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Effect of pyrene-induced changes in root activity on growth of Chinese cabbage (*Brassica campestris* L.), and the health risks caused by pyrene in Chinese cabbage at different growth stages

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Abstract

Background: Polycyclic aromatic hydrocarbons (PAHs) pose a potential risk to ecological safety and human health. They have a range of effects on plant growth and there have been few reports on the health risks associated with ingestion of vegetable crops at different growth stages.

Methodology: In this study, a pot experiment in which Chinese cabbage (*Brassica campestris* L.) were grown in a greenhouse for 75 days was used to investigate the dose–effect relationship of pyrene with plant growth and also the exposure risk for adults of ingestion of Chinese cabbage at different growth stages.

Results: The results showed that low doses of pyrene (5–45 mg kg^{-1}) promoted plant growth (20–220% and 55–97% higher than control treatment for the root biomass and shoot biomass, respectively), but significant inhibition was observed at a high dose (405 mg kg^{-1}) (41–66% and 43–91% lower than control treatment for the root biomass and shoot biomass, respectively). High doses of pyrene reduced soil bacterial abundance and diversity during the growth of Chinese cabbage, and increased malondialdehyde (MDA) levels in the plant. The effects of pyrene on plant biomass were mainly attributed to changes in root activity induced by pyrene, as the relationship between soil pyrene concentration and biomass was similar to that between soil pyrene concentration and root activity. Furthermore, structural equation modeling analysis showed that pyrene altered growth of the vegetable by directly affecting root activity. The incremental lifetime cancer risk for adults is highest for ingestion of Chinese cabbage at the seedling stage, followed in decreasing order by the rosette stages and heading stages.

Conclusions: The health risk of consumers who have the possibility to ingest the Chinese cabbage planted in pyrene-contaminated soil would be decreased with the increasing growth periods. However, further studies are required to confirm the dose–effect relationship between pyrene concentration and Chinese cabbage growth on a field scale.

Keywords: Pyrene, Chinese cabbage, Root activity, Malondialdehyde, Microbial community, Incremental lifetime cancer risk

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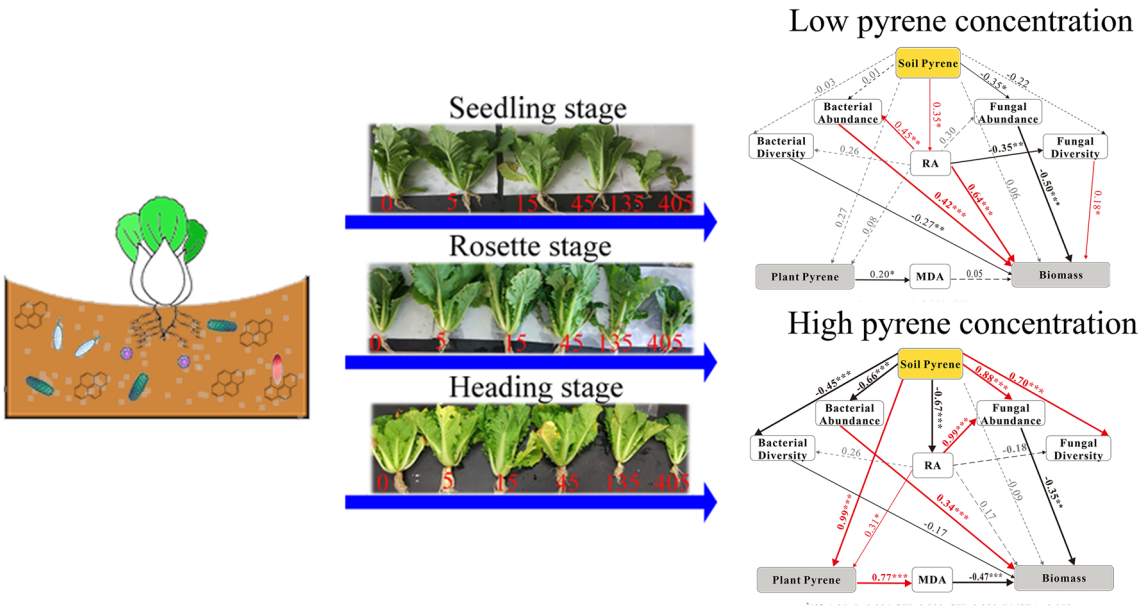
Graphical Abstract



Background

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous and toxic pollutants that are primarily released into the environment through the incomplete combustion of carbonaceous materials [1]. Due to their high rate of bioconcentration, PAHs in soils can quickly enter the food chain, where they pose a threat to ecosystems and human health (a 0.4- to 6-fold increase in cancer risks) [2–4]. According to the national survey of soil pollution status, PAHs are major organic pollutants of soil in China [5]. Thus there is an urgent need to understand how PAHs in agricultural soils affect crop growth and potential health risks [6, 7].

PAHs in soils exert a range of toxic effects on plants through the soil–root–shoot pathway, including root damage [8], decreased biomass [9], disruption of the antioxidant system [10], and DNA damage [11]. However, some studies have reported that PAHs had no effect on plant growth, or that even promoted it [12–14]. These inconsistent findings could be related to several factors, including oxidation reactions, hydrolysis, pollutant-degrading endophytes in plants [15], soil PAH levels [12], duration of exposure [7], and soil properties [16]. However, it has not yet been established whether it is only the direct effects of PAHs that inhibit or promote plant growth or whether these compounds may have some other indirect effects.

Root systems, which have a vital role in terrestrial plants, are directly exposed to and hence severely affected by PAHs in soils [17]. Root activity (RA) reflects the strength of the overall metabolism in the roots, and it includes respiration, oxidation, and enzyme activities, which are closely related to life activities [18]. Previous studies have shown that high concentrations (50 g/kg) of petroleum hydrocarbons mainly composed of PAHs could inhibit the root activity of *Spartina anglica* as a result of the destruction of the root duct tissue by PAHs, thereby inhibiting plant growth [19]. Malondialdehyde (MDA), which is produced in response to cell membrane injury, is considered to be one of the key indicators of accumulation of reactive oxygen species (ROS) and lipid peroxidation and is routinely used to assess plant sensitivity to oxidative stress [20, 21]. Therefore, a knowledge of the effects of PAHs on plant root activity and MDA levels is important for understanding the impact of these compounds on plant growth. The rhizosphere is rich in root exudates such as sugar, amino acids, phenols, aromatic acids, aliphatic acids, fatty acids, and terpenes, and it also contains significant populations of bacteria and fungi [22, 23]. These induce the enzymes responsible for PAH degradation in soil [24], and could therefore have a significant influence on plant nutrition [25], and allow plant growth to occur despite PAH stress [26].

Recently, extensive studies have been conducted on the health risks posed by dietary exposure to PAHs in vegetables. However, these studies focused mainly on a single growth stage in the plant life cycle [27, 28]. The health risks associated with PAHs in Chinese cabbage (*Brassica campestris* L.) at different growth stages have received very little attention. Pyrene, a four-ringed and

widespread PAH, is strongly associated with 16 PAHs that have been identified as a priority by the U.S. Environmental Protection Agency (EPA), and termed the EPA PAHs [29, 30]. For this reason it is usually selected as the target PAH. Chinese cabbage is one of the most popular vegetable crops grown in China. The cultivated area of this vegetable crop was reported to be 2.62 million hm^2, which represents 14.4% of the total cultivated area of vegetables [31]. Due to its significant economic value, Chinese cabbage was considered to be a suitable vegetable for this study. The objectives of the study were as follows: (1) to investigate the change in biomass, root activity and MDA content in Chinese cabbage, and soil microbial community at different growth stages under pyrene stress; (2) to analyze how pyrene influence the biomass of Chinese cabbage; (3) to assess the potential health risks to adults of ingesting Chinese cabbage at different growth stages after the vegetable had been grown in pyrene-polluted soil.

