Influence of C14 alkane stress on antioxidant defense capacity, mineral nutrient element accumulation, and cadmium uptake of ryegrass

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Abstract
In order to explore the influence of C14 alkane on physiological stress responses, mineral nutrient elements uptake, cadmium (Cd) transfer, and uptake characteristics of Lolium perenne L. (ryegrass), a series of pot trials were conducted which included a moderate level of Cd (2.182 mg·kg⁻¹) without (control) and with five levels of C14 alkane (V/m, 0.1%, 0.2%, 0.5%, 1%, 2%). Biomass and Cd content in the root and shoot, chlorophyll content, antioxidant enzymes activity, and mineral nutrient elements in the shoot of ryegrass were determined at the end of the experiment. The results indicated that Cd uptake significantly elevated at 0.1% C14 alkane treatment, then gradually decreased with the increase of C14 alkane concentration. Compared with the control, chlorophyll content was significantly suppressed and malondialdehyde (MDA) concentration obviously increased. Superoxide dismutase (SOD) activity and catalase (CAT) activity significantly increased to prevent the C14 alkane stress. With the increase of C14 alkane, the Mn concentration gradually increased; Mg and Fe significantly decreased. Correlation analysis showed that Mn was positively correlated with SOD (with the exception of 2% treatment) and CAT (p < 0.01), and negatively correlated with Cd uptake (p < 0.01). It implied that the increase of Mn induced by C14 alkane stress was an important reason for the decrease of Cd uptake.

Keywords C14 alkane · Cd uptake · Antioxidant enzymes · Mineral nutrient elements

Introduction
With the rapid industrialization and urbanization, soil contaminated with heavy metals and organic pollutants has become a severe environmental and human health concern (Dong et al., 2013; Agnello et al., 2016). Among these heavy metals, cadmium (Cd) is one of the most toxic elements, which can induce renal dysfunction, cytotoxicity, and carcinogenicity in humans upon persistent exposure (Zeng et al., 2020). Petroleum hydrocarbon pollutants are recalcitrant compounds and are classified as priority pollutants (Varjani, 2017). The effects of Cd and petroleum on fauna and flora are difficult to predict and require urgent remediation of the co-contaminated soil. Phytoremediation, which has been recognized as a green emerging technology, is a cost-effective, environment-friendly, and esthetically pleasing approach for removing toxic contaminants from polluted soils (Li et al., 2021). The main mechanism involved in the phytoremediation of petroleum is biodegradation by microorganisms stimulated by the rhizosphere (Wang et al., 2011). The phytoremediation of heavy metals is mainly based on the plants to uptake and accumulate contaminants from soil to plant tissues (Chaney et al., 1997).

Cd and petroleum (especially low molecular weight petroleum components) contamination usually leads to impaired growth, decreased production of photosynthetic pigments, imbalanced nutrient uptake, and oxidative damage in plants, which are mainly caused by the production of excess reactive...
oxygen species (ROS) (Adam and Duncan, 2002; Cui et al., 2016; Xie et al., 2018; Zeng et al., 2020). Plants can actively regulate antioxidant activities to defend against stress conditions (Ahammad et al., 2015). Several antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are used to regulate the presence of ROS in plant tissues (Gao et al., 2018). Meanwhile, phytoremediation processes might be influenced by the interactive effects of multiple pollutants on soil processes, plant growth, and rhizosphere biota (Wang et al., 2012a). For example, Lu et al. (2014) found that the presence of heavy metals (Cu, Cd, and Pb, 100–2000 mg·kg⁻¹) significantly reduced both plant growth and pyrene dissipation in soils. It was proved that heavy metals decreased microbial biomass and shifted the community structure, thereby decreased the degradation of organic contaminants (Chigbo et al., 2013). The presence of organic pollutants could either inhibit or favor metal accumulation by plants (Lin et al., 2008; Lu et al., 2014), depending on the pollutant characteristics, plants species, plant growth stages, and experimental conditions (Zhang et al., 2011). Alkio et al. (2005) reported that PAHs can passively penetrate the root cell membranes of plants without any carrier, which can therefore facilitate the penetration of metal or metal complexes into the cells.

