

Combined Effects of Inoculating *Serendipita Indica* on Soybean Growth and Soil Health Under Cd Stress

Xiaohui Wang

Heilongjiang University

Xiaoxu Fan

Heilongjiang University

Weidong Wang

Heilongjiang Bayi Agricultural University

Fuqiang Song (✉ 0431sfq@163.com)

Heilongjiang University <https://orcid.org/0000-0001-7754-5058>

Research Article

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Abstract

Cadmium (Cd) pollution in the soil is a global environmental problem. Plants-microbial technology has been regarded as a potential technique for the remediation of Cd polluted soils. Here, we aimed to explore the combined effects of inoculating (*Serendipita indica*) *S. indica* on soybean growth and soil health under Cd stress. Therefore, a pot experiment was conducted to investigate the *S. indica* on soybean growth and the soil enzyme activities, pH and chemical forms of Cd in the soil under Cd 0, 10, 20, 30 mg/kg soil treatments. Results reflected that compared to non-inoculated ones, the application of *S. indica* can still enhance the dry weight (66.57%), shoot height (90.35%) and promote the net photosynthesis rate (72.18%), transpiration ratio (80.73%), and stomatal conductance (119.05%) photosynthesis of soybean under Cd 30 mg/kg soil. Furthermore, The pH, phosphatase (116.39%) and catalase (4.17%) activities in the *S. indica* treatments were increased under Cd 10 mg/kg soil. Meanwhile, inoculated *S. indica* treatments significantly shifted Cd from exchangeable fraction to other more stable fractions, primarily decreased Cd concentrations (23.66%) under Cd 20 mg/kg soil. The Cd pollution assessment in soil indicated that *S. indica* could effectively reduce Cd pollution in the Cd 10 mg/kg soil treatments. This work suggests that *S. indica* may be a potential method for not only promoting plant growth, but also relieving the phytotoxicity of Cd and remediating Cd contaminated soil.

Introduction

Soil heavy metal pollution is one of the main factors restricting the safety and development of agricultural products, which has caused great ecological damage to the environment. On the one hand, the heavy metals in soil come from the waste water, waste residue and waste gas discharged in the process of industrial production, on the other hand, they come from the excessive use of pesticides and chemical fertilizers by farmers. Cd is one of the most dangerous elements to the environment and human health because of its dynamic migration and easy accumulation. It is also an unnecessary, undegradable and harmful element to plants and humans (Satarug et al. 2004). When the accumulation of Cd in the soil exceeds the tolerance range of plants, the physiological and respiratory processes of plants will be disordered and plant growth will be hindered (Sebastian et al. 2019).

Soybean (*Glycine max* (Linn.) Merr.), as the main crop in Asian countries, plays a crucial role in providing proteins because it can fix nitrogen by endosymbiotic nodule bacteria and improve soil fertility. However, Cd seriously affects the growth of soybean and potentially leads to severe impacts on public health (Xian et al. 2020; Zhi et al. 2020). Furthermore, oxalate accumulation induced by Cd contributes to the inhibition of root growth in soybean. Overexpression of wild soybean Oxalyl-CoA synthetase can reduce oxalate accumulation and increase Cd tolerance of hairy soybean roots (Xian et al. 2020). Therefore, we hope that Cd can be fixed in the soil and not easily absorbed by plants. Also, even if plants absorb Cd, it can be enriched in the non edible parts, so that the edible parts of plants have no or low concentrations of Cd. The technology of symbiosis between endophytic fungi and plants has the potential to solve these problems. (Li et al. 2020).

The root endophytic fungus, *S. indica*, can colonize in the roots of plenty of plant species and promote the absorption of nutrients. Besides, the antioxidant defense system of the plant can be enhanced by the fungus, which has a vital role in resistance against biotic and abiotic stresses (Valente 2019). *S. indica* can regulate the hormone level of plants to resist Cd stress by mediating the signal transduction of salicylic acid, ethylene, jasmonic acid and other plant hormones. (Akum et al. 2015; Alga et al. 2011; Ruchika et al. 2018; Hilbert et al. 2013). A successful *S. indica* colonization can enhance the secretion of defensive secondary metabolites, such as catalase, ascorbic acid and glutathione to remove the active oxygen caused by Cd toxicity (Padash et al. 2016; Shayan et al. 2017; Shahabivand et al. 2017; Vahabi et al. 2016). Moreover, *S. indica* can accumulate Cd in the plant roots and restrict their movement to aerial parts (Shahabivand et al. 2017).

The symbiotic relationship between fungi and host plants can produce a synergistic effect on Cd phytoremediation. There were few reports on the physiological characteristics and soil health of soybean under Cd stress. Therefore, in this research, we hypothesized that *S. indica* could promote soybean growth and transform Cd into a form, which is not easily utilized by plants under high Cd stress, thus reducing the risk of Cd flowing into the food chain. We conducted pot experiments to determine the effects of Cd stress and *S. indica* on soybean growth, soybean physiological characteristics, the enzyme activities and the accumulation of Cd in the soil. In addition, the ecological risk of soybean soil inoculated with *S. indica* was analyzed to reveal the application prospect of *S. indica* in the remediation of Cd contaminated farmland.

Materials And Methods

Plant and fungal materials

Heinong 48 soybean seeds purchased from seed station, Harbin, China. Seeds were surface sterilized by soaking in 70% ethanol for 2 min and 4% sodium hypochlorite for 10 min, and then washed with double distilled water for 4 times. *S. indica*, is a strain preserved and propagated in the laboratory, was cultured in Petri dishes on a Hill & Käfer (2001) medium at $30 \pm 1^\circ\text{C}$ in the dark for 14 days. The mycelium plugs of *S. indica* (10 mm) were taken from the edge of the fungus culture plates (7.3×10^4 spore / plug). The fungal plugs of non-*S. indica* treatments were the same as *S. indica* treatments but it was autoclaved.

Soil preparation

The soil of pot experiment was collected from farmland soil around Nangang District of Harbin City, Heilongjiang Province, PR China, is a typical black soil, and then physicochemically characterized: The soil pH was 7.8, organic matter content was 24.3 g/kg, total nitrogen was 1.8 g/kg, available nitrogen was 50.3 mg/kg, available potassium was 213.1 mg/kg, available phosphorus was 9.2 mg/kg, and background Cd concentration is 0.128 mg/kg soil. Air-dried soil was sieved with a 2 mm sieve and autoclaved sterilized 3 times at 100°C for 1 h to eliminate AM fungal spores and other microorganisms activities. After that, four concentrations of Cd (0, 10, 20 and 30 mg/kg soil) were added to the soil in the

form of $\text{CdCl}_2 \cdot 5\text{H}_2\text{O}$ aqueous solution. Then the soil samples were incubated at 20°C for 30 days to make the Cd evenly distributed and stabilized in the soil solid phase.

