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RESEARCH ARTICLE

Elevated CO_2 and nitrogen addition diminish the inhibitory effects of cadmium on leaf litter decomposition and nutrient release

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Abstract

Aims Rising atmospheric CO_2 concentrations and nitrogen (N) deposition alter litter decomposition processes that control soil carbon (C) and nutrient cycles. However, few studies have explored such impacts on litter decomposition and micronutrient and macronutrient (C, N, phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)) release in a heavy-metal-contaminated environment.

Methods We performed an open-top chamber experiment to explore the effects of 15-month elevated CO_2 and N addition on leaf litter decomposition rate and nutrient release of *Cinnamomum camphora* (non-N-fixing species) and *Acacia auriculiformis* (N-fixing species) during litter decomposition in cadmium (Cd)-contaminated environment.

Results We found that Cd addition consistently reduced leaf litter nutrient (C, N, P, K, Ca, and Mg) loss, while these negative effects were offset by elevated CO_2 (average 10.6%) and N addition (average 23.9%). The mitigative effects of elevated CO_2 and N addition together ($\beta = -0.78$) far exceeded the effects of each ($\beta = -0.15$ for elevated CO_2 and $\beta = -0.42$ for N addition) separately. Such mitigative effects were related to higher litter quality (the increased N, P and Ca in the initial litter), and higher soil microbial activities (higher ligninase and cellulase activities). Additionally, these mitigative effects on leaf litter nutrient release were greater in *C. camphora* litter than in *A. auriculiformis* litter, due to its higher C:N and cellulose: N ratios.

Conclusions Our results suggest that N addition and elevated CO_2 concentration may diminish the negative effects of Cd addition on leaf litter decomposition and increase nutrient cycle, especially in non-N fixing trees under the global change.

Keywords Leaf litter decomposition · Enzyme activities · Cd contamination · N-fixing species

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Introduction

Atmospheric nitrogen (N) deposition is expected to increase up to ~200 T g N yr^{-1} by 2050 over the globe (Gallo et al. 2004; Galloway et al. 2008). The increase rate may be especially rapid in subtropical forests (Zheng et al. 2017; Sun et al. 2010; Li et al. 2012). During the same period, atmospheric CO_2 concentration may increase up to 540-970 μmol mol^{-1} by the year 2100 (IPCC 2013; https://www.co2.earth/). The effects of these global change on litter decomposition involved in forest carbon (C) and nutrient cycles have been intensively reported in regions without heavy-metal contamination, but have seldom been considered in regions with heavy-metal pollution (Ferreira et al. 2016; Xue et al. 2018). However, soils have been suffering from heavy metal contamination in many industrialized or urbanized regions such as the Pearl River Delta in south China (Sun et al. 2010; Hou et al. 2014). More importantly, heavy metals in the soil have long-lasting effect on soil microbial activities and further influence litter decomposition and nutrient release (Xue et al. 2018). Litter decomposition is a fundamental ecological process, which controls carbon and nutrient cycles and contributes to the maintenance of soil fertility in forest ecosystems, and ultimately plays a major role in regulating the earth's climate system (Ochoa-Hueso et al. 2019). Therefore, research is now needed to explore the combined effects of elevated CO_2 and N deposition on litter decomposition and nutrient release in heavy-metal-contaminated soils.

Elevated CO_2 potentially accelerates litter nutrient mineralization not only by improving forest-floor environmental conditions i.e., increasing soil moisture and temperature, but also increasing litter quality (increasing litter P) (Liu et al. 2015). N addition stimulates litter decomposition and nutrient release in N-poor ecosystems by increasing soil N supply or by reducing litter cellulose: N or lignin: N ratio (Song et al. 2015). Soil microbial biomass end enzyme activities are generally stimulated by increasing soil N availability or soil moisture (Cenini et al. 2016; He et al. 2016). Therefore, elevated CO_2 and N addition would increase litter decomposition and nutrient release by increasing soil cellulose-degrading enzyme activities of β-1,4-glucosidase (BG) and cellobiohydrolase (CBH), and the soil lignin-degrading enzyme activities of phenol oxidase (PPO) and peroxidase (POD) (Sinsabaugh et al. 2002; Andersson et al. 2004; Alster et al. 2013). Unlike the positive effects of N addition and elevated atmospheric CO_2, Cd addition generally reduced litter decomposition by inhibiting the activities of BG, CBH, PPO, and POD (Siegenthaler et al. 2010; Chen et al. 2014; Xue et al. 2018). To date, the effects of N addition, elevated CO_2, and Cd addition have been generally investigated as a single and independent factor. In fact, however, increases in N deposition, elevated atmospheric CO_2 concentration, and heavy-metal-contamination occur simultaneously in many industrialized or urbanized areas (Sun et al. 2010). In addition, the N-fixing plants can fix the atmospheric N_2, and have high N concentrations in the plant leaf litters, resulting in distinctive litter quality from non-N-fixing plants. Thus, the response of litter decomposition and nutrient release to N addition and elevated CO_2 in Cd-contaminated soil may differ between two species, and may vary between the early and later stage of litter decomposition (Cenini et al. 2016; Xia et al. 2017; Zhou et al. 2018; Luo et al. 2019a; Zang et al. 2022). These mounting evidences motivate us to solve the question: whether and how elevated atmospheric CO_2 concentration and N deposition offset the detrimental effect of Cd addition on leaf litter decomposition and nutrient release in forest ecosystems which improve the prediction of future C and nutrient cycles in heavy-metal-contaminated areas.

To solve this issue, we conducted a 15-month field experiment in open-top field chamber to address whether N addition and elevated CO_2 concentration mitigate the detrimental effects of Cd addition on litter decomposition and nutrient release for N-fixing and non-N-fixing trees litter. We tested three hypotheses: (1) N addition and its combination with elevated CO_2 would offset the detrimental effects of Cd addition on leaf litter decomposition and nutrient release; (2) the mitigative effect of N addition and its combination with elevated CO_2 is greater in non-N-fixing species (*Cinnamomum camphora*) than in N-fixing species (*Acacia auriculiformis*), due to higher litter N content in *A. auriculiformis*; (3) the mitigative effects on litter nutrient release would be different at the early (less than 6 moths of incubation) vs. later stage of litter decomposition, due to the rapid loss of litter simple compounds at the early stage of litter decomposition.

