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Root Litter Mixing with That of Japanese Cedar Altered CO₂ Emissions from Moso Bamboo Forest Soil

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Abstract: Research Highlights: This study examined the effect of mixing fine roots of Japanese cedar with moso bamboo on soil carbon dioxide (CO₂) emissions with nitrogen (N) addition treatment. Background and Objectives: Moso bamboo expansion into adjacent forests and N deposition are common in subtropical China. The effects of litter input on soil CO₂ emissions, especially fine root litter input, are crucial to evaluate contribution of moso bamboo expansion on greenhouse gas emissions. Materials and Methods: An in situ study over 12 months was conducted to examine mixing fine roots of Japanese cedar with moso bamboo on soil CO₂ emissions with simulated N deposition. Results: Fine root litter input of Japanese cedar and moso bamboo both impacted soil CO₂ emission rates, with mixed litter, positively impact soil CO₂ emission rate with N addition treatment. Moso bamboo fine root litter input decreased the sensitivity of soil CO₂ emission rate to soil temperature. Conclusions: The encroachment of moso bamboo into adjacent forests might benefit soil C sequestration under warming climate, which will also benefit the mitigation of global climate change.

Keywords: moso bamboo expansion; simulated nitrogen deposition; soil CO₂ emission; litter decomposition; fine root

1. Introduction

Soils are important source of atmospheric carbon dioxide (CO₂) [1]. CO₂ emissions from soil account for a substantial percent of global CO₂ [2]. Slight changes in soil CO₂ emissions will generate profound alterations in atmospheric compositions. As one of the largest components of greenhouse gas, emission measurement and mitigation of CO₂ emission are both crucial in global climate change mitigation [3]. Therefore, a factor impacting soil CO₂ emissions will further influence global warming and climate change [4]. In general, microbial activities, soil temperature, soil moisture, and substrate availabilities control soil CO₂ emissions [5,6]. In forest ecosystems, litter decomposition impacted both biotic and abiotic factors that are associated with soil CO₂ emissions [7], which is thereby an important topic in studies of ecosystem CO₂ emissions.

Litter decomposition is an important process that is associated with carbon (C) and nutrients cycling in forest ecosystems [8]. During litter decomposition process, C and nutrients are released from their bounded organic matter, impacting surrounding the soil environment, element cycling

process [9], and, hence, soil CO₂ emissions. Indeed, most C input into belowground during litter decomposition process [10], providing more C substrate for microbial activities that are associated with soil CO₂ emissions. Therefore, factors impacting litter decomposition process will also impact soil CO₂ emissions both directly or indirectly. Litter quality has been demonstrated to be one of the important factors regulating litter decomposition rate [11,12], which varied with litter types or plant species, except for nutrient addition or other abiotic factors. Under the natural conditions, litter with different quality might be mixed by species, producing altered litter quality and litter types, which will generate altered effects on litter decomposition process and soil CO₂ emissions. While aboveground litter decomposition has been studied, belowground fine root litter has not been thoroughly studied, especially their effects on soil CO₂ emissions.

In forest ecosystems with more than one species, both above- and below-ground litter input by growing plant will decompose in litter mixture [13], potentially impacting soil CO₂ emissions by mixing decomposed fine root litter, especially under the context of N deposition or other global change factors. Below-ground litter input, i.e., fine root litter, and their effects on soil CO₂ emissions are still not clearly understood when compared with above-ground leaf litter input, due to limited visibility and difficulties in measurement and monitoring. Here, we conducted in situ studies in Jiangxi province to investigate fine root litter mixing effects on soil CO₂ emissions in Japanese cedar forest encroached by moso bamboo while using fine root litter of both Japanese cedar and moso bamboo as well as mixed fine root litter of both species.

We aimed to answer the following question: (1) How does decomposition of litter produced by Japanese cedar and moso bamboo impact soil CO₂ emissions? (2) Will there any effects on soil CO₂ emissions by mixing litter of both Japanese cedar and moso bamboo? (3) How do different litter treatments impact response of soil CO₂ emission to soil temperature and moisture?

2. Materials and Methods

2.1. Study Sites and Focal Species

This study was conducted in Lu Mountain in Jiangxi province (115°53'51"~116°05'55"E, 29°24'54"~29°39'48"N), where Japanese cedar (*Cryptomeria japonica*) and moso bamboo (*Phyllostachys edulis*) coexisted. During the prolonged coexistence, moso bamboo expanded into Japanese cedar forests, providing ideal platform for studying of litter mixing effects on soil CO₂ emissions. Jiangxi province lies in subtropical China. The area where Lu Mountain lies in is characterized by annual average precipitation of 1917 mm, and annual mean temperature between 15–18 °C. The soil in the studied area is highly weathered, with lower pH (3.86–4.23) and soil organic matter content (72.88–159.59 g kg⁻¹) or total nitrogen (1.94–7.38 g kg⁻¹).