Experimental design

The greenhouse experiment was conducted in a 2 × 4 factorial design with a completely random design. Two *S. indica* treatments were - S (non-inoculation / control) and + S (*S. indica*); Four soil Cd concentrations were Cd 0 mg/kg soil (CK), Cd 10 mg/kg soil (LH), Cd 20 mg/kg soil (MH), and Cd 30 mg/kg soil (HH). Each treatment was repeated 10 times.

Sampling

Each experimental plastic pot (30×15×15 cm³) was filled with 5 kg four added Cd concentrations soil and five soybean seeds with one fungal plug below. Every pot was watered once every four days. The soybean plants were harvested after about 120 days of planting (June 4th - October 4th, 2018). The shoots and roots were rinsed with distilled water, wiped with tissue paper and weighted. Then, shoot height was determined, and finally dried at 75°C for 48 h to determine the dry weights and Cd concentrations. Furthermore, root subsamples were stored in 50% ethanol for root colonization assessment. The rhizosphere soil samples were collected from each basin, thoroughly mixed, passed through 10, 60 and 100 mesh nylon screens, and divided into two parts for storage: one part was air-dried for the determination of soil pH, Cd content and chemical form, and one part was preserved at 4°C for the determination of soil enzyme activity.

Root colonization

The trypan blue was used to estimated root colonization according to Phillip's (1970) method. After 60 days of co culture, 3 samples were randomly selected from each treatment. The roots were washed with distilled water, cut into 1 cm root pieces, and put in 10% KOH solution overnight, then washed with distilled water for 3–5 times. After soaking in 1% HCl for 3–5 min, 0.05% trypan blue staining was performed for 1 min, After that, the root pieces were washed in distilled water for 8–10 times. Slides were prepared and observed under the light microscope, and took photographs.

Measurement of photosynthesis

At the blooming stage of soybean, the net photosynthetic rate (Pn), stomatal conductance (Tr), intercellular CO₂ concentration (Ci), and transpiration rate (Gs) of the flag leaves were measured by an ultra-light portable photosynthesis system (CI-340, CID Inc., USA) during 9:00 ~ 11:00 am, July 20th, 2018.

Soil enzyme activities

The soil urease activity was assayed by phenol sodium colorimetric method, and the results were expressed as the number of milligrams of NH₃-N released in 1 g of soil after incubation at 37 °C for 24 h (mg / g). The soil sucrase activity was measured by 3, 5 - dinitrosalicylic acid colorimetry (DNS method), and the results were expressed as the number of milligrams of glucose hydrolyzed in 1 g of soil after incubation at 37 °C for 24 h (mg / g). The soil phosphatase activity was assayed by sodium diphenyl

phosphate colorimetry method, and the results were expressed as the number of milligrams of phenol released in 1 g of soil after incubation at 37 °C for 24 h (mg / g). The soil catalase activity was determined by UV spectrophotometry (240 nm), and the results were expressed as the number of milligrams of hydrogen peroxide consumed in 1 g of soil after incubation at 20 °C for 0.5 h (mg / g) (Yang et al. 2007; Ge et al. 2017; Trasar. 1999).

Cd determination

Cd content was analyzed according to Vieira et al. (2005). The dried soil samples (0.25 g) were digested with HNO₃ and HClO₄ (5:1) in a microwave oven. The Cd concentrations were estimated by an inductively coupled plasma mass spectrometry (ICP-MS, XSeries2, ThermoFisher Scientific, Waltham, MA, USA).

Determination of Cd chemical forms

According to Tessier method (1979), all the soil samples were extracted in sequence to determine the form of Cd in the soil. Cd were divided into five different forms: exchangeable form (MgCl₂, pH = 7); carbonate form (NaOAc, pH = 5); reducible iron and manganese form (NH₄OH + HCl); organic matter bound form (HNO₃ + H₂O₂, H₂O₂, NH₄OAc); and residual form (HF + HNO₃ + H₂O₂). The supernatant liquid was separated from the solid phase by centrifugation at 1917 g for 20 min after each extraction step to determine the concentrations of Cd (mg / kg soil).

Assessment methods of heavy metal pollution

The single pollution index method (Hakanson 1980) was used to evaluate the risk of heavy metal pollution in soybean soil. The formula of the single pollution index method was as follows:

$$I_j = C_j / C_0$$

In the formula:

I_j represents the single factor index of the pollution;

C_j is the measured concentration of the pollution (mg/kg);

C_0 is the assessment standard of the pollution (mg/kg).

The contribution of *S. indica* in reducing Cd pollution was expressed by the decrease rate of Cd.

$$R_p = (R_s - R_c) / R_s$$

In the formula:

R_p represents the decrease rate of Cd;

R_s is the concent of total Cd in soil inoculated with *S. indica* (mg/kg);

R_c is the content of total Cd in non inoculated *S. indica* soil (mg/kg).

Statistical analysis

The experimental data were statistically analyzed by Statistical Product and Service Solutions (SPSS) 22.0 software version. Two-way analysis of variance (ANOVA) was used to statistically analyze the significance of each treatment. The primary factors (*S. indica* and Cd levels) and their interactions were evaluated. Duncan's Multiple Range Test was used to compare the mean values of 0.05 and 0.01 probability levels. Data were expressed as the means of replicates \pm standard error (SE). Redundancy analysis (RDA) in CANOCO for Windows (version 5.0, Microcomputer power, Ithaca, NY, US; ter Braak et Šmilauer, 2019) was used to draw the relationships between variables.

Result

Establishment of the symbiotic relationship between *S. indica* and soybean

S. indica can infect soybean roots, especially establish symbiotic relationships with mature root soybean (Fig. 1). The spore in soybean roots is a typical pear type. The results showed that with the CK, LH, MH, and HH treatments, the colonization rate of *S. indica* was 83.81%, 67.46%, 47.62% and 34.92% after inoculation (Table 1). The root colonization rate decreased significantly with the increase of Cd content in soil ($P < 0.01$). The infection rate of *S. indica* was the lowest under HH treatment (Table 1). Though the colonization rate decreased, the *S. indica* was still functional. Still, the promotion effect of *S. indica* on soybean growth, soil enzyme activity and so on was limited at high Cd concentration (Table 1).

