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Effects of Elevated CO_2 Concentration and Nitrogen Addition on Soil Respiration in a Cd-Contaminated Experimental Forest Microcosm

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Abstract: Forests near rapidly industrialized and urbanized regions are often exposed to elevated CO_2, increased N deposition, and heavy metal pollution. To date, the effects of elevated CO_2 and/or increased N deposition on soil respiration (Rs) under heavy metal contamination are unclear. In this study, we firstly investigated Rs in Cd-contaminated model forests with CO_2 enrichment and N addition in subtropical China. Results showed that Rs in all treatments exhibited similar clear seasonal patterns, with soil temperature being a dominant control. Cadmium addition significantly decreased cumulative soil CO_2 efflux by 19% compared to the control. The inhibition of Rs caused by Cd addition was increased by N addition (decreased by 34%) was partially offset by elevated CO_2 (decreased by 15%), and was not significantly altered by the combined N addition and rising CO_2. Soil pH, microbial biomass carbon, carbon-degrading hydrolytic enzymes, and fine root biomass were also significantly altered by the treatments. A structural equation model revealed that the responses of Rs to Cd stress, elevated CO_2, and N addition were mainly mediated by soil carbon-degrading hydrolytic enzymes and fine root biomass. Overall, our findings indicate that N deposition may exacerbate the negative effect of Cd on Rs in Cd-contaminated forests and benefit soil carbon sequestration in the future at increasing atmospheric CO_2 levels.

Keywords: soil respiration; elevated atmospheric CO_2; nitrogen addition; cadmium-polluted soil; fine root; carbon-degrading enzyme

1. Introduction

Owing to human activity, unprecedented concentrations of atmospheric carbon dioxide (CO_2) and increasing deposition rate of nitrogen (N) have been two major global changes and are expected to influence the structure and function of ecosystems by altering the carbon (C) and N cycles. The CO_2 concentration of the atmosphere has rapidly reached 403 ppm in 2016, and is expected to rise to ~800 ppm at a staggering rate by 2100 [1,2]. Meanwhile, the deposition rate of N has increased three to five times over the past century and will continue to keep a steady rise in the future because of large amounts of artificial N application [3,4]. These increases in atmospheric CO_2 concentrations and N deposition are occurring simultaneously with heavy metal pollution of soil, which has become a

global environmental problem [5,6]. However, the regrettable reality is that the response of heavy metal-stressed ecosystems to global change still lacks attention.

Soil respiration (Rs) represents the maximal C flux from the soil to the atmosphere, and the input of C into the atmosphere is more than 10-fold greater from Rs than from fossil fuel burning [7]. It follows that even a minor variation in Rs in response to environmental change has the potential to greatly alter atmospheric CO_2 concentrations and to thereby affect global climate change. Many researches have confirmed that the responses of Rs to elevated CO_2 and/or N addition in forests can be uncertain. Sustained increases in Rs under elevated CO_2 have been generally reported from previous CO_2 enrichment experiments [8–10]. Increased Rs under elevated CO_2 is mainly caused by increases in plant residues, root biomasses, root exudates, and root turnover rates [11–13]. Nevertheless, some notable exceptions were also reported [9,14], suggesting that a positive Rs response to elevated CO_2 may not be universal. Similarly, the response of Rs to N addition was also ambiguous, including promotion [15,16], inhibition [17,18], and no effect [19]. Zhong et al. [20] suggested that the responses of Rs to elevated CO_2 or N addition may vary among soil and vegetation types.

Although the effects of rising CO_2 concentration and N addition on Rs have been widely studied as single independent factors, only a few studies have examined both independent and interactive effects of elevated CO_2 and N addition on Rs [21,22]. In addition, most previous studies have been conducted in non-polluted sites, such that the responses of Rs to elevated CO_2 and/or N addition in polluted ecosystems are still not clear.

In forests located near sites with intensive industrialization and agriculture, the concentration of some heavy metals in soil have become abnormally high [23,24]. Because heavy metals are toxic and persistent, excessive concentrations of heavy metals in soil will cause long-term adverse effects on forests and humans [25]. Cadmium (Cd) is a ubiquitous heavy metal element in soil and has high activity and toxicity [26]. High concentrations of Cd in soil can have direct and indirect effects on Rs, i.e., Cd pollution can inhibit basal Rs and enzyme activity [27,28], decrease microbial diversity [5], and reduce root biomass and root growth [29]. Under global change (i.e., elevated atmospheric CO_2 concentration and rising N deposition), the responses of Rs to soil Cd contamination may become more complex, i.e., there may be interactions between global change factors and excessive Cd in soil. However, little information is available concerning how these combinations will affect Rs.

In the present study, we established 15 open-top chambers to investigate the responses of Rs to elevated CO_2 and/or N addition in model subtropical forest with Cd pollution in a subtropical region, southern China. The objectives were (1) to clarify the separate and combined effects of elevated CO_2 and N addition on the Rs in a Cd-contaminated forest; and (2) to determine how the responses of Rs to elevated CO_2 or/and N addition in Cd-contaminated soil are related to environmental factors (e.g., soil pH, temperature, and moisture) and biotic factors (e.g., fine root biomass, litterfall, microbial biomass, and enzyme activity).

2. Materials and Methods

2.1. Study Site

The experiment was conducted at South China Botanical Garden (SCBG), Chinese Academy of Science, Guangzhou, China (23°20′ N and 113°30′ E). The SCBG is an important ex-situ plant protection base surrounded by urban forests. The area belongs to typical monsoon and humid climate with annual precipitation of 1600~1900 mm and a mean annual temperature of 21.5 °C [30]. The N deposition rate of this area (39–49 kg N ha^{-1} yr^{-1}) is well above the global average (10 kg N ha^{-1} yr^{-1}) due to a large amount of artificial N application [3,31]. Meanwhile, the area is facing serious heavy metal pollution issues as a result of rapid urbanization and industrialization. Sun et al. [24] reported that the soil Cd content had far exceeded the critical load of the plant.

