b). In the current study, we found that the toxic effects of light REEs (La and Ce) are approximately equal at the molecular level as deduced from comparing the change of the global metabolites profiles.

3.2.2. Disturbed metabolic pathways in T. aestivum exposed to REEs

The results presented above indicate that the intensity of toxic effects of La and Ce on wheat shoots is similar. Furthermore, in order to explore if similar toxicology mechanisms are shared among different lanthanides, the up/down-regulated SCMs were quantified for La and Ce treatments separately and they are shown in Tables S1 and S2. The determined SCMs in positive and negative mode were integrated and

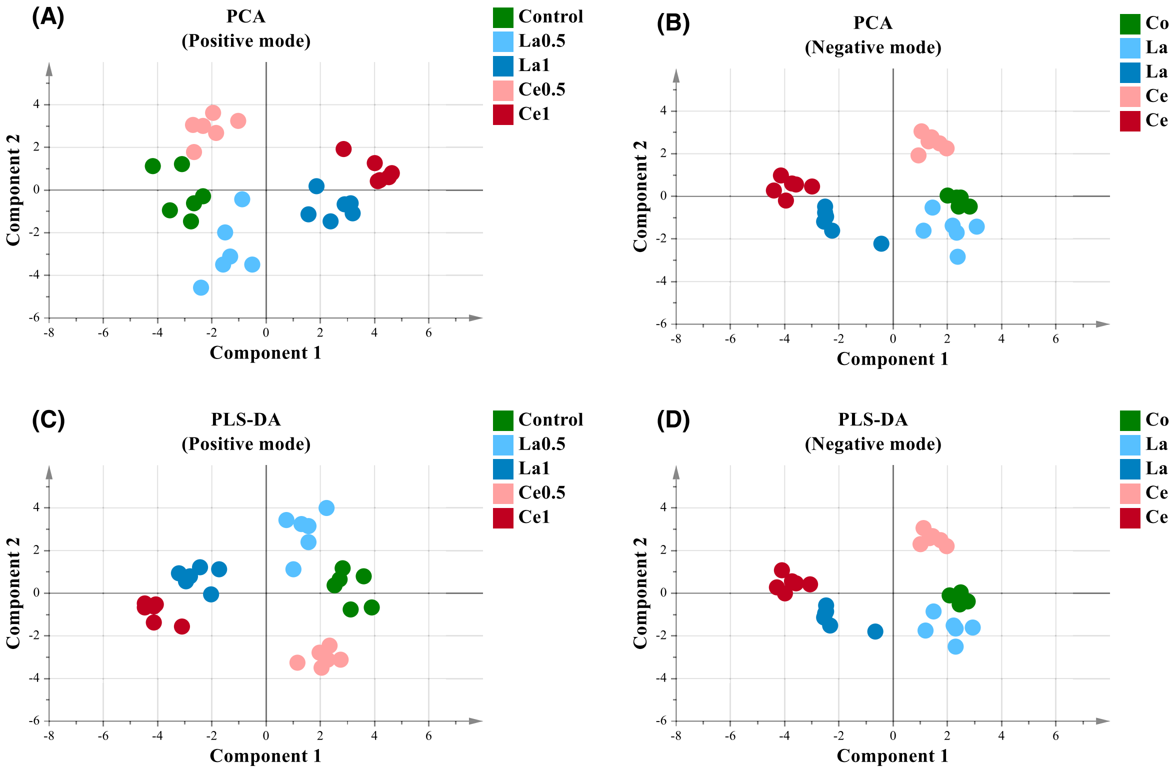

Fig. 2. Analysis of the metabolomic responses of wheat shoots (*Triticum aestivum*) exposed to a low (0.5 μM) and a high level (1.0 μM) of La and Ce, respectively. (A, B) PCA score plots in positive and negative modes; (C, D) PLS-DA score plot in positive and negative mode. Hierarchical clustering of the significantly changed metabolites (SCMs) in positive and negative modes are shown in the heatmap (Fig. S1-S2).

prepared for Venn analysis. Totally, 76 (41.3%) common SCMs responded to both La and Ce treatments, 63 (34.2 %) and 45 (24.5 %) specific SCMs responded to La and Ce treatment, respectively (Fig. 3A). Moreover, the SCMs were imported into MetaboAnalyst 4.0 software for enrichment analysis to determine the corresponding biological pathways. Totally, six metabolomic pathways were determined as significantly (enrichment ratio>2, p value<0.05) disturbed pathways by both La and Ce exposure and one pathway only for Ce exposure (Fig. 3B, C). Overall, the high overlap ratio of SCMs and matched disturbed pathways induced by La and Ce indicates that the underlying toxic mechanisms of La and Ce in wheat shoots are similar to each other. Accordingly, the potential toxicity mechanisms in the entire lanthanide series were assessed by using a toxicogenomics approach, and similar trends were observed among lanthanides (La, Pr, Nd and samarium) of low-molecular weight, while a distinct characteristic behavior was found between lanthanides of low- and high-molecular weight (Pallares et al., 2021).