Materials and methods

Site description

The experiment was conducted at South China Botanical Garden, Guangzhou (23°20'N and 113°30′E), Guangdong Province, China. This area has a monsoonal climate with four distinct seasons. The amount of N deposition from rainfall in a pine forest in Guangzhou (Guangdong Province) is 39 - 49 kg N ha^{-1} y^{-1} (Li et al. 2012). The mean annual temperature at the experimental site is 21.5 °C, the mean relative air humidity is 77%, and the mean annual precipitation is 1750 mm.

Experimental design

A total of 15 open-top chambers (cylindrical, 3.0 m in diameter and 4.5 m in height) were constructed for the current experiment. The experiment had five treatments, with three replicate chambers per treatment: Cd addition (Cd, 10 kg ha^{-1} yr^{-1}); Cd and N addition (Cd + N; 10 kg Cd ha^{-1} yr^{-1} and 100 kg N ha^{-1} yr^{-1}); Cd addition with elevated CO_2 (Cd + CO_2; 10 kg Cd ha^{-1} yr^{-1} and 700 µmol mol^{-1} CO_2); Cd and N addition under elevated CO_2 (Cd + CO_2 + N; 10 kg Cd ha^{-1} yr^{-1}, 700 µmol mol^{-1} CO_2, and 100 kg N ha^{-1} yr^{-1}), and a control (ambient Cd, CO_2, and N). We used a N addition rate of 100 kg N ha^{-1} yr^{-1} for two considerations. One is atmospheric N deposition will increase from 46 kg N ha^{-1} yr^{-1} in 1988 to 109 kg ha^{-1} yr^{-1} in 2030 in China (Ren et al. 2000; Li et al. 2012), which is close to the addition rate used in our study. The other is that a doubled N addition rate enables us to detect significant N addition effects in a short time. We used an elevated CO_2 concentration of 700 µmol mol^{-1}, because the atmospheric CO_2 concentration will reach 700 µmol mol^{-1} by the end of this century (IPCC 2019). We used a Cd addition rate of 10 kg Cd ha^{-1} yr^{-1} for following consideration. The concentration of Cd in soil is more than 3 mg kg^{-1} in Pearl River Delta (Duan et al. 2016), and then the calculated Cd stock is 9.6 kg Cd ha^{-1} (3 mg Cd kg^{-1} × 2000 kg soil/ 6.25 m^2 cultivation box) in each chamber. Therefore we set the Cd addition rate as 10 kg Cd ha^{-1} yr^{-1} (which is equal to 3.125 mg Cd kg^{-1} soil). The Cd source was chemically pure $CdCl_2$, and the N source was chemically pure NH_4NO_3. The solutions (18L) of Cd and N were sprayed monthly on soil surface of the corresponding chambers, and the same amount of water was sprayed for the control.

The above-ground of chamber was wrapped with impermeable and transparent plastic sheets, and the top of chamber were totally open. Light intensity in the chamber was 97% of full sunlight and no spectral changes were detected. Air temperature was recorded inside or outside of chamber, and no significant difference was found. In each chamber, the addition CO_2 was supplied from a tank, and was distributed by transparent pipe. The transparent pipe with pinholes (0.1 cm diameter) was hung 2.0 m in height. The distance between two pinholes was 3 cm. To ensure that CO_2 was equally distributed in the entire chamber, the pipe was connected to a fan. CO_2 fumigation was applied with fans from 8:30 am to 4:30 pm everyday except on rainy days. The CO_2 flux from the tank was controlled by a flow meter and CO_2 concentration on the five planes (0.5 m, 1 m, 1.5 m, 2.0 m, and 2.5 m in height) in the chambers were weekly checked using a Licor-6400 (LI-COR Inc., Lincoln, NE, USA). The treatments started in March 2017. Soil from the organic layer was collected from a primary broadleaf forest. The soil is Ultisol, and its pH ranged from 5.06 to 5.22 before treatment (Table S1). Two years old seedlings were transplanted into the chambers. Each chamber was uniformly planted with three seedlings for each of the following five species: *A. auriculiformis*, *Castanopsis hystrix*, *C. camphora*, *Liquidambar formosana* and *Syzygium hainanense*. These species are widely planted and grown tree species in subtropical China.

Litter decomposition

In October 2017, freshly fallen leaf litters was collected from the floor of *A. auriculiformis* and *C. camphora* forest without any treatment in Heshan, Guangdong, China; neither the forest soil nor the litter had been subjected to any experimental treatment before the litter was collected. *A. auriculiformis* is a N-fixing species, and *C. camphora* is a non-N-fixing species. The leaf litter samples were oven-dried at 65 °C to a constant weight and were then ground for chemical analysis. The initial chemical characteristics of the two leaf litter were shown in Table 1.

Nylon mesh litter bags were used to conduct leaf litter decomposition experiment. Each nylon mesh bag (10 cm × 15 cm, mesh size 1 mm) was filled

Table 1 Initial leaf litter chemical properties of the non-N-fixing species (*C. camphora*) and N-fixing species (*A. auriculiformis*)

Species	*C. camphora*	*A. auriculiformis*
C (g kg^{-1})	527.3 ± 5.7a	556.7 ± 6.3b
N (g kg^{-1})	13.3 ± 0.8a	16.1 ± 0.7b
P (g kg^{-1})	0.71 ± 0.04a	0.61 ± 0.02a
K (g kg^{-1})	4.22 ± 0.12a	4.16 ± 0.12a
Mg (g kg^{-1})	1.19 ± 0.06a	0.98 ± 0.09a
Ca (g kg^{-1})	12.89 ± 0.67b	5.10 ± 0.12a
Lignin (g kg^{-1})	340.0 ± 11.5a	400 ± 8.7b
Cellulose (g kg^{-1})	240.0 ± 8.7a	260.0 ± 5.8a
C:N ratio	39.8 ± 1.6a	34.6 ± 0.8b
Lignin: N ratio	25.6 ± 0.5a	24.9 ± 0.4a
Cellulose: N ratio	18.1 ± 0.3a	16.2 ± 0.2b

Values are means ± standard error (SE) ($n = 3$). Means in a column followed by the same letters are not significantly different ($p > 0.05$). C: carbon; N: nitrogen; P: phosphorus; K: potassium; Mg: magnesium; Ca: calcium

with 10 g of dried leaf litter. At the beginning of the study in November 2017, the litterbags containing *A. auriculiformis* or *C. camphora* litter were positioned on the surface of the soil organic layer in the chambers. The litterbags were harvested after 3, 6, 12, and 15 months. In total, there were 120 litterbags (2 species × 5 treatments × 3 replicates × 4 sampling times). Replicate litter bags in each chamber were collected at each harvest time and were transported to the laboratory as soon as possible. The harvested leaf litter sample was dried at 65 °C to a constant mass and weighed to determine the total dried litter mass and its chemical characteristics.