In the previous researches about the interactive effects of petroleum and heavy metals on phytoremediation, total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAHs) are the main target pollutants of petroleum (Zhang et al., 2011; Lu et al., 2014; Steliga and Kluk, 2020). Petroleum is a complex mixture of hydrocarbons, and related compounds are generally classified into four fractions: aliphatics (alkanes), aromatics, polar or resins, and asphaltenes (Harayama et al., 1999). Alkanes can constitute 50 to 95% of crude oil, depending on the oil source (Rojo, 2009). Compared to alkanes of larger chain length (C20–C40), those of shorter chain length (C10–C20) are more toxic (Rojo, 2009; Savage et al., 2010; Xia et al., 2014), since they are easily absorbed and act at the cellular level (Baruah et al., 2014). On the other hand, previous studies showed that Chinese soils were mainly polluted by moderate and light Cd levels (<3 mg·kg⁻¹) (Chen et al., 2015), which presented no or slight inhibitory effects on plants growth (Shentu et al., 2008; Wang et al., 2012a). However, in the presence of moderate concentration Cd-contaminated soil, the effect of highly toxic short-chain alkanes (such as C14 alkane) on plant growth and Cd accumulation has not been reported.

Therefore, this study intends to highlight the physiological stress responses, mineral nutrient elements uptake, Cd transfer, and uptake characteristics of *Lolium perenne* L. (ryegrass) to different levels of highly toxic short-chain alkanes (take C14 alkane as an example). Ryegrass is selected based on previous studies about the survival capability and remediation potential of these plants (Rees et al., 2015; Habibul et al., 2019). The main aims are as follows: (1) to determine the impact of C14 alkane on the physiological response of ryegrass, including photosynthetic pigments, lipid peroxidation (MDA), and antioxidative enzymes system (SOD, CAT, and POD); (2) to examine the influence of C14 alkane on the uptake of main mineral nutrient elements (K, Na, Ca, Mg, Fe, Mn, Cu, and Zn); and (3) to elucidate the influence of C14 alkane on phytoextraction potential of ryegrass in Cd-contaminated soil.

### Materials and methods

#### Chemicals and soil

All the chemicals used in this study were analytical grade or higher, which were obtained from Sinopharm Chemical Reagent Co., Ltd (China). Standard solutions of Cd and mineral nutrient elements were purchased from the National reference material (RM) Resources Network. All the solutions were prepared used ultrapure water (Milli-Q, IQ 7000, Millipore, France).

The unpolluted soil was obtained from arable surface soil (10–20 cm depth) near the Institute of Applied Ecology in Liaoning province, China. The main physicochemical properties of the unpolluted soil are analyzed and presented in Table S1 of the Supporting Information. The soil type was loamy clay soil.