Effects of *S. indica* on soybean growth and development

If there is excessive Cd accumulated in the soil, the growth and development of plants will be incredibly restricted. However, these studies have suggested that inoculated *S. indica* can significantly alleviate this inhibition (Table 1). Compared with the control, CK, LH, MH and HH treatments with *S. indica* increased the plant height of soybean by 11.00%, 13.52%, 31.52% and 90.35%. After treatment with *S. indica*, the dry weights of soybean under Cd concentration gradient were increased by 137.35%, 116.60%, 84.07% and 66.57%, respectively. The maximum dry weight of soybean was 43.91 g, obtained after treatment with CK and *S. indica*. The effect of the *S. indica* on the dry weight and plant height of soybean had different trends. Cd treatment and fungal treatment had significant main effects and interaction effects ($P < 0.01$) on root colonization and growth parameters of soybean (Table 1).

Table 1

Effects of *S. indica* and Cd treatment on root colonization, plant height, and dry weight in soybean

Fungal treatment	Cd treatment	Root colonization (%)	Shoot length (cm)	Dry weight (g/plant)
-S	CK	0 ± 0 e	63.92 ± 0.47 ab	18.50 ± 0.44 c
	LH	0 ± 0 e	56.50 ± 0.50 ab	11.74 ± 0.20 e
	MH	0 ± 0 e	46.00 ± 0.70 c	9.23 ± 0.75 f
	HH	0 ± 0 e	30.47 ± 1.75 d	6.97 ± 0.35 g
+S	CK	83.81 ± 0.33 a	70.95 ± 1.58 a	43.91 ± 1.49 a
	LH	67.46 ± 0.36 b	64.14 ± 0.42 ab	25.43 ± 1.03 b
	MH	47.62 ± 0.41 c	60.50 ± 0.45 b	16.99 ± 0.72 d
	HH	34.92 ± 0.14 d	58.00 ± 0.57 b	11.61 ± 0.66 e
Main effect				
-S		0 ± 0 b	52.16 ± 1.37 b	11.61 ± 0.45 b
+S		58.45 ± 0.95 a	62.77 ± 0.61 a	24.48 ± 1.28 a
Main effect				
	CK	41.90 ± 4.56 a	66.73 ± 0.51 a	31.20 ± 13.95 a
	LH	33.73 ± 3.70 b	61.85 ± 0.51 a	18.58 ± 7.52 b
	MH	23.81 ± 2.62 c	55.67 ± 0.88 b	13.11 ± 4.30 b
	HH	17.46 ± 1.91 d	48.82 ± 1.46 c	9.29 ± 2.59 b
ANOVA				
S		--	**	**
Cd		--	**	**
S × Cd		--	**	**

- S: non-inoculation (control), + S: *S. indica*. The value represents the mean ± SE. The same letter in the same row of data in the table indicates significant no differences among treatments using Duncan's Multiple Range Test in the level of $P < 0.05$.

** $P < 0.01$.

Effects of *S. indica* on photosynthesis of soybean

Understanding the photosynthetic response of plants is central to comprehending the physiological response. Table 2 showed the effects of *S. indica* and Cd on photosynthetic parameters of soybean.

Compared with CK, LH, MH, and HH treatments significantly reduced the net photosynthetic rate (Pn), stomatal conductance (Tr), intercellular CO₂ concentration (Ci), and transpiration rate (Gs) of soybean ($P < 0.05$). Further, Pn, Tr, Ci, and Gs were significantly different across CK, LH, MH, and HH treatments ($P < 0.05$). The application of *S. indica* increased Pn, Tr, Gs, but reduced Ci, and there was no significant difference in the intercellular CO₂ concentration ($P < 0.05$). Compared with the uninoculated treatment, *S. indica* led to a maximum increase under HH in the Pn (72.18%), Tr (80.73%), and Gs (119.05%), and maximum reduction under CK in Ci (12.93%). Data from Table 2 revealed that Cd treatment and fungal treatment had significant main effects and interaction effects ($P < 0.01$) on photosynthetic physiological parameters of soybean.

Table 2

Effects of *S. indica* and Cd treatment on photosynthetic physiological indexes of soybean leaves

Fungal treatment	Cd treatment	Pn (um/m ² /s)	Tr (mmol/m ² /s)	Ci (ppm)	Gs (mmol/m ² /s)
-S	CK	14.47 ± 0.30 c	3.79 ± 0.29 b	366.40 ± 25.48 a	257.80 ± 24.30 bc
	LH	12.45 ± 0.96 de	3.57 ± 0.21 b	339.67 ± 7.37 b	237.00 ± 5.57 cd
	MH	11.30 ± 1.02 e	2.30 ± 0.14 c	323.00 ± 8.11 c	211.75 ± 13.84 d
	HH	6.65 ± 0.33 f	1.68 ± 0.26 d	314.78 ± 16.44 cd	143.00 ± 29.26 e
+S	CK	17.28 ± 0.11 a	4.43 ± 0.30 a	319.00 ± 5.39 cd	322.67 ± 15.47 a
	LH	15.95 ± 0.71 b	3.77 ± 0.36 b	318.44 ± 4.90 cd	294.67 ± 35.17 ab
	MH	13.14 ± 0.66 d	3.68 ± 0.12 b	317.88 ± 11.41 cd	270.20 ± 18.46 bc
	HH	11.45 ± 0.69 e	3.68 ± 0.09 b	303.89 ± 7.94 d	258.44 ± 17.75 bc
Main effect					
-S		11.86 ± 2.79 b	2.97 ± 0.88b	331.38 ± 23.31a	202.53 ± 53.81 b
+S		14.28 ± 2.34 a	3.83 ± 0.35a	314.45 ± 9.95b	283.88 ± 33.32 a
	Main effect				
	CK	15.64 ± 1.46 a	4.02 ± 0.43 a	327.21 ± 14.70 b	293.18 ± 38.776 a
	LH	13.95 ± 1.98 b	3.67 ± 0.30 b	320.72 ± 6.91 a	275.44 ± 40.153 a
	MH	12.47 ± 1.20 c	2.99 ± 0.73 c	338.75 ± 29.12 b	244.22 ± 34.514 b
	HH	9.53 ± 2.54 d	3.01 ± 1.00 c	309.33 ± 13.72 c	207.94 ± 63.317 c
ANOVA					
S		**	**	**	**
Cd		**	**	**	**

Fungal treatment	Cd treatment	Pn (um/m ² /s)	Tr (mmol/m ² /s)	Ci (ppm)	Gs (mmol/m ² /s)
S × Cd		**	**	**	**

- S: non-inoculation (control), + S: *S. indica*. The value represents the mean ± SE. The same letter in the same row of data in the table indicates significant no differences among treatments using Duncan's Multiple Range Test in the level of $P < 0.05$.