Analytical methods

The total organic C concentrations of the leaf litter samples were determined using the $K_2Cr_2O_7$ oxidation method (Nelson and Sommers 1982). Litter total N concentrations were measured with the Kjeldahl method, and total phosphorus (P) concentrations were measured with the molybdate-ascorbic acid method (Bremner and Mulvaney 1982; Liu et al. 1996) using a UV4800 spectrophotometer (Shimadzu, Japan). The concentration of litter potassium (K), calcium (Ca), and magnesium (Mg) were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Optima-2000 DV, PerkinElmer, USA) after nitric acid digestion. The Cd concentration of leaf and soil samples were determined with flame atomic absorption spectrophotometer (AAS, contrAA800, Analytikjena, Germany). Concentrations of cellulose and lignin in the leaf litter samples were determined using the acid-detergent fiber method (Rowland and Roberts 1994; Goering and Van Soest 1970), and with the follow formulas:

$$\text{Cellulose} = \text{acid detergent fiber} - \text{acid detergent lignin} \quad (1)$$

$$\text{Lignin} = \text{acid detergent lignin} - \text{ash content} \quad (2)$$

In soil, microbial biomass C (MBC) was determined using the chloroform fumigation-extraction method, and the experimentally-derived conversion factors was 0.45 for MBC (Vance et al. 1987). Soil β-1,4-glucosidase (BG) and cellobiohydrolase (CBH) contribute to cellulose decomposition, and both phenol oxidase (PPO) and peroxidase (POD) contribute to lignin decomposition. The activities of these four C-degrading enzymes (i.e., BG, CBH, PPO, and POD) in soil were determined following the procedures of Allison and Vitousek (2005) and Freeman et al. (1995). Additional details were provided in our previous study (Luo et al. 2019b).

Statistical analysis

At each harvest time, the total remaining litter was weighed. The dried litter mass remaining in the bags was expressed as a percentage of the initial leaf litter weight in each litter bag. The single-exponential decay model (Olson 1963) was used as follows, which was used to calculate the annual decomposition rate constant k (yr^{-1}).

$$M_t/M_0 = \exp(-kt) \quad (3)$$

where M_t is the mass (g) remaining at time t (year), and M_0 is the initial mass (g). All sample mass was converted to the equivalent mass at 65 °C, and t is the incubation time (year). The turnover time (T, k^{-1}) was also calculated for each treatment and litter type.

The quantity of nutrients release via litter decomposition was expressed as a proportion of the initial nutrient content, which was calculated by determining the nutrient content at each sampling time and dividing it by the initial nutrient content (Pancotto et al. 2003; Song et al. 2015).

$$\text{Nutrient release}\ (\%) = \left[\left(M_t \times C_t\right)/\left(M_0 \times C_0\right)\right] \times 100 \quad (4)$$

where M_t is the oven-dry mass (g) at time t (year); C_t is the nutrient concentration (g kg^{-1}) at time t (year); M_0 is the initial oven-dry mass (g); and C_0 is the initial nutrient concentration (g kg^{-1}).

Litter C:N:P ratios were calculated based on mass. Differences in parameters among treatments, including litter annual decomposition rate, concentration and content of litter lignin and cellulose, and basic soil properties, were determined by one-way analyses (ANOVAs) at $p < 0.05$. The effects of litter type, treatment, time and interactions on litter enzyme activities were assessed by Multi-way ANOVAs. Student's t test was used to test for statistically significant differences of initial leaf chemical properties between *A. auriculiformis* and *C. camphora*. All data were analyzed using SPSS 19.0 software (SPSS. Inc., Chicago, IL, USA), and the figures were drawn with Origin 2015 (Origin Lab. Inc., Massachusetts, USA).

A structural equation model (SEM) was used to assess the relative importance of Cd addition, elevated CO_2, N addition, elevated CO_2 and N addition, MBC, ligninase (PPO and POD), cellulase (BG and CBH), litter quality (cellulose: N ratio and lignin: N ratio) on litter nutrient release (litter C, N, P, K, Mg and Ca remaining in litter (% of initial)) using AMOS 24.0 (IBM Corporation, New York, US). Of the variation in nutrient release, 84% and 75% were explained by the first component of a principal component analysis (PCA) conducted with litter C, N, P, K, Mg and Ca (% of initial) in *C. camphora* and *A. auriculiformis*, respectively; Of the variation in litter quality, 95% and 97% were explained by the first component of a PCA conducted with litter cellulose: N ratio and lignin: N ratio in *C. camphora* and *A. auriculiformis*, respectively; Of the variation in cellulase, 89% and 88% were explained by the first component of a PCA conducted with β-glucosidase (BG) and cellobiohydrolase (CBH) in *C. camphora*) and *A. auriculiformis*, respectively; Of the variation in ligninase, 88% and 87% were explained by the first component of a PCA conducted with phenol oxidase (PPO) and peroxidase (POD) in *C. camphora*) and *A. auriculiformis*, respectively. Therefore, principal component analysis (PCA) was used to reduce each of these four groups of variables to one variable (litter quality, cellulase, ligninase, and nutrient release), respectively, which were introduced into the model as new variables. The SEM results were presented as a typical path diagram. The fit of the model was evaluated using the χ^2 test and the root mean squared error (RMSE) of approximation, and the fit was confirmed using Akaike information criterion (AIC).

Results

Initial leaf litter chemistry and soil properties

The concentration of leaf litter P, K, Mg and cellulose have no significant difference between the non-N-fixing species (*C. camphora*) and the N-fixing species (*A. auriculiformis*) (Table 1). However, the concentrations of organic C, total N, and lignin were significantly higher in *A. auriculiformis* than in *C. camphora*, while the C:N ratio, cellulose: N ratio, and the concentration of Ca were significantly lower in *A. auriculiformis* than in *C. camphora* (Table 1). Initial soil properties including soil pH, electrical conductivity, bulk density, total C, total N, total P, and available Cd were similar among the different treatments (Table S1).