3.2.2.1. Carbohydrate Metabolism. The significant changes of relative abundance of a number of metabolites relevant to carbohydrate metabolism were determined, mainly including: sucrose, fructose, glucose-6P, maltose, trehalose, and raffinose. These metabolites are involved in the biological pathways of starch and sucrose metabolism, galactose metabolism, and the pentose phosphate pathway (Fig. 4**,** S3-S5). Most of these SCMs were upregulated under REEs exposure, especially at a high exposure level. Consistently, increased contents of carbohydrates, e.g., sucrose, fructose, maltose, trehalose and raffinose, were also observed in leaves of wheat exposed to ionic and nanoparticulate Mo (Huang et al., 2021). Significant accumulation of maltose was detected in green alga (*Chlamydomonas reinhardtii*) treated with a sublethal concentration of mercury (Slaveykova et al., 2021). Under heat stress and freezing stress, the amount of total leaf maltose was increased in *Arabidopsis thaliana* (Kaplan and Guy, 2004). Carbohydrates are produced during photosynthesis and utilized as a source of energy and as precursors for the synthesis of many structural and metabolic components (Ögren, 2000). It has been proven that non-structural carbohydrates, e.g., glucose and starch, could represent important functional traits of plants adaptation to changes in their external environment (Li et al., 2016). The alteration of the contents of carbohydrates indicated the disturbance of photosynthetic system and carbon metabolism of wheat under stress.

Sucrose is a carbohydrate formed from the combination of fructose and glucose, which are important products of photosynthesis (Bahaji et al., 2014). Sucrose can be transported to sink tissues for use as a source of energy or as assimilated carbon in the form of starch for storage reserves (Vriet et al., 2014). The amount and composition of starch deposited in wheat grains is controlled primarily by sucrose transport from source tissues to the grain and its conversion to starch (Kumar et al., 2018). Maltose is produced from starch in plants by hydrolysis and can be further hydrolyzed to glucose and trehalose. The content of maltose in leaves could be elevated when starch breakdown is induced. It was assumed that maltose metabolism in plants makes a bridge between transitory starch breakdown and the adaptation of plants to changes in environmental conditions (Lu and Sharkey, 2006). The obvious change of the contents of sucrose, fructose and maltose detected in this study suggested the potential impact on starch, which could be considered as the major determinant of wheat grain yield and quality (Kumar et al., 2018). Moreover, the role of the precursor of trehalose, trehalose-6P, was shown to be affected and this is a signal for impacts on the regulation of plant metabolism and development (Wingler et al., 2012). Above all, it can be ascertained that exposure to excess concentrations of La and Ce will cause adverse effects on the growth of crop plants.

3.2.2.2. Amino acid metabolism. In total, three biological pathways related to amino acid metabolism were disturbed by REE exposure, including arginine biosynthesis, D-glutamine and D-glutamate metabolism, alanine, aspartate and glutamate metabolism (Fig. 5**,** S6-S8). The disturbance of amino acid metabolism in plants after exposure to contaminants has been reported previously. The levels of amino acids (glycine, serine, L-threonine, L-aspartic acid, L-glutamic acid, and thymine) were, for instance, changed by exposure to nanoparticles of Ag (Huang et al., 2020a). Metabolites, e.g., serine, threonine, aspartic acid and glutamic acid were remarkably affected in *Oryza sativa* L. leaves exposed to pesticides, and six amino acid metabolism pathways were disturbed (Liu and Zhu, 2020). In this study, all of the quantified SCMs involved in the amino acid metabolism were significantly down-regulated under La and Ce treatments, including L-glutamine, L-glutamate, L-aspartate, L-alaine, argininosuccinate and citrate.

Nitrogen is critical to the synthesis of amino acids in plants. Glutamine is the primary product of nitrogen assimilation from inorganic nitrogen sources and is a central metabolite in nitrogen metabolism (Chellamuthu et al., 2014). An altered ability to acquire nitrogen compounds was assumed, as decreased glutamine levels in corn leaves were found when exposed to Mo nanoparticles (Huang et al., 2021). The