Japanese cedar has been cultivated since the last century and formed density monospecific in Lu Mountain. Moso bamboo is one of the important economy species that has been widely cultivated in subtropical China. However, moso bamboo has been seriously expanding its historic ranges to adjacent forests due to conservation practice and other potential reasons [14]. The expanding of moso bamboo has caused series changes in soil C and N cycling process. Above-ground litter input effects following moso bamboo expansion into adjacent forests on soil CO₂ and nitrous oxide emissions has been reported, while, however, fine root litter effects on soil CO₂ emissions have not been studied. While Japanese cedar fine root litter was higher in N, it was lower in the C:N ratio when compared with moso bamboo (Table 1), indicating potential differences in the decomposition rate and, hence, their effects on soil CO₂ emissions.

Table 1. Carbon (C), nitrogen (N), and phosphorus (P) concentration (g kg^{-1}) and their stoichiometric characteristics of cedar and bamboo fine root litter used in this study.

Litter Treat	C (g kg^{-1})	N (g kg^{-1})	C:N
cedar	461.54 \pm 10.37	12.47 \pm 0.21	37.00 \pm 0.24
bamboo	508.67 \pm 3.15	9.58 \pm 0.37	53.34 \pm 2.05

2.2. Experimental Design and Soil CO₂ Emission Measurement

In situ studies were conducted over fourteen-month in Lu Mountain. A full factorial randomized experimental design was used for experiments that were established in August 2018. Fine root litter was collected from mixed forests with both Japanese cedar and moso bamboo and prepared by removing dirt and then being air dried. The sub-samples were oven dried to constant weight to obtain water content of fine root litter. Mixed fine root litter was prepared by mixing both Japanese cedar and moso bamboo fine root litter at 1:1 ratio. All of the root litter was deployed back to soil by control, single species, or mixed species treatments by root mass based on fine root biomass investigated when the fine root litter was collected. The studied area from where the soil CO₂ emission rate was measured and root litter decomposed was thoroughly cleared for original root litter, especially the top 40 cm soil layer where fine root litter mainly distributed. All of the fine root litter samples were deployed and then left decomposed in situ simultaneously before the study. Simulated N deposition treatment was applied by spraying urea solutions to the studied area accumulated to the rate of 8 g N m⁻² in September and October, 2018. The N control treatment received equal quantity of deionized water when N was added.

The soil CO₂ emission rate was measured by the static chamber and gas chromatography method. One month before measurement, nylon collars with groove were installed by N and litter treatments to the depth of 20 cm [15]. An opaque column with the height of 1 m was covered on the collar when the soil CO₂ emission rate was measured. When measurement began, collar groove was filled with water for an airtight purpose. An air sample from the head space of the column was collected that the time when column was closed and then collected at 7, 14, 21 min. after the column closed. The concentration of CO₂ in air samples was determined by gas chromatography (Agilent 7890B, Santa Clara, CA, USA that was equipped with flame ionization detector (FID). The soil CO₂ emission rates were calculated from changes in CO₂ concentration with time following the Equation (1), below [16]:

$$E = P \times V \times \frac{d\text{CO}_2}{dt} \times \frac{1}{RT} \times M \times \frac{1}{S} \quad (1)$$

where E refers to soil CO₂ emission rates ($\mu\text{g g}^{-1} \text{h}^{-1}$), P stands for standard atmospheric pressure (Pa), V refers to headspace volume of the closed column (m^3), R is universal gas constant, T stand for absolute air temperature (K), M refers to the molecular mass of CO₂ (g mol^{-1}), and S is the interior bottom area of the column (m^2).

The soil CO₂ emission rates were measured for 23 times from August 2018 to November 2019. At times when soil CO₂ emission rate was measured, soil temperature and soil moisture of the measured location were both also recorded by a portable soil moisture detector (HydroSense II, CAMPBELL SCIENTIFIC, Logan, UT, USA). The soil CO₂ emission rates were measured during days without substantial precipitation to avoid the potential effects of water that was stored in collars. Cumulative soil emissions were obtained by summing up total CO₂ emissions during the studied time [17].