Litter decomposition rate and mass loss

The decomposition rates (k-values) in the control treatment were 0.64 yr^{-1} for *C. camphora* litter, and 0.66 yr^{-1} for *A. auriculiformis* litter (Table 2). The

Table 2 Litter decomposition rates for two plant leaf litter

Treatment	*C. camphora*		*A. auriculiformis*	
	k-value ($year^{-1}$)	R^2	k-value ($year^{-1}$)	R^2
Control	0.64 ± 0.01c	0.96	0.66 ± 0.03a	0.95
Cd	0.51 ± 0.04a	0.85	0.54 ± 0.04a	0.90
Cd + CO_2	0.56 ± 0.01ab	0.90	0.64 ± 0.05a	0.96
Cd + N	0.68 ± 0.05bc	0.83	0.69 ± 0.03ab	0.87
Cd + CO_2 + N	0.83 ± 0.05c	0.72	0.80 ± 0.07b	0.88

Values are means ± standard error (SE) ($n = 3$). Means in a column followed by the same letters are not significantly different ($p > 0.05$). The annual decomposition rates (k-values) were calculated using the first-order exponential decay model ($X_t/X_0 = e^{-kt}$). The correlation coefficient R^2 indicate the linear regression of the fraction of initial remaining vs. incubation time. *C. camphora* is a non-N-fixing species and *A. auriculiformis* is an N-fixing species

decomposition rates were decreased by the Cd treatment alone, but this decreases was somewhat alleviated by elevated CO_2 (Table 2). N addition significantly offset the negative effect of Cd addition on decomposition rates by 33.3% and 27.8% in *C. camphora* and *A. auriculiformis* litter, respectively. The single-exponent decomposition model provided good fits for the fraction of the initial mass remaining over time for both types of litter; R^2 values ranged from 0.72 to 0.90 for *C. camphora* litter and ranged from 0.87 to 0.95 for *A. auriculiformis* litter (Table 2).

After 15 months of incubation, Cd addition decreased litter mass loss by 17.6% and 13.2% in *C. camphora* and *A. auriculiformis*, respectively, compared to control. Combination of elevated CO_2 and N addition offset these negative effect of Cd addition, and increased the mass loss by 18.7% for *C. camphora* litter and by 21.1% for *A. auriculiformis* litter (Fig. 1). Overall, litter mass loss of the two plant was rapid in the first 6 months of incubation and then slowed; decomposition during the first 6 months accounted for 54.6-82.8% of the mass loss over 15 months (Fig. 1).

Litter nutrient release

After 15 month of decomposition of *C. camphora* and *A. auriculiformis* litter, the proportions of the initial lignin, cellulose, C, N, P, K, Mg, and Ca remaining in the litter decreased, and the decrease was greater for litter lignin, cellulose, C, K and Mg than for N, P, and Ca (Figs. 2 and S3). The concentration of litter lignin, cellulose, C, K, and Mg remaining in litter decreased and reached a stable state after 15 months of incubation, while the concentration of litter N, P, and Ca remaining in litter increased and reached peak (Fig. S1 and Table S2). Cd addition significantly reduced litter lignin, cellulose, C, N, P, K, Mg, and Ca release in *C. camphora* and *A. auriculiformis* litter, especially at the later state of decomposition (after 6 months of incubation, Figs. 2 and S3). However, the negative effect of Cd addition was slightly alleviated by elevated CO_2 and substantially alleviated by N addition. N addition greater alleviated the Cd detrimental effects on litter lignin, cellulose, C, P, Mg and Ca release by 27%, 25%, 31%, 24%, 38%, and 42% for *C. camphora* than by 23%, 18%, 22%, and 13% for *A. auriculiformis*, respectively (Figs. 2 and S3).

The litter C: N ratios, C: P ratios, lignin: N ratios, and cellulose: N ratios in both *C. camphora* and *A. auriculiformis* litter decreased during litter decomposition, but the litter N: P ratios did not significantly change (Fig. S2 and Fig. S3e, f, g, h). N addition significantly offset the detrimental effects of Cd addition, and deceased litter C: N ratios, lignin: N ratios,

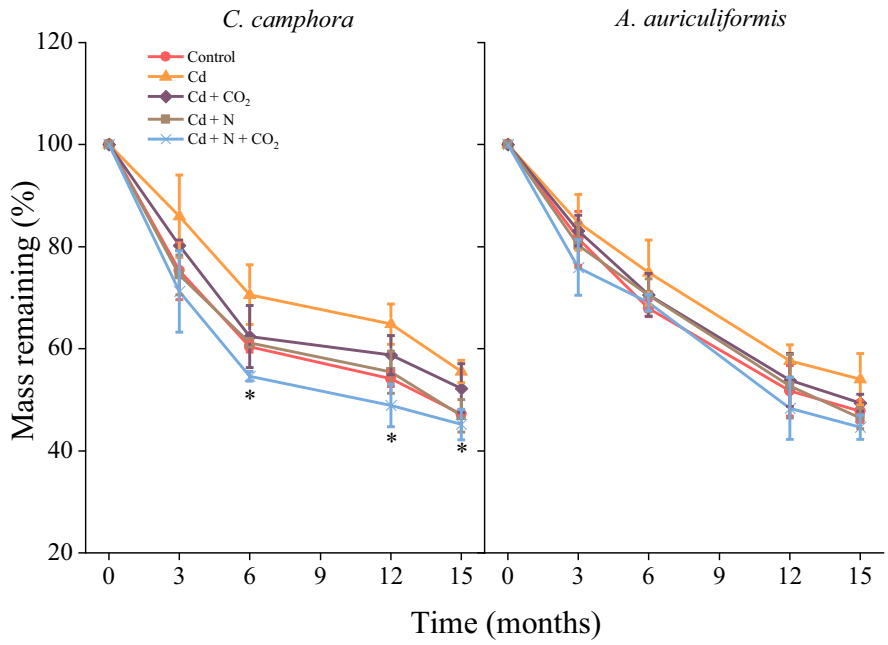

Fig. 1 Change in litter mass of a non-N-fixing species (*C. camphora*) and an N-fixing species (*Acacia auriculiformis*) as affected by treatment and incubation time. Values are means ± standard error (SE) ($n=3$). An asterisk (*) indicates a significant difference between Cd addition and other treatments (including elevated CO_2 and Cd addition, N and Cd addition, or N and Cd addition under elevated CO_2) at $p<0.05$