#### Experimental design

The soil sample was air-dried and grounded to pass through a 0.84-mm sieve. Cadmium alone and Cd with various concentrations of C14 alkane–co-contaminated soil were obtained by spiking the cadmium nitrate and C14 alkane (CAS: 629–59-4, >98% purity) with a standard solution of cadmium nitrate prepared (2.5 mg·L⁻¹), and soil was mixed thoroughly with the rate of soil: solution (m/V) = 1:1 and maintained in a pot at room temperature for 1 month. Thereafter, the Cd-contaminated soil was air-dried again and sieved through a 0.84-mm sieve. The total concentration of Cd in the soil was analyzed and the value was 2.182 mg·kg⁻¹. Then, measured amounts of C14 alkane were dissolved in n-hexane and thoroughly mixed into a measured amount of Cd-contaminated soil. To ensure uniform distribution of the C14 alkane and complete volatilization of n-hexane, the soil was agitated in a fume hood once a day for 2 weeks. The concentrations of C14 alkane in the experimental soils were expressed as the ratio of C14 alkane volume (ml) to soil mass (g) and the final concentrations were 0.1%, 0.2%, 0.5%, 1.0%, and 2.0%.
A total of six treatments were conducted including five treatments with Cd and different concentrations of C14 alkane (0.1%, 0.2%, 0.5%, 1.0%, and 2.0%)—co-contaminated soil and a control treatment with Cd alone—contaminated soil (Table S2). The experiment tests were carried out in a rectangular pot which has dimensions of 22 cm (length) × 16 cm (width) × 12.5 cm (height). Each treatment was conducted with three pot replications. Each pot was loaded with approximately 4.5 kg of contaminated dry soil and the soil was irrigated with tap water. Ryegrass seeds were obtained from Suqian Sunrise Seed Industry Co., Ltd (China). The seeds were sterilized with H2O2 (30%, V/V) for 30 min, and then rinsed several times with Milli-Q water before being sown. Then, the seeds were sown directly into the soil when the soil water content declined to approximately 18%. Six rows of ryegrass were evenly sown in each pot and 2.0 g seeds were planted in each row. The pots were placed under an artificial light source which was provided from fluorescent lamps with an average light intensity of 54 µmol·m−2·s−1. It was run with an automatic timer to provide a 16/8 h light/dark cycle. The temperature of the greenhouse was maintained at 24–26 °C in light and 18–20 °C in dark. The pots were watered every other day and no fertilizers were added.

After 60 days of growth of plants, the leaves were collected for chlorophyll and antioxidant enzyme analysis. Then, roots and shoots of plants were harvested separately from each pot. The fresh samples were washed with tap water and rinsed with purified water, then the moisture on the samples was gently removed with blotting paper. After being washed with purified water, the roots were soaked in 20-mM ethylenediaminetetraacetic acid disodium salt (Na2 EDTA) for 15 min to remove metal ions in the root surface, then rinsed three times with purified water. The washed plant samples were heated at 105 °C for 20 min and dried to constant weight in an oven at 70 °C. Then, the dry plant biomass was measured and recorded.

**Analytical method**

**Soil pH**

Soil pH was measured using a pH meter (S220 seven compact, Mettler—Toledo International Inc., Switzerland) by preparing slurries with soil to water ratio of 1:2.5 (Yuan et al., 2021).

**Chlorophyll content**

The total chlorophyll, chlorophylls a and b, and carotenoid contents were extracted with 80% ethanol. Absorbance was determined at 470 nm, 665 nm, and 649 nm using an ultraviolet–visible spectrophotometer. The concentrations of chlorophylls a, chlorophylls b, and carotenoid (mg·g−1 fresh leaf weight (FW)) were calculated using the equations of a previous study (Lichtenthaler and Wellburn, 1983).

**Malondialdehyde and enzyme activity**

The level of lipid peroxidation was expressed as the content of MDA (nmol·g−1 FW). The fresh leaves were homogenized in trichloroacetic acid (TCA) solution, then reacted with thiobarbituric acid (TBA). The absorbance of the supernatant was measured at 440 nm, 532 nm, and 600 nm, and the MDA content was calculated according to a previous study (Hodges et al., 1999). The SOD activity was measured using the nitro blue tetrazorium (NBT) method (Beauchamp and Fridovich, 1971). The fresh leaves were homogenized with 0.05 M sodium phosphate buffer and centrifuged at 4000 g for 10 min for POD and CAT activity analysis. The POD activity was determined on the basis of guaiacol oxidation by hydrogen peroxide (Zhang et al., 2007). The CAT activity was assayed with a method as described by a previous study (Zhang et al., 2005).

**Cd and mineral nutrient elements**

The dry shoots and roots of ryegrass were digested with HNO3 + HClO4 (Zhang et al., 2005), and the supernatant was filtered through a 0.22-µm filter. Then, the concentrations of Cd and mineral nutrient elements were analyzed by an inductively coupled plasma mass spectrometer (ICP-MS, iCAP RQ, Thermo Fisher Scientific, USA).

**Statistical analyses**

The Cd uptake amount of shoot or root was calculated using the equation suggested by Wang et al. (2020):

\[
U_{Cd} = C_{Cd} \times M
\]

where \(U_{Cd}\) (ug·pot−1, dry weight) is the Cd uptake amount of shoot or root; \(C_{Cd}\) (mg·kg−1, dry weight) is the Cd concentration of shoot or root. \(M\) (g) is the dry weight of shoot or root.