2.2. Experimental Design

We set up 15 cylindrical open-top chambers (OTCs, 3.0 m in diameter and 4.5 m in height; 5 treatments with 3 replicates per treatment), and established experimental plantation forests close to the natural condition in this experiment. The above-ground section of each chamber was wrapped with transparent and impermeable plastic sheets, and the below-ground section was surrounded by a stainless-steel panel to prevent root encroachment and the lateral or vertical movement of water and/or elements from the surrounding soils. Light conditions and air temperatures did not significantly differ inside vs. outside of a chamber. To better mimic the natural forest, the soils applied in our study were collected from a nearby forested site located within Nankunshan natural reserve. Three soil layers (0–10, 10–30, and 30–60 cm depth) were collected separately and homogenized by soil depth and then placed in the below-ground part of each chamber in correspondence with their original depth. As backgrounds, the soil samples presented in Table 1 were collected prior to the treatment application. The initial values of soil chemical parameters of the top 10 cm soil layer among all the different treatments were not statistically different. The soil was poor due to soil erosion and strong eluviation. Five tree species that are generally distributed and widely planted in southern China were selected, including *Syzygium hainanense*, *Liquidambar formosana*, *Cinnamomum camphora*, *Castanopsis hystrix*, and *Acacia auriculiformis*. Three 1-year-old seedlings of uniform size per species were planted adjacently with different species in each chamber, such that there were 15 equally spaced seedlings per chamber.

Table 1. Soil physicochemical properties of the surface 10 cm soil layers before treatments. SOC, soil organic carbon; TN, total nitrogen content of soil; TP, total phosphorus content of soil; TCd, total cadmium content of soil; NH_4^+-N, ammonium-N content of soil; NO_3^-N, nitrate content of soil. The five treatments individually referred as CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Values are means ± SE (n = 3).

Treatments	pH	SOC (mg g^{-1})	TN (mg g^{-1})	TP (mg g^{-1})	TCd (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Coarse sand content (%)	Fine sand content (%)	Silt and clay content (%)
CK	5.44±0.13	2.24±0.12	0.16±0.01	0.12±0.01	0.96±0.17	2.23±0.17	1.03±0.31	50.8±1.1	21.5±0.0	27.7±1.1
Cd	5.26±0.03	2.37±0.27	0.19±0.02	0.12±0.00	0.97±0.16	2.45±0.23	0.99±0.26	54.6±0.8	18.5±0.3	26.9±0.6
CdC	5.23±0.01	2.34±0.08	0.20±0.01	0.12±0.01	0.91±0.05	2.30±0.18	1.06±0.28	53.7±0.2	19.8±0.1	26.5±0.1
CdN	5.34±0.05	2.63±0.18	0.18±0.01	0.12±0.01	0.97±0.13	2.53±0.12	0.99±0.32	52.0±2.2	19.1±0.7	28.8±1.6
CdCN	5.25±0.08	2.50±0.33	0.16±0.03	0.13±0.01	0.94±0.06	2.31±0.34	1.09±0.15	50.1±0.6	21.7±0.4	28.2±0.2

In March 2017, after the plants had grown for 1 year under ambient conditions, each OTC was subjected to one of five treatments: CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). The elevated CO_2 treatments were set at 700 μmol mol^{-1} from 8:00 to 17:00 every day (except rainy days). The additional CO_2 concentrations of chambers were adjusted by a flow regulator and regularly re-examined via a Li-Cor 6400 (Li-COR Inc., Lincoln, Nebraska, USA). A survey found that the soil's total Cd content is 2.1 mg kg^{-1} in the suburbs of Guangzhou city due to industrial sewage irrigation, and even 640 mg kg^{-1} in the most heavily polluted areas [32]. Thus, we simulated relatively serious soil Cd pollution by adding monthly, for a total amount of $CdCl_2$ solution at 10 kg Cd ha^{-1} yr^{-1} using backpack sprayers. In addition, atmospheric N deposition in Guangzhou city reached 49 kg N ha^{-1} yr^{-1} in 2009 [31]. Hence, considering the ability to detect the response of the forest in a relatively short time (~3 years), N addition at 100 kg N ha^{-1} yr^{-1} in this study represents twice the N deposition rates of this area. N addition was performed by spraying the ground monthly with NH_4NO_3 solution. The same amount of water was applied to the control chambers.

2.3. Measurement of Rs, Soil Temperature, and Soil Moisture

Rs was measured in situ every 10 days from September 2017 to October 2018 using LI-8100 (Li-COR Inc., Lincoln, Nebraska, USA). Two polyvinyl chloride collars of 7 cm height (inserted ~4 cm into the soil) and 20 cm inner diameter for Rs measurements were permanently installed in each chamber 3 months before the first measurement to minimize disturbance. Rs was measured between 9:00 and 11:00 am (local time) on rainless mornings. For each collar, Rs was determined twice, and the average was used as the measured value for the collar. Each Rs measurement in elevated CO_2 chambers was carried out at least 2 h after the CO_2 enrichment was switched off because a higher headspace concentration in elevated CO_2 chambers could suppress the diffusion of CO_2 from soils [33]. During each Rs measurement, 10 cm underground soil temperature and moisture were measured at three random places in each chamber. Soil temperature was determined by a Li-COR thermocouple probe, and soil moisture was measured using a TDR 300 (Spectrum Technologies Inc., South Bend, IN, USA). Rs in each chamber was represented by the average of measurements from two collars. Both soil temperature and moisture in each chamber were expressed as the average of measurements from three places.

2.4. Sampling and Measurements

The surface 10 cm soil layer was sampled in mid-February and mid-August 2018. The litter layer was carefully cleared, and eight soil cores were randomly collected using a 5.0 cm inner diameter auger in each chamber. After removing the visible stones and plant roots by hand, the soil samples were passed through a sieve (2 mm) and split into halves: One portion was used to measure soil physicochemical attributes after air drying, and the other portion was stored at 4 °C for 2 weeks, at which time it was used for analysis of the microbial attributes and available N.

Three litter traps (0.5 m × 0.5 m basket) were regularly deployed in each open-top chamber to estimate annual litterfall production. We collected the aboveground litterfall monthly from September 2017 to October 2018. Subsequently, the samples of litterfall were oven-dried 48 h at 65 °C and weighed to calculate the dry mass. In mid-August 2018, the fine root biomass of surface 10 cm was surveyed by a 5.0 cm inner diameter auger. Eight soil cores were taken in each chamber. Fine roots (less than 2 mm) were picked up from soil by washing the soil cores over a 0.25-mm sieve, oven-dried at 65 °C for 48 h, and weighed.

