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Three Different Fertilizers Enhance Spinach Growth and Reduce Spinach Cd Concentration in Cd Contaminated Alkaline Soil

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Abstract: In order to investigate the impact of peach branch derived fertilizer (PB), cow manure derived fertilizer (CM) and silicon liquid fertilizer (Si) on the growth and Cd uptake of the *Spinacia oleracea* L. in the Cd contaminated soil, a pot experiment was conducted. The fertilizers were applied with low (L), medium (M) or high (H) levels, leading to nine treatments and a control group (CK). As a result, compared to CK, PB increased shoot dry mass by 15 to 46% and reduced shoot Cd by 19 to 56%; CM increased shoot dry mass by 6.1 to 162% and reduced shoot Cd by 38 to 55%; Si showed no effect on plant biomass but significantly reduced the root Cd bioconcentration factor. The CMM and CMH significantly reduced soil-available Cd by 6.5 and 7.5%, respectively, compared to CK. The CM enhanced the plant biomass dilution of Cd and decreased soil-available Cd, but led to higher total shoot Cd accumulation. PB led to simultaneous decline of the shoot Cd and total shoot Cd accumulation, indicating a stronger plant Cd "rejection" effect, independent from biomass accumulation. Si reduced plant root Cd with the sacrifice of biomass accumulation.

Keywords: alkaline soil; dilution effect; mineral nutrients; soil organic matter; vegetable production

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1. Introduction

Due to disordered mining, sewage irrigation and application of substances containing heavy metals, serious farming land cadmium (Cd) contamination has occurred in China [1,2]. A survey, conducted by the China Ministry of Ecology and Environment in 2014, showed that the farmland soil defined as Cd contamination soil exceeded 7% of total farming land [3]. The biomass of crops growing in Cd contaminated soil can be reduced by 15% compared to soil with proper amendments [4,5], threatening land productivity and market supply. It is difficult to naturally remove Cd; however, it is highly toxic and easy to transfer and enrich in edible parts of the agricultural products [6]. Particularly true for leafy greens and compared with root vegetables and fruit vegetables, it is much easier for leafy vegetables to absorb and bioconcentrate Cd from contaminated soil [7]. The Cd content in the aboveground part of spinach can reach up to 4.24–6.94 mg kg^{-1} [8].

In general, using alkaline bulking agents can promote Cd precipitation by forming insoluble molecule or aggregating with other substances [9,10]. Especially in southern China, where the farming soil tends to be acidic, alkaline soil amendments are widely used to increase the pH value of the Cd contaminated soil. This thus decreases soil Cd bioavailability and crop Cd uptake [11,12]. However, in northern China, the farming soil tends to be alkaline. There, using the same method to reduce the bioavailable Cd of the Cd contaminated soil is infeasible [13].

Applying fertilizer with a high organic content is a good way to reduce crop Cd uptake in Cd contaminated soil [14]. The difference in the amount of soil organic matter content is a driving factor of different Cd accumulation in crop [14]. For leafy vegetables, the change of soil pH and soil organic matter contents can explain more than 65% of the plant bioconcentration of Cd [15]. The adsorption of organic substance to Cd is one of the important processes in Cd solidification. Through this process, the organic substance reduces Cd mobility [16,17]. In addition, though Cd ions tend to allocate in smaller soil aggregates, the distribution and adhesion of Cd ions were more influenced by organic matter distribution, but not by soil aggregates size [18]. Some organic soil amendments applied as Cd adsorbent may also stimulate the activity of phosphatase and reduce the organic acids concentration. This will extensively increase the solidification of Cd [19], but cause elevation of soluble organic carbon [20] or organic acids [16] in soil, which may elevate Cd bioavailability. From another perspective, Hu et al. [21] concluded that adding organic fertilizers into heavy metal-polluted soil can significantly increase soil nitrogen, available phosphorus and availability to potassium. The negative influence of soil Cd on crop growth and the crop tolerance to Cd stress can be relieved and enhanced by optimizing nutrient supply at rhizosphere, respectively [22]. For instance, biochar made from cow manure can significantly improve the barley shoot biomass under Cd stress [23], and phosphorus fertilizer can promote wheat shoot dry mass in Cd contaminated soil [24].

Silicon benefits plants in numerous biotic and abiotic stress, and plants with higher Si deposits have higher physical strength and faster hypersensitive reaction under stresses [25]. The increase of antioxidation capacity and reactive oxygen species after silicon amendment may be a reason why heavy metal stress could be relieved in growth of crops [26]. Silicon application can also reduce soil-available Cd. At the same time, it can block plant Cd uptake by reducing active metal ions, co-precipitation toxic metal ions and retarding the metal transporter gene expression [27]. The plant root can deposit silicon in apoplast, thereby, blocking the entry of Cd to cell wall and the precipitation of silicon to Cd suppress its transportation to different plant organs [28]. For example, the root applied silicon can increase the rice biomass in Cd contaminated soil and reduce the shoot Cd concentration [4]. It also can enhance the biomass dilution effect of Cd in wheat, reducing its biological toxicity [29].

In order to investigate the effect of fertilizers with different effective components on the spinach growth promotion and Cd uptake reduction, a pot experiment was designed. The experiment target is to explore the direct impacts of different fertilizers to the soil physicochemical properties, the plant nutrients uptake and the Cd movement between soil and plant, as well as the efficacy of different fertilizers when they are applied in different rates. The plant uptake of Cd and macronutrients, plant biomass accumulation and soil nutrients availability were analyzed, and the potential connection between the plant growth, soil mineral nutrients and Cd movement between plant and soil were also assessed. We hypothesized that the fertilizers would increase the key macronutrients in the soil and reduce the Cd absorption through root, thereby, increasing plant biomass and reducing plant Cd concentration.