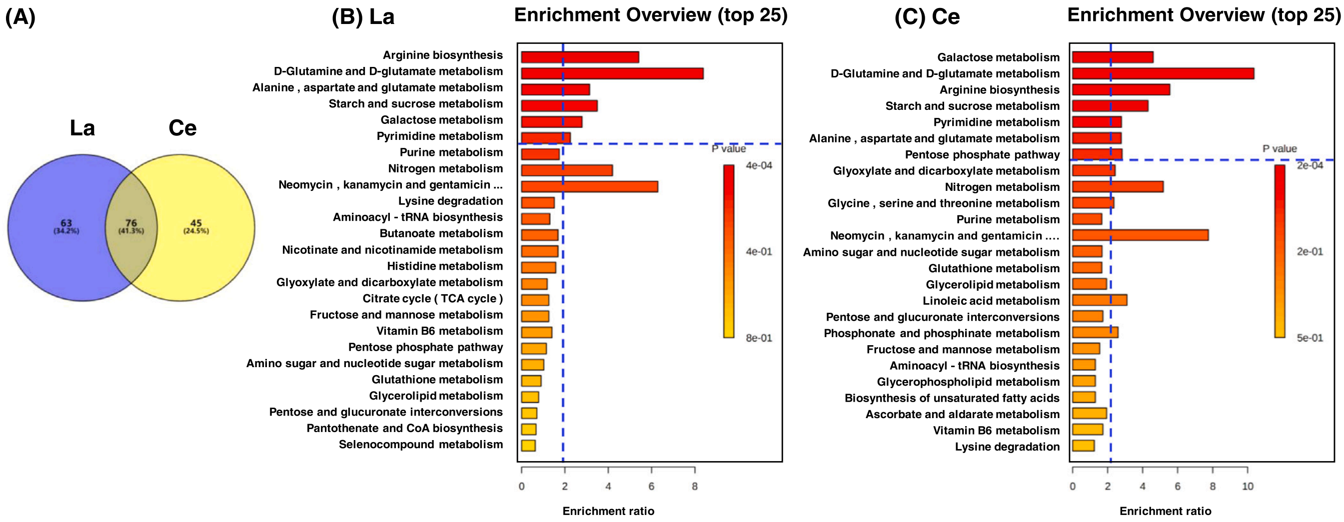

Fig. 3. Venn diagram of significantly changed metabolites (SCMs) in shoot of wheat (*Triticum aestivum*) exposed to La and Ce (A); the enrichment analysis of the SCMs in La (B) and Ce (C) treatment using MetaboAnalyst 4.0 software. Every pillar represents a metabolic pathway, the pathways with p value < 0.05 and enrichment ration > 2 were determined as significantly disturbed.

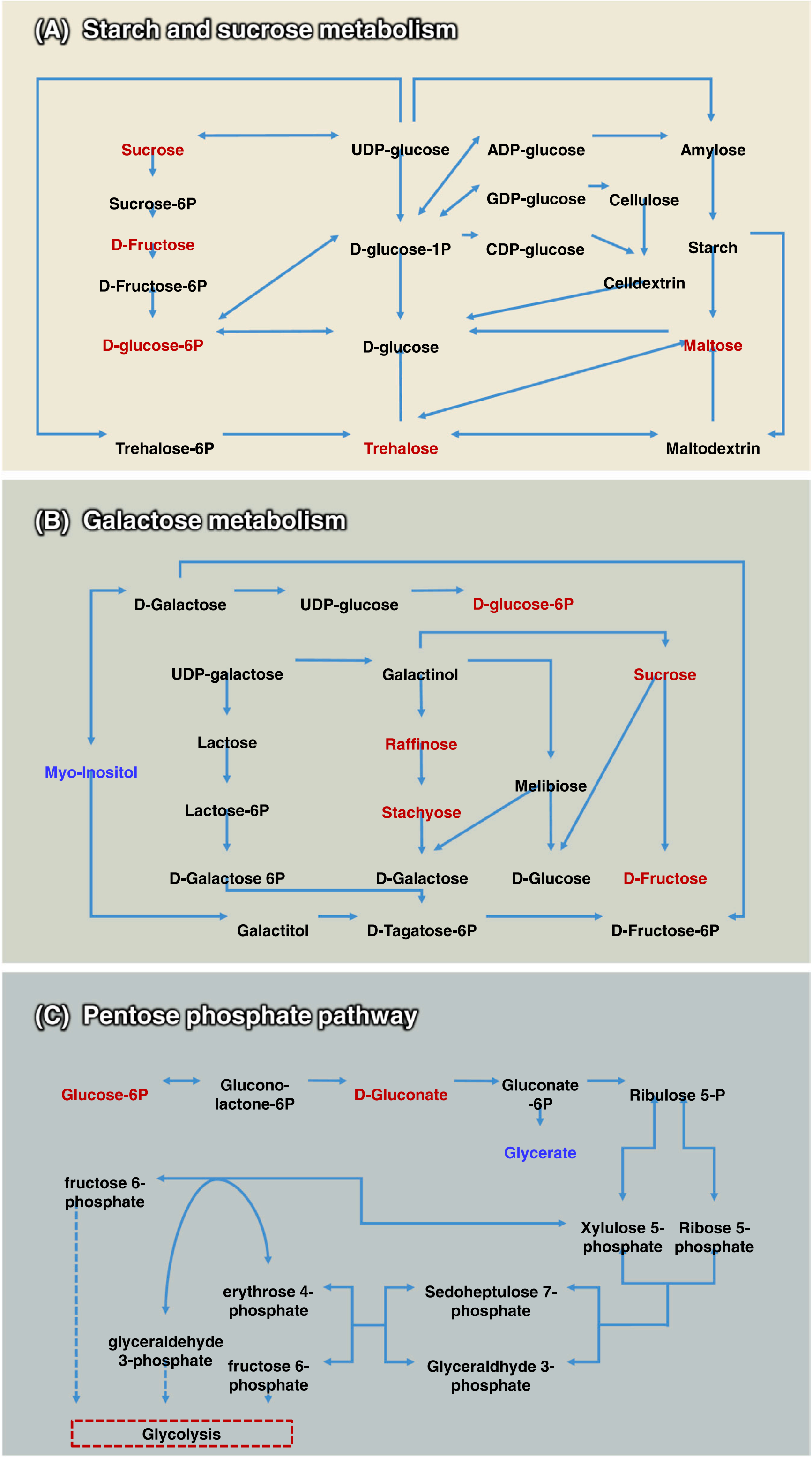

Fig. 4. The perturbed biological pathways related to carbohydrate metabolism in the shoots of wheat (*Triticum aestivum*), including (A) starch and sucrose metabolism, (B) galactose metabolism and (C) the pentose phosphate pathway. The significantly upregulated and downregulated metabolites were marked red and blue, respectively. The change of the relative abundance of the involved significantly changed metabolites (SCMs) exposed to different concentrations of La and Ce are shown in box charts (Fig. S3-S5).

decreased content of glutamine and glutamate in this study could indicate perturbations of nitrogen metabolism, which is pivotal for maintaining metabolic homeostasis and sustaining growth of plants (Nunes-Nesi et al., 2010). Furthermore, amino acids could serve as the building elements of proteins, nucleotides, chlorophyll, signaling molecules and other metabolites in mammalians and plants (Häusler et al., 2014). Aspartate is the precursor for threonine, methionine, lysine, pyrimidines, pantothenate, nicotinamide adenine dinucleotide (NAD) and serves as the nitrogen donor for arginine synthesis (Reitzer, 2014). L-Alanine can be used as the source of numerous nitrogen compounds in plants and acts as the precursor for D-alanine, which is a major component in the biosynthesis of cell wall (Moscoso et al., 2018). The reduced levels of aspartate and alanine further suggested the disturbance of nitrogen metabolism and possible damage of cell membranes.