Each treatment was conducted in triplicate and the results were reported as mean ± SD. Analysis of variance ANOVA was used to detect the differences among treatments. Statistical significance was defined as \(p < 0.05\) (IBM SPSS Statistics 22, New York, USA). Bivariate correlations analyses were performed and significance was accepted at \(p < 0.05\) in all cases (Person’s correlation). Principal component analysis (PCA) was performed to assess the possible correlations among mineral nutrient elements and chlorophyll content and antioxidant enzyme activity. Redundancy analysis (RDA) was performed to determine the factors that
influenced the Cd uptake. PCA and RDA were conducted using R (version 4.0.2). Origin software (version 8.0, Origin Lab Corporation, Northampton, USA) was used to prepare graphs.

**Results and discussion**

**Effect of C14 alkane on biomass of ryegrass**

Biomass yield was measured to examine the effects of C14 alkane on the health of ryegrass. As shown in Fig. 1, the dry biomass yield of shoot and root of ryegrass gradually decreased with the increase of C14 alkane concentration. A significant decrease of shoot biomass was occurred at 0.2% C14 alkane treatment, while at 0.5% C14 alkane treatment, a more dramatically inhibitory effect was observed and the percentage of inhibition was approximately 58.67% of the control treatment. The ryegrass root biomass also significantly decreased when C14 alkane was added to soil; the percentage of decrease at 2% C14 alkane treatment was approximately 46.34% of the control treatment. Toxic effects of organic compounds on plant growth and biomass have already been observed in many plants (Xie et al., 2017; Gao et al., 2018; Xi et al., 2018), which was consistent with this research.

**Effect of C14 alkane on Cd concentration and accumulation of ryegrass**

After different concentrations of C14 alkane treatments, the Cd concentration and uptake amount in the shoot and root of ryegrass are shown in Fig. 1. The results indicated that the presence of C14 alkane in soil significantly affected the Cd concentration and uptake amount of ryegrass. Compared with the control treatment, Cd concentration in shoot and root of ryegrass significantly increased at 0.1% C14 alkane treatment, then gradually decreased with the increase of C14 alkane concentration. When C14 alkane concentration increased to 1%, Cd
content in the shoot of ryegrass was significantly lower than that in the control treatment. However, Cd content in the root of ryegrass at 2% C14 alkane treatment presented no significant difference with control treatment.

With the increase of C14 alkane concentration, the variation trend of the Cd uptake amount in shoot and root of ryegrass was similar with that of Cd concentration. The maximum value of the Cd uptake amount was observed at 0.1% C14 alkane treatment, which was 30.17 μg·pot⁻¹ in shoot and 4.44 μg·pot⁻¹ in the root. Compared with the control, the Cd uptake amount at 0.1% C14 alkane treatment was increased by 69.9% in shoot and 48.8% in the root. A similar phenomenon was found by Lu et al. (2014) that the pyrene addition significantly increased Cu, Cd, and Pb concentrations of both roots and shoots. Similar to PAHs, C14 alkane can passively penetrate the root cell membranes of ryegrass, then facilitate the penetration of metal or metal complexes into the cells, which may be one of the explanations for the increased concentration of Cd in root and shoot of ryegrass (Lu et al., 2014). However, the Cd uptake amount gradually decreased with the increase of C14 alkane concentration. When the C14 alkane concentration increased to 0.5%, the Cd uptake amount in shoots of ryegrass was significantly lower than that at the control treatment. Compared with the control treatment, the Cd uptake amount at 2% C14 alkane treatment was reduced by 85.1% in shoot and 47.8% in the root. At 0.5% C14 alkane treatment, the decrease of ryegrass biomass was the main reason for the decrease of the Cd uptake amount in the shoot. When C14 alkane concentration increased to 1%, both biomass and Cd concentration had a significant effect on the decrease of the Cd uptake amount in the shoot. Gao and Zhu (2004) also reported that the concentrations of phenanthrene and pyrene above certain levels (133 and 172 mg·kg⁻¹ DW) decreased the dry weight of 12 plant species.