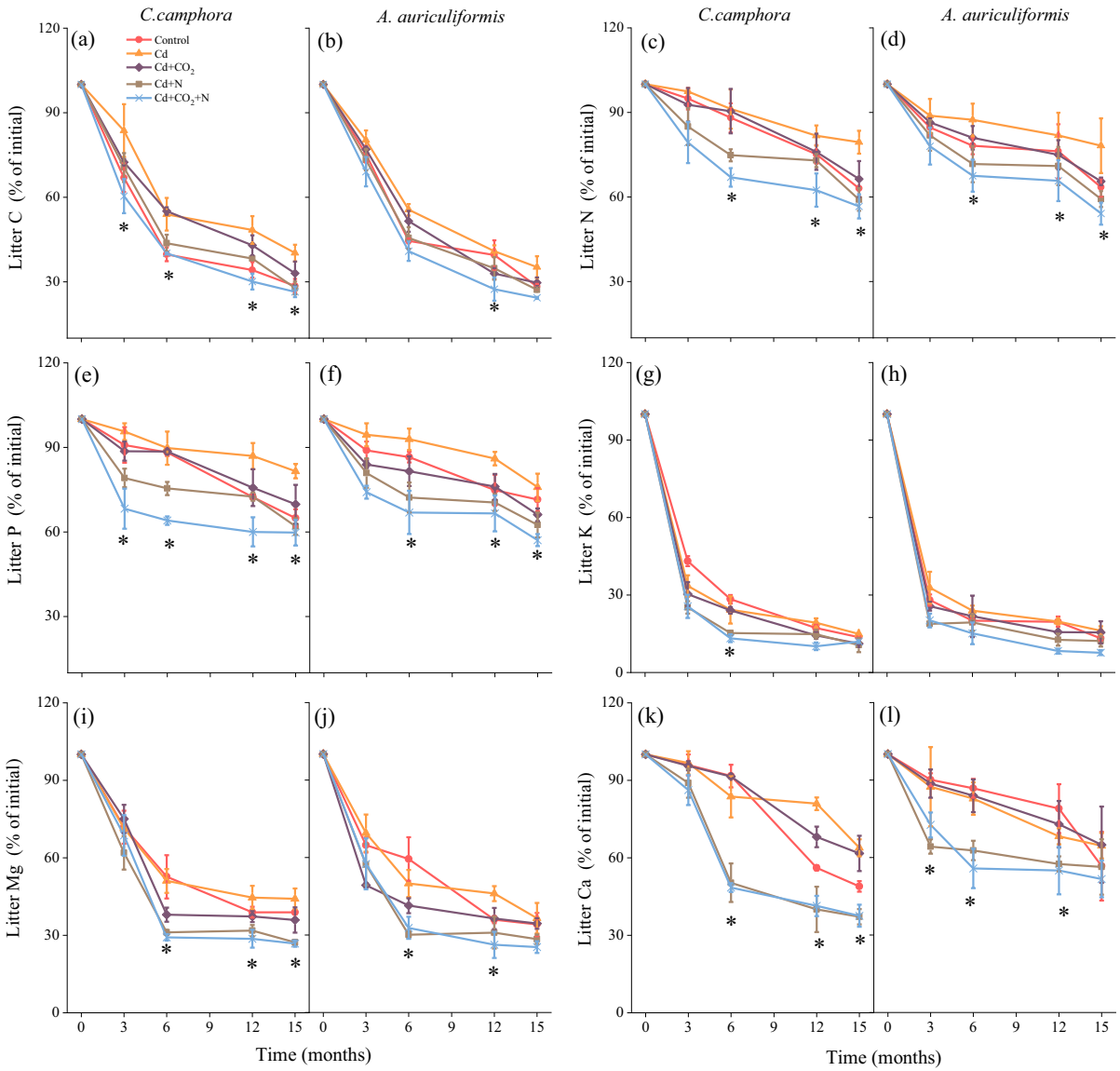

Fig. 2 Changes in the contents of organic carbon (C), nitrogen (N), and phosphorous (P) (% of initial) in the litter of a N-fixing species (*C. camphora*) and a non-N-fixing species (*A. auriculiformis*) as affected by treatment and incubation time. Values are means ±SE ($n=3$). An asterisk (*) indicates a significant difference between Cd addition and other treatments (including elevated CO_2 and Cd addition, N and Cd addition, or N and Cd addition under elevated CO_2) at $p<0.05$.

cellulose: N ratios, improving litter quality (Figs. S2 and S3).

Soil MBC and enzyme activities

During 15-month litter decomposition, the activities of four degrading enzymes tended to increase over incubation time (Fig. 3). Cd addition decreased the activity of BG and CBH, while N addition and its combination with elevated CO_2 increased the activity of BG and CBH, compared to Cd addition at the early stage of decomposition, and the effect was greater for *C. camphora* litter than for *A. auriculiformis* litter (Fig. 3a–d). After declining during the first 12 months of incubation, the activities of cellulase (BG and CBH) increased at the later