Materials and methods

Experimental materials

The experimental soil was cinnamon soil that had been collected from typical vegetable production land in Beijing. The air-dried soil at room temperature was crushed and passed through a 3-mm sieve prior to the experiment. Its main chemical properties were as follows: soil organic matter, 5%; pH, 8.42; total nitrogen, 1.09 g kg^{-1}; total phosphorus, 0.93 g kg^{-1}. Seeds of *Brassica campestris* L. were obtained from the Institute of Vegetable and Flowers, Chinese Academy of Agricultural Sciences, China. Pyrene (> 98% purity) was purchased from Aldrich Chemical Cooperation.

Experimental methods

Preliminary experiment

A preliminary experiment was performed in order to determine appropriate concentration ranges of pyrene for testing in a definitive dose–response study [32]. Briefly, the test plants were exposed to a pyrene concentration series of 1, 10, 100, and 1000 mg kg^{-1}. A 50 g soil containing the required concentration of pyrene was added to a 10-cm petri dish, and 15 seeds of the test plant were then placed in the soil. Each treatment was replicated three times. The petri dishes were placed in an incubator and maintained at 25 ± 0.5℃ and 80% humidity, in total darkness. Water loss from petri dishes was monitored daily by weighing, and distilled water was added if necessary. The preliminary experiment was terminated when the length of the growing radicle in the control reached 20 mm. The percentage germination of seeds was determined and the lengths of the roots were measured. The pyrene concentrations that caused 50% inhibition (EC_{50}) and 10% inhibition (EC_{10}) were used to evaluate toxicity. EC_{50} (470.46 mg kg^{-1}) and EC_{10} (2.83 mg kg^{-1}) were determined based on the concentration of pyrene as a function of the rate of inhibition of root elongation (Additional file 1: Fig. S1), and pyrene concentrations within this range were applied to the soil in the main experiment.

Main experiment

Based on the results of the preliminary experiment, pyrene concentrations of 0, 5, 15, 45, 135 and 405 mg kg^{-1} were used in the main experiment. To obtain the soil with above-mentioned pyrene concentration, pyrene was first dissolved in acetone and added to the soil to 3500 mg kg^{-1}. The treated soil was then placed under a laboratory fume hood until the soil become dry, after which it was stored in the dark at 4℃. Treated soil was subsequently homogenized with non-contaminated soil in proportions that would give a final soil pyrene concentration of 5, 15, 45, 135 and 405 mg kg^{-1}. The soil samples containing fixed concentrations of pyrene were then passed through a 3-mm sieve to ensure homogeneity of the mixture, before being packed in pots (23-cm diameter top, 18-cm diameter base), with 7 kg dry weight of soil per pot, and adjusted to 50% water-holding capacity [33]. Soil without pyrene addition was used as the control treatment (CK). For removing the surface microbes, seeds of Chinese cabbage were sterilized in 75% (v/v) alcohol for 5 min, washed with ultrapure water, and soaked in water for 1 h. A total of 15 uniform seeds were selected and planted in each pot. One seedling was retained at the cotyledon stage. The positions of pots were changed randomly in order to avoid the effects of non-uniform illumination, and each treatment was replicated nine times. Plants were grown for 25, 50 and 75 days (corresponding to the seedling, rosette and heading stages, respectively) [34], and plant growth characteristics were then evaluated. Three pots for each treatment were harvested at the seedling stage, the rosette stage and the heading stage.

Plant samples collected at each developmental stage were carefully removed from the soil, washed with ultrapure water, weighed, and separated into root and above-ground parts. A part of plant samples was freeze-dried, pulverized and stored − 20℃ for the analysis of pyrene. Another part of plant samples was for the analysis of root activity and MDA. Soil samples were collected and divided into two portions, one was stored at − 80℃ for microbial analysis. Another was freeze-dried, ground to a powder and placed at − 20℃ for the analysis of pyrene.

Determination of pyrene in soil and plants

Pyrene in soil and plant samples was extracted and purified as described by Zhu and Zhang [35]. Ultrasonic method was used to extract pyrene in soil and plant samples. Solid-phase extraction columns were used to clean the extracts, and the pyrene concentration was detected by gas chromatography–mass spectrometry (Thermo Fisher Trace1300 ISQ). Detailed extraction and clean-up procedures are described in the supporting information (Additional file 1: Text S1).

Determination of root activity and malonaldehyde (MDA) levels

Root activity was determined by measuring oxidation of alpha-naphthylamine (α-NA) [36]. MDA levels were measured using a plant MDA assay kit (Beijing Leagene Biotech Co., Ltd, Beijing, China), according to the manufacturer's instructions. The detailed procedures are described in Additional file 1: Text S2.

Soil DNA extraction, PCR amplification and sequencing

Soil microbial genomic DNA was extracted from 0.25 g samples of soil using a DNA extraction kit (MOBIO PowerSoil® DNA Isolation Kit USA). DNA concentration and purity were determined using a NanoDrop One spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Bacterial and fungal community structure in soil was determined by high-throughput sequencing technology. The detailed procedures are described in Additional file 1: Text S3.

Health risks associated with pyrene in Chinese cabbage

The potential effects of PAHs on health were quantitatively analyzed using the toxicity equivalency factor (TEF) calculated from the toxicity of benzo[a] pyrene (B[a]P) [37]. The carcinogenic risk of pyrene was expressed in terms of its B[a]P equivalent concentration (BEC) as:

$$\mathrm{BEC} = \mathrm{C} \times TEF_P,$$

where C is the concentration of pyrene in Chinese cabbage shoot tissue, and $\mathrm{TEF_p}$ is the corresponding toxic equivalency factor for pyrene (TEFp = 0.001) [38].

Daily dietary pyrene exposure level (E_D) was calculated as:

$$\mathrm{E}_D = \mathrm{BEC} \times \mathrm{IR},$$

where BEC is the $\mathrm{B[a]P_{eq}}$ in Chinese cabbage (μg/kg), and IR is the amount of Chinese cabbage ingested daily (g/day). According to Zhai and Yang [39], the average amount of dark green leafy vegetables ingested was 76.2 g/day for men and 75.5 g/d day for women. Chinese cabbage accounts for approximately 10% of dark leafy greens ingested in the daily diet. Thus the average amount of Chinese cabbage ingested daily is estimated to be 7.62 g/day for men and 7.55 g/day for women.

The incremental lifetime cancer risk (ILCR) associated with dietary exposure to PAH was calculated as:

$$\mathrm{ILCR} = E_D \times EF \times ED \times SF \times CF/(BW \times AT),$$

where EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), SF is the oral cancer slope factor for benz[a]pyrene (7.3 mg/kg/day) [40], CF is a conversion factor (10^{-6} mg/ng), BW is the body weight, and AT is the average lifespan (25,550 days); the average body weight is estimated to be 62.82 kg for men and 54.73 kg for women [41].

Data analysis

One-way ANOVA followed by Duncan's multiple comparison was used to analyze the effects of pyrene on root and shoot fresh weight, root activity, MDA, and soil microbial abundance and diversity. All statistical analyses were performed using Microsoft Excel 2007 and SPSS 19 (SPSS Inc., Chicago, USA). The results were expressed as mean values ± standard deviation.