Soil pH was an important factor affecting Cd solubility in soil and its availability to plants (Wang et al., 2020). Thus, the soil pH before and after ryegrass planted are presented in Fig. S1. The results indicated that C14 alkane concentration just had a slight effect on soil pH before the ryegrass was planted. After the ryegrass was planted, soil pH significantly decreased at the control treatment. Plant rhizosphere exuded organic acids to soil which may be an important reason for soil pH decrease at the control treatment (Yuan et al., 2021). With the increase of C14 alkane concentration, soil pH gradually increased. And soil pH presented no significant difference before and after the ryegrass was planted at 2% C14 alkane treatment. The results indicated that the presence of C14 alkane inhibits the growth of ryegrass and reduces the release of organic acids to soil. The change of soil pH was a reason for the reduction of Cd concentration in ryegrass.

Effect of C14 alkane on chlorophyll content of ryegrass

Chlorophyll a, chlorophyll b, and carotenoid concentrations in leaves of ryegrass grown in different concentrations of C14 alkane–contaminated soil are presented in Fig. 2. The results revealed that chlorophyll contents in ryegrass were gradually diminished with increasing concentration of C14 alkane, although differences in chlorophyll contents for some treatments were not significant. Compared with the control treatment, chlorophyll a, chlorophyll b, and carotenoid concentrations of ryegrass at 2% C14 alkane treatment were reduced by 16.37%, 23.16%, and 16.60%, respectively. The concentration of C14 alkane had a more significant effect on chlorophyll b than that on chlorophyll a and carotenoid.

Effect of C14 alkane on membrane lipid peroxidation and antioxidant enzyme activities of ryegrass

The MDA concentration was used as the general indicator of the extent of membrane lipid peroxidation (Ahamed et al., 2012). Elevation of the MDA contents indicated higher lipid peroxidation and over-production of reactive oxygen species (ROS) resulting from environment stress (Wang et al., 2006; Choudhary et al., 2011; Xi et al., 2018). The MDA contents in leaves of ryegrass grown in different concentrations of C14 alkane–contaminated soil are presented in Fig. 3a. Compared with the control treatment, the MDA contents in ryegrass significantly increased in all the C14 alkane treatments. When the C14 alkane concentration was less than 0.5%, the increased percentage of MDA concentrations was approximately 60% of control treatment, and no significant disparities among different C14 alkane treatments were shown. When the C14 alkane concentration increased to 1%, the MDA concentrations sharply increased. The highest MDA content was observed at 2% C14 alkane treatment, which was 160.2% higher than that in the control treatment. To prevent cell damage, antioxidant enzyme systems were elevated among plants for coping with environmental stress. SOD, CAT, and POD were three vital enzymes that can scavenge ROS in plant cells. SOD was the first defense against ROS since it can catalyze the conversion of superoxide radicals into hydrogen peroxide (Gao et al., 2018). The SOD activity in the leaves of ryegrass is shown in Fig. 3b. The SOD activity first gradually increased with an increase of C14 alkane concentration. The maximum value of SOD activity was observed at 1% C14 alkane treatment, which was increased by 4082% compared with that in the control. While, the superoxide radicals as well as other radicals can inactivate antioxidant enzymes (Gao et al., 2017). The SOD activity significantly decreased when the C14 alkane concentration was higher than 1%. It suggested that the
superoxide radical production exceeded the ability of SOD to scavenge it. The CAT activity was the primary $H_2O_2$ scavenging enzyme in plant cells; $H_2O_2$ can be reduced to $H_2O$ and $O_2$ by it (Li and Yi, 2012). Compared with the control treatment, the CAT activity had a slight increase when C14 alkane concentration was in the range of 0.1 to 1%, then significantly increased at 2% C14 alkane treatment (Fig. 3c). The CAT activity at 2% C14 alkane treatment increased by 121.8% compared with that in the control. The CAT results were consistent with the increase of MDA contents at 2% C14 alkane treatment as shown above. The elevated CAT activity was associated with the increase of ROS, which was a signal molecule inducing the expression of the CAT gene (Wang et al., 2012b).