** $P < 0.01$.

Effects of *S. indica* on the activities of soil enzymes under Cd stress

Because soil enzymes participate in the catalytic process of various complex biochemical reactions, the soil enzyme activities can reflect the severity of soil pollution to a certain extent. Soil enzymes include urease, sucrase, catalase and so on. The activities of urease, sucrase, phosphatase and catalase in soil decreased as Cd concentrations rising during soybean harvest. The activities of soil urease (53.48%) and phosphatase (52.40%) were significantly between CK and the control ($P < 0.05$); the activities of phosphatase (116.39%) and catalase (4.17%) in LH were significantly higher than those of the control ($P < 0.05$). Simultaneously, there was no significant difference in sucrase activities between ML, HH and the control.

Effects of *S. indica* on pH and the decreasing rate of Cd in soil

The contents of Cd and pH in the soil after harvest have shown in Fig. 3. The increase of Cd content decreased the soil pH, and the soil pH of *S. indica* inoculated treatments were significantly higher than those of the control. The results were indicative that the Cd content in all *S. indica* treatments was lower than that of control, especially in the MH treatment. There was a significant difference between the inoculated *S. indica* treatment and the control ($P < 0.01$), inoculated *S. indica* decreased the Cd content in soil by 23.66%.

Effects of *S. indica* on soil Cd fraction

Figure 3 showed the distribution of Cd in the soil tested by the Tessier sequential extraction. The result was reflected that soil Cd mainly combined with the exchangeable form, accounting for 40.78% ~ 54.86% of the total Cd in the rhizosphere soils. However, the exchangeable Cd in the rhizosphere soil was transformed into carbonate-bound and reducible iron and manganese forms in the *S. indica* treatments. Especially in the soil treatment of MH, the decline rate of the control group was the largest (Fig. 4): exchangeable form (41.76%), carbonate form (- 9.05%), reducible iron and manganese form (- 11.48%), organic matter bound form (23.81%) and residual form (4.00%).

Redundancy analysis of soybean growth index and environmental factors

The relationship of soybean physiological indexes, soil enzyme activities and Cd chemical forms were analyzed through redundancy analysis (Fig. 5). The exchangeable form Cd accounted for 67.4%, which can be considered as the main driver, the cumulative rate was 80.8%, which could explain all variables. The growth indexes (dry weight, shoot height) of soybean and photosynthesis indexes (Gs, Pn, Tr) were in the second quadrants; The soil enzyme activities indexes (urease, phosphatase, sucrase and catalase) and pH were in the third quadrants. The chemical forms of Cd in soil were negatively correlated with growth, photosynthesis and soil enzymes and had a greater impact on pH and soil enzyme activities.

Assessment of heavy metal pollution in soil

The assessment of the contents of Cd in soybean soil revealed that the contents of Cd in the soil of inoculated and uninoculated *S. indica* treatments were higher compared to the limitation standards. Besides, the contents of Cd in inoculated *S. indica* treatments were significantly lower than those in control (Table 3). According to the limitation standard, the risk assessment of Cd pollution in soil indicated that: Cd content in CK, MH and HH treatments reached extremely low, low and medium pollution levels, respectively; in LH treatment, inoculation of *S. indica* reached extremely low pollution level, and non-inoculation of *S. indica* reached low pollution level. According to the actual needs of the five alternatives, the risk of pollutant concentration assessment is divided into five levels according to the risk screening value of relevant standards (Ministry of ecological environment, China): extremely low (25 mg/kg soil), very low (45 mg/kg soil), low medium (65 mg/kg soil), high (85 mg/kg soil), and extremely high (105 mg/kg soil).

Table 3
Heavy metal risk assessment results in soybean soil

Fungal treatment	Cd treatment	Measured value (mg/kg soil)	pollution index	Pollution level
-S	CK	0.31 ± 0.09e	6.58	Extremely low
	LH	2.91 ± 0.55d	25.33	Low
	MH	5.73 ± 0.47b	44.09	Low
	HH	7.08 ± 0.70a	60.21	Medium
+S	CK	0.21 ± 0.04e	6.29	Extremely low
	LH	2.28 ± 0.52d	19.34	Extremely low
	MH	4.23 ± 0.14c	35.73	Low
	HH	5.05 ± 0.77b	54.40	Medium

Discussion

Effects of *S. indica* on soybean growth and development

Cd has an extremely inhibitory effect on the growth and development of plants. *S. indica* can alleviate the stress of Cd on plants. In this research, the addition of exogenous Cd decreased the dry weight and plant height of soybean and the total dry weight (6.97 g) and the plant height (30.47 cm) were the lowest in the HH treatments. However, *S. indica* treatments increased the dry weight and plant height of soybean and promoted the growth and development of Soybean (Table. 2), which are consistent with the findings of Wu et al. (2018). The study of Yun Ping et al. (2018) found that under salt alkali stress, the *S. indica* could regulate the transfer rate of K to the aboveground part, which was beneficial to the growth and development of plants. Thus we suspected that the increase of soybean biomass was due to the fact that *S. indica* promoted the absorption of nutrients and photosynthesis to improve the adaptability of host plants to Cd stress.

Photosynthesis plays a decisive role in the growth and development of plants. Therefore, plants will have withering and yellow leaves and other growth performance in the presence of Cd pollution in the soil. The inhibition of Cd on photosynthesis was the decrease of photosynthetic pigment and stomatal number. At present, the research on the effect of *S. indica* on plant photosynthesis mainly focused on non stomatal conductance factors such as chlorophyll synthesis, PSII photochemistry and electron transport rate. However, Pn, Tr, Ci and Gs rate can also be used to estimate the photosynthetic physiological process of plants (Hui et al. 2015; Malkowski et al. 2020). There were many reports proved that *S. indica* can help plant resist saline alkali stress not only from promoting plant nutrient absorption, but also from improving stomatal conductance to grow and develop, which is good agreement with the results of ours (Yun et al. 2018; Liu et al. 2020).