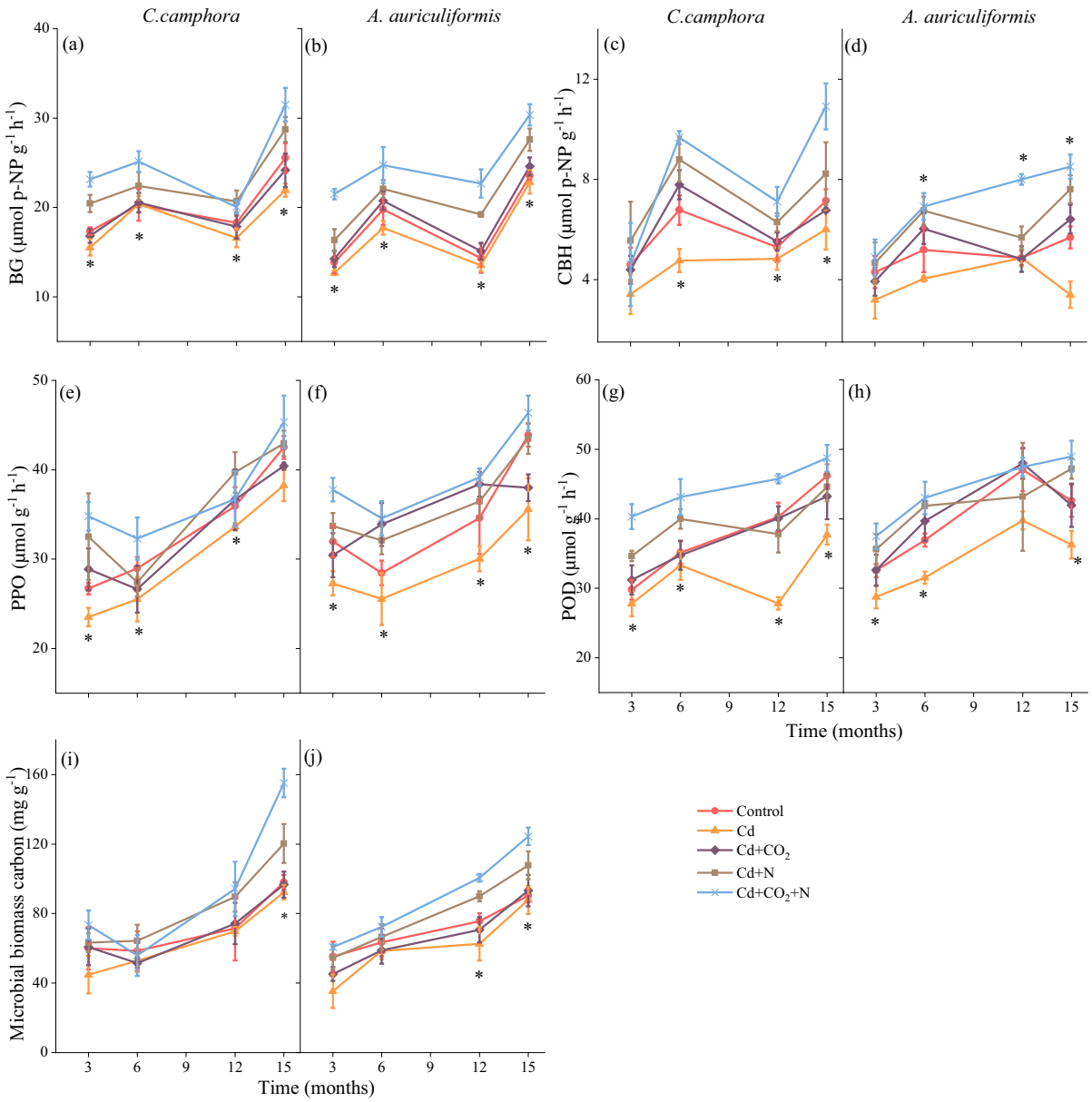

Fig. 3 Activities of two cellulase enzymes (β-glucosidase and cellobiohydrolase) and two ligninase enzymes (phenol oxidase and peroxidase) in the litter of a non-N-fixing species (*C. camphora*) and an N-fixing species (*A. auriculiformis*) as affected by treatment and incubation time. Values are means ±SE ($n = 3$). An asterisk (*) indicates a significant difference between Cd addition and other treatments (including elevated CO_2 and Cd addition, N and Cd addition, or N and Cd addition under elevated CO_2) at $p < 0.05$.

decomposition stage (12-15 months of incubation). The activities of PPO and POD increased throughout the decomposition period (Fig. 3e–h). N addition and its combination with elevated CO_2 significantly alleviated the inhibitory of Cd addition, and increased the activities of PPO and POD for both *C. camphora* and *A. auriculiformis* litter. For both types of litter, soil MBC increased during litter decomposition and greater increased at the later stage of decomposition (after 6 months of incubation) than at the early stage of decomposition (Fig. 3i, j). The treatment and time individually significantly not only affected litter

element release, but also influenced soil microbial activities (Table 3).

Relationship between nutrient release and litter quality or enzyme activities

According to the Pearson correlation analysis, the proportions of the initial C, N, P, K, Mg, and Ca remaining in the litter were significantly positively correlated with each other in *C. camphora* and *A. auriculiformis* (Table S3). While the proportions of the initial C, N, P, K, Mg, and Ca remaining in the litter were negatively correlated with the activities of degrading enzymes (BG, CBH, PPO, and POD) (Table S3).

The goodness-of-fit model revealed that Cd addition inhibited litter nutrient release, i.e., increased the proportions of the initial C, N, P, K, Mg, and Ca remaining in the litter ($\beta = 0.19$ for *C. camphora* and $\beta = 0.05$ for *A. auriculiformis*, Fig. 4c, d). Elevated CO_2, N addition, and their combination alleviated the detrimental effects of Cd addition, and accelerated litter nutrient release. The combination of elevated CO_2 and N addition (average $\beta = -0.78$) greater offset the inhibitory of Cd addition on litter nutrient release than elevated CO_2 (average $\beta = -0.15$) or N addition (average $\beta = -0.42$), separately (Fig. 4). The positive standardized total effect on the litter nutrient release decreased in the following order: litter quality ($\beta = 0.56$) > Cd addition ($\beta = 0.19$) in *C. camphora*, and litter quality ($\beta = 0.45$) > Cd addition ($\beta = 0.05$) in *A. auriculiformis.* The negative standardized total effect decreased in the following order in *C. camphora:* elevated CO_2 + N addition ($\beta = -1.10$) > ligninase ($\beta = -0.80$) > N addition ($\beta = -0.70$) > elevated CO_2 ($\beta = -0.18$) > cellulase ($\beta = -0.10$) > MBC ($\beta = -0.01$) (Fig. 4c). The negative standardized total effect decreased in the following order in *A. auriculiformis:* MBC ($\beta = -0.74$) > elevated CO_2 + N addition ($\beta = -0.45$) > cellulase ($\beta = -0.33$) > ligninase ($\beta = -0.20$) > N addition ($\beta = -0.14$) > elevated CO_2 ($\beta = -0.12$) (Fig. 4d).

Discussion

As hypothesized, Cd inhibited while N addition and it combination with elevated CO_2 accelerated the litter decomposition and nutrient release in *C. camphora* and *A. auriculiformis*, especially at the later stage of

Table 3 Effects of litter type, treatment, time, and their interactions on element release and the activities of enzyme during litter decomposition as indicated by Multi-way ANOVAs (*F* statistics)

Source of variation	Litter type	Treatment	Litter type ×Treatment	Time	Litter type ×Time	Treatment ×Time	Litter type ×Treatment ×Time
Element release							
C	0.2	15.5**	0.3	244.7**	1.6	0.5	0.5
N	1.7	13.6**	0.1	23.3**	1.0	0.8	0.3
P	0.01	24.9**	0.6	23.8**	0.2	0.5	0.2
Cellulose	6.5*	11.3**	0.2	143.3**	3.0*	0.7	0.5
Lignin	7.5**	9.7**	1.3	100.9**	1.9	0.6	0.8
K	2.7	11.0**	1.3	36.3**	1.8	0.8	1.0
Mg	0.5	13.7**	0.3	47.3**	0.3	0.9	0.5
Ca	0.2	17.7**	0.4	29.4**	3.1*	1.0	0.8
Microbial activities							
BG	12.8**	40.7**	1.6	102.1**	1.9	0.4	0.9
CBH	20.2**	18.5**	0.3	23.7**	3.5*	1.9*	1.1
PPO	1.8	13.9**	0.8	62.5**	1.1	1.0	1.0
POD	6.2*	18.2**	0.4	31.5**	4.0*	0.8	0.6
MBC	2.7	9.5**	0.03	54.9**	1.5	1.3	0.5