Structural equation modeling (SEM) analysis was used to investigate the direct and indirect effects of pyrene on Chinese cabbage biomass. The first step of SEM analysis required the building of a priori model [42] based on the known effects and relationships between different factors that affect biomass. SEM analysis was performed in IBM SPSS Amos 21 (SPSS Inc., Chicago, USA) using maximum likelihood. The adequacy of the model fitting was assessed via Chi-square (χ^2) test ($P > 0.05$), Chi-square/degree of freedom (χ^2/df; traditionally χ^2/df < 3), comparative fit index (CFI; traditionally CFI > 0.90), goodness-of-fit (GFI; traditionally GFI > 0.90) and root square mean error of approximation (RSMEA; the model has a good fit when RMSEA is near 0).

Results and discussion

Pyrene dissipation in soil and uptake by plants

The concentration of residual pyrene in soil decreased (Fig. 1a, Additional file 1: Table S1), while the dissipation ratio of pyrene increased irrespective of pyrene dose with increasing duration of plant growth (Fig. 1b). This increased dissipation ratio of pyrene in soil over time was mainly due to the biodegradation of pyrene by soil microorganisms. Similarly, El Amrani et al. reported that the removal of soil PAHs might be a result of rhizodegradation of these compounds [43]. Although plant could also uptake pyrene, it only accounted for small fraction [14]. Therefore, microbial degradation was a major contributor to the dissipation of pyrene. Sun et al. reported that the pyrene concentration in nonsterile soil was

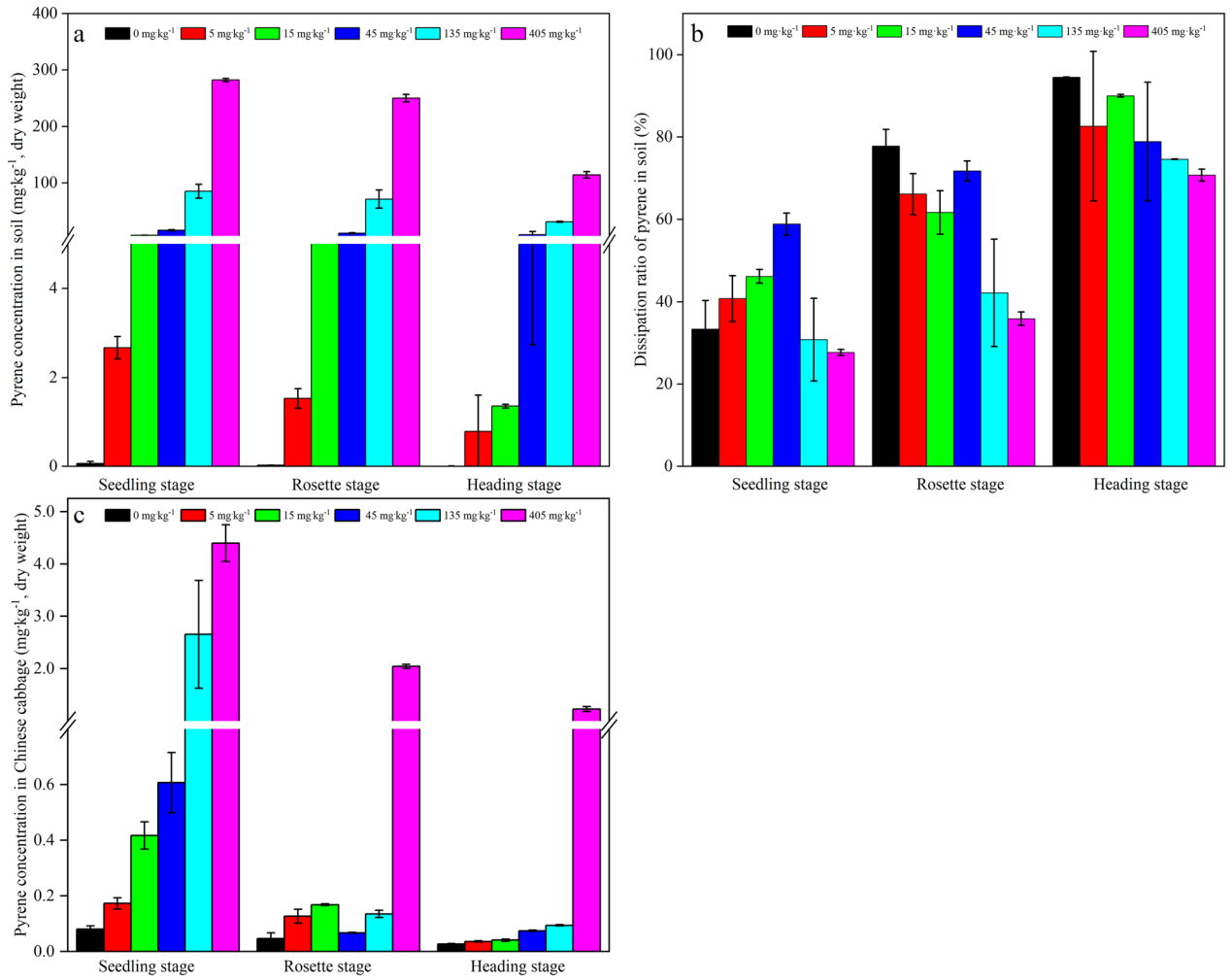


Fig. 1 Residual pyrene concentration (**a**), dissipation ratio of pyrene (**b**) in soil and pyrene concentration in Chinese cabbage (**c**) at different growth stages of Chinese cabbage. Values are means ± standard errors of three replicates

significantly lower than that in sterile soil [44], which suggested that indigenous microorganisms play a key role in the degradation process. In addition, the soil in which Chinese cabbage were growing had higher indigenous bacterial relative abundance and diversity compared with the unplanted soil (Table 1, Additional file 1: Table S2). Thus, the degradation of pyrene was accelerated. In fact, plant roots could release exudates into the soil, resulting in an increase in microbial activity and diversity [45], as these exudates contain soluble organic and inorganic compounds that provide a carbon source for the microbial population [46].

As shown in Fig. 1c, the pyrene concentration in Chinese cabbage increased as the dose of pyrene applied to the soil increased, irrespective of developmental stage, but that it decreased with growing time irrespective of the pyrene dose in the soil. This result was similar to the findings of previous studies [22, 35]. One possible explanation for it was the growth dilution effect caused by an increase in plant biomass with growth time (Fig. 3c, d). Another possible reason was that the extractable proportion of pyrene in the soil decreased as the growth time increased (Fig. 1a).

Effect of pyrene on the microbial community

Soil microbial abundance and diversity

We observed that soil microbial abundance had obvious differences, which were associated to pyrene dose and plant growth stage (Table 1). The soil bacterial abundance, as expressed by the Chao1 index, reached at a maximal value with the pyrene dose of 5 mg kg^{-1}, and then declined with increasing pyrene concentration at all growth stages. This could be explained by the fact that the low pyrene concentration promoted the secretion of

Table 1 Chao1 and Shannon–Weiner indices showing diversity of soil microbial communities at different developmental stages of Chinese cabbage