The pH value of soil was measured in a soil water suspension (1:2.5 w/v). Soil C (SOC) was measured by the K_2CrO_7-H_2SO_4 oxidation method, and soil N (TN) was measured using a semi-micro-Kjeldahl method [16]. The content of total Cd in soil (TCd) was determined according to Huang et al. [34]. Soil NH_4^+-N and NO_3^--N were extracted with 2 M KCl, and the filtrates were measured by an auto flow-injection analyzer (Lachat Instruments, Mequon, WI). Soil microbial biomass C (MBC) was measured by a method of chloroform fumigation-extraction [35], following the detailed procedures described in Wang et al. [36]. In light of Saiya-Cork et al. and Bell et al. [37,38], the potential activities of the hydrolytic C-degrading enzymes β-glucosidase (βG) and cellobiohydrolase (CBH) and of the oxidative C-degrading enzymes phenol oxidase (PPO) and peroxidase (POD) were determined.

2.5. Data Analysis

An exponential function and a linear function were used to fit the relationship between Rs and soil temperature, and Rs and soil moisture, respectively [39]. Cumulative soil CO_2 efflux was calculated as described by Li et al. [40]. The effects of treatments on Rs, soil temperature, moisture, and litterfall were determined by repeated measures ANOVAs with least significant difference (LSD) tests. The effects of treatments on microbial biomass C (MBC), enzyme activities, fine root biomass, and other soil physical and chemical attributes were determined by one-way ANOVAs with LSD tests. Pearson's correlations were assessed between Rs and soil physicochemical attributes, microbial biomass, and enzyme activities. All statistical analyses in this study were conducted using SPSS 17.0

(SPSS. Inc., Chicago, IL, USA), and graphs were drawn with SigmaPlot 11.0 (Systat Software Inc., Chicago, IL, USA). The significance level was set at $\alpha = 0.05$.

To analyze the hypothetical pathways regulating the responses of Rs to Cd stress, N addition, and CO_2 enrichment, structural equation modeling (SEM) was performed using AMOS 22.0 software (IBM SPSS, Armonk, NYC, USA). Predicted causal relationships between variables were based on the relationships between variables in the present study and on prior knowledge of how soil biotic and abiotic properties affect Rs. Data was fitted to the SEM model applying the maximum likelihood estimation method. The model was considered to have a good fit when $p > 0.05$, RMSEA < 0.08, and values of χ^2 and AIC were low [41].

3. Results

3.1. Soil Properties

Neither soil temperature nor soil moisture content were significantly changed by the treatments (Figure 1a and b). However, both soil temperature and soil moisture exhibited an obvious pattern of seasonality for all treatments ($p < 0.01$, Figure 1a and b). The mean soil temperature was 23.18 ± 0.36 °C (range: 10.7 °C in February to 29.6 °C in September), while the mean soil moisture was 19.1 ± 0.3% (range: 7.7% to 29.2%) during the measurement period across all chambers.

Solely Cd addition significantly increased the total Cd content (TCd) in soil and significantly reduced soil MBC but did not markedly affect soil pH, SOC, TN, C:N ratio, or available N content in summer or in winter (Table 2). Under Cd stress, elevated CO_2 significantly decreased soil TCd while N addition and the combined CO_2 and N enrichment significantly enlarged the soil available N content and decreased soil pH and TCd in summer and winter (Table 2). Moreover, compared with Cd treatment, the CdCN treatment increased soil TN and reduced the soil C:N ratio in winter (Table 2).

Table 2. Soil properties (0–10 cm depth) in the summer (August 2018) and the winter (February) as affected by the five treatments: CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Values are means ± SE of three replicates. Means in a row followed by different letters are significantly different ($p < 0.05$) based on a one-way ANOVA followed by an least significant difference (LSD) test.

Variable	Season	Treatments				
		CK	Cd	CdC	CdN	CdCN
pH	summer	5.46±0.24a	5.33±0.11a	5.36±0.11a	4.68±0.08b	4.64±0.02b
	winter	5.37±0.00a	5.31±0.04a	5.25±0.01a	4.66±0.04b	4.66±0.07b
SOC	summer	4.08±0.48a	4.33±0.54a	3.71±0.11a	3.53±0.28a	3.75±0.25a
(mg g^{-1})	winter	3.19±0.24a	3.63±0.18a	3.94±0.36a	3.33±0.08a	3.31±0.25a
TN	summer	0.28±0.03a	0.34±0.01a	0.31±0.00a	0.28±0.04a	0.29±0.01a
(mg g^{-1})	winter	0.24±0.02ab	0.23±0.00b	0.26±0.01ab	0.26±0.00ab	0.29±0.02a
C:N ratio	summer	14.85±2.15a	12.65±1.32a	12.07±0.31a	12.69±1.35a	12.80±0.40a
	winter	13.47±0.46ab	16.01±0.84a	15.23±1.93a	12.67±0.19ab	11.62±0.51b
TCd	summer	1.02±0.12d	85.39±6.40a	70.10±5.80b	21.22±5.77c	20.49±1.62c
(mg kg^{-1})	winter	0.57±0.03d	87.43±6.35a	75.34±1.98b	24.24±1.05c	25.00±3.03c
MBC	summer	84.80±11.05a	52.06±1.92b	52.80±3.92b	48.73±7.98b	38.43±2.25b
(mg kg^{-1})	winter	80.78±9.09a	40.09±6.99b	48.01±6.66b	44.98±12.19b	52.66±2.46b
NH_4^+-N	summer	5.90±0.55b	5.76±0.44b	5.61±0.54b	10.56±1.62a	10.94±1.24a
(mg kg^{-1})	winter	3.60±0.73b	5.79±0.78b	6.61±0.22b	11.97±1.49a	14.63±3.26a
NO_3^--N	summer	0.86±0.34b	0.98±0.23b	1.68±0.47b	6.51±1.41a	7.12±1.29a
(mg kg^{-1})	winter	3.07±0.41b	4.15±0.64b	5.69±1.29b	10.52±1.08a	12.67±0.98a