POD was a defense enzyme that can scavenge highly toxic ROS major produced by SOD to plant cells (Zhou et al., 2016). POD had been reported to reduce $H_2O_2$ using phenolic compounds or flavonoids as donors of electrons (Mitsou et al., 2006). The POD activity in the leaves of ryegrass at different C14 alkane concentration treatments is shown in Fig. 3d. Compared with the control treatment, the POD activity showed a slight increase trend at 0.1% C14 alkane treatment, then gradually decreased at the following two C14 alkane treatments (0.2% and 0.5%), and finally showed an increasing trend again when the C14 alkane concentration was higher than 0.5% (Fig. 3d). The minimum value of POD activity was observed at 0.5% C14 alkane treatment, which was 26.06% lower than that in the control treatment. The results indicated that C14 alkane concentration just had a little impact on the POD activity of ryegrass. Based on the above analysis, we can conclude that SOD and CAT were the main antioxidant enzymes in ryegrass to prevent the C14 alkane stress.

**Effect of C14 alkane on mineral nutrient elements uptake of ryegrass**

The concentrations of mineral nutrient elements (K, Na, Ca, and Mg, Fe, Mn, Cu, and Zn) in shoots of ryegrass grown in different concentrations of C14 alkane–contaminated soil are shown in Fig. 4. The results indicated that the presence of C14 alkane in soil affected the uptake of all the measured elements. As shown in Fig. 4a, the K concentration in shoots of ryegrass showed a fluctuant trend with the increase of C14 alkane concentration. While the Na concentration decreased in the ryegrass grown in all the C14 alkane–contaminated soil. The Ca concentration showed a slight decrease, and no conspicuous changes were observed between different concentrations of C14 alkane treatment. The Mg concentration obviously decreased in different C14 alkane treatments and showed a fluctuant trend with the increase of C14 alkane concentration. The Fe concentration significantly decreased at all the C14 alkane treatments compared with the control treatment. The Mn concentration presented a gradually increasing trend with the increase of C14 alkane concentration. The variation trend of Cu and Zn concentration in
ryegrass was similar and showed no pronounced changes with the increase of C14 alkane concentration.

The correlation of chlorophyll content, antioxidant enzyme activities, and mineral nutrient elements in the shoots are analyzed and listed in Table S3. In addition, PCA was used to assess the relationship among the chlorophyll content, antioxidant enzyme activities, and mineral nutrient elements (Fig. 5). The cumulative contribution ratio of PCA from the first two principal components was 74.5%. As the results showed, chlorophyll content and antioxidant enzyme activities were correlated with many mineral nutrient elements. Specifically, chlorophyll contents were positively correlated with K, Na, Ca, Mg, Fe, and negatively correlated with Mn. MAD was positively correlated with Mn and negatively correlated with K, Na, Mg, and Fe. POD activity was positively correlated with Cu and Zn. CAT activity was positively correlated with Mn and negatively correlated with K and Na. SOD was negatively correlated with K, Ca, and Mg. As mentioned above, the influence of C14 alkane on the root cell membranes of ryegrass increased the concentration of Cd in the root and shoot of ryegrass. However, for mineral nutrient elements, the influence of C14 alkane just facilitated the uptake of Mn in the shoot. As C14 alkane directly destructed the root cell membranes of ryegrass, the uptake of mineral nutrient elements in the root may be increased. It will be carried out in further study.