2. Materials and Methods

2.1. Experimental Design and Cultivation Method

The pot experiment was conducted from 15 January 2022 to 16 March 2022 in the gut-connected glass greenhouse of the Beijing Academy of Agriculture and Forestry Sciences, located in Haidian District, Beijing, China. The average temperature from January 2022 to March 2022 was 2.3 °C, and the average daylength was 10 h 45 min.

The experiment was a completely randomized block design with five replicates, one pot per replication, for each treatment. The contaminated soil was surface soil (0–20 cm depth) collected from cadmium contaminated fields soils, and the previous crop was maize. The soil was defined as cinnamon soil with the following basic physicochemical properties: pH—7.74, organic matters—11.1 g·kg^{-1}, electric conductivity—0.0147 s·m^{-1}, total nitrogen—1 g·kg^{-1}, total phosphorus—1.5 g·kg^{-1}, total potassium—16 g·kg^{-1}, available phosphorus—23 mg·kg^{-1}, available potassium—155 mg·kg^{-1}. Three types of fertilizers were tested: peach branch derived fertilizer (PB), cow manure derived fertilizer (CM) and silicon liquid fertilizer (Si). Each of the fertilizers were applied with three different levels. Designed application rates for PB and CM were 1 t ha^{-1} (low), 3 t ha^{-1} (medium) and 5 t ha^{-1} (high), equivalent to 1.92 g (low), 5.77 g (medium) and 9.62 g (high) per pot, respectively. The designed application rates for Si were 43 kg (low), 86 kg (medium) and 172 kg (high) per hectare, equivalent to 0.065 g (low), 0.165 g (medium) and 0.330 g (high) per pot, respectively. The equivalent rates were transferred based on the mass ratio of the 0–20 cm depth top layer soil. Therefore, including a control group, there were 10 treatments in total: no organic fertilizer (CK), and the PB, CM and Si with three application levels of each (PBL, PBM, PBH, CML, CMM, CMH, SiL, SiM and SiH). All fertilizers were thoroughly premixed with the soil before potting.

The peach branch derived fertilizer was provided by Beijing Dahua Fertilizer Industry Co., Ltd. (Beijing, China), and its basic physicochemical properties were as follows: pH—6.76, EC—1600 µs cm^{-1}, organic matter—20%, TN—1.28%, P_2O_5—1.03% and K_2O—1.87%. The cow manure derived fertilizer was provided by Beijing Organic Biotechnology Co., Ltd. (Beijing, China), and its basic physicochemical properties were as follows: pH—7.33, EC—1200 µs cm^{-1}, organic matter—23.1%, TN—1.39%, P_2O_5—2.14% and K_2O—1.84%. The silicon liquid fertilizer was provided by Xi'an Fengcai Biotechnology Co., LTD, and its basic physicochemical properties were as follows: Si $\geq$ 24%, K $\geq$ 12%, pH—9.5 to 11 (with 1:100 dilution), liquid specific gravity:1.28.

The *Spinacia oleracea* L. Hongfu hybridization generation I, provided by Beijing Juping Xingli Agricultural Technological Co., Ltd. (Beijing, China), was chosen as the model crop. The planting pots were 13 cm tall with a diameter of 16 cm. Air-dried soil was screened with 2 mm sieves. Then, a mass of 5 kg was weighed for each pot. Before sowing, the pots with soil were soaked in a shallow basin filled with water to make sure the soil inside reached maximum water holding capacity. After the excessive water was totally drained, 15 seeds were sowed in each pot. After the seedlings had spread two true leaves, they were thinned to 5 plants per pot. The pots were weighed daily to check the water consumption and the necessity of watering. They were then watered with deionized water to ensure that the soil water content in pots could be kept around 75% of maximum water holding capacity. The arrangement of the pots' positions within the experiment plot were rotated daily to ensure uniform light conditions for each pot.

2.2. Plant Biomass and Nutrients Uptake

The plants were harvested after 60 days of sowing. The plants were cut at the soil level, at the boundary line between leaves and main root. The parts above the cut were defined as shoot, and the parts below the cut were defined as root. The root parts were cleaned with tap water and rinsed with distilled water and then wiped dried with absorbent paper. After weighing the fresh mass of shoot and root, the plants were preheated in the oven under 105 °C for 10 min, and then, dried under 75 °C for 48 h. The dry mass was weighed.

Dried samples were ground with a ball mill and then sealed for subsequent use. For plant total nitrogen, total phosphorus and total potassium (TN, TP and TK, respectively), 0.5 g of the dry sample was weighed and digested with the sulfuric acid–hydrogen peroxide for each measurement. For plant total calcium and total magnesium (TCa and TMg), 1 g dry sample was digested with hydrofluoric acid and perchloric acid for each measurement. Plant TN was tested with the Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (KDY-9820, KETUO, Beijing, China), whilst the plant TP, TK, TCa and TMg were tested by the inductively coupled plasma emission spectrometry with oscillating extraction (NexION 350 ICP-MS, PerkinElmer, Waltham, MA, USA).

2.3. Soil Physiochemical Properties

After the plants harvest, the soil in each pot was well mixed, and the plant debris was screened out and sampled for further analysis of its physiochemical properties. For the pH of soil, 10 g of the dry sample was suspended in 25 mL distilled water. Immediately after the particles settled, the pH was measured with PHS-3C pH meter (Leici, INASE Scientific Instrument Co., LTD, Shanghai, China). For the EC of soil and organic fertilizers, 10 g of the dry sample was suspended in 50 mL distilled water. After the particles settled down, the EC was measured with TZS-ECW-GA soil salinity meter (Zhejiang Top Cloud-agri Technology Co., Ltd., Hangzhou, China). The organic matter content of soil was analyzed following the potassium dichromate volume-external heating method.