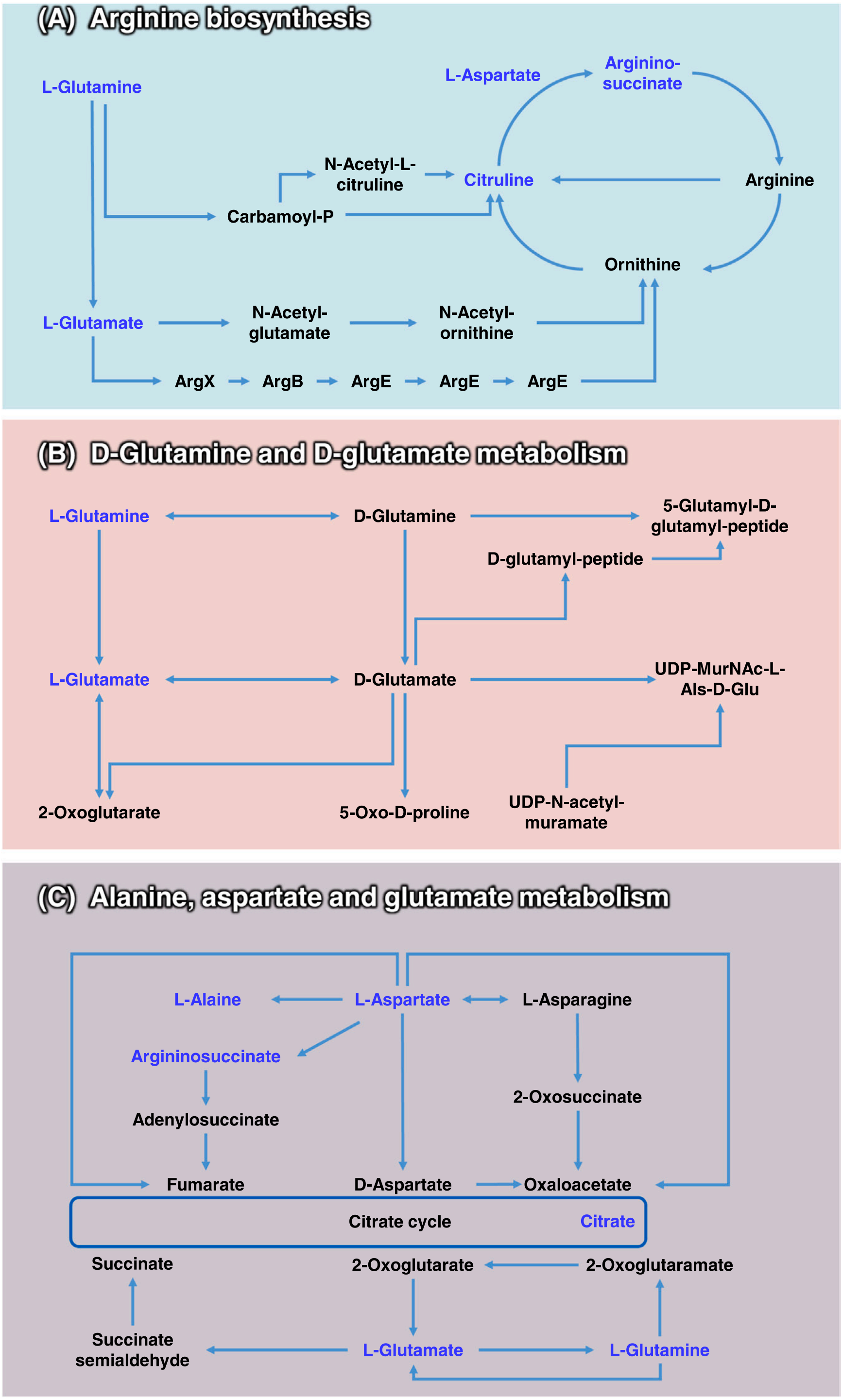

Fig. 5. The perturbed biological pathways related to amino acid metabolism in shoot of wheat (*Triticum aestivum*), including (A) arginine biosynthesis, (B) D-glutamine and D-glutamate metabolism (C) alanine, aspartate and glutamate metabolism. The significantly upregulated and downregulated metabolites were marked red and blue, respectively. The change of relative abundance of the involved significantly changed metabolites (SCMs) exposed to different concentrations of La and Ce are shown in box charts (Fig. S6-S8).