With the increase of C14 alkane concentration, the Mn concentration in the shoots of ryegrass gradually increased (Fig. 4f). Correlation analysis showed that the Mn concentration was significantly and positively correlated with MDA (Table S3 and Fig. 5). These results indicated that the oxidative stress of C14 alkane induced the increase of Mn concentration in the shoot of ryegrass. Carvalho et al. (2019) also reported a similar phenomenon that Mn concentration significantly increased after Cd exposure. Previous studies had proved that supplemental Mn can reduce plants’ oxidative damage induced by Cd or salt (Cramer and Nowak, 1992; Rahman et al., 2016). Manganese played a vital role as a cofactor in Mn-SOD and Mn-CAT, which participate in the plant’s defense against oxidative stress (Pittman, 2005). Meanwhile, Mn can act as a scavenger of superoxide and hydrogen peroxide (Pittman, 2005; Millaleo et al., 2010). In the present study, Mn concentration was significantly correlated (p < 0.01) with CAT activity (Table S3, Fig. 5), which was consistent with previous researches (Rahman et al., 2016). However, no significant correlation was observed between Mn concentration and SOD activity in shoots of ryegrass. In the 2% C14 alkane treatments, SOD activity in ryegrass dramatically decreased (Fig. 3b), which may be inactivated by superoxide radicals and other radicals (Ahamed et al., 2012). With the exception of the 2% treatment, Mn concentration was significantly and positively correlated with SOD activity (r = 0.752, p < 0.01). We can conclude that the increased Mn concentration in shoots of ryegrass was a self-protection mechanism to reduce C14 alkane toxicity.

When the ryegrass was grown in the C14 alkane–contaminated soil, Mg and Fe concentrations in the shoot significantly decreased (Fig. 4d, e). The increasing uptake of Mn may be a reason for inhibiting the uptake of Mg and
Fe (Heenan and Campbell, 1981). The results were similar to the previous studies which found that Mn concentration in the stem tissues of Lactuca spp. significantly increased but other essential micronutrients decreased under the stress of Cd (Ramos et al., 2002). Mg was an essential macro-element participating in the enzymatic reactions and photosynthesis as a structural component of the chlorophyll molecule (McSwain et al., 1976; Wang et al., 2017). Fe, which is located mainly in the photosynthetic membranes, had a significant influence on the structure and function of chloroplasts (Zembala et al., 2010; Liu et al., 2017). Combined with the results of the correlation analysis, we can conjecture that the decrease of Mg and Fe concentrations in shoots of ryegrass under C14 alkane stress was an important reason for the decrease of chlorophyll content and ryegrass growth.

The Cd uptake amount in the shoot of plants was an important parameter for determining the efficiency of phytoremediation, since this part can be harvested easily and treated. Biomass and Cd content in plants were two important indices that affected the Cd uptake amount. As indicated above, the Cd uptake amount obviously increased at 0.1% C14 alkane treatment. The bioremediation of C14 alkane
may decrease soil pH with a consequent solubilization of metals (Chen et al., 2004). In addition, Alkio et al. (2005) reported that PAHs can passively penetrate the root cell membranes of plants without any carrier, which can therefore facilitate the penetration of metal or metal complexes into the cells. The C14 alkane might also facilitate the penetration of metal into the cells in a similar way as PAHs did. However, the Cd uptake amount gradually decreased with the increase of C14 alkane concentration. In order to reveal the primary factors that affected the Cd uptake, the relationship between the Cd uptake indices (biomass, Cd content, Cd uptake amount) and chlorophyll content, antioxidant enzyme activities, and mineral nutrient elements was studied using an RDA (Fig. 6) and Pearson's correlation (Table S4). According to the RDA of the Cd uptake with respect to the mineral nutrient elements (Fig. 6a), the first two ordination axes explained 95.48% of the total variation in the Cd accumulation, with the first axis explaining 93.87% and the second axis explaining 1.61%. The Cd uptake amount showed a positive correlation with Fe and Mg, but a negative correlation with Mn. According to the RDA of the Cd uptake with respect to the chlorophyll content and antioxidant enzyme activities (Fig. 6b), the first two ordination axes explained 88.25% of the total variation in the Cd uptake, with the first axis explaining 86.92% and the second axis explaining 1.33%. The Cd uptake amount showed a positive correlation with chlorophyll content, but a negative correlation with MAD.