For the soil TN, 0.5 g of the dry sample was weighed and digested with sulfuric acid–hydrogen peroxide for each measurement. It was then tested following the Kjeldahl method with automatic Kjeldahl nitrogen analyzer (KDY-9820, KETUO, Beijing, China). For the available phosphorus (AP), available potassium (AK), exchangeable calcium (ECa) and exchangeable magnesium (EMg) of soil, 0.5 g of the dry sample was extracted with sodium bicarbonate and ammonium acetate for each measurement, respectively. Soil AP was tested following vanadium molybdate yellow colorimetric method with spectrophotometer (Model 722, Modern Science Ltd., Shanghai, China) [30]; soil AK was tested following the flame photometer method (6400A, Precision Scientific Instrument Co., Ltd., Shanghai, China); and soil ECa and EMg were determined with the inductively coupled plasma emission spectrometer (ICAP-6300, Thermo Fisher, Waltham, MA, USA).

2.4. The Cadmium Concentration in the Soil, Organic Fertilizers and Plant, as well as the Plant Cadmium Biological Concentration and Transportation

After the plant harvest, the soil in each pot was well mixed, and the plant debris was screened out and sampled for Cd concentration. For the available Cd (ACd), the dry sample was immersed in DTPA for 2 h with a ratio of soil:liquid of 1:2.5 (mass:volume). It was then filtered with quantitative filter paper (Fan et al., 2020) [31]. The filtrate was used for testing. For the total Cd (TCd) of soil, the dry sample were digested by a mixture of concentrated hydrochloric acid, concentrated nitric acid, hydrogen peroxide and hydrofluoric acid; it was dissolved, filtrated and diluted to a constant volume of 100 mL [32]. This was the final supernatant used for testing. The Cd concentration in the test liquid samples were measured following the procedures indicated by Xie et al., [33] with an inductive coupling plasma mass spectrograph (NexION 350 ICP-MS, PerkinElmer, Waltham, MA, USA). Then, the dry mass based TCd and ACd concentrations were calculated from the test results.

In addition, before the start of the experiment, the pre-test soil and organic fertilizers were well mixed and sampled to determine the initial soil ACd, soil TCd and TCd of the fertilizer products. According to the national soil quality standards for vegetable field issued in 2006 (HJ333-2006), the safety threshold of total soil Cd content for safety vegetable production with soil pH > 7.5 was 0.4 mg kg^{-1} [32]. The soil had an initial TCd of 1.87 mg·kg^{-1} and an initial ACd of 0.60 mg·kg^{-1}; therefore, the soil was defined as highly-contaminated soil. The TCd of the PB and CM were 0.31 mg kg^{-1} and 0.27 mg kg^{-1}, respectively, which were within the safety range. Cd presence in the silicon liquid fertilizer has not been detected.

The measurement procedure for plant Cd concentration was the same as for the soil TCd: the concentration was calculated based on the dry mass. The plant Cd biological concentration level was assessed based on the bioconcentration factor (BF). The calculations of both factors are shown as follows:

$$BF_{shoot} = TCd_{shoot}/TCd_{soil} \tag{1}$$

$$BF_{root} = TCd_{root}/TCd_{soil} \tag{2}$$

2.5. Analysis and Statistics

The influence of different fertilizers and application level on the plant growth, mineral nutrients and Cd absorption, as well as soil physicochemical properties, were compared through one-way ANOVA analysis. The Pearson correlation was performed for the plant growth traits and Cd uptake traits. A principal components analysis was performed for the plant biomass, Cd uptake and soil physiochemical properties. The ANOVA was performed with JMP 16 Pro (SAS Institute Inc., Cary, NC, USA). The Pearson correlation, principal components analysis, violin chart and line chart were produced with Origin Pro 2020 (OriginLab Corporation, Northampton, MA, USA). All data were shown as mean ± standard error.

3. Results

3.1. Plant Cadmium Absorption, Biomass Accumulation and Mineral Nutrient Uptake

The influence of different fertilizers and their application levels on plant Cd absorption are shown in Figure 1. The shoot Cd concentration was 3.83 mg kg^{-1} for CK. Compared with CK, the shoot Cd in the PBM and PBH decreased by 46%, and 56%, respectively; the shoot Cd in the CML, CMM, and CMH groups decreased by 38%, 48% and 55%, respectively; the shoot Cd in SiH decreased by 48.6%. For root Cd concentration, only Si showed a significant effect. When compared to CK, the SiL, SiM and SiH significantly reduced the root Cd from 2.92 mg kg^{-1} to 2.57 mg kg^{-1}, 2.18 mg kg^{-1} and 2.43 mg kg^{-1}, respectively. All the fertilizers applied in medium and high levels showed significant effects in decreasing the plant shoot Cd bioconcentration factor (BF_{shoot}), except for SiM. The PBM, PBH, CMM, CMH and SiH reduced the BF_{shoot} by 48.3%, 53.7%, 48.8%, 54.2% and 60.7%, correspondingly. However, only SiH significantly reduced the root Cd bioconcentration factor (BF_{root}) from 1.53 to 0.83, compared to CK.