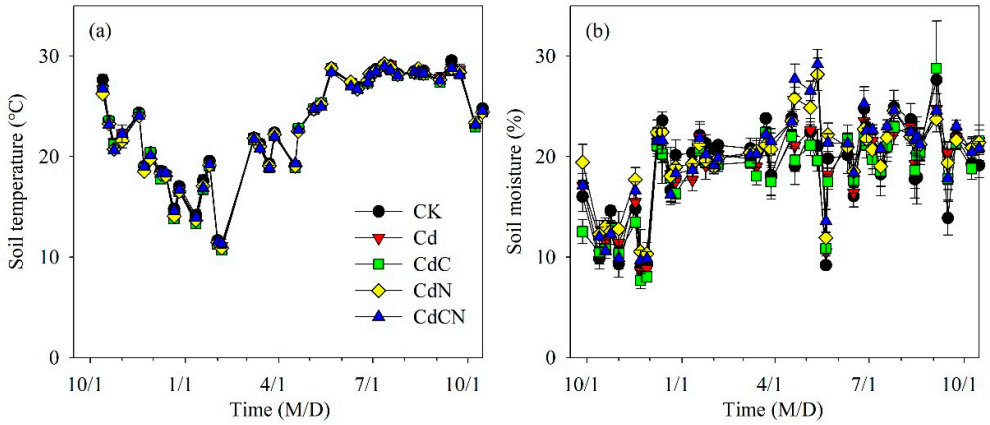

Figure 1. Seasonal dynamics of soil temperature at the 10-cm depth (**a**) and soil moisture content within the top 10 cm of the soil profile (**b**) as affected by five treatments: CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Treatment effects were not statistically significant for soil temperature ($p = 0.77$) or soil moisture ($p = 0.56$). Values are means ± SE ($n = 3$).

3.2. Litterfall and Fine Root Biomass

Cd addition did not significantly affect the annual litterfall of model forest (Figure 2a). Under Cd stress, neither elevated CO_2 nor N addition obviously changed the annual litterfall. However, elevated CO_2 plus N addition largely promoted annual litterfall production (Figure 2a). Litterfall showed double peaks both in December and June for all treatments ($p < 0.01$, Figure 2b).

Cd addition reduced the fine root biomass by 60% (Figure 2c). Relative to the Cd treatment, fine root biomass was further decreased by 41% in the CdN treatment but was not significantly changed by the CdC or CdCN treatment (Figure 2c).

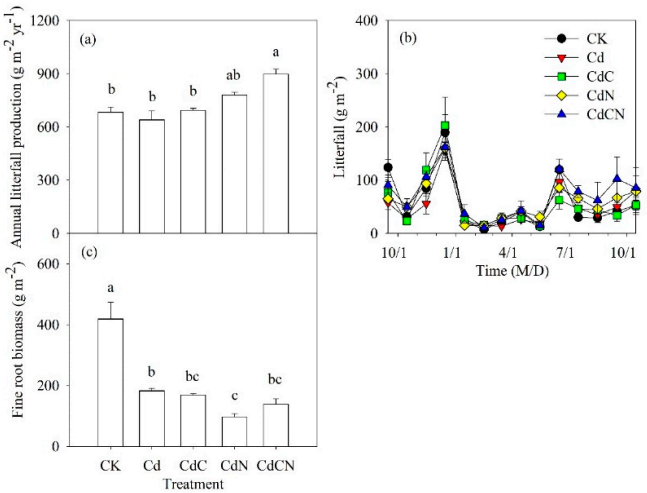

Figure 2. Annual litterfall production (**a**); seasonal variations of litterfall (**b**); and fine root biomass from the top 10-cm soil layer (**c**) as affected by five treatments: CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Vertical lines represent the standard error of the mean ($n = 3$). Means with different letters are significantly different ($p < 0.05$) based on a one-way ANOVA followed by an LSD test.

3.3. Carbon-degrading Enzymes

Relative to the control, the Cd treatment significantly reduced the potential activities of the labile C-degrading enzymes βG and CBH by 41% and 39%, respectively, in summer, and by 63% and 50%, respectively, in winter (Figure 3a and b). Under Cd stress in winter, βG and CBH activities were not significantly altered by elevated CO_2, N addition, or by both elevated CO_2 and N addition (Figure 3a and b). Under Cd stress in summer, however, N addition further reduced βG and CBH activities; elevated CO_2 and the combined elevated CO_2 and N addition did not obviously change βG or CBH activities in Cd-contaminated soil in summer (Figure 3a and b). None of the five treatments significantly changed the potential activities of the recalcitrant C-degrading enzymes POD and PPO in summer or in winter (Figure 3c and d).