C carbon, *N* nitrogen, *P* phosphorus, *K* potassium, *Mg* magnesium, *Ca* calcium, *BG* β-1,4-glucosidase, *CBH* cellobiohydrolase, *PPO* phenol oxidase; *POD* indicates peroxidase; *MBC* microbial biomass carbon. * and ** indicate $p < 0.05$ and $p < 0.01$, respectively

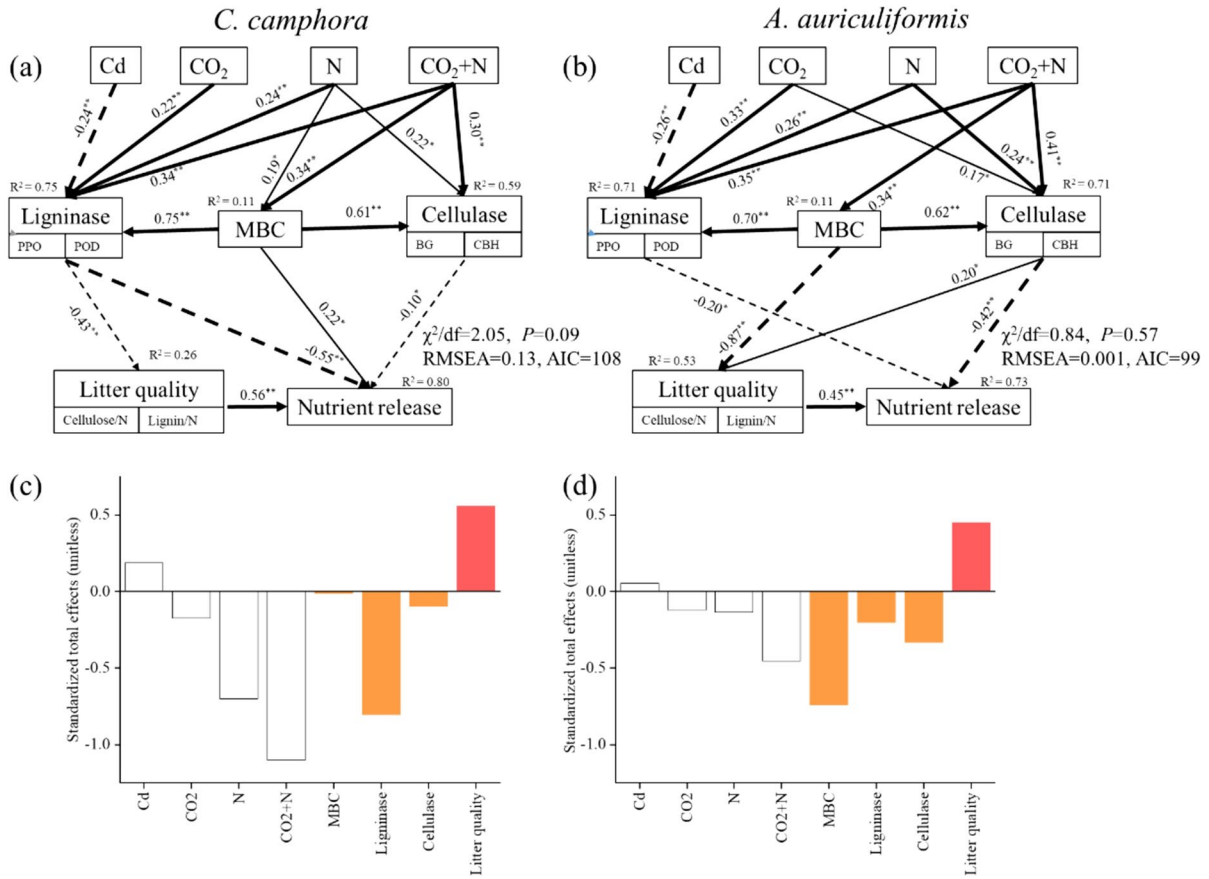

Fig. 4 Structural equation model (SEM) results for the effects of the environmental variables on litter nutrient release in *C. camphora* (**a**) and *A. auriculiformis* (**b**) and standardized total effects (direct plus indirect effects) derived from them (c and d). Numbers adjacent to arrows are standardized path coefficients, which are analogous to relative regression weights and which indicate the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. Arrow width is proportional to the strength of the relationship. Goodness-of-fit statistics for the model are shown in the right corner (df, degrees of freedom). R^2: the proportion of variance explained. Cd: Cadmium addition; CO_2: elevated CO_2; N: N addition; CO_2+N: elevated CO_2 and N addition; BG: β-1,4-glucosidase; CBH: cellobiohydrolase; PPO: phenol oxidase; POD: peroxidase; MBC: microbial biomass carbon; nutrient release including litter C, N, P, K, Mg and Ca (% of initial), and is indexed by the first component of a PCA conducted with litter C, N, P, K, Mg and Ca (% of initial)

litter decomposition. N addition and its combination with elevated CO_2 increased litter nutrient release not only by improving litter quality and increasing the N, P, Ca concentration in litter, but also by increasing soil microbial activities. These results suggest that the detrimental effects of Cd addition on litter nutrient release in Cd-contaminated environments, can be offset by combined N addition and elevated CO_2. Furthermore, the mitigative effects of N addition and its combination with elevated CO_2 on litter decomposition, nutrient release, and soil microbial activities were greater in non-N-fixing species *C. camphora* than N-fixing species *A. auriculiformis*. These results suggest that the nutrient cycle of non-N-fixing species *C. camphora* in Cd- contaminated regions can be accelerated by rising atmospheric CO_2 and increasing N deposition in the future.

N addition and its combination with elevated CO_2 offset the detrimental effect of Cd addition on litter decomposition and nutrient release

In the 15-month decomposition experiment reported here, litter decomposition and nutrient release were

significantly reduced by Cd addition. Consistent with our first hypothesis, the negative effect of Cd addition on litter decomposition and nutrient release was substantially alleviated by N addition, and greater by the combined N addition and elevated CO_2 which was further confirmed by our SEM that elevated CO_2 + N addition ($\beta = -0.78$) > N addition ($\beta = -0.42$) > elevated CO_2 ($\beta = -0.15$).