Treatment	16S		ITS	
	Chao1	Shannon	Chao1	Shannon
Seedling stage				
0	1970 ± 37a	8.09 ± 0.02a	402 ± 31a	1.66 ± 0.13c
5	2008 ± 54a	8.05 ± 0.17a	369 ± 33a	1.17 ± 0.16d
15	1964 ± 67a	7.60 ± 0.30b	391 ± 14a	1.14 ± 0.30d
45	1773 ± 56b	7.43 ± 0.15b	407 ± 49a	1.14 ± 0.09d
135	1988 ± 17a	8.06 ± 0.24a	399 ± 7a	2.09 ± 0.18b
405	1722 ± 8b	7.57 ± 0.29b	382 ± 6a	3.35 ± 0.22a
Rosette stage				
0	1837 ± 39b	7.75 ± 0.51ab	431 ± 35a	1.90 ± 0.11ab
5	2183 ± 66a	8.35 ± 0.15a	392 ± 44a	1.52 ± 0.35b
15	2189 ± 11a	7.93 ± 0.63ab	402 ± 28a	1.68 ± 0.39b
45	2149 ± 23a	8.10 ± 0.28ab	400 ± 34a	1.32 ± 0.36b
135	1823 ± 56b	7.76 ± 0.62ab	396 ± 18a	1.88 ± 0.26ab
405	1716 ± 82c	7.36 ± 0.37b	387 ± 11a	2.35 ± 0.41a
Heading stage				
0	2001 ± 16c	8.07 ± 0.18ab	378 ± 25ab	1.89 ± 0.01ab
5	2353 ± 51a	8.62 ± 0.07a	395 ± 12a	1.77 ± 0.17ab
15	2102 ± 88b	8.05 ± 0.78ab	367 ± 73ab	1.67 ± 0.41ab
45	2069 ± 50bc	7.93 ± 0.50ab	292 ± 14c	1.79 ± 0.34ab
135	2084 ± 27bc	8.20 ± 0.65ab	276 ± 44c	1.51 ± 0.30b
405	1658 ± 49d	7.44 ± 0.57b	321 ± 19bc	2.27 ± 0.54a

Values are means ± standard errors of three replicates. Different letters indicate significant differences between treatments at the same developmental stage ($p < 0.05$)

root exudates [47], which may serve as a source of nutrients for the growth and survival of soil microorganisms [22]. Root exudates have been reported to have an important influence on the soil bacterial community, and may increase soil bacterial richness [48]. The bacterial communities from the soil that had been treated with 405 mg kg^{-1} pyrene at all three plant growth stages had the lowest Chao 1 index compared with the control ($p < 0.05$) (Table 1), which indicated that high doses of pyrene decrease soil bacterial richness. These results were consistent with previous findings [49, 50], which could be ascribed to the toxic effects of high doses of PAHs on microbes [51]. The soil bacterial diversity expressed by Shannon index in the treatment of 15, 45 and 405 mg kg^{-1} was significantly lower compared with CK at the seedling stage. However, no significant differences were observed at the rosette and the heading stages.

No significant differences in soil fungal abundance were observed between each of the treatments at the seedling and rosette stages. The level of soil fungal abundance was slightly higher for the treatment with 5 mg kg^{-1} pyrene than for CK at the heading stages, and significantly lower for the treatments with 45 and 135 mg kg^{-1} pyrene than for CK at the heading stage (Table 1). This finding suggested that low doses of pyrene have a slight stimulatory effect on fungal activity, whereas the stress of long-term high doses significantly inhibits such activity. The marked change in soil fungal abundance was observed in the heading stage but not in the earlier stages, and could be related to exposure time. Compared with the control, lower soil fungal Shannon index at seedling stage were found with the treatments of 5, 15 and 45 mg kg^{-1}, but the higher Shannon index were observed with the treatments of 135 and 405 mg kg^{-1} (Table 1). There were no significant differences between the control and treatments at the rosette and heading stages.

Soil microbial community composition

To determine whether specific phylotypes are associated with the fate of pyrene, the relative abundance of and net differences among the phylotypes were further summarized at the taxonomic level of phylum (Fig. 2, Additional file 1: Fig. S2). Bacterial phyla included *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Nitrospirae*, *Firmicutes*, *Patescibacteria* and *Gemmatimonadetes* (Additional file 1: Fig. S2a). *Proteobacteria* was the most abundant, consistent with previous study in which this phylum dominated the bacterial community [52]. All of the fungi identified in this study belong to five distinct phyla, namely *Ciliophora*, *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, and *Chlorophyta* (Additional file 1: Fig. S2b). Members of the *Ascomycota* were the most abundant fungi across all soil samples (Additional file 1: Fig. S2b), which was consistent with previous findings [53].

Pyrene had a relatively stronger effect on the microbial phylotypes, and the effect was dose and time dependent (Fig. 2). For bacteria, 6 of the 12 phyla showed a significant increase in relative abundance with at least one dose of pyrene at seedling stage. Specifically, the relative abundance of *Actinobacteria* and *Chloroflexi* was significantly increased at a pyrene concentration of 15 mg kg^{-1}, that of *Cyanobacteria*, *Nitrospirae* and *Firmicutes* was significantly increased at 135 mg kg^{-1}, and that of *Patescibacteria* was significantly increased at 405 mg kg^{-1}. Three phyla showed a significant decrease in relative abundance with at least one dose of pyrene. For example, *Bacteroidetes* and *Acidobacteria* were significantly decreased at pyrene concentrations of 15, 45 and 135 mg kg^{-1}, whereas only *Acidobacteria* exhibited a significant decrease at a pyrene concentration of 405 mg kg^{-1}; *Planctomycetes* were significantly reduced at 405 mg kg^{-1}. Until the rosette stage, the effect of low-dose pyrene (5-45 mg kg^{-1}) on the relative abundance of bacteria was very weak, with the exception of *Cyanobacteria*,

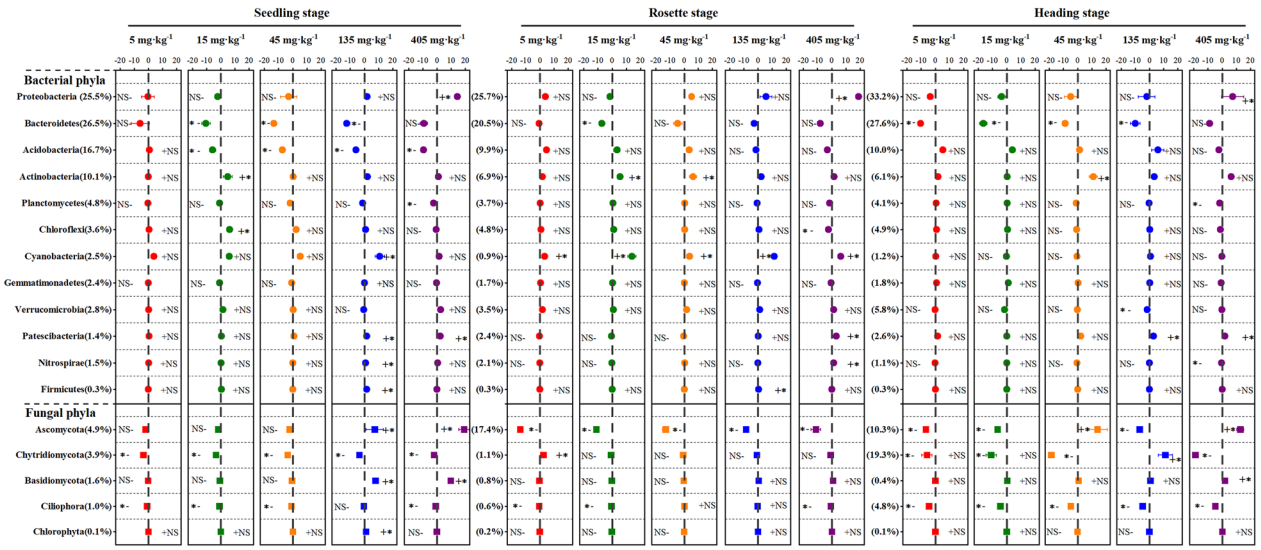


Fig. 2 Differences in relative abundance of fungi and bacteria at the phylum level between pyrene-treated soils and control soils. The net difference was calculated by the relative abundance under pyrene-treated soils minus that under control soils. Error bars represent standard error of the means (n = 3). Percentage values in parentheses denote the relative abundance in the control treatment. +, increase in relative abundance compared with the control; –, decrease in relative abundance compared with the control. *, significant differences at $p < 0.05$; NS, no significant difference ($p > 0.05$)

which showed a significant increase in relative abundance. A total of four phyla, namely *Proteobacteria*, *Cyanobacteria*, *Patescibacteria* and *Nitrospirae*, exhibited a significant increase in relative abundance at a pyrene concentration of 405 mg kg^{-1}, whereas *Chloroflexi* showed a significant reduction. After the heading stage, low-dose pyrene (5-45 mg kg^{-1}) had little effect on the relative abundance of any of the bacterial phyla except for *Bacteroidetes*. However, significant changes were still observed in six phyla at pyrene concentrations of 135 and 405 mg kg^{-1}.