3.2.2.3. Nucleotide/side metabolism. The pathway of pyrimidine metabolism was disturbed, with cytidine 5'-diphosphate (CDP), cytidine 5'-monophosphate (CMP), cytidine, L-glutamine, deoxycytidine and thymidine, being dysregulated (Fig. 6 and S9). Accordingly, the change of nucleotide metabolites has been reported in freshwater snail exposed to the pesticide imidacloprid, with decreasing levels of inosine, uridine and guanosine (Tufi et al., 2015). The pyrimidine nucleobases and corresponding nucleotide/sides, including cytosine, uracil, CMP and uridine, were accumulated in green alga exposed to Hg (Slaveykova et al., 2021). Under environmental stress, e.g., salts, water and light, the change of synthesis and utilization of nucleotides were found in maize, pepper and safflower (Stasolla et al., 2003). The key reaction in pyrimidine synthesis represents the reaction between carbamoyl phosphate and aspartate to form carbamoyl aspartate (Bhagavan and Ha, 2015). The downregulation of nucleosides (cytidine) and nucleotides (CMP and CDP) is consistent with the above detected reduction of aspartate. As is well known, purine and pyrimidine nucleotides are basic component units of nucleic acids in plants, and involved in the synthesis of glycogen and membrane phospholipids (Geigenberger et al., 2005). Therefore, the disturbance of pyrimidine metabolism will subsequently affect the growth and development of plants.

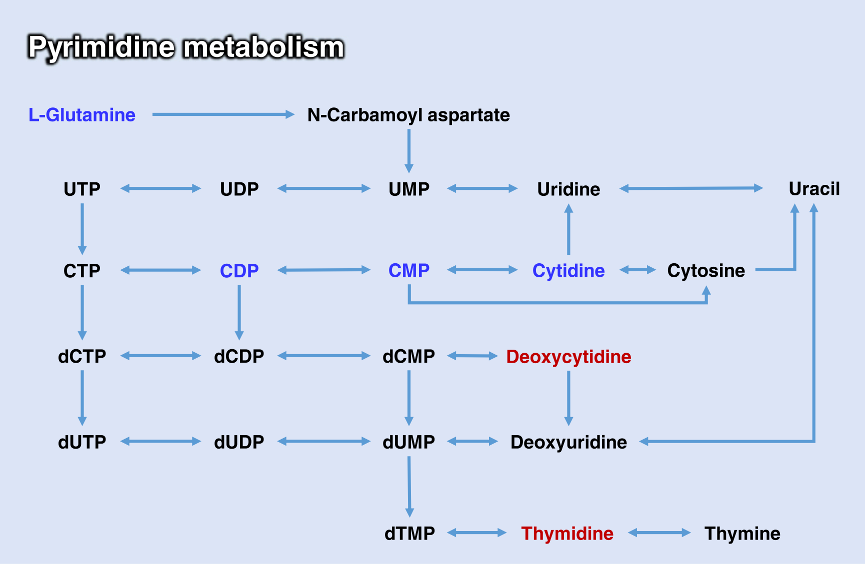

Fig. 6. The perturbed biological pathway related to nucleotide/side metabolism in the shoots of wheat (*Triticum aestivum*), mainly including pyrimidine metabolism. The significantly upregulated and downregulated metabolites were marked red and blue, respectively. The change of the relative abundance of the involved significantly changed metabolites (SCMs) exposed to different concentrations of La and Ce are shown in box charts (Fig. S9).

4. Conclusion

The current study, to our knowledge, for the first time investigated the toxicological mechanism of REEs in the crop plant, *T. aestivum* by combining physiological and nontargeted metabolomic approaches. Generally, changes of metabolite profiles were detected in the shoots of wheat under REEs exposure, with an obvious dose-dependent trend. The determined SCMs and involved biological pathways in La and Ce highly overlapped, indicating their similar mode of action. Through bioinformatics analysis, it turned out that mainly the metabolism of carbohydrates, amino acids and nucleotides/sides were disturbed. These disturbances are indicative of the potential disturbance of the carbon and nitrogen cycles, the damage of cell membranes, and finally the adverse effects on the development and growth of plants. This was further proven by the alterations of key physiological and biochemical indexes of the photosynthetic and antioxidant systems. Therefore, a precise understanding of the ecological and human health risks associated with REEs is desired before their agricultural applications. The choice of appropriate REE dose in fertilizers is especially important for crop safety. Overall, the present work improved our understanding of the underlying toxicity mechanisms of the observed inhibited growth of crop plants induced by REEs. While we focused only on two lanthanide elements, La and Ce, in this study, future toxicology studies should be conducted for more REEs to obtain a comprehensive mechanistic interpretation across the entire series of lanthanides. In this way, it is possible to conclude whether REEs can be considered as a uniform group of elements with a similar mode of action and this finding will facilitate the risk assessment of REEs in mixture scenarios.

CRediT authorship contribution statement

Erkai He: Data curation; Methodology; Formal analysis; Writing - original draft. **Willie J.G.M. Peijnenburg**: Writing - review & editing. **Hao Qiu**: Conceptualization; Writing - review & editing; Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113922.

References

Aggarwal, A., Sharma, I., Tripathi, B.N., Munjal, A.K., Sharma, V., 2011. Metal toxicity and photosynthesis. In: Shigeru, I., Prasanna, M., Guruprasad, K.N. (Eds.), Photosynthesis: Overviews on recent progress & future perspective. IK International Publishing House, New Delhi, pp. 229–236.

Bahaji, A., Li, J., Sánchez-López M., Á., Baroja-Fernández, E., Muñoz, F.J., Ovecka, M., Almagro, G., Montero, M., Ezquer, I., Etxeberria, E., Pozueta-Romero, J., 2014. Starch biosynthesis, its regulation and biotechnological approaches to improve crop yields. Biotechnol. Adv. 32, 87–106.