Nitrogen addition alleviated the detrimental effect of Cd addition on litter decomposition and nutrient release in *C. camphora* and *A. auriculiformis* which attributed to the following two possible reasons. First, N addition alleviated the detrimental effects of Cd addition on litter decomposition and nutrient release, which possibly be explained the changes in litter chemistry (Cleveland et al. 2014). Litter quality parameters of N content and C:N ratio are generally recognized as important variables related to litter decomposition and nutrient release. P, Ca, K, and Mg contents remaining in litter also positively affected litter decomposition and nutrient release in tropic and subtropical forests (Waring 2012). In the current study, N addition reduced the negative effect of Cd addition on litter decomposition and greater increased the decomposition of litter cellulose and lignin than litter N. These results suggest that N addition slowed the release of litter N, and decreased litter cellulose: N and lignin: N ratios, which improved litter quality, and consequently accelerating litter decomposition (Koopmans et al. 1998). On the other hand, N addition increased N, P and Ca concentration in litter (Fig. S1), which also led to the greater litter decomposition and nutrient release. Second, N addition can increase the availability of soil N for microorganisms and can thereby increase the production of enzymes that degrade cellulose and lignin, especially in low-N soils (Zhou et al. 2018). In this study, N addition alleviated the toxic effects of Cd, and increased the activities of soil ligninase (PPO and POD) and cellulase (BG and CBH), which is consistent with Sinsabaugh et al. (2015) reported that N deposition significantly increased PPO, POD and CBH activities. N addition increased the activities of ligninase and cellulose in *C. camphora* and *A. auriculiformis* litter, probably because the external N inputs induced priming effects on the activities of microbial community and enzyme activities (Lü et al. 2013).

Combination of N addition and elevated CO_2 strongly offset Cd effects on litter nutrient release not only by changing litter chemistry, but also increasing soil N availability and soil moisture. Elevated CO_2 accelerate litter nutrient mineralization by improving litter quality (Liu et al. 2015). In our study, elevated CO_2 slightly alleviated the inhibitory effect of Cd addition on litter decomposition and nutrient release in two species. Perhaps because elevated CO_2 can increase the N, P, and Ca concentration in litter (Waring 2012) and also increases soil moisture which increases soil enzyme activities, consequently accelerating litter decomposition and nutrient release (Adair et al. 2009; Liu et al. 2015). However, elevated CO_2 increase the accumulation of lignin in litter to reduce litter decomposition (Cotrufo et al. 1999), which would counteract the positive effect of elevated CO_2 on nutrient release. Moreover, combination of N addition and elevated CO_2 increase cellulose and lignin decomposition by improving litter quality (e.g., by decreasing litter cellulose: N and lignin: N ratios) (Koopmans et al. 1998; Mo et al. 2006).

Additionally, the mitigated effects of N addition and its combination with elevated CO_2 were greater in *C. camphora* than *A. auriculiformis*, which is consistent with our secondary hypothesis. A possible explanation for the different responses between the two types of litter is the difference in the N-fixing ability of the two species. As a non-N fixing species, *C. camphora* produces litter with a relatively low N concentration such that decomposition of its litter may benefit from added N. In contrast, *A. auriculiformis* is an N-fixing species that produces litter with a relatively high N concentration and low lignin: N ratio, such that decomposition of its litter does not apparently benefit from added N, especially at the later stage of litter decomposition (Parton et al. 2007; Hobbie et al. 2012; Lü et al. 2013). Our SEM also confirmed that N addition and it combination elevated CO_2 greater improve litter quality in *C. camphora* ($\beta = 0.56$) than in *A. auriculiformis* ($\beta = 0.45$), and greater increase the activities of soil ligninase in *C. camphora* ($\beta = -0.80$) than *A. auriculiformis* ($\beta = -0.2$), which finally greater accelerate litter nutrient release *of C. camphora* than *A. auriculiformis.*

Divergent mitigations on litter nutrient release at the early vs. later stage of litter decomposition

As our third hypothesis, N addition and its combination with elevated CO_2 greater alleviated the effects

of Cd addition on nutrient release at later (after 6 months of incubation) than early stage of litter decomposition. A possible reason is that the toxic of Cd (Table S4) and the supply of N availability to soil microorganisms may have been delayed and thus resulted in the effects of Cd addition and N addition on litter decomposition and nutrient release became apparent over the time of litter incubation (Shen et al. 2005). N addition and elevated CO_2 affected litter nutrient release by differently changing soil enzyme activities and litter lignin/cellulose: N ratio at the early vs. later stage of litter decomposition (Hobbie et al. 2012; Zhou et al. 2018). In our study, N addition and its combination with elevated CO_2 greater alleviated the negative effects of Cd addition on the activities of ligninase than cellulase, and greater decreased litter lignin: N ratio than cellulose: N ratio at the later decomposition stage. The phenomena was consistent with previous study that the effects of N addition on microbial activity varied over the time of litter decomposition (Andersson et al. 2004). Litter cellulose and other simple carbohydrates in litter were preferentially decomposed at the early stage of litter decomposition and that lignin and other recalcitrant compounds were preferentially latterly decomposed by the high activities of ligninase during the later stage of litter decomposition (Ge et al. 2013). In addition, N addition and its combination with elevated CO_2 increase soil N availability and soil moisture, and then increased the activities of soil ligninanse and soil microbial biomass to increase litter nutrient release at the later stage of litter decomposition. On the other hand, N addition and its combination with elevated CO_2 decrease litter lignin and increase litter N concentration to decrease lignin: N, and consequently accelerate litter nutrient release at the later stage of decomposition.

Conclusions

The results of this study showed that N addition and/or elevated CO_2 offset the detrimental effects of Cd addition on litter decomposition and litter C, N, P, K, Ca, and Mg release. The combined mitigative effects of elevated CO_2 and N addition far exceeded the effect of each factor separately. The mitigative effects of N addition and its combination with elevated CO_2 were greater in non-N-fixing species *C. camphora* than in N-fixing species *A. auriculiformis* at the later stage of decomposition. These results, therefore, suggest that N addition and its combination with elevated CO_2 reduce Cd toxicity to accelerate ecosystem biogeochemical cycling, especially for the non-N-fixing trees. The stimulation of nutrient release under N addition and elevated CO_2 not only was associated with increases in the N, P, Ca in litter for improving litter quality, but also was related to increases in soil microbial biomass and enzyme activities. Findings from this study will help understand the separate or combined effects of N and elevated CO_2 on terrestrial ecosystem C and nutrient dynamics in Cd-contaminated environments. Collectively, we recommend that N-fixing species *A. auriculiformis* can be used to improve soil fertility and ecological restoration in Cd-contaminated sites, while the application of *C. camphora* should be associated with N fertilizer.

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Declarations

Conflict of interest The authors have no conflict of interest to declare.

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