Although a high dose (405 mg kg^{-1}) of pyrene reduced the bacterial abundance, some specific phylotypes (e.g., *Proteobacteria* and *Patescibacteria*) still showed strong enrichment (Table 1; Fig. 2). This suggested that these phyla could use the pyrene as a carbon or energy source for growth, resulting in pyrene degradation. This finding was consistent with the study by Ren et al., who observed that soil bacterial richness decreased at high doses of pyrene, while contrary result was found that the bacterial richness of some specific phylotypes, such as *Betaproteobacteria* and *Gammaproteobacteria*, were significantly increased at high doses of pyrene [54]. *Proteobacteria* have been found to be predominant in PAH-polluted soils, and are capable of degrading complex organic molecules, enabling these bacteria to adapt to different environments [55]. The decrease in abundance of *Acidobacteria* at seedling stage (Fig. 2) demonstrated that they were more sensitive to pyrene, but their level of abundance remained stable over time, indicating that *Acidobacteria* gradually adapt to pyrene-induced stress.

At the taxonomic level of genus, a total of 44 bacterial genera were identified from all soil samples (Additional file 1: Fig. S3). Among them, four conventional genera related to pyrene removal were identified, namely *Mycobacterium* [56], *Bacillus* [57], *Rhodococcus* [58] and *Pseudoxanthomonas* [59]. However, in the present study these genera were not dominant in soil treated with low doses (5–45 mg kg^{-1}) of pyrene; their relative abundance was 0.09–0.18% for *Bacillus*, 0.06–0.11% *for Mycobacterium*, 0.03–0.12% for *Pseudoxanthomonas* and 0.02–0.04% for *Rhodococcus*. Furthermore, the relative abundance of these four genera was not significantly different from that of the control (Additional file 1: Fig. S5), demonstrating that these four genera may not be active in pyrene dissipation. However, there was significant dissipation of pyrene in soil that had been treated with low doses of pyrene (Fig. 1b). Bacosa and Inoue also observed that pyrene degradation occurred in a sediment in the absence of pyrene-degrading genera [60]. This might be due to the PAH dioxygenase gene, which has a key role in PAH degradation, being carried by other dominant bacterial groups [49]. This gene has been reported to spread rapidly in bacteria by horizontal gene transfer [61]. The dissipation of pyrene in soil that has been treated with high doses of that compound can be attributed to the enrichment of *Mycobacterium*, *Rhodococcus*, *Pseudoxanthomonas* and other genera belonging to the *Proteobacteria*, as the

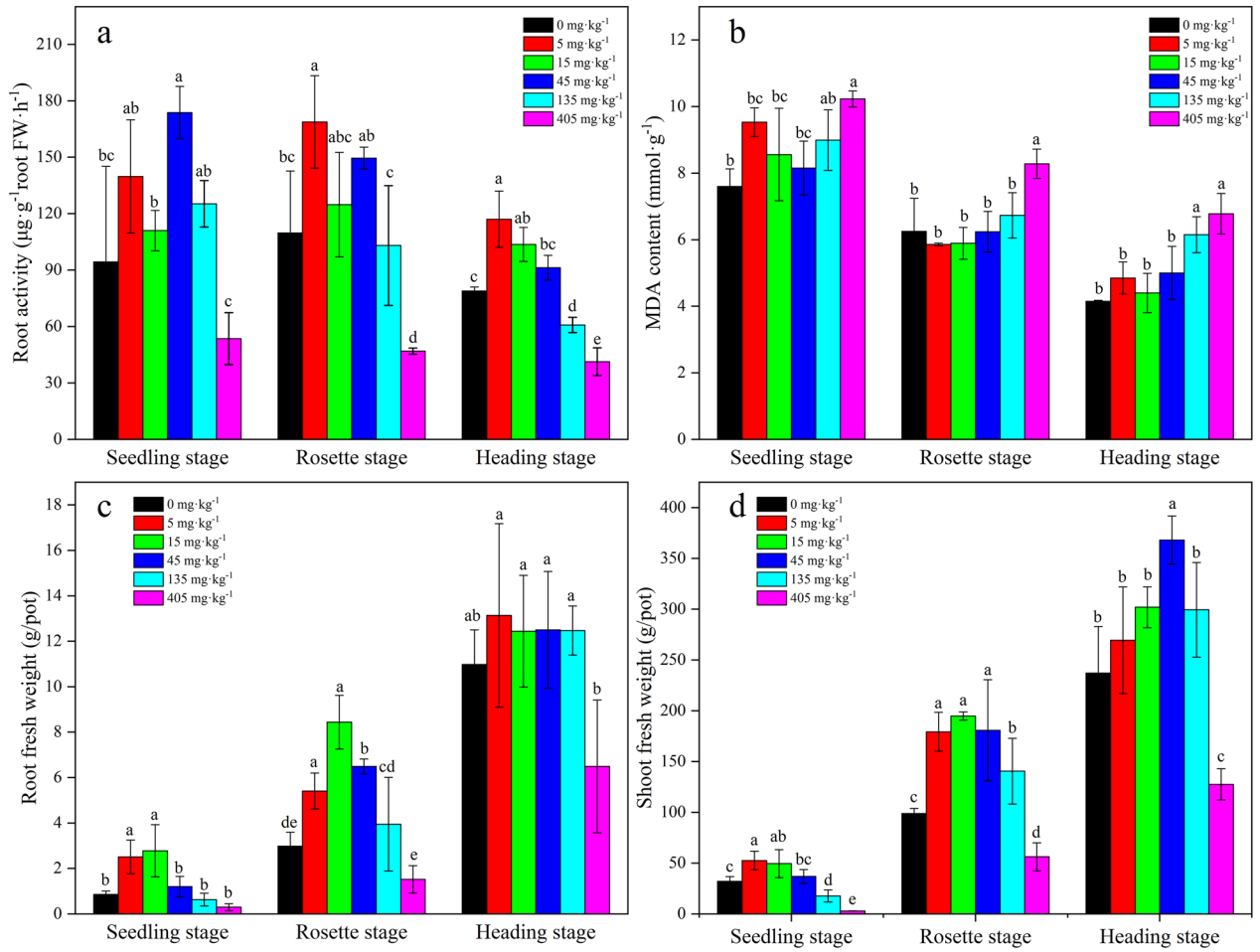


Fig. 3 Effects of pyrene dose on root activity (**a**), MDA content (**b**), root fresh weight (**c**) and shoot fresh weight (**d**) of Chinese cabbage at seedling, rosette and heading stages. Values are means ± standard errors of three replicates. Lower-case letters indicate significant differences between treatments at the same growth stage ($p < 0.05$)

relative abundance of these three genera and other *Proteobacteria* in soil treated with high doses of pyrene was significantly greater than that in other soils (Fig. 2, Additional file 1: Fig. S5).