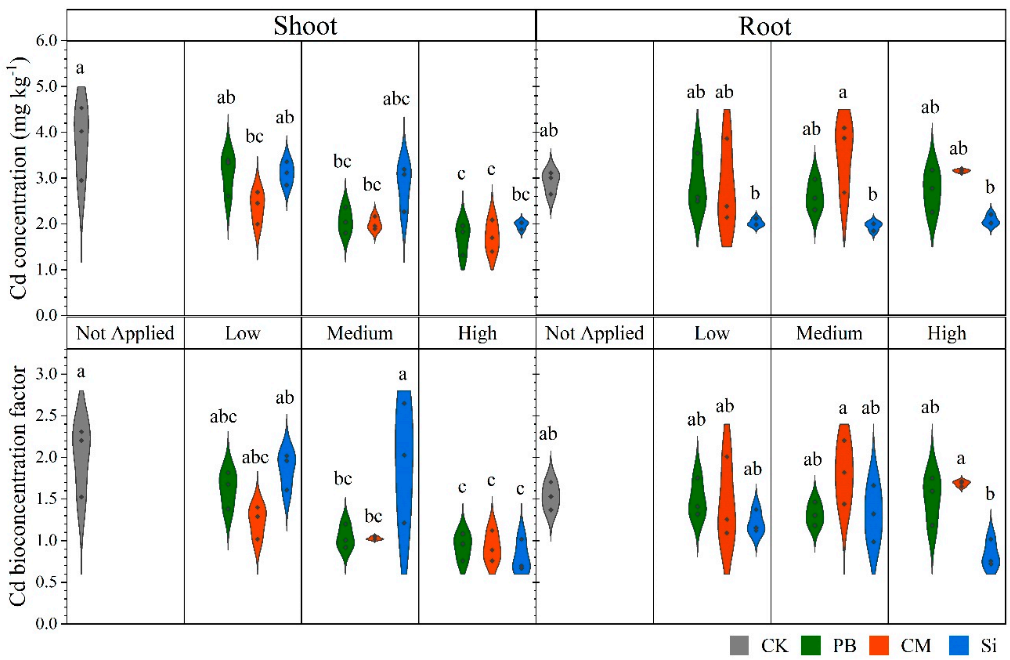

Figure 1. Influence of different fertilizers and application level on the total Cd content and bioconcentration factor of spinach shoot and root in the Cd contaminated soil. The boxes that do not share the same letters are in different homological subgroups, as compared by Tukey HSD.

The root to shoot Cd transfer factor and the total shoot Cd accumulation are shown in Table 1. The PBM, PBH, CMM and CMH significantly reduced the Cd transfer factor. The CMH had the best performance, reducing the factor by 58.0%. For the shoot Cd accumulation, all the treatments' averages fall in the same homogenous subset under Tukey's HSD all pairs multiple comparison; the differences between the averages were big.

Table 1. The influence of different fertilizers and application level on the root to shoot Cd transfer factor and the amount of shoot Cd accumulation. The data are shown in the average of 3 replicates ± standard error. The numbers that do not share any letters are in different homological subgroups, as compared by Tukey HSD.

		Cd Transfer Factor	Shoot Cd Accumulation
		Shoot Cd/Root Cd	mg
CK	0	1.31 ± 0.11 ab	29.47 ± 5.99 a
PB	L	1.10 ± 0.09 abc	27.06 ± 2.84 a
	M	0.80 ± 0.07 cd	20.81 ± 3.11 a
	H	0.63 ± 0.10 d	18.88 ± 2.82 a
CM	L	0.89 ± 0.10 bcd	19.01 ± 1.41 a
	M	0.58 ± 0.08 d	25.69 ± 2.86 a
	H	0.55 ± 0.06 d	34.04 ± 3.31 a
Si	L	1.53 ± 0.08 a	32.88 ± 1.56 a
	M	1.45 ± 0.11 a	31.48 ± 3.67 a
	H	0.95 ± 0.03 bcd	26.82 ± 1.00 a

The influence of the treatments on the plant shoot dry mass accumulation and water content are shown in Figure 2. Not all the treatments showed positive effect, but the PBM, PBH, CMM and SiH significantly increased the spinach shoot water content compared to CK, from 84.7% to 91.2%, 91.0%, 89.8% and 90.5%, respectively. Only CMM and CMH significantly increased spinach shoot dry mass, which were, correspondingly, 71.9% and 162% higher than CK.

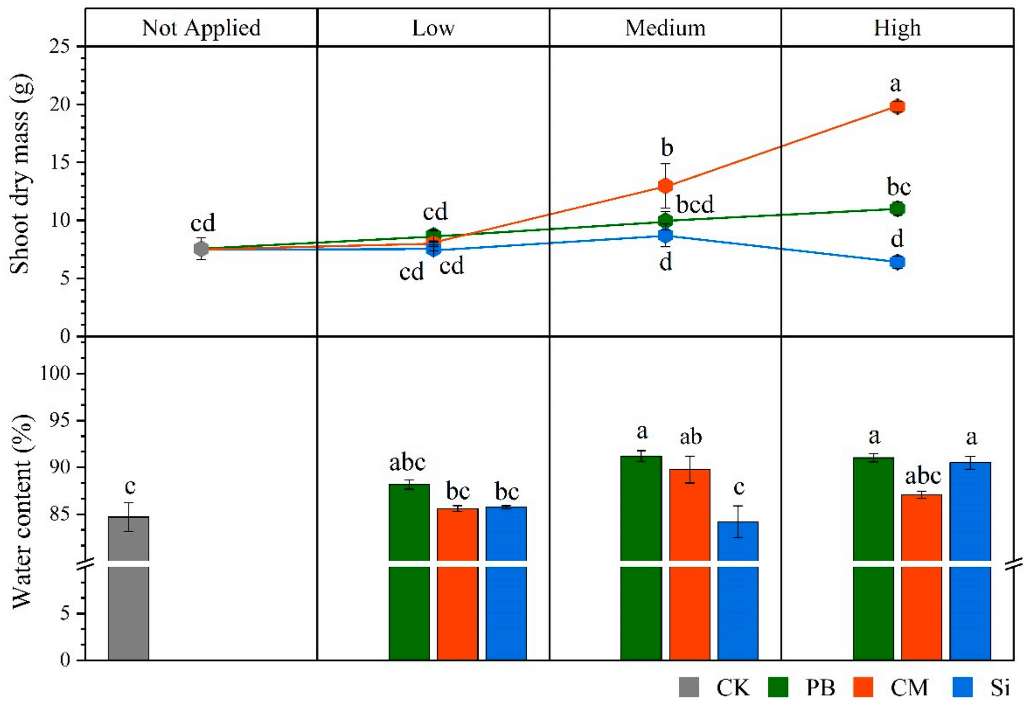

Figure 2. The influence of different fertilizers and application levels on spinach shoot water content percentage and shoot dry mass in the Cd contaminated soil. The data are shown as the averages of 5 replicates ± standard error. The numbers that do not share any letters are in different homological subgroups, as compared by Tukey HSD.