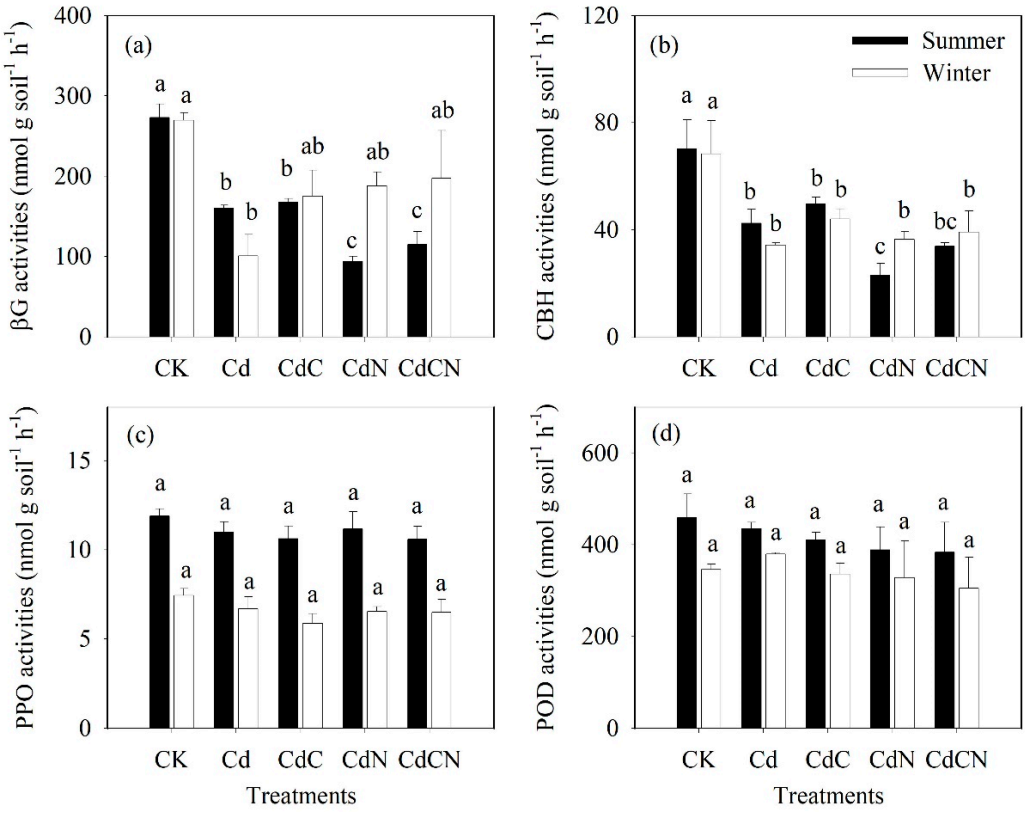

Figure 3. Potential activities for the C-degrading enzymes βG (**a**), CBH (**b**), PPO (**c**), and POD (**d**) as affected by five treatments in summer and in winter. βG, β-glucosidase; CBH, cellobiohydrolase; PPO, phenol oxidase; POD, peroxidase. The five treatments individually referred as CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Within the same season and for each enzyme, means with different letters are significantly different ($p < 0.05$) based on a one-way ANOVA followed by an LSD test.

3.4. Soil Respiration

Soil respiration showed an obvious pattern of seasonality as well ($p < 0.01$), and was highest during summer (from June to September) when soil temperatures were high and lowest during the winter (from December to following March) when soil temperatures were low (Figure 1a and Figure 4a). The annual mean soil respiration rates for CK, Cd, CdC, CdN, and CdCN treatments were 6.17 ± 0.44, 5.01 ± 0.34, 5.26 ± 0.36, 4.10 ± 0.29, and 4.76 ± 0.32 μmol CO_2 m^{-2} s^{-1}, respectively. Repeated measure ANOVA showed that Cd addition significantly decreased Rs ($p < 0.05$). Under Cd stress, Rs

was further reduced by N addition ($p < 0.05$), and yet was not significantly altered by elevated CO_2 or combined elevated CO_2 and N addition (Figure 4b). Treatment effects on Rs were more significant in the summer than in the winter (Figure 4b).

Over the measurement period, cumulative soil CO_2 efflux was 19% lower in the Cd treatment relative to the control ($p < 0.05$, Figure 4c). Under Cd stress, cumulative soil CO_2 efflux was 5% higher ($p > 0.05$) with elevated CO_2 (treatment CdC) than with the Cd treatment alone but was 18% lower ($p < 0.05$) with N addition (treatment CdN) and 5% lower ($p > 0.05$) with elevated CO_2 plus N addition (treatment CdCN) than with the Cd treatment alone (Figure 4c).

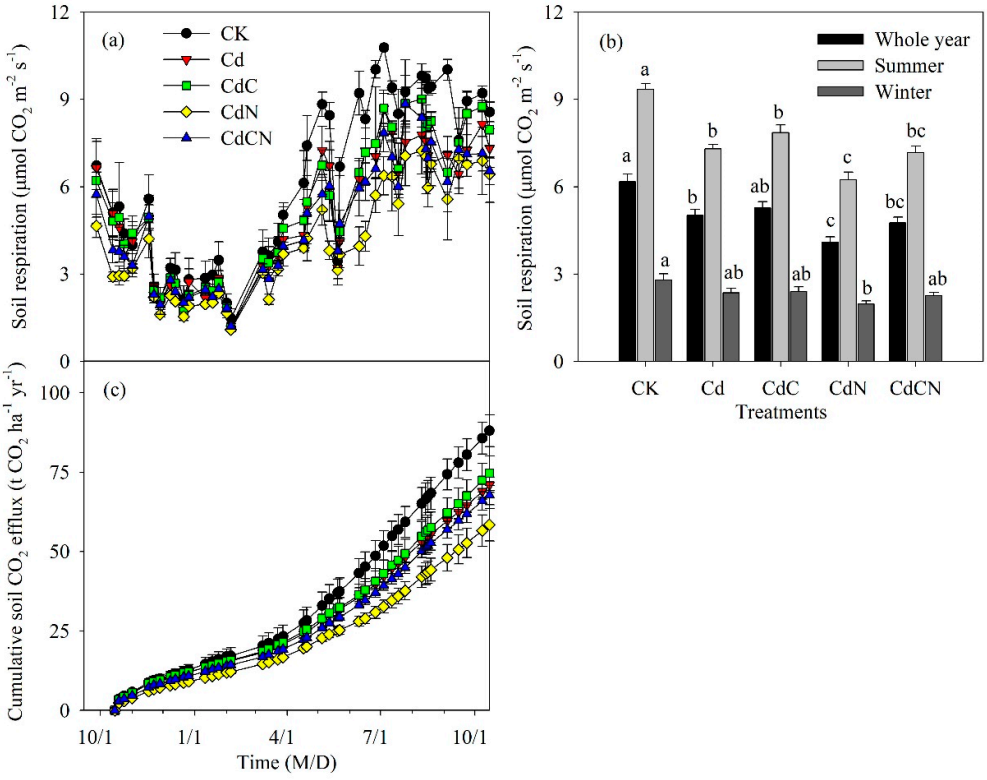

Figure 4. Seasonal variations of soil respiration (**a**); mean soil respiration rate for the whole year, the summer, and the winter (**b**); and cumulative CO_2 efflux (**c**) as affected by five treatments: CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Vertical lines represent the standard error of the mean ($n = 3$). Within the same period, means with different letters are significantly different ($p < 0.05$) based on repeated measures ANOVA.