With regard to fungal phyla, the relative abundance of *Chytridiomycota* and *Ciliophora* was significantly lower at all of the pyrene doses compared with the control, with the exception of *Ciliophora* at a pyrene dose of 135 mg kg^{-1} (Fig. 2), indicating that these fungi were more susceptible to pyrene. The other three phyla, namely *Ascomycota*, *Basidiomycota* and *Chlorophyta*, showed a significant increase in relative abundance at high doses of pyrene. These three phyla may have mechanisms that enable them to resist toxic PAHs. For example, some genera in the *Ascomycota* are able to degrade hydrocarbon contaminants by secreting non-specific fungal enzymes [62]. At rosette stage, the relative abundance of *Ascomycota* was significantly reduced by all doses of pyrene; the abundance of *Ciliophora* was significantly decreased at 5 mg kg^{-1}, 15 mg kg^{-1} and 405 mg kg^{-1}. At heading stage, the relative abundance of *Ascomycota*, *Chytridiomycota* and *Ciliophora* was significantly decreased at low doses (5–45 mg kg^{-1}) of pyrene, with the exception of *Ascomycota* at 45 mg kg^{-1}, which showed a significant increase in relative abundance. *Ascomycota*, *Chytridiomycota* and *Basidiomycota* were significantly increased at specific high doses of pyrene (*Ascomycota* and *Basidiomycota* at 405 mg kg^{-1}, and *Chytridiomycota* at 135 mg kg^{-1}). At genus level, some fungal genra, such as *Fusarium* and *Aspergillus* (Additional file 1: Fig. S4), belong to plant mycorrhizal symbiotic fungus [63].

Effect of pyrene on root activity, MDA content and biomass

Root activity

The effects of pyrene on root activity of Chinese cabbage at different growth stages are shown in Fig. 3a. At seedling stage, the root activity of plants grown in soils that had been treated with a pyrene dose of less than 45 mg kg^{-1} showed an initial increase followed by a slight decrease. The root activity of Chinese cabbage increased to a maximum value of 173.8 μg g^{-1} root FW·h^{-1} at treatment with 45 mg kg^{-1} pyrene, and subsequently the root activity decreased as the pyrene dose increased further. At rosette stage, root activity showed a similar change to that observed at seedling stage, However, it reached a maximum value (168.7 μg g^{-1}root FW·h^{-1}) at a pyrene dose of 5 mg kg^{-1}. At heading stage, the root activity initially increased and then decreased, and it reached a maximum value (117.0 μg g^{-1}root FW·h^{-1}) for the treatment with 5 mg kg^{-1} pyrene. Zhen et al. also observed that lower doses of PAHs promoted plant root activity [19]. This could be attributed to PAH degradation processes.

For all growth stages, the lowest root activity was observed for the treatment with 405 mg kg^{-1} pyrene. Moreover, for the rosette and heading stages, but not the seedling stage, there were significant differences ($p < 0.05$) in root activity between the control and the treatment with 405 mg kg^{-1} pyrene (Fig. 3a). There were two possible reasons for the decrease in root activity at higher pyrene concentrations. First, pyrene could permeate the plant roots, destroy the root vascular tissue, and thus inhibit plant growth [64]. Second, the accessibility of soil inorganic nitrogen might be decreased. Jiang et al. have noted that the presence of PAHs in soil usually decreases the availability of dissolved inorganic nitrogen to plants, thus impairing root growth and decreasing root activity [65].

Malondialdehyde (MDA) content

As a product of membrane lipid peroxidation, MDA is an important indicator of cell membrane injury [20]. Its accumulation is a physiological response of plants to higher concentrations of PAHs [10]. In the present study, MDA levels in the leaves of Chinese cabbage at rosette and heading stages increased with rising pyrene concentration, whereas at seedling stage the MDA levels initially increased, then decreased, and finally increased again (Fig. 3b). The MDA content of plants exposed to 405 mg kg^{-1} pyrene was significantly higher than the control value at all growth stages ($p < 0.05$), indicating that lipid peroxidation had occurred. Jafari et al. reported that accumulation of high levels of PAHs and overcoming antioxidant system in plants can increase lipid peroxidation and MDA content [66]. In response to oxidative stress caused by ROS, chloroplast ascorbate peroxidase (APX1) plays a key role at the molecular level [67]. In addition, high ROS levels may have caused the observed decrease in plant biomass. Furthermore, MDA levels decreased for all soil treatments during the growth period, suggesting that the stress effects of pyrene on Chinese cabbage gradually diminished.

Biomass

The root fresh weight of Chinese cabbage increased with all treatments during the growth period (Fig. 3c). At seedling stage and rosette stage, the maximum values of root fresh weight were observed at treatment with 15 mg kg^{-1} pyrene (2.8 g/pot for seedling stage, 220% higher than CK; 13.1 g/pot for rosette stage, 183% higher than CK) and it decreased with the increasing pyrene dose afterwards. At heading stage, the root fresh weight showed a similar change with those observed at seedling and rosette stage, however it reached a maximum value (13.1 g/pot, 20% higher than CK) for the treatment with 5 mg kg^{-1} pyrene. Significant differences in root fresh weight at seedling and rosette stages were observed between the control and the treatment with 15 mg kg^{-1} pyrene (Fig. 3c). The minimum root fresh weight at all growth stages was observed for the treatment with 405 mg kg^{-1} pyrene (66, 43 and 41% lower than CK for seedling stage, rosette stage and heading stage, respectively) (Fig. 3c).

The shoot fresh weight also increased with all treatments during the growth period (Fig. 3d). The maximum values of soot fresh weight were 52.6 g/pot (63% higher than CK) at the seedling stage with the treatment of 5 mg kg^{-1} pyrene, 194.9 g/pot at the rosette stage (97% higher than CK) with the treatment of 15 mg kg^{-1} pyrene, and 368.1 g/pot (55% higher than CK) at the heading stage with the treatment of 45 mg kg^{-1} pyrene. The minimum shoot fresh weight at all growth stages was observed for the treatment with 405 mg kg^{-1} pyrene (91%, 43% and 46% lower than CK for seedling stage, rosette stage and heading stage, respectively) (Fig. 3d).

There was a significant dose–effect relationship between pyrene concentration and biomass, with an increase in biomass observed at intermediate pyrene concentrations (5 mg kg^{-1} at seedling stage, 15 mg kg^{-1} at rosette stage, and 45 mg kg^{-1} at heading stagey) but a decrease at high pyrene concentrations (405 mg kg^{-1} for all growth stages) (Fig. 3c, d). These findings were in contrast to those reported by Gao and Zhu, who observed that the root and shoot biomass of Chinese cabbage (*Brassica parachinensis* Bailey)

decreased as the soil pyrene concentration (0–489 mg kg^{-1}) increased after 45 days of incubation [33]. These conflicting findings might be partly attributed to the use of different varieties of Chinese cabbage. It was possible that the stimulatory effect of low concentrations of pyrene (5–45 mg kg^{-1}) on the biomass of Chinese cabbage (Fig. 3c, d) was due to pyrene acting as a plant growth promoter [12], as PAHs show some structural similarities to endogenous plant hormones. On the one hand, low PAH concentrations could increase root activity (Fig. 3a), enabling adequate uptake of nutrients and water to the aerial parts of the plant, and thus stimulating plant growth. On the other hand, low levels of PAHs could promote the secretion of organic acids by the roots [68], which in turn increase soil nutrient solubility [69], and enhance nutrient transfer from the soil environment to roots by ion exchange [70]. The significant reduction in root and shoot biomass of plants grown in soil treated with a high concentration of pyrene (405 mg kg^{-1}) (Fig. 3c, d) could be attributed to the inherent toxicity of pyrene. Contamination of soils with high levels of PAHs reduces the ability of plants to take up water and nutrients, impairs transport across cell membranes, and inhibits photosynthetic activity and electron transport, thereby leading to a decline in biomass production [71].