The growth characteristics of plants were one of the most important indicators used for the phytoremediation of contaminated soil (Zeng et al., 2020). As Table S4 and Fig. 6 show, significant positive relationships were discovered between biomass and chlorophyll contents. The chlorophyll contents reflect the photosynthesis ability of plants, which was one of the most essential processes of plant growth and development (Ahammed et al., 2012; Zeng et al., 2020).
Meanwhile, biomass was positively correlated with K, Na, Ca, Fe, and Mg. As analyzed above, chlorophyll contents were also positively correlated with K, Na, Ca, Mg, and Fe, and we conjectured that the decrease of Mg and Fe was an important reason for the decrease of chlorophyll content. In other words, the decrease of Mg and Fe was an important reason for the decrease of biomass, which was consistent with previous results (Nazar et al., 2012).

In this study, the Cd concentration in the shoot of ryegrass was positively correlated with K, Na, and chlorophyll a and negatively correlated with Mn, MAD, and CAT (Table S4 and Fig. 6). Previous studies had proved that the addition of K as KCl or K2SO4 increased Cd concentrations in plant shoots of both Brookton and Krichauff (Zhao et al., 2004). But K and Na concentration in the shoot of ryegrass just had a slight decrease when C14 alkane concentration was in the range of 0.1 to 0.5% (Fig. 4a, b). In addition, Cd did not have a known biological function in plants and enters plant cells mainly via cation channels of Ca, Mg, and Fe or transporters of other divalent cations (Huang et al., 2020). Therefore, we believed that the influence of K and Na on the Cd concentration in the shoot of ryegrass was limited.

Many researchers observed competitive uptake between Cd and Mn, Ca, Zn, and Fe, because Cd and Mn, Ca, Zn, and Fe use some common transporters for their uptake and translocation in plants (Rahman et al., 2016; Huang et al., 2020). As mentioned above, Mn concentration in the shoot of ryegrass significantly increased with the increase of C14 alkane concentration (Fig. 4f). Previous studies using distinct plant species evidenced that the increased shoot Mn accumulation reduced Cd uptake (Carvalho et al., 2019). In the presence of Cd, Peng et al. (2008) proved that adding Mn to the solution significantly reduced the concentrations of Cd in all organs of the plant. Thus, the increase of Mn in the shoots of ryegrass might be an important reason for the decrease of Cd concentration (Fig. 7).

**Conclusions**

In this study, the obtained results indicated that 0.1% C14 alkane treatment significantly increased the Cd uptake amount, then gradually decreased with the increase of C14 alkane concentration. Under the stress of C14 alkane, reactive oxygen species in the shoot of ryegrass significantly increased. To prevent C14 alkane toxicity, SOD and CAT activity significantly increased. Meanwhile, C14 alkane stress induced the increase of Mn content, which might play a critical role in defense C14 alkane toxicity. Cadmium, Fe, and Mg contents in the shoot of ryegrass significantly decreased as the competitive uptake with Mn. The decrease of Mg and Fe concentrations was an important reason for the decrease of chlorophyll content and ryegrass growth. Thus, we conclude that the increase of Mn concentration induced by the stress of C14 alkane directly or indirectly affected the growth and Cd content of ryegrass, and ultimately influenced the Cd phytoremediation efficiency. This study will help us to understand the influence mechanism of C14 alkane on the phytoremediation efficiency of Cd.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-16806-x.

**Author contribution** Lizhu Yuan: performed the experiments, data processing, draft preparation. Penghong Guo: reviewing, revising, editing the manuscript. Shuhai Guo: conceived and designed experiments. Jianing Wang: conceptualization, methodology. Yujie Huang: participated in some tests. All authors read and approved the final manuscript.

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**Fig. 7** The influence mechanism of C14 alkane stress on Cd uptake of ryegrass. A: The increase of Mn induced by C14 alkane increased SOD and CAT to reduce C14 alkane toxicity to ryegrass. B: The competitive uptake with Mn decreased the Cd, Fe, and Mg contents; C: The decrease of Mg and Fe decreased chlorophyll content and inhibited the growth.
Data availability The authors declare that (the/all other) data supporting the findings of this study are available within the article (and its supplementary information files).

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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