3.5. Factors Controlling Soil Respiration

Regression models indicated that Rs was significantly correlated with soil moisture via a linear function and with soil temperature via an exponential function in all treatments (Table 3). Soil temperature and moisture explained about 75% and 15%, respectively, of the variation in Rs during the study period (Table 3). In summer, Rs was positively and linearly related to soil pH ($p < 0.01$; Figure 5a), soil MBC ($p < 0.01$; Figure 5b), βG activity ($p < 0.01$; Figure 5c), and CBH activity ($p < 0.01$; Figure 5d). In winter, Rs was not related to soil pH or βG activity ($p = 0.053$ and $p = 0.34$, respectively) but was significantly related to soil MBC and CBH activity ($p < 0.05$ and $p < 0.01$, respectively) (Figure 5 a, b, c, and d). Moreover, there was no correlation between mean annual soil respiration and annual litterfall production ($p = 0.12$; Figure 6a), but the mean annual soil respiration was significantly and positively related to the biomass of fine root ($p < 0.01$; Figure 6b).

The structural equation model (SEM) revealed that 74% of the variation in Rs was directly driven by soil C-degrading hydrolytic enzymes, soil MBC, fine root biomass, and soil pH, but only the effects of CBH and fine root biomass were significant (pathway coefficient = 0.91 and 0.68, respectively; $p < 0.05$ for both, Figure 7). Via effects on CBH activity and fine root biomass, Rs was indirectly decreased by Cd stress but was indirectly increased by CO_2 enrichment (Figure 7). N addition directly decreased soil ßG activity and increased soil acidification, and the latter also indirectly decreased Rs by inhibiting CBH activity (Figure 7).

Table 3. Regression models for the relationships of soil respiration (*Rs*) with soil temperature (*T*) and soil moisture (*M*) among the five treatments. The five treatments individually referred to as CK (the control; with ambient Cd, ambient CO_2, and ambient N deposition); Cd (with Cd addition, ambient CO_2, and ambient N deposition); CdC (with Cd addition, elevated CO_2, and ambient N deposition); CdN (with Cd addition, N addition, and ambient CO_2); and CdCN (with Cd addition, elevated CO_2, and N addition). Values in parentheses are SE ($n = 3$), R^2 is the determination of coefficient. * and ** indicate $p < 0.05$ and < 0.01, respectively.

Treatment	$Rs = a\ \exp^{bT}$			$Rs = k\ M + c$		
	a	***b***	**R^2**	***k***	***c***	**R^2**
CK	0.6283	0.0917	0.72**	0.2072	2.2626	0.11*
Cd	0.5941	0.0863	0.75**	0.2211	0.8834	0.17**
CdC	0.5921	0.0891	0.77**	0.1948	1.7273	0.13*
CdN	0.5159	0.0842	0.74**	0.1849	0.3509	0.16**
CdCN	0.5439	0.0885	0.79**	0.2050	0.6976	0.21**

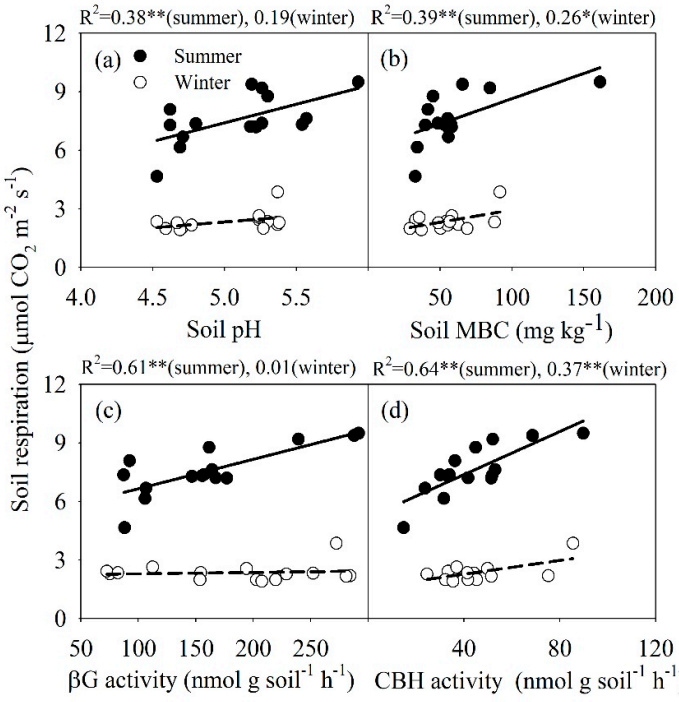

Figure 5. Dependence of soil respiration on soil pH (**a**), soil MBC (**b**), ßG activity (**c**), and CBH activity (**d**) in the summer season (solid line) and winter season (dashed line). * and ** indicates significant difference at $p < 0.05$ and $p < 0.01$.

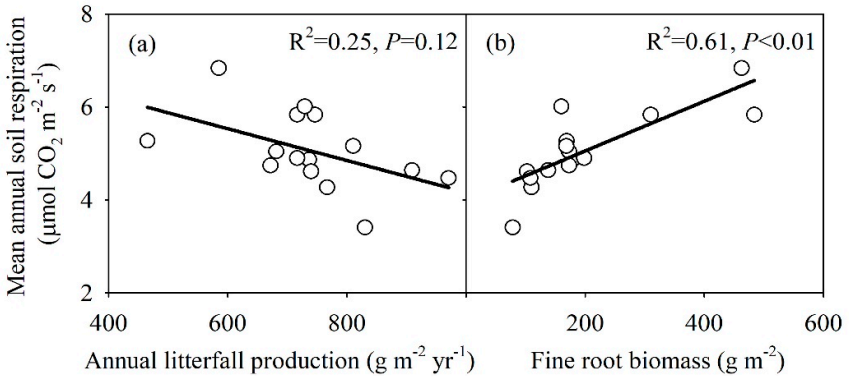

Figure 6. Relationships between annual litterfall production (**a**), fine root biomass (**b**), and mean annual soil respiration.