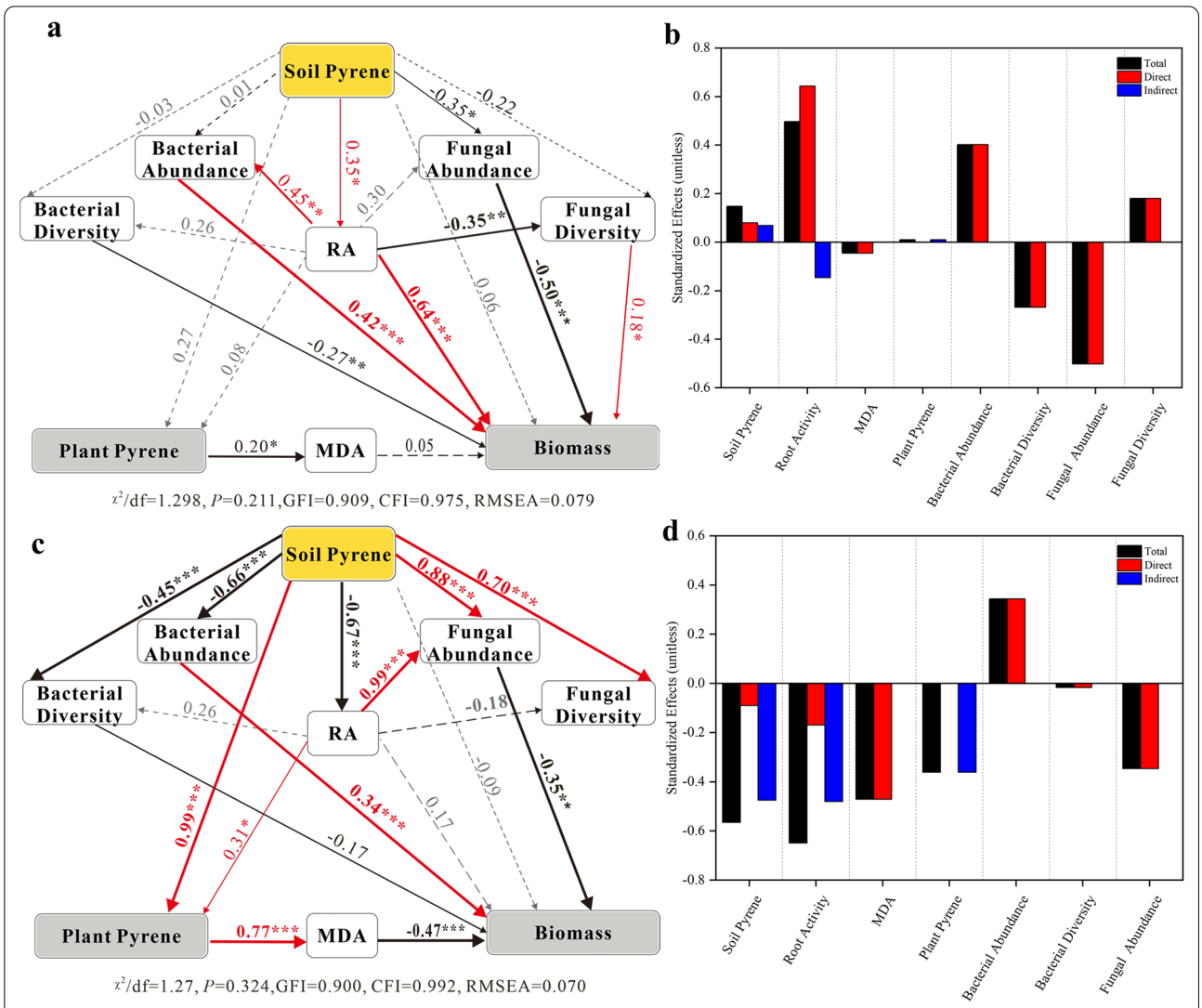


Fig. 4 Structural equation model describing the effects of low doses (5–45 mg kg^{-1}) (**a**, **b**) and high doses (135–405 mg kg^{-1}) (**c**, **d**) of pyrene on growth of Chinese cabbage. Arrows depict casual relationships, red lines indicate positive effects, and black lines indicate negative effects. Arrow widths are proportional to *p* values. Dotted grey arrows indicate nonsignificant paths ($p > 0.05$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Biomass is the sum of root fresh weight and shoot fresh weight. RA, root activity; MDA, malondialdehyde

Structural equation modeling (SEM) analysis of the effects of pyrene on biomass

SEM was used to gain a more detailed understanding of the direct and indirect effects of soil pyrene, root activity, MDA levels and the microbial community on the biomass of Chinese cabbage. The results indicated that low doses of pyrene (5–45 mg kg^{-1}) increased the biomass, whereas high doses (135–405 mg kg^{-1}) reduced it. Therefore all treatments were divided into low-dose and high-dose groups for SEM analysis. A satisfactory fit was confirmed for the two SEMs ($\chi^2/df = 1.298$, $P = 0.211$, CFI = 0.909, GFI = 0.975, RSMEA = 0.079 for low doses of pyrene; $\chi^2/df = 1.270$, $P = 0.324$, CFI = 0.900, GFI = 0.992, RSMEA = 0.070 for high doses of pyrene) (Fig. 4).

SEM analysis identified four effects of low doses of pyrene (5–45 mg·kg^{-1}) on growth of Chinese cabbage (Fig. 4a). (1) Low doses of pyrene in the soil directly induced an increase in root activity ($\lambda = 0.35$, $p < 0.05$), and this in turn directly promoted plant growth ($\lambda = 0.64$, $p < 0.001$). (2) The soil pyrene-induced increase in root activity had a direct positive effect on bacterial abundance ($\lambda = 0.45$, $p < 0.01$), whereafter bacterial abundance directly promoted plant growth ($\lambda = 0.42$, $p < 0.01$). (3) The soil pyrene-induced increase in root activity had a negative effect on fungal diversity ($\lambda = -0.35$, $p < 0.01$), which in turn directly increased plant growth ($\lambda = 0.18$, $p < 0.05$). (4) Low doses of pyrene in the soil induced a decrease in fungal abundance ($\lambda = -0.35$ $p < 0.01$), which in turn inhibited plant growth ($\lambda = -0.53$, $p < 0.001$). For the total standardized effects, the most important positive factor promoting plant growth was root activity, followed by bacterial abundance. The most important negative factor inhibiting plant growth was fungal abundance, followed by bacterial diversity (Fig. 4b). Thus low doses of pyrene appear to influence plant growth predominantly by directly affecting root activity. Interestingly, the decrease in fungal abundance had a negative effect on plant biomass. This result might be due to fungal pathogens competing with the plant for nutrients. In the present study, the fungal genus *Fusarium*, which is an important plant pathogen, was identified (Additional file 1: Fig. S4). Plant pathogens can directly utilize nutrients in plant roots, reducing the ability of the plant to absorb nutrients and thus having a negative effect on plant growth [72].