Bhagavan, N.V., Ha, C.-E., 2015. Nucleotide metabolism. In: Bhagavan, N.V., Ha, C.-E. (Eds.), Essentials of Medical Biochemistry. Academic Press, San Diego, pp. 465–487.

Blaise, C., Gagné, F., Harwood, M., Quinn, B., Hanana, H., 2018. Ecotoxicity responses of the freshwater cnidarian *Hydra attenuata* to 11 rare earth elements. Ecotoxicol. Environ. Saf. 163, 486–491.

Blinova, I., Lukjanova, A., Muna, M., Vija, H., Kahru, A., 2018. Evaluation of the potential hazard of lanthanides to freshwater microcrustaceans. Sci. Total Environ. 642, 1100–1107.

Carpenter, D., Boutin, C., Allison, J.E., Parsons, J.L., Ellis, D.M., 2015. Uptake and effects of six rare earth elements (REEs) on selected native and crop species growing in contaminated soils. PLOS One 10.

Chao, L., Bofu, P., Weiqian, C., Yun, L., Hao, H., Liang, C., Xiaoqing, L., Xiao, W., Fashui, H., 2008. Influences of calcium deficiency and cerium on growth of spinach plants. Biol. Trace Elem. Res. 121, 266–275.

Chellamuthu, V.-R., Ermilova, E., Lapina, T., Lüddecke, J., Minaeva, E., Herrmann, C., Hartmann, Marcus, D., Forchhammer, K., 2014. A widespread glutamine-sensing mechanism in the plant kingdom. Cell 159, 1188–1199.

Chen, J., Le, X.C., 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.

Chen, S.-A., Zhao, B., Wang, X., Yuan, X., Wang, Y., 2004. Promotion of the growth of *Crocus sativus* cells and the production of crocin by rare earth elements. Biotechnol. Lett. 26, 27–30.

d'Aquino, L., de Pinto, M.C., Nardi, L., Morgana, M., Tommasi, F., 2009. Effect of some light rare earth elements on seed germination, seedling growth and antioxidant metabolism in *Triticum durum*. Chemosphere 75, 900–905.

Dinali, G.S., Root, R.A., Amistadi, M.K., Chorover, J., Lopes, G., Guilherme, L.R.G., 2019. Rare earth elements (REE) sorption on soils of contrasting mineralogy and texture. Environ. Int. 128, 279–291.

Fiehn, O., 2002. Metabolomics – the link between genotypes and phenotypes. Plant Mol. Biol. 48, 155–171.

Foyer, C.H., Noctor, G., 2011. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155, 2–18.

Foyer, C.H., Shigeoka, S., 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol. 155, 93–100.

Geigenberger, P., Regierer, B., Nunes-Nesi, A., Leisse, A., Urbanczyk-Wochniak, E., Springer, F., van Dongen, J.T., Kossmann, J., Fernie, A.R., 2005. Inhibition of de novo pyrimidine synthesis in growing potato tubers leads to a compensatory stimulation of the pyrimidine salvage pathway and a subsequent increase in biosynthetic performance. Plant Cell 17, 2077–2088.

Gong, B., He, E., Qiu, H., Li, J., Ji, J., Zhao, L., Cao, X., 2019. Phytotoxicity of individual and binary mixtures of rare earth elements (Y, La, and Ce) in relation to bioavailability. Environ. Pollut. 246, 114–121.

Gonzalez, V., Vignati, D.A., Leyval, C., Giamberini, L., 2014. Environmental fate and ecotoxicity of lanthanides: are they a uniform group beyond chemistry? Environ. Int. 71, 148–157.

Gonzalez, V., Vignati, D.A., Pons, M.N., Montarges-Pelletier, E., Bojic, C., Giamberini, L., 2015. Lanthanide ecotoxicity: first attempt to measure environmental risk for aquatic organisms. Environ. Pollut. 199, 139–147.

Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., Sanganyado, E., 2018. Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci. Total Environ. 636, 299–313.

Häusler, R.E., Ludewig, F., Krueger, S., 2014. Amino acids – a life between metabolism and signaling. Plant Sci. 229, 225–237.

He, E., Qiu, H., 2022. Lanthanum and cerium disrupt similar biological pathways and interact synergistically in *Triticum aestivum* as revealed by metabolomic profiling and quantitative modeling. J. Hazard. Mater. 426, 127831.

He, E., Qiu, R., Cao, X., Song, L., Peijnenburg, W.J.G.M., Qiu, H., 2020. Elucidating toxicodynamic differences at the molecular scale between ZnO nanoparticles and $ZnCl_2$ in *Enchytraeus crypticus* via nontargeted metabolomics. Environ. Sci. Technol. 54, 3487–3498.

Herrmann, H., Nolde, J., Berger, S., Heise, S., 2016. Aquatic ecotoxicity of lanthanum – a review and an attempt to derive water and sediment quality criteria. Ecotoxicol. Environ. Saf. 124, 213–238.

Hu, X., Ding, Z., Chen, Y., Wang, X., Dai, L., 2002. Bioaccumulation of lanthanum and cerium and their effects on the growth of wheat (*Triticum aestivum* L.) seedlings. Chemosphere 48, 621–629.

Hu, Z., Richter, H., Sparovek, G., Schnug, E., 2004. Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: a Review. J. Plant Nutr. 27, 183–220.