Our result expounded that compared with the control, treatments with exogenous Cd significantly decreased Pn, Tr, Gs, and Ci (Table 2). This change may be caused by the decrease of stomatal conductance and the obstruction of CO₂ entering leaves when soybeans were stressed by Cd, which led the decrease of Pn, which was the stomatal limitation of photosynthesis (Liu et al. 2020). The Ci value of the treatment with inoculation of *S. indica* decreased may be because CO₂ was the raw material of photosynthesis, the less CO₂ between cells, the more carbon dioxide consumed in photosynthesis, the greater Pn, which was opposite to Tsai et al. (2020). In the study of rice, *S. indica* can reduce the toxic effect of Cd by stomatal closure and oxidative stress reduction. The main reason for this result may be that the host selected was different (Klichowska et al. 2019). There was research also indicated that the decrease of photosynthesis under Cd stress was related to the decrease of carboxylase and ribose 1, 5-diphosphate carboxylase activities (Song et al. 2019). However, the application of *S. indica* enhanced Gs, Pn, Tr of soybean (Table 2). *S. indica* can effectively improve the stability of Cd and reduce the inhibition of Cd on photosynthesis.

Effects of *S. indica* on the activities of soil enzymes under Cd stress

The soil enzyme is an important biocatalyst in soil, which reveals ecosystem perturbations and plays an irreplaceable role in the detoxification process of pollutants. It can also facilitate the biogeochemical cycle of nutrients, maintain soil structure, and produce the necessary compounds for microorganisms

and plants (Gelsomino et al. 2006; Topac et al. 2009; Hu et al. 2014). Many studies demonstrated that Cd had adverse effects on soil enzyme activities (Ali et al. 2020). On the one hand, pollutants inhibit enzyme activity by silencing catalytic active groups that led to protein conformational denaturation. On the other hand, pollutants may compete with the enzyme's substrate, thus hindering the enzyme from functioning (Kizilkaya and Bayrakli 2005). Among the different soil enzymes, soil urease, sucrase and phosphatase are often used to evaluate the nutrient absorption of plants and organic matter transformation, and catalase was often used to evaluate the detoxification ability of soil ecosystem.

In this study, we also observed that Cd contamination had adverse effects on soil enzyme activities. The activities of urease, sucrase and catalase in the LH, MH and HH treatments were significantly lower than those of control. The decrease of soil enzyme activities was due to the increase of heavy metal content and decreased pH value (Fig. 5). A result showed that the soil enzyme activities decreased with the increase of heavy metal concentration, and Cd inhibited the activities of urease and catalase (Wang et al. 2020). On the one hand, the reason for *S. indica* to played a role might be that *S. indica* promoted the growth of soybean (Table 2), stimulated the secretion of plant root metabolites, and directly enhanced soil enzyme activity. Similar to endophytic plant growth promoting bacteria and arbuscular mycorrhizal fungi, *S. indica* might improve the activity of urease, sucrase and phosphatase in soil to absorb sufficient C / N / P from soil, and significantly increased soybean biomass (Table 2), which can make the root system absorb and accumulate Cd; on the other hand, *S. indica* stimulated soil microorganisms, which increased the biomass and activity of microorganisms, and indirectly increased the activity of soil enzymes. The effect of microorganism on soil enzyme activity is more complex. We observed that the soil enzyme activity decreased with the increase of Cd concentration, and plants inoculation with *S. indica* in the root, which increased soil enzyme activities, in turn. The phosphatase and catalase activities in soybean soil, which inoculated *S. indica* were significantly increased under MH. It is similar to the conclusion of Xiao et al. (2021), which in highly Cd-polluted soils, *Trifolium repens* with mycorrhizal inoculation and straw treatment, phosphatase activity and catalase activity were promoted, and reduced Cd toxicity via a dilution effect.

Heavy metal ecotoxicity and soil enzyme activity are closely related to soil physical and chemical properties (Heidari et al. 2020), especially can be significantly affected by soil pH value. RDA results demonstrated a positive correlation between soil enzyme activity and soil pH value. That is, the decrease of soil pH value could represent the adverse effect of Cd on soil enzyme activity. The change of soil pH value in *S. indica* treatment was the result of multiple factors. The increase of Cd content led to more organic acids secreted by plant roots, decreased soil pH and increased metal availability, making plants absorb more heavy metals (Zeng et al. 2020). Notably, in HH treatments, the soil pH was enhanced from 7.60 to 7.68 with *S. indica*, indicating that *S. indica* may reduce the content of soil organic acid to effectively inhibit of soil acidification process. (Fig. 5d). The research of Yang et al. (2020) found that the citric and malic acids in rhizosphere soil of inoculating AMF were significantly higher than the control under Cd stress. Besides, the decrease of exchangeable Cd form may be due to the significant reduction of organic acid release from soybean roots by *S. indica* (Fig. 4).

Effects of *S. indica* on soil Cd fraction

The toxicity of heavy metals was mainly related to the exchangeable form, which was highly mobile and easy to enter into plants. On the contrary, carbonate form and reducible iron and manganese form were relatively stable components, which were not easy to enter the plants (Liu et al. 2014). Figure 3 illustrated that the Cd component has been gradually transformed into a more stable component from an exchangeable form and effectively immobilized in the soil. Also, the changes of chemical forms of Cd indicated that the application of *S. indica* could significantly reduce the bioavailability of Cd.

Moreover, the form of Cd in soil and the proportion of various forms are the key factors to determine its impact on the environment and the surrounding ecosystem (Lee et al. 2015). Previous studies also reported that the exchangeable fraction of Cd accounted for the highest proportion of Cd concentration in soil (Nemati et al. 2011). Generally, soil pH, organic matter and redox conditions all affected the forms of heavy metals in soil, but pH is the most important factor. It was found that the decrease of pH value by only 0.2 unit will lead to the increase of exchangeable Cd by 3–5 times (Zhu et al. 2016; Meng et al. 2020). The results showed that the application of *S. indica* to soils could contribute to higher pH, which increased the contents of carbonate bound and residual form Cd to immobilize Cd in the soil. According to the single pollution index method, under the treatment of 10 mg / kg Cd, inoculation of *S. indica* could effectively reduce the level of Cd pollution, indicating that the use of *S. indica* could reduce the risk of Cd pollution in the soil.

These results were confirmed by RDA analysis. The chemical forms of Cd in soil were negatively correlated with the content of DW, SH and photosynthetic parameters of soybean, indicating that the accumulation of Cd in soil greatly inhibited the physiological indexes of soybean. Indeed, the photosynthetic parameters of soybean was positively correlated with urease, sucrase, phosphatase and catalase in soil. In this study, *S. indica* caused a positive effect on the growth and development of soybean to attenuate the toxic effects of Cd on soybean, and ultimately enhanced soil enzyme activities of soybean soil to reduce the accumulation of Cd.