SEM analysis identified three effects of high doses of pyrene (135–405 mg·kg^{-1}) on growth of Chinese cabbage (Fig. 4c). (1) High doses of pyrene had a direct negative effect on root activity ($\lambda = -0.67$, $p < 0.001$), and this in turn directly affected plant pyrene levels ($\lambda = 0.31$, $p < 0.05$), exacerbating cell membrane damage as indicated by high levels of MDA ($\lambda = 0.77$, $p < 0.001$), which in turn inhibited plant growth ($\lambda = -0.47$, $p < 0.001$). (2) High doses of pyrene induced a decrease in bacterial abundance ($\lambda = -66$, $p = 0.001$), which in turn inhibited plant growth ($\lambda = 0.34$, $p = 0.001$). (3) High doses of pyrene directly affected fungal abundance ($\lambda = 0.888$, $p < 0.001$) and also indirectly affected it by inhibiting root activity ($\lambda = -0.67$, $p < 0.001$), which in turn inhibited plant growth ($\lambda = -0.35$, $p < 0.01$). For the total standardized effects, the most important factors inhibiting plant growth were bacterial abundance and fungal abundance (Fig. 4d). Although root activity had a non-significant direct effect on plant biomass, it was still a major factor, because root activity had a strong influence on fungal abundance and plant pyrene levels, and its total effects for biomass were highest (Fig. 4c, d).

Health risks associated with ingestion of Chinese cabbage

Chinese cabbage is one of the most popular vegetables consumed on a daily basis in China. Vegetables grown on farmland polluted with PAHs have been found to present a major health risk to local people. The incremental lifetime cancer risk (ILCR) has been widely used to assess the potential health hazards for people who are exposed

Table 2 Incremental lifetime cancer risk (ILCR) for adults associated with ingestion of Chinese cabbage at different growth stages

Growth stage	Treatment	ILCR	
		Male	Female
Seedling stage	0	5.31×10^{-7}	6.04×10^{-7}
	5	1.53×10^{-6}	1.74×10^{-6}
	15	3.69×10^{-6}	4.20×10^{-6}
	45	5.37×10^{-6}	6.11×10^{-6}
	135	2.35×10^{-5}	2.67×10^{-6}
	405	3.89×10^{-5}	4.43×10^{-5}
Rosette stage	0	7.62×10^{-7}	8.66×10^{-7}
	5	1.12×10^{-6}	1.28×10^{-6}
	15	1.49×10^{-6}	1.69×10^{-6}
	45	5.93×10^{-7}	6.11×10^{-6}
	135	1.20×10^{-6}	1.36×10^{-6}
	405	1.81×10^{-5}	2.06×10^{-5}
Heading stage	0	2.39×10^{-7}	2.71×10^{-7}
	5	3.19×10^{-7}	3.63×10^{-7}
	15	3.63×10^{-7}	4.13×10^{-7}
	45	6.55×10^{-7}	7.45×10^{-7}
	135	8.32×10^{-7}	9.47×10^{-7}
	405	1.09×10^{-5}	1.24×10^{-5}

Values are means of three replicates

to contaminants through the ingestion of food [73]. An ILCR value of less than 10^{-6} is considered to represent an acceptable or negligible level of risk, values between 10^{-6} and 10^{-4} indicate a potential risk, and values higher than 10^{-4} indicate a serious risk [74].

There were clear differences in ILCR values, which were dependent on the soil pyrene concentration and the plant growth stage, with ILCR decreasing as the plant growth period increased (Table 2). At the seedling stage, the ILCR values associated with dietary exposure to pyrene were in the range 10^{-6}–10^{-4}, and suggested that there was a potential risk to health regardless of gender. At the rosette stage, the ILCR values for dietary exposure to pyrene were in the range 10^{-6}–10^{-4}, except for the treatment with 135 mg kg^{-1} pyrene, for which the ILCR value was lower, at 10^{-6}. At the heading stage, the ILCR values estimated for ingestion of Chinese cabbage grown in soils treated with pyrene at a dose of 405 mg kg^{-1} were higher than the risk threshold (10^{-6}), indicating a potential risk to health irrespective of gender. However, the ILCR values estimated for ingestion of plants grown in soils treated with pyrene doses of less than 135 mg kg^{-1} were lower than the risk threshold (10^{-6}), indicating that the health risk was acceptable or negligible regardless of gender. On the whole, the estimated ILCR values for ingestion of Chinese cabbage that had been exposed to pyrene decreased as the growth period increased. In other words, the health risk of consumers who have the possibility to ingest the Chinese cabbage planted in pyrene-contaminated soil would be decreased with the increasing growth periods.

Conclusion

Different doses of pyrene cause substantial changes in the biomass of Chinese cabbage plants. Low doses of pyrene (5–45 mg kg^{-1}) promoted plant growth, but the highest dose of pyrene (405 mg kg^{-1}) caused significant inhibition of growth. The potential mechanisms associated with these effects on plant growth include changes in root activity, soil bacterial abundance and diversity, and MDA levels induced by pyrene. However, the effects of pyrene on Chinese cabbage growth were mainly attributed to changes in root activity induced by pyrene, because the relationship between pyrene dose and plant biomass was similar to that between pyrene dose and root activity. The SEM analysis further demonstrated that pyrene affected the growth of Chinese cabbage by directly altering root activity. The ILCR for ingestion of Chinese cabbage by adults generally varied according to the plant growth stage, being highest at the seedling stage, followed in decreasing order by the rosette and heading stages. This suggests that the longer time for Chinese cabbage grown in pyrene-contaminated soil, the lower the risk associated with ingestion of this vegetable. These results have implications for assessment of the risks of pyrene to ecological safety and human health. However, further studies are required to confirm the dose–effect relationship between pyrene concentration and Chinese cabbage growth on a field scale.

Abbreviations

MDA: Malondialdehyde; PAHs: Polycyclic aromatic hydrocarbons; RA: Root activity; ROS: Reactive oxygen species; TEF: Toxicity equivalency factor; B[a]P: Benzo[a]pyrene; BEC: B[a]P equivalent concentration; ILCR: Incremental lifetime cancer risk; SEM: Structural equation modeling; $\chi 2$: Chi-square; $\chi 2$/df: Chi-square/degree of freedom; CFI: Comparative fit index; GFI: Goodness-of-fit; RSMEA: Root square mean error of approximation.

Supplementary Information

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Additional file 1: Text S1. Determination of pyrene in soil and plants. **Text S2.** Determination of Root activity and malonaldehyde (MDA) levels. **Text S3**. Soil DNA extraction, PCR amplification and sequencing. **Table S1**. Measured concentration of pyrene after one week of aging (mg·kg-1). **Table S2**. Chao1 and Shannon–Weiner indices showing diversity of soil microbial communities without Chinese cabbage cultivation. **Fig. S1**. Effect of pyrene concentration on rate of inhibition of root elongation. **Fig. S2**. Composition of bacterial (a) and fungal (b) phyla in soils treated with different pyrene doses, at the seedling, rosette and heading stages. SS, seedling stage; RS, rosette stage; HS, heading stage. **Fig. S3**. Relative abundance (%) of detected bacteria at the genus level in soils treated with different doses of pyrene (Pyr) during the Chinese cabbage growth period. **Fig. S4**. Relative abundance (%) of detected fungi at the genus level in soils treated with different doses of pyrene (Pyr) during the Chinese cabbage growth period. **Fig. S5**. Dynamics of four pyrene-degrading bacterial genera (Mycobacterium, Bacillus, Rhodococcus and Pseudoxanthomonas) in soils treated with different concentrations of pyrene during the Chinese cabbage growth period.

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Authors' contributions

XY: conceptualization, methodology, formal analysis, investigation and writing—original draft. ZH: conceptualization, writing—review and editing. XX: investigation. LH: investigation. RZ: investigation. BD: investigation. YL: conceptualization, funding acquisition, project administration, resources, supervision, writing—original draft, writing—review and editing.

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Availability of data and materials

All the data are included in manuscript and supplementary material.

Declarations

Ethics approval and consent to participate

This study does not involve any human, animal or endangered species.

Consent for publication

All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

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

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