Huang, M., Keller, A.A., Wang, X., Tian, L., Wu, B., Ji, R., Zhao, L., 2020a. Low concentrations of silver nanoparticles and silver ions perturb the antioxidant defense system and nitrogen metabolism in N_2-fixing cyanobacteria. Environ. Sci. Technol. 54, 15996–16005.

Huang, X., He, E., Qiu, H., Zhang, L., Tang, Y., Zhao, C., Li, M., Xiao, X., Qiu, R., 2020b. Do toxicokinetic and toxicodynamic processes hold the same for light and heavy rare earth elements in terrestrial organism *Enchytraeus crypticus*? Environ. Pollut. 262, 114234.

Huang, X., Cervantes-Avilés, P., Li, W., Keller, A.A., 2021. Drilling into the metabolomics to enhance insight on corn and wheat responses to molybdenum trioxide nanoparticles. Environ. Sci. Technol. 55, 13452–13464.

Kaplan, F., Guy, C.L., 2004. β-Amylase induction and the protective role of maltose during temperature shock. Plant Physiol. 135, 1674–1684.

Khan, A.M., Bakar, N.K.A., Bakar, A.F.A., Ashraf, M.A., 2017. Chemical speciation and bioavailability of rare earth elements (REEs) in the ecosystem: a review. Environ. Sci. Pollut. Res. 24, 22764–22789.

Kumar, R., Mukherjee, S., Ayele, B.T., 2018. Molecular aspects of sucrose transport and its metabolism to starch during seed development in wheat: a comprehensive review. Biotechnol. Adv. 36, 954–967.

Li, J., Hong, M., Yin, X., 2008. Accumulation and geochemical characteristics of exogenous rare earths in soil of leeward area of tailingsdam of Baotou iron & steel (group) company. Chin. Rare Earths 29, 57–62.

Li, J., Verweij, R.A., Van Gestel, C.A.M., 2018. Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. Chemosphere 193, 412–420.

Li, J., He, E., Romero-Freire, A., Cao, X., Zhao, L., Qiu, H., 2020. Coherent toxicity prediction framework for deciphering the joint effects of rare earth metals (La and Ce) under varied levels of calcium and NTA. Chemosphere 254, 126905.

Li, N., He, N., Yu, G., Wang, Q., Sun, J., 2016. Leaf non-structural carbohydrates regulated by plant functional groups and climate: Evidences from a tropical to cold-temperate forest transect. Ecol. Indic. 62, 22–31.

Li, X., Chen, Z., Chen, Z., Zhang, Y., 2013. A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China. Chemosphere 93, 1240–1246.

Liang, T., Zhang, S., Wang, L., Kung, H.-T., Wang, Y., Hu, A., Ding, S., 2005. Environmental biogeochemical behaviors of rare earth elements in soil–plant systems. Environ. Geochem. Health 27, 301–311.

Lin, F., Sun, J., Liu, N., Zhu, L., 2020. Phytotoxicity and metabolic responses induced by tetrachlorobiphenyl and its hydroxylated and methoxylated derivatives in rice (*Oryza sative* L.). Environ. Int. 139, 105695.

Lipiec, J., Siczek, A., Sochan, A., Bieganowski, A., 2016. Effect of sand grain shape on root and shoot growth of wheat seedlings. Geoderma 265, 1–5.

Liu, D., Lin, Y., Wang, X., 2012. Effects of lanthanum on growth, element uptake, and oxidative stress in rice seedlings. J. Plant Nutr. Soil Sci. 175, 907–911.

Liu, N., Zhu, L., 2020. Metabolomic and transcriptomic investigation of metabolic perturbations in *Oryza sativa* L. triggered by three pesticides. Environ. Sci. Technol. 54, 6115–6124.

Lu, Y., Sharkey, T.D., 2006. The importance of maltose in transitory starch breakdown. Plant Cell Environ. 29, 353–366.

Majumdar, S., Keller, A.A., 2021. Omics to address the opportunities and challenges of nanotechnology in agriculture. Crit. Rev. Environ. Sci. Technol. 51, 2595–2636.

Moscoso, M., García, P., Cabral, M.P., Rumbo, C., Bou, G., 2018. A D-Alanine auxotrophic live vaccine is effective against lethal infection caused by Staphylococcus aureus. Virulence 9, 604–620.

Nunes-Nesi, A., Fernie, A.R., Stitt, M., 2010. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol. Plant 3, 973–996.

Ögren, E., 2000. Maintenance respiration correlates with sugar but not nitrogen concentration in dormant plants. Physiol. Plant. 108, 295–299.

Oukarroum, A., Bras, S., Perreault, F., Popovic, R., 2012. Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. Ecotoxicol. Environ. Saf. 78, 80–85.

Pagano, G., Aliberti, F., Guida, M., Oral, R., Siciliano, A., Trifuoggi, M., Tommasi, F., 2015. Rare earth elements in human and animal health: State of art and research priorities. Environ. Res. 142, 215–220.

Pallares, R.M., Faulkner, D., An, D.D., Hébert, S., Loguinov, A., Proctor, M., Villalobos, J. A., Bjornstad, K.A., Rosen, C.J., Vulpe, C., Abergel, R.J., 2021. Genome-wide toxicogenomic study of the lanthanides sheds light on the selective toxicity mechanisms associated with critical materials. Proc. Natl. Acad. Sci. USA 118, e2025952118.