Conclusions

Pot experiments were conducted to examine *S. indica* on soil enzyme activities, physiological characteristics of soybean and the potential risks of heavy metal pollution to soybean. According to the investigation results, the following conclusions can be drawn:

(1) Cd contamination in the soil caused physiological dysfunctions of plants. Cd reduced root colonization and growth and also affected the photosynthesis of soybean plants. Inoculation of *S. indica* mitigated the negative impact of Cd by enhancing growth and improvement in Pn, Tr, Gs.

(2) The increase of Cd concentration lowered soil pH, which not only inhibited the activities of soil enzymes, but also increased the risks of heavy metals pollution. Besides, *S. indica* can reduce Cd toxicity by enhancing soil pH, promote soil enzyme activities, and reduce exchangeable Cd in soil.

(3) In our study, the Cd content in the soil inoculated with *S. indica* significantly decreased. According to the single pollution index method analysis, *S. indica* minimized the risk of soil heavy metal pollution in LH treatment, which indicated that these *S. indica* treatments could improve soil health.

We suggest *S. indica* can be used as a microbial fertilizer and heavy metal remediation agent in Cd-contaminated soils to realize sustainable agriculture.

Abbreviations

Cd, Cadmium, *S. indica*, *Serendipita indica*, Pn, net photosynthetic rate, Tr, stomatal conductance, Ci, intercellular CO₂ concentration, Gs, transpiration rate.

Declarations

Author contribution

Song F. Q.: Funding acquisition, Conceptualization, Proofreading, Methodology, Writing - review & editing.

Wang, X.H.: Investigation, Writing - review, Performed experiments, Writing - original draft, Data analysis & editing. **Fan X.X.:** Proofreading, Writing - review & editing. **Wang W.D.:** Investigation and data analysis. All authors read and approved the manuscript.

Ethics approval The ethical consent was approved by the Research Ethics Committee of the Life Sciences College at Heilongjiang University (Harbin, China)

Consent to participate: All the patients provided their informed consent for participation to the study.

Consent for publication: All the authors have read and approved the manuscript and accorded the consent for publication.

Competing interests: The authors declare no competing interests.

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Data availability

Supporting data set will be made available upon reasonable request.

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Figures

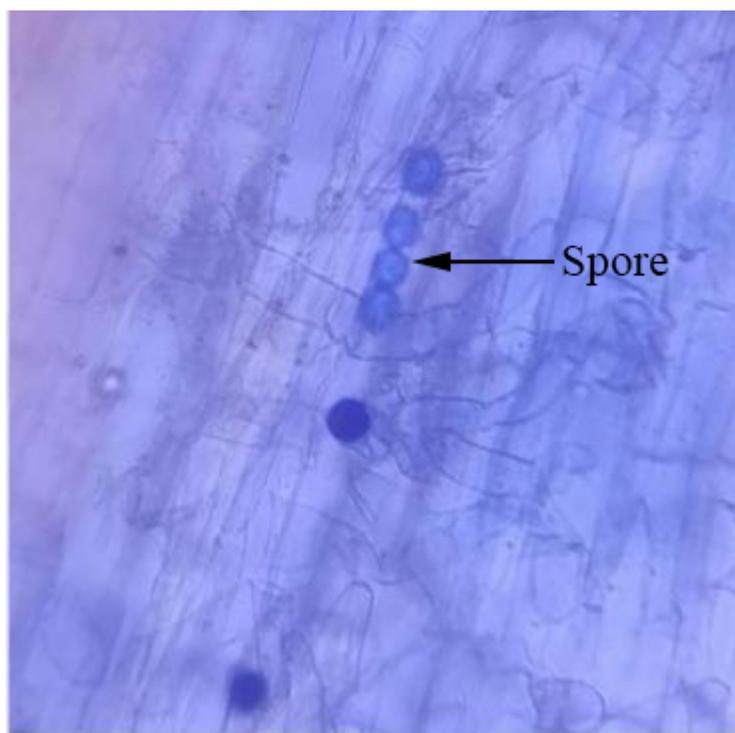


Figure 1

Infection of *S. indica* on soybean observed under the microscope. Hyphae, and chlamydospores of *S. indica* under the light microscope, magnification 200 \times . Trypan blue (0.001 mg/ml) method.