The influence of the treatments on plant shoot nutrients uptake are shown in Table 2. The total nitrogen (plant N), potassium (plant K), phosphorus (plant P), calcium (plant Ca) and magnesium (plant Mg) concentrations of the plant shoot were also significantly impacted. The plant N and plant P of PBM, PBH, CMM and CMH were significantly improved compared to CK, whereas SiH only improved plant N. On the contrary, the application of the fertilizers at medium and high levels tended to reduce the plant K and Ca; compared to CK, the PBM, PBH, CMM, CMH and SiH reduced the plant Ca by 45.2%, 40.2%, 61.2%, 70.6% and 39.0%, respectively, and reduced the plant K by 32.5%, 21.7%, 34.2%, 32.8% and 33.2%, respectively. All treatments reduced plant Mg with a percentage higher than 45%, compared to CK.

Table 2. The influence of different fertilizers and application levels on the nutrients of spinach in the Cd contaminated soil, including the total nitrogen (N), potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) concentrations of plant shoot. The data are shown in the average of 5 replicates ± standard error. The numbers that do not share any letters are in different homological subgroups, as compared by Tukey HSD.

		N	**P**	**K**	**Ca**	**Mg**
		g kg^{-1}	**mg kg^{-1}**	**g kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**
CK	0	13.03 ± 1.21 d	23.90 ± 1.85 b	2.95 ± 0.23 ab	119.69 ± 10.19 a	96.66 ± 12.44 a
PB	L	14.38 ± 0.31 cd	75.43 ± 3.95 ab	3.03 ± 0.20 ab	103.51 ± 15.10 abc	31.09 ± 0.91 b
	M	27.58 ± 0.78 ab	72.03 ± 16.30 ab	1.99 ± 0.11 c	65.56 ± 6.49 cd	43.39 ± 2.28 b
	H	26.31 ± 2.27 ab	112.87 ± 13.43 a	2.31 ± 0.04 bc	71.57 ± 1.90 bcd	30.80 ± 1.57 b
CM	L	13.40 ± 0.03 d	85.50 ± 17.43 ab	3.41 ± 0.09 a	105.79 ± 8.67 ab	20.85 ± 3.48 b
	M	30.19 ± 2.28 a	99.27 ± 9.05 a	1.94 ± 0.28 c	46.46 ± 6.17 d	32.84 ± 4.59 b
	H	28.28 ± 0.17 ab	64.33 ± 12.31 a	1.98 ± 0.04 c	35.23 ± 2.29 d	52.76 ± 4.99 b
Si	L	13.2 ± 0.13 d	57.7 ± 10.80 ab	3.03 ± 0.15 ab	127 ± 3.16 a	34.7 ± 5.29 b
	M	13.1 ± 0.83 d	69.3 ± 3.74 ab	2.93 ± 0.24 ab	127 ± 3.53 a	31.6 ± 2.83 b
	H	21.8 ± 3.48 bc	56.3 ± 12.75 ab	1.97 ± 0.19 c	73.0 ± 9.34 bcd	45.5 ± 12.71 b

3.2. Soil Physicochemical Properties, Mineral Nutrient and Cadmium Content

Effects of the fertilizers and their application levels on the soil physicochemical properties are shown in Table 3. Though still alkaline, all treatments significantly reduced soil pH compared to CK; SiH had the most effective influence. PBM, PBH, CMM and CMH significantly increased soil EC, but SiL, SiM and SiH reduced the soil EC. PBH significantly elevated the soil organic matter (OM) by 90% compared to CK. Similarly, the PBH and CMH increased soil TN by 68% and 62%, respectively, compared to CK. Both PB and CM increased soil AP and AK, with the exception of PBL; the enhancement of soil AP and AK also increased with the increase of application rate, all compared to CK. The CMM and CMH reduced the soil ACd by 6.5 and 7.5%, compared to CK; none of the treatments had a significant influence on soil ECa. The Si tended to reduce soil EMg, and the SiL significantly reduced soil EMg by 43.7% compared to CK.

3.3. The Relation between the Plant Growth, Soil Nutrients and the Cd Migration in Soil-Plant System

The Pearson correlation between the traits of plant Cd absorption and plant growth is shown in Figure 3. The spinach shoot Cd concentration and Cd transfer factor showed significant negative correlations with shoot water content, shoot dry mass, shoot N and shoot P, but also showed significant positive correlations with shoot K and Ca. Shoot Cd bioconcentration showed a similar trend, except for shoot dry mass and shoot P. Shoot Cd accumulation was only negatively correlated to shoot water content. The root Cd and root Cd bioconcentration factor had significant positive correlation with shoot dry mass, and the root Cd was also positively and negatively correlated to shoot N and shoot Ca.