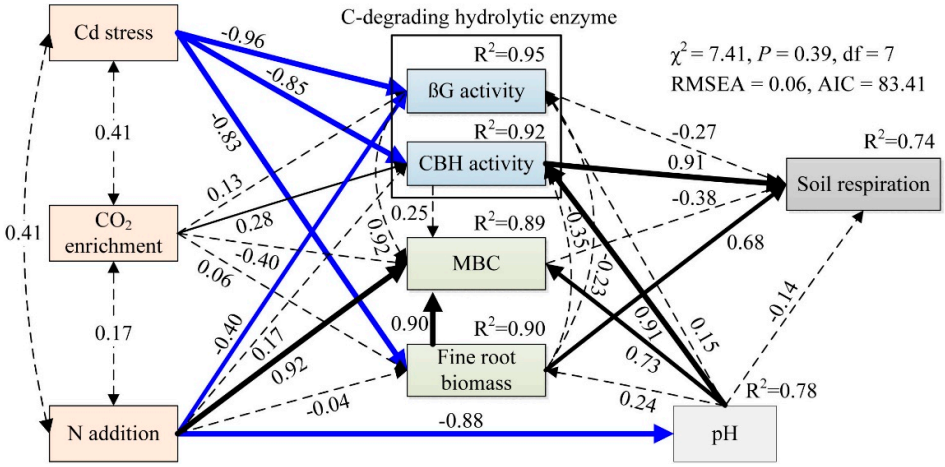

Figure 7. Structural equation model (SEM) for soil respiration under Cd stress, CO_2 enrichment, and N addition treatments using data from the summer. Arrows indicate the hypothesized direction of causation, and the arrow width is proportional to the strength of the path coefficients. Black and blue solid arrows represent significant positive and negative pathways, respectively, and black dashed arrows indicate non-significant pathways. Numbers at arrows are standardized path coefficients. R^2 values above response variables indicate the proportion of variation explained by relationships with other variables. RMSEA: root mean square error of approximation, AIC: Akaike information criteria.

4. Discussion

4.1. Seasonal Dynamics of Rs

Rs in all treatments displayed a similar and clear seasonal dynamic, with a peak in summer and the lowest value in winter (Figure 4a). Similar results have been found in other studies of forests from subtropical China [39,42]. The area of these studies belong to a typical monsoon climate, where the air temperature is significantly higher in the summer than in the winter. Higher air temperatures induce faster plant growth and higher microbial activity and can consequently increase Rs. Soil temperature and moisture are generally regarded as two main drivers determining the temporal dynamics of Rs [43,44]. Similar to previous research [42,45], we found positive exponential relationships between Rs and soil temperature, and positive linear relationships between Rs and soil moisture in all treatments (Table 3). Soil temperature, however, explained more of the variation (72%–79%) in Rs than soil moisture (11%–21%) (Table 3), suggesting that the seasonal variation of Rs was basically affected by fluctuations in the soil temperature.

4.2. Effect of Cd Addition on Rs

Cd addition in this study reduced Rs, and the cumulative CO_2 efflux of the Cd treatment was 19% lower than that of the controls (Figure 4b and c), which agrees with several previous studies [46,47]. Rs primarily reflects plant root respiration and microbial respiration [48]. On the one hand, excess Cd usually suppresses root growth and reduces root biomass [29], thereby inhibiting Ra. Likewise, Cd addition reduced fine root biomass in this study (Table 2), resulting in the reduction in Rs (Figure 7), which was supported by the positive relationship between Rs and fine root biomass (Figure 6b). On the other hand, the properties of soil microorganisms are often considered as indicators of metal pollution because of their high sensitivity to heavy metal stress [49]. Adverse effects of heavy metals on soil organisms have been documented in both laboratory experiments and long-term field experiments [50,51]. A high Cd concentration in soil can inhibit Rh via severe negative effects on microbial and enzyme activity and soil microbial biomass [52,53]. In our experiment, the activity of the labile C-degrading enzymes βG and CBH was obviously lower in the Cd treatment than in the control (Figure 3a and b), and the activities of both enzymes were positively correlated with Rs (Figure 5 c and d), supporting the inference that Rs is reduced by Cd addition (Figure 7). As the most active part of SOM, MBC is an important C pool for Rs (microbial respiration) and participates in SOM decomposition. We also found that Cd addition decreased soil MBC (Table 2) and that Rs was positively correlated soil MBC (Figure 5b).

4.3. Effect of Elevated CO_2 on Rs in Cd-contaminated Soil

Most studies involving elevated CO_2 treatments have found that such treatments increase Rs in unpolluted forests [8,10,54], although there have been some exceptions [9,14]. A meta-analysis also showed that elevated CO_2 increased Rs by 28.6% [55]. Forest ecosystems exposed to elevated CO_2 generally exhibit enhanced primary productivity (both aboveground and belowground) and increased C inputs into soil [56], often leading to a higher soil respiration rate. In contrast to the results of these earlier experiments, which were conducted with unpolluted forest soils, we failed to find a strong positive effect of elevated CO_2 on Rs in Cd-contaminated soil (Figure 4a and b). Similar to the study by Guo et al. [57], rising CO_2 levels can increase the absorption of Cd in trees, so that soil TCd in the CdC treatment was lower than the Cd treatment in this study (Table 2). Both soil MBC, litterfall, and fine root biomass showed no significant difference between the Cd and CdC treatments in the current study (Table 2, Figure 2a and c), which suggested that, although soil C inputs may have increased under elevated CO_2 in the non-polluted soils of earlier studies, this was not the case in the Cd-polluted soil of the current study. Therefore, we suggest that a lack of increased soil C inputs may explain the absence of a positive response of Rs to elevated CO_2 in Cd-contaminated soil. Additionally, elevated CO_2 may increase soil moisture via decreased plant stomatal conductance and transpiration, which could increase SOM decomposition and result in greater Rs [58,59]. There were no obvious differences in soil moisture between the Cd and CdC treatments, further indicating that the influence of elevated CO_2 on Rs in Cd-contaminated soil was small.