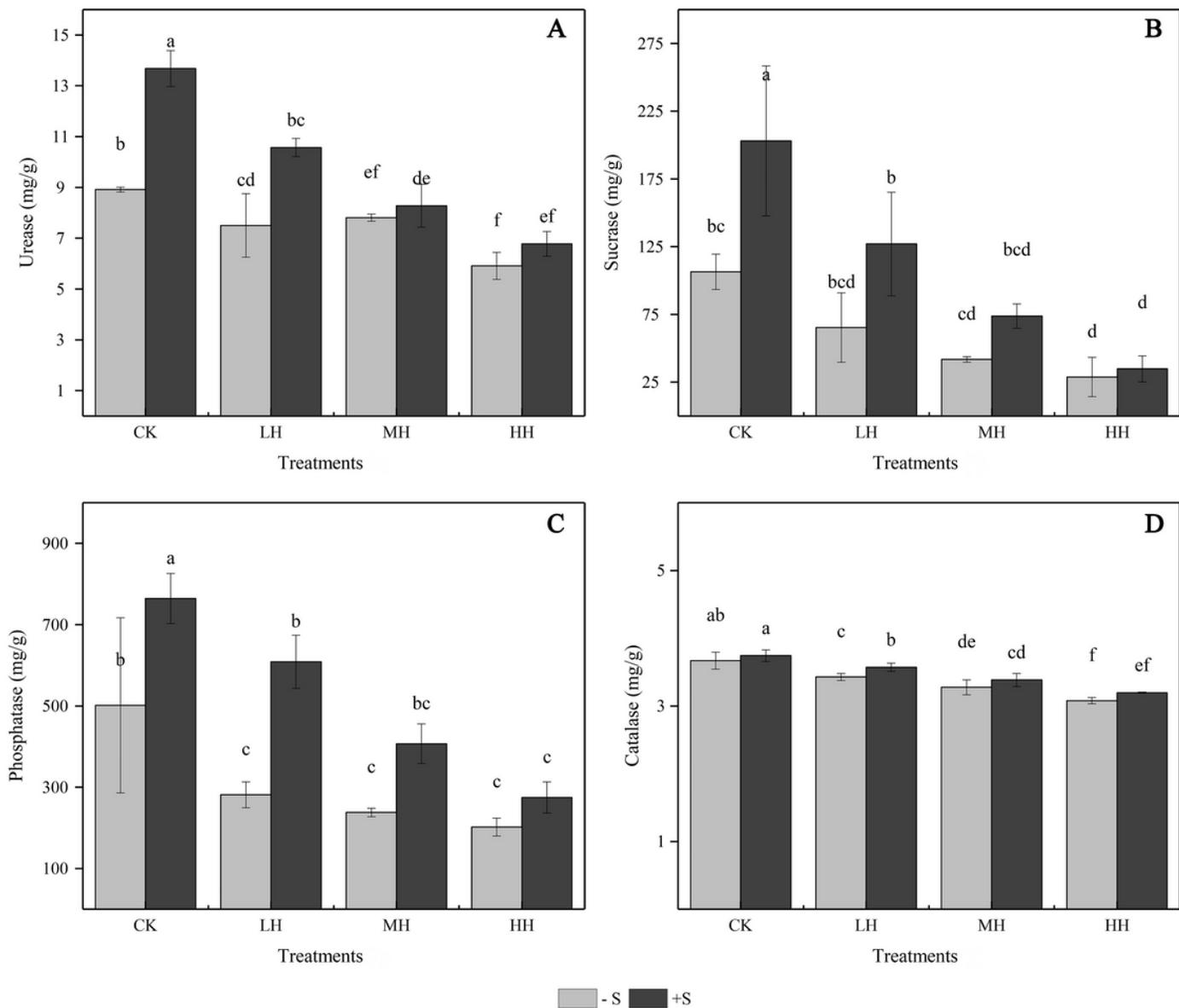


Figure 2

Effects of *S. indica* on soil urease (A), sucrase (B), phosphatase (C) and catalase (D) activities as well as Cd residue in Cd contaminated soil

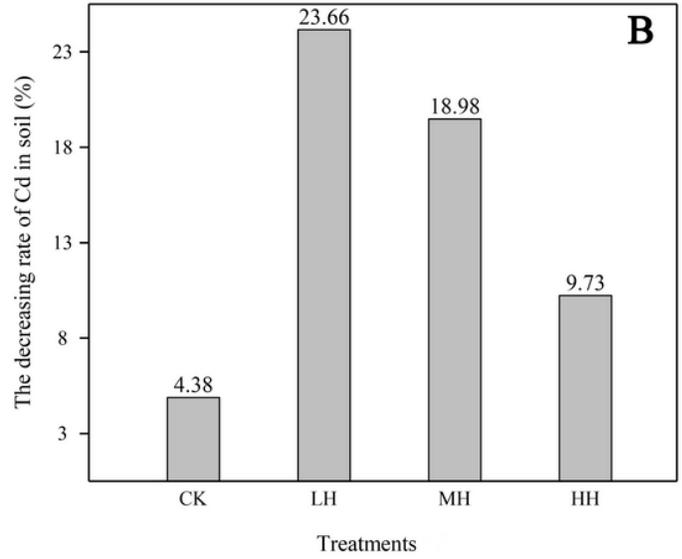
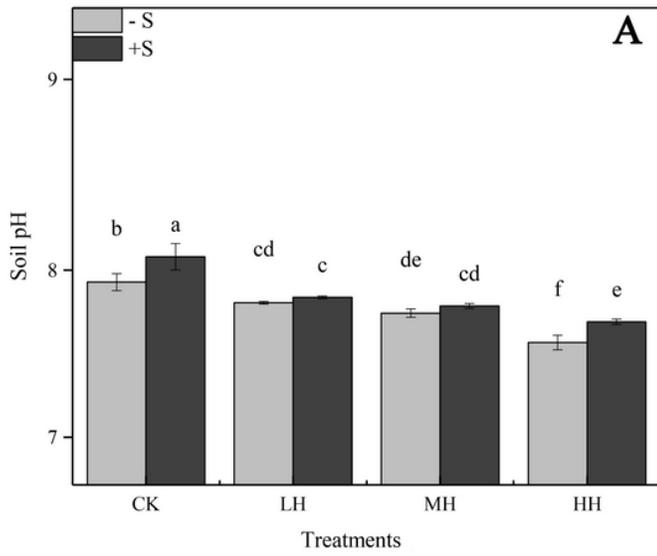


Figure 3

Effects of *S. indica* on soil pH and the decreasing rate of Cd in soil, (A) Effects of *S. indica* on soil pH under Cd pollution, (B) Contribution of *S. indica* reduce Cd pollution.