Table 3. The influence of different fertilizers and application level on the soil physicochemical properties, including pH, EC, organic matter, total nitrogen concentration (Total N), total cadmium concentration (TCd), available phosphorus concentration (AP), available potassium concentration (AK), available cadmium (ACd), exchangeable calcium (Ca^{2+}) and exchangeable magnesium (Mg^{2+}). The data are shown as the average of 5 replicates ± standard error. The numbers that do not share any letters are in different homological subgroups, as compared by Tukey HSD.

		pH	EC	Organic Matters	Total N	Soil TCd	AP	AK	ACd	Ca^{2+}	Mg^{2+}
			μs cm^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}
CK	0	8.31 ± 0.06 a	243 ± 4 d	18.23 ± 0.42 b	1.12 ± 0.01 c	1.91 ± 0.04 ab	9.00 ± 0.12 f	161 ± 3 e	0.93 ± 0.01 a	6.49 ± 0.68 a	1.03 ± 0.09 abc
PB	L	7.99 ± 0.06 bc	246 ± 3 cd	19.92 ± 0.66 b	1.21 ± 0.01 bc	1.92 ± 0.06 ab	15.10 ± 0.27 f	172 ± 7 e	0.92 ± 0.00 a	5.96 ± 0.08 a	1.03 ± 0.05 ab
	M	7.61 ± 0.05 de	283 ± 3 bc	21.98 ± 0.11 ab	1.35 ± 0.03 abc	1.98 ± 0.02 ab	40.01 ± 2.60 d	216 ± 7 de	0.93 ± 0.01 a	5.65 ± 0.02 a	1.15 ± 0.03 a
	H	7.81 ± 0.02 bc	287 ± 2 bc	34.60 ± 8.71 a	1.88 ± 0.41 a	1.82 ± 0.05 b	57.30 ± 2.12 c	285 ± 33 bc	0.93 ± 0.01 a	5.79 ± 0.26 a	1.20 ± 0.09 a
CM	L	8.01 ± 0.04 c	246 ± 38 cd	21.47 ± 0.41 ab	1.32 ± 0.03 abc	1.93 ± 0.02 ab	29.40 ± 0.91 e	246 ± 15 cd	0.92 ± 0.01 a	5.50 ± 0.08 a	1.08 ± 0.04 a
	M	7.83 ± 0.03 cd	294 ± 5 b	26.06 ± 0.85 ab	1.61 ± 0.04 abc	1.95 ± 0.09 ab	68.00 ± 3.03 b	337 ± 9 b	0.87 ± 0.00 b	5.89 ± 0.26 a	1.35 ± 0.07 a
	H	8.01 ± 0.04 c	383 ± 18 a	31.98 ± 0.20 ab	1.81 ± 0.07 ab	1.87 ± 0.02 ab	97.60 ± 0.91 a	448 ± 3 a	0.86 ± 0.01 b	6.72 ± 0.62 a	1.42 ± 0.23 a
Si	L	7.89 ± 0.04 bc	200 ± 8 e	18.90 ± 0.43 b	1.07 ± 0.01 c	2.03 ± 0.05 ab	10.00 ± 1.29 f	190 ± 15 de	0.92 ± 0.00 a	5.65 ± 0.47 a	0.58 ± 0.01 d
	M	7.85 ± 0.02 bc	188 ± 12 e	18.80 ± 0.44 b	1.07 ± 0.01 c	1.95 ± 0.05 ab	9.96 ± 0.87 f	176 ± 6 e	0.93 ± 0.03 a	5.44 ± 0.06 a	0.58 ± 0.01 cd
	H	7.51 ± 0.02 e	215 ± 5 de	18.10 ± 0.22 b	1.10 ± 0.01 c	2.08 ± 0.06 a	11.50 ± 2.27 f	184 ± 4 de	0.94 ± 0.01 a	5.51 ± 0.10 a	0.58 ± 0.00 bcd

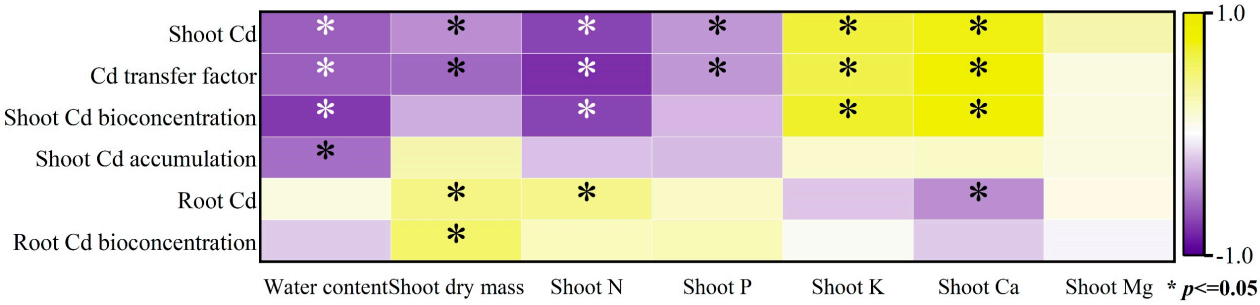

Figure 3. The Pearson correlation between the Cd uptake traits and the growth traits of spinach. The Cd uptake parameters were lined vertically, including the Cd concentration in shoot and root, the Cd bioconcentration factor of shoot and root, the total amount of shoot Cd accumulation, and the root to shoot Cd transfer factor. The growth traits were lined horizontally, including shoot water content, shoot dry mass, and the concentration of nitrogen, phosphorus, potassium, calcium and magnesium of shoot.

The principal components analysis of the traits of plant growth, nutrients absorption, Cd absorption and soil properties are shown in Figure 4. The principal component (PC) 1 explained 52.3% of the total variance of the traits assessed. The soil pH, plant root Cd bioconcentration factor, shoot dry mass, soil ECa, root Cd, soil AK, soil EMg, soil EC, soil AP, soil OM, soil TN and plant shoot water content were positively influenced by the PC1, whereas the shoot Cd accumulation, shoot Cd bioconcentration factor, shoot Cd, root to shoot Cd transfer factor and soil ACd were negatively influenced by PC1. PC2 explained 20.4% of the total variance of the traits assessed. The soil ACd and shoot water content were negatively influenced by PC2, whilst all other traits were positively influenced by PC2. The cluster of Si data points showed distinct separation from the data points clusters of PB and CM.