Pang, X., Li, D., Peng, A., 2002. Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. Environ. Sci. Pollut. Res. 9, 143.

Reitzer, L., 2014. Amino Acid Synthesis, Reference Module in Biomedical Sciences. Elsevier.

Rico, C.M., Hong, J., Morales, M.I., Zhao, L., Barrios, A.C., Zhang, J.-Y., Peralta-Videa, J. R., Gardea-Torresdey, J.L., 2013. Effect of cerium oxide nanoparticles on rice: A Study involving the antioxidant defense system and in vivo fluorescence imaging. Environ. Sci. Technol. 47, 5635–5642.

Severini, A.D., Wasson, A.P., Evans, J.R., Richards, R.A., Watt, M., 2020. Root phenotypes at maturity in diverse wheat and triticale genotypes grown in three field experiments: relationships to shoot selection, biomass, grain yield, flowering time, and environment. Field Crops Res. 255, 107870.

Slaveykova, V.I., Majumdar, S., Regier, N., Li, W., Keller, A.A., 2021. Metabolomic responses of green alga Chlamydomonas reinhardtii exposed to sublethal concentrations of inorganic and methylmercury. Environ. Sci. Technol. 55, 3876–3887.

Stasolla, C., Katahira, R., Thorpe, T.A., Ashihara, H., 2003. Purine and pyrimidine nucleotide metabolism in higher plants. J. Plant Physiol. 160, 1271–1295.

Sun, D., He, N., Chen, Q., Duan, S., 2019. Effects of lanthanum on the photosystem ii energy fluxes and antioxidant system of *Chlorella vulgaris* and *Phaeodactylum tricornutum*. Int. J. Environ. Res. Public Health 16, 2242.

Tommasi, F., Thomas, P.J., Pagano, G., Perono, G.A., Oral, R., Lyons, D.M., Toscanesi, M., Trifuoggi, M., 2021. Review of rare earth elements as fertilizers and feed additives: a knowledge gap analysis. Arch. Environ. Contam. Toxicol. 81, 531–540.

Tufi, S., Stel, J.M., de Boer, J., Lamoree, M.H., Leonards, P.E., 2015. Metabolomics to explore imidacloprid-induced toxicity in the central nervous system of the freshwater snail *Lymnaea stagnalis*. Environ. Sci. Technol. 49, 14529–14536.

Viant, M.R., Ebbels, T.M.D., Beger, R.D., Ekman, D.R., Epps, D.J.T., Kamp, H., Leonards, P.E.G., Loizou, G.D., MacRae, J.I., van Ravenzwaay, B., Rocca-Serra, P., Salek, R.M., Walk, T., Weber, R.J.M., 2019. Use cases, best practice and reporting standards for metabolomics in regulatory toxicology. Nat. Commun. 10, 3041.

Vijver, M.G., van Gestel, C.A.M., Lanno, R.P., van Straalen, N.M., Peijnenburg, W.J.G.M., 2004. Internal metal sequestration and its ecotoxicological relevance: a review. Environ. Sci. Technol. 38, 4705–4712.

Vriet, C., Edwards, A., Smith, A.M., Wang, T.L., 2014. Sucrose and starch metabolism. In: Tabata, S., Stougaard, J. (Eds.), The Lotus Japonicus Genome. Springer Berlin Heidelberg, Berlin Heidelberg, pp. 97–115.

Wang, L., Liang, T., 2015. Geochemical fractions of rare earth elements in soil around a mine tailing in Baotou, China. Sci. Rep. 5, 12483.

Wang, Y.-M., Kinraide, T.B., Wang, P., Zhou, D.-M., Hao, X.-Z., 2013. Modeling rhizotoxicity and uptake of Zn and Co singly and in binary mixture in wheat in terms of the cell membrane surface electrical potential. Environ. Sci. Technol. 47, 2831–2838.

Wen, B., Yuan, D.-A., Shan, X.-Q., Li, F.-L., Zhang, S.-Z., 2001. The influence of rare earth element fertilizer application on the distribution and bioaccumulation of rare earth elements in plants under field conditions. Chem. Speciat. Bioavailab. 13, 39–48.

Wingler, A., Delatte, T.L., O'Hara, L.E., Primavesi, L.F., Jhurreea, D., Paul, M.J., Schluepmann, H., 2012. Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. Plant Physiol. 158, 1241–1251.

Zhang, P., He, E., Romero-Freire, A., Xia, B., Ying, R., Liu, Y., Qiu, H., 2022. Plant intelligence in a rapidly changing world: implementation of plant-plant communications in managed plant systems. Sustain. Horiz. 2, 100008.

Zhao, L., Huang, Y., Keller, A.A., 2017. Comparative metabolic response between cucumber (*Cucumis sativus*) and Corn (*Zea mays*) to a $Cu(OH)_2$ nanopesticide. J. Agric. Food Chem. 66, 6628–6636.

Zhao, L., Huang, Y., Paglia, K., Vaniya, A., Wancewicz, B., Keller, A.A., 2018. Metabolomics reveals the molecular mechanisms of copper induced cucumber leaf (*Cucumis sativus*) senescence. Environ. Sci. Technol. 52, 7092–7100.

Zicari, M.A., d'Aquino, L., Paradiso, A., Mastrolitti, S., Tommasi, F., 2018. Effect of cerium on growth and antioxidant metabolism of *Lemna minor* L. Ecotoxicol. Environ. Saf. 163, 536–543.