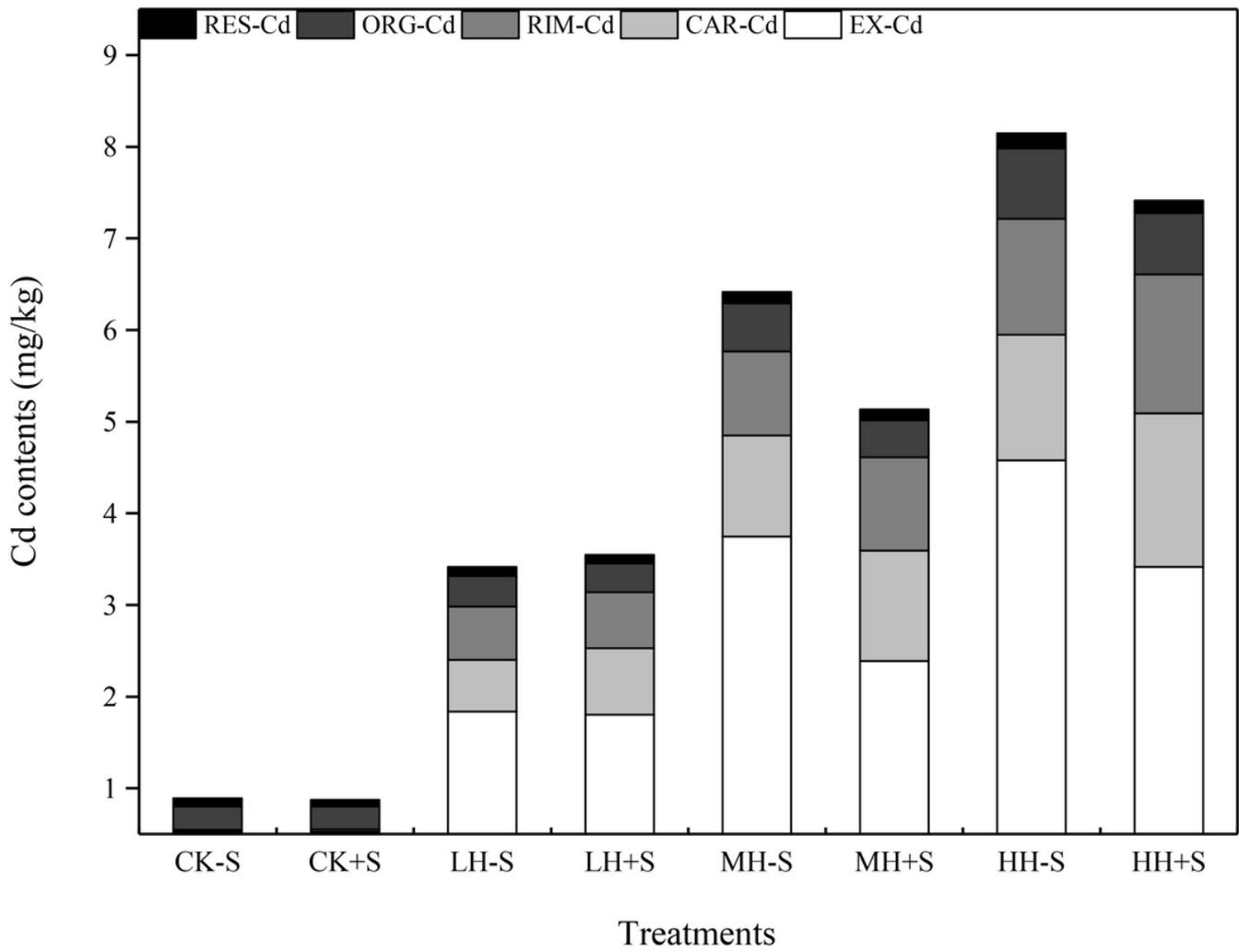


Figure 4

Fractionation of Cd in the rhizosphere soil of soybean at the harvest time (EX-Cd: exchangeable form, CAR-Cd: carbonate form, RIM-Cd: reducible iron and manganese form, ORG-Cd: organic matter bound form, RES-Cd: residual form.)

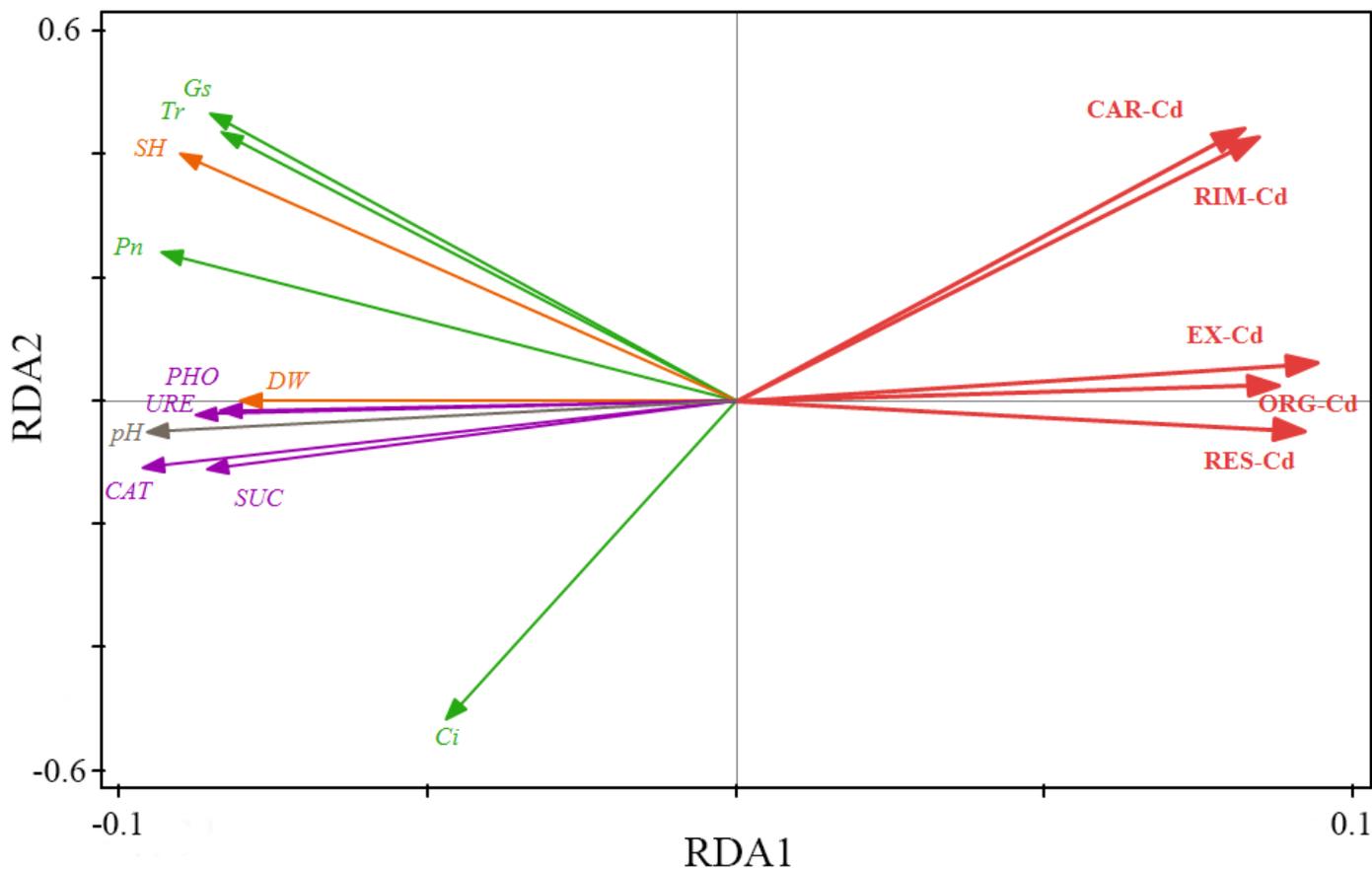


Figure 5

Redundancy analysis of soybean growth and soil health. EX-Cd: exchangeable form, CAR-Cd: carbonate form, RIM-Cd: reducible iron and manganese form, ORG-Cd: organic matter bound form, RES-Cd: residual form, represents, DW: dry weight, SH: shoot height, URE: urease, PHO: phosphatase, SUC: sucrase, CAT: catalase, Tr: transpiration ratio, Gs: stomatal conductance, Pn: net photosynthesis rate, Ci: intercellular CO₂ concentration.

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