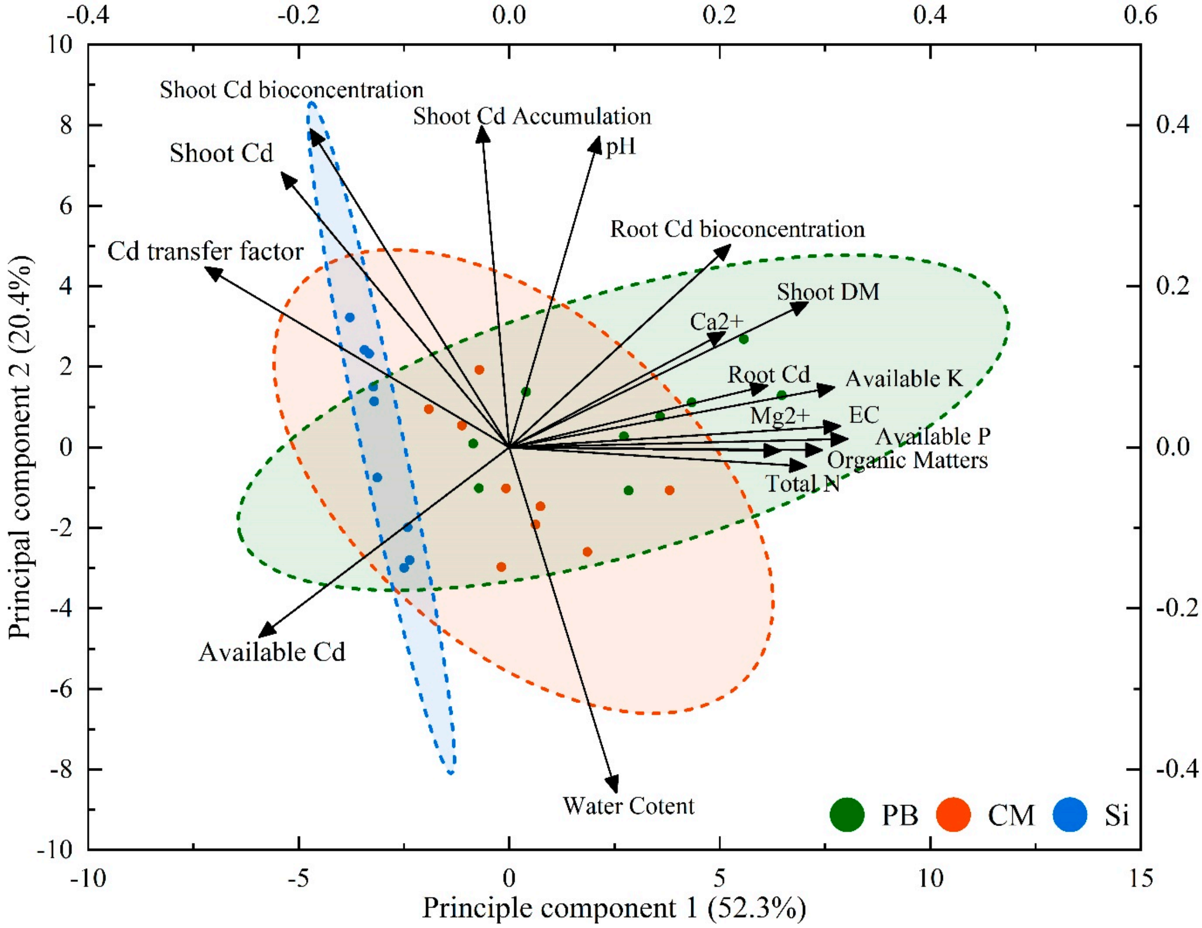

Figure 4. The principal components analysis of plant biomass, nutrients uptake, Cd absorption and soil physiochemical properties. DM—dry mass, Ca^{2+}—soil exchangeable calcium, Mg^{2+}-soil exchangeable magnesium.

4. Discussions

The amendments of fertilizers have been widely studied for their potential applications in decreasing the crop Cd concentration, but the differences brought by different effective components and how they influence the plant Cd absorption and inner plant Cd transportation were not researched well. Here, the influence of the different fertilizers on the plant growth, Cd uptake of and soil physicochemical properties were addressed, and the potential relations between them were discussed.

4.1. The Improvement of Peach Branch Fertilizer and Cow Manure Fertilizer on Plant Biomass, Plant Cd Concentration and Total Cd Accumulation

The alleviation of Cd toxicity was firstly reflected in biomass improvement [4,23,24,34], which was consistent with our results. Both PB and CM increased plant shoot biomass, and the effect increased with the dosage, especially the CM, which showed a higher efficacy on the biomass improvement with the application rate increasing. Optimizing mineral nutrients supply can relieve the plant from Cd stress and promote growth [22]. The PCA results also indicated that the change of soil macronutrients availability and shoot DM were mainly driven by the same factors. The CM extensively increased the soil AP and AK compared to PB, which could be the reason for its superior effect on plant growth than PB. Specifically, the extensively higher AP in CM contributed to the lower shoot Cd (Figure 4); higher phosphorus availability had been widely proven to benefit biomass accumulation and the dilution effect of systems under Cd stress [35–37].

Therefore, the reduction of shoot Cd after soil amended with PB and CM could be a dilution effect of biomass to the Cd concentration, which was first described by Rizwan et al. [29]. The aerial part biomass instead of Cd concentration determines the total accumulation of Cd in the shoot [34]. For this reason, the total Cd accumulation in CM plants were significantly higher than those of PB, despite the extensive decline of shoot Cd concentration observed in CM compared to PB. Thus, CM treatments had a better efficacy on Cd removal per production area, whilst the PB had higher efficacy on reducing the Cd content in spinach products: the PB treatment did not require such a high biomass such as CM to achieve the reduction of shoot Cd. Furthermore, the simultaneous occurrence of the biomass increase and Cd accumulation decline in PB implied a better performance in minimizing the plant Cd contamination risk.