4.4. Effect of N Addition on Rs in Cd-contaminated Soil

Adding N to forests of N limitation could increase the soil N availability and reduce the ratio of soil C:N, which favors the growth of plants and microbes, and thus increases Rs [60]. However, we found that N addition showed significant inhibition of soil respiration in Cd-contaminated soil (Figure 4a and b), which was similar to previous reports from tropical and subtropical forests [18,42,61]. N in soil could reach beyond the needs of vegetation and microbes and lead to N saturation under continued large amounts of N input, which may change the magnitude and orientation of the effects of N addition on Rs [62,63]. Enhanced Rs with a low N addition rate and reduced Rs with a high N addition rate has been observed in several studies from subtropical forests [15,64]. The rate of N addition (100 kg N ha^{-1} yr^{-1}) in our research was far more than the critical load of N deposition (10–20 kg N ha^{-1} yr^{-1}) for forests [65], and inevitably exceeded the demands of plant and soil microbes. Moreover, soil acidification following N addition might inhibit Rs by altering plant root growth and

the microbial community structure by decreasing microbial activity [66,67]. In our experiment, N addition also clearly reduced the value of soil pH and the activity of the labile C-degrading enzymes βG and CBH in Cd-contaminated soil (Table 2, Figure 3a and b). In addition, N fertilization can reduce fine root biomass and thus inhibit rhizosphere respiration [68]. We also found that fine root biomass in the CdN treatment was obviously lower than in the Cd treatment (Table 2), resulting in a reduction in Rs, which was supported by the positive relationship between Rs and fine root biomass (Figure 6b). Variable partition analysis showed that Cd pollution, N addition, and the interaction of them explained 53.8%, 20.4%, and 3.1% variation of the fine root biomass, respectively. The biomass of fine root was reduced by the addition of N to Cd-polluted soil because an increase in N availability could lead to increased C allocation to aboveground tissues and to decreased C allocation to roots [69,70]. Increased absorption of Cd in aboveground tissues with increased aboveground biomass may lead to the lower soil TCd in the CdN treatment than in the Cd treatment (Table 2). This is reinforced by the results of the annual litterfall. Annual litterfall was 22% higher with N addition (CdN treatment) than with the Cd treatment alone in the current study (Figure 2a).

4.5. Effect of Combined Elevated CO_2 and N Addition on Rs in Cd-contaminated Soil

Some studies have shown that CO_2 enrichment and N addition reciprocally affect plant growth [71,72], which suggested that simultaneous changes in atmospheric CO_2 levels and N deposition may result in complicated effects on Rs. A recent meta-analysis showed that the effect of combined CO_2 and N enrichment (51.6%) was significantly positive on Rs to a greater extent than those of a single one (28.6% and 8.8% respectively) [55]. The synergistic interaction of CO_2 enrichment and N addition on Rs may be mainly explained by the increased Rs of elevated CO_2 or N addition [21,22]. However, the response of Rs to high CO_2 levels was often inconsistent with the response induced by N addition. As a result, their combined effects on Rs might be inconsistent. Butnor et al. [73] showed that rising CO_2 concentrations promoted Rs but that N addition reduced Rs in plots treated with an ambient or elevated level of CO_2.

In this experiment, the combined CO_2 enrichment and N addition did not significantly affect Rs under Cd stress, and cumulative CO_2 efflux in the CdCN treatment was only 5% less than that in the Cd treatment (Figure 4a and b). These results may be explained by a weak positive effect of elevated CO_2 on Rs and a strong negative effect of N addition on Rs in Cd-contaminated soil (Figure 4a and b). Moreover, the increase (5%) of Rs in the CdC treatment relative to the Cd treatment was less than the increase (16%) in Rs in the CdCN treatment relative to the CdN treatment, showing that soil available N restricted the effects of elevated CO_2 on Rs under Cd stress. Deng et al. [22] suggested that higher CO_2 levels would promote photosynthetic assimilation, and seedlings exposed to elevated CO_2 required more N from soil for growth. Consequently, fine root biomass and annual litterfall increased 42% and 40% in the CdCN treatment than in the CdC treatment, respectively (Figure 2a and c), which might be responsible for the higher Rs in the CdCN treatment. In addition, the nutrient effect on Rs induced by N addition under elevated CO_2 can offset the acidification effect induced by the addition of N without elevated CO_2 in Cd-contaminated soil, further indicating an interactive effect of these two factors on Rs under Cd stress. This might also explain why the response of Rs was similar in the CdCN treatment and the Cd treatment in our study.

Compared to the control, cumulative soil CO_2 efflux in Cd, CdC, CdN, and CdCN treatments decreased by 19%, 15%, 34%, and 23%, respectively. This suggests that the negative effects of Cd alone on Rs may exceed the effects of other global change factors alone or collectively. This finding also suggests that the widely applied models of the forest C cycle (i.e., Century model [74], forest-denitrification-decomposition (forest-DNDC) model [75], and biome-bio geochemical cycles (Biome-BGC) model [76]), which ignore soil Cd stress, could overestimate Rs, especially in areas with high N deposition. In line with previous results obtained by Deng et al. [39], N addition (especially under elevated CO_2) significantly increased annual litterfall production via the promotion of aboveground biomass, thereby increasing soil C input (Figure 2a). Therefore, N addition will increase soil carbon storage in the future at rising atmospheric CO_2 levels due to inhibition of Rs and increased litterfall input.

5. Conclusions

Our study demonstrated that soil Cd pollution could significantly inhibit Rs. Under Cd stress, N addition could reinforce the adverse effect of Cd on Rs by decreasing the activity of C-degrading hydrolytic enzymes and by acidifying soils and thereby decreasing fine root biomass. Elevated CO_2, however, can partly compensate the negative effect of Cd on soil respiration by increasing CBH activity. The combined effect of CO_2 and N enrichment on Rs was not remarkable. Our findings indicate that N deposition may be beneficial to Cd-contaminated soil carbon sequestration in the future at rising atmospheric CO_2 levels.

Author Contributions: D.W. and B.Y. conceived and designed the study. B.Y., G.Z., Y.Y., and M.X. performed the experiments. B.Y., Y.Y., and M.X. contributed to the sample measurement and data analysis. B.Y., Q.H. and D.W. contributed in writing original draft, editing and revising the text. All authors have read and agreed to the published version of the manuscript.

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