Many researchers demonstrated that leafy vegetables may use the Ca^{2+} channel in the root to absorb and transport the Cd from contaminated soil [38,39]. The PCA results did show that the change of soil ECa and root Cd were driven by the same factors. The higher calcium availability in rhizosphere can suppress the Cd intake through plant root, but also stimulates the xylem loading of Cd, increasing root to shoot translocation [40]. On the contrary, although the low calcium supply would exacerbate the Cd toxicity to the root, it reduces the shoot calcium concentration together with the Cd translocation to the shoot [41]. In our experiment, neither PB nor CM reduced root Cd; however, both significantly reduced the shoot Cd and Cd transfer factors. Therefore, the significant reduction of shoot Ca appeared together with the significantly reduction of Cd transfer factor, without any change in root Cd, indicating the importance of Ca^{2+} in Cd inner plant translocation. The function of Ca^{2+} and the influence of shoot Ca^{2+} concentration on root to shoot Cd translocation deserve further study. The target could not only be suppressing Cd concentration of the crop-edible part, but could also be increasing the crop Cd accumulation for soil Cd removal.

4.2. The Influence of Silicon Liquid Fertilizer on Plant Cadmium Concentration and Inner Plant Cd Transportation

The reduction of plant Cd under Si application was not related to soil ACd. Researchers concluded that the change of soil organic matter and total organic carbon were highly associated with the soil Cd availability [42]. Since the silicon fertilizer in our experiment did not contain organic content, the soil ACd was not shifted after its application. Normally, plant shoot is more sensitive than root to the fluctuation of Cd stress [34]. That is why plant responses to the change of soil Cd bioavailability or the relief of Cd stress are more easily observed in the shoot but not the root; the reduction of Cd concentration and the improvement of biomass tend to appear solely in shoot [4,20]. The proper soil amendment would significantly reduce the Cd translocation from root to shoot, but not decrease the root Cd concentration [4,20]. However, in our experiment, the Si showed an effect on root Cd reduction and did not improve the shoot biomass, which was totally different from the influence brought by PB and CM. Hence, the blocking of Si to the Cd may take place in the root cell wall. The competition between the heavy metal ions and Si on the same transporter protein may also influence the entry of ions [28]. The reduction of root Cd had not benefited plant biomass accumulation but suppressed the growth of whole plant; the mechanism deserves further study.

4.3. The Influence of Fertilizers on Soil Physiochemical Properties and Cd Availability

The soil pH decreased after amending PB, CM and Si in the soil. However, such a reduction of pH was limited (only by 0.3~0.8) and soil pH after treatments was still higher than 7.5. However, the pH of Si was higher than 9, and soil pH was still reduced. Neither the PB soil, nor Si soil, significantly changed the soil ACd concentration. On the contrary, CMM and CMH significantly reduced the soil ACd. Compared to cow manure, peach tree branch has a much higher lignin content, and silicon liquid fertilizer was an industrial product, these differences could lead to the different impacts on the soil physiochemical properties.

In most cases, the increase of soil pH was the key function brought by soil amendments targeting Cd retardation [16,43,44]. However, some organic substance, including lignin made products, could significantly elevate the soil pH and available Cd at the same time due to the higher soluble organic carbon it brought into the soil [20]. Specifically, for alkaline soil, such as in our experiment, the beneficials of the lignin products on Cd solidification would be diminished due to the increase of soluble hydrophilic organic matters [45]. Thus, the lower pH in PB soil had no advantages on Cd precipitation, but even increased the competition between the H^+ and the Cd^{2+} on the bonding sites of the organic particle surfaces [46].

Adding organic fertilizers into heavy metal polluted soils is able to improve soil quality by significantly increasing organic matters and AN, AP and AK contents in soils, as well as decreasing Cd availability in soils [21]. The higher TP content in the fertilizer derived from cow manure than the one derived from peach branch and silicon liquid fertilizer could be the reason why the CM soil had higher AP than the PB and Si did. In our experiment, the soil OM, TN, AP and AK were increased by adding PB and CM; Only CM had a significant effect on soil ACd reduction when applied at a higher ratio. The significantly higher phosphorus content in CM and the soil after applied CM could be the reason for reduced soil ACd. Much research has addressed the importance of increasing phosphorus for Cd retardation, especially in the assistance for the organic matter adsorption to Cd [16,19,47].

5. Conclusions

Our hypothesis that fertilizer with different effective contents could alleviated spinach Cd stress with different mechanisms was not fully accepted. The fertilizers derived from organic sources had much better effects on the improvement of soil organic matter, soil macronutrients concentration and plant biomass; there was an alleviation of plant Cd toxicity and soil-available Cd in which cow manure derived fertilizer had higher efficacy than peach branch derived fertilizer when their application rates were increased. However, CM treatments increased the total shoot Cd accumulation, whereas PB also led to the total shoot Cd accumulation decline, indicating a stronger plant Cd "rejection" that is independent from biomass accumulation. Si was effective on the reduction of plant root Cd bioconcentration factor, but also led to plant biomass accumulation. Hence, the fertilizers derived from organic sources had a better influence on the plant biomass accumulation and soil Cd removal. Fertilizer with silicon as the effective component could block the plant Cd absorption from the root. The distinguishing reasons of the three fertilizers on plant inner balance between Cd absorption and biomass dilution are valuable for vegetable production on Cd contaminated soil. Further studies that target the function of silicon on plant root Cd uptake and its relationship with macronutrient absorption deserve further study.

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