2.3. Litter and Soil C and N Measurement

The air-dried litter and soil samples were passed through a 0.149 mm sieve for the determination of organic C and N. Organic C was determined by the potassium dichromate ($\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$) oxidation method [18]. Litter N was $\text{H}_2\text{SO}_4\text{-HClO}_4$ digested and measured by automatic discrete chemical

analyzer (Smart Chem 200, Westco, Rome, Italy). We calculated soil C and N stoichiometric ratio while using dry weight basis concentrations.

2.4. Data Analyses

Analysis of variance (ANOVA) were conducted to analyze the dependence of soil temperature, soil moisture, and soil CO₂ emission rates on N deposition, litter treatments, and their interactions with measured time (days) as random effects. Analysis of variance was also used to determine the dependence of cumulative soil CO₂ emissions on N and litter treatment and their interactions. Tukey's post-hoc tests were used to examine the differences among means when significant results were observed. The single positive exponential model was used to examine correlations between the soil CO₂ emission rate and soil temperature, as affected by N and litter treatment. The quadratic function was applied to examine the correlations between soil CO₂ emission rate and soil moisture as affected by N and litter treatment.

All of the statistical analyses were conducted by JMP 9.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Soil CO₂ Emission Rates as Affected by N and Litter Treatment

The soil CO₂ emission rates were significantly affected by N and litter treatment, as well as their interactions (Table 2; Figure 1). In addition, while N and litter treatments did not influence soil temperature, soil moisture was significantly influenced by both N and litter treatments (Table 2). Specifically, simulated N deposition decreased both soil CO₂ emission rates and cumulative CO₂ emissions (Tables 2 and 3; Figure 1).

Table 2. The dependence of soil temperature (°C), soil moisture (%), and soil CO₂ emission rates (mg m⁻² h⁻¹) on N and fine root litter treatments in analysis of variance with measure time (days) as random effects.

Treatment	df	Soil Temperature (°C)		Soil Moisture (%)		CO ₂ Rate (mg m ⁻² h ⁻¹)	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
N treat	1	0.8	0.3642	6.1	0.0137	9.7	0.0020
Litter treat	3	0.1	0.9555	6.5	0.0003	13.2	<0.0001
N treat × Litter treat	3	2.5	0.0580	26.8	<0.0001	7.1	0.0001
Days & Random	22	279.6	<0.0001	140.9	<0.0001	22.9	<0.0001

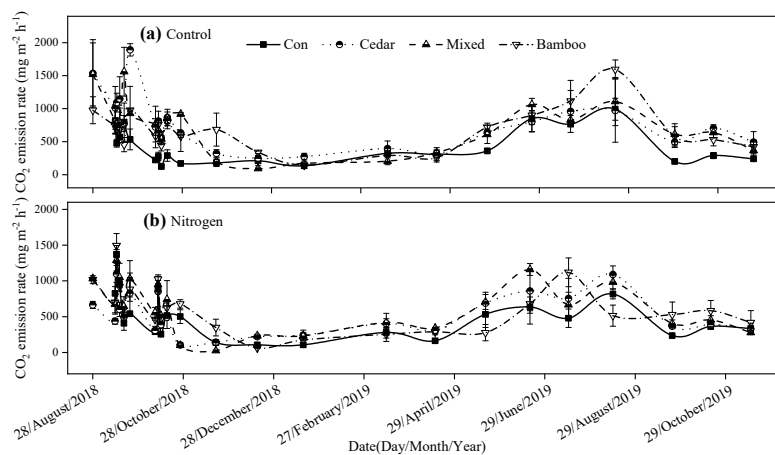


Figure 1. Soil CO₂ emission dynamics as affected by N deposition (a, Control; b, N deposition) and different fine root litter treatments over 14-month in situ study. Con, no root litter; Cedar, cedar fine root litter; Bamboo, bamboo fine root litter; Mixed, mixed fine root litter by both Cedar and Bamboo at 1:1 ratio.

Table 3. The dependence of cumulative soil CO₂ emissions (kg m⁻²) on N and fine root litter treatments in two-way analysis of variance.

Treatment	df	Soil CO ₂ Emissions (kg m ⁻²)		
		SS (10 ¹²)	F	p
N treat	1	5.87	4.6939	0.0457
Litter type	3	15.3	4.0703	0.0251
Litter type × N treat	3	2.03	0.5402	0.6616

While the litter effects varied with species, the litter mixing effects on soil CO₂ emissions depended on N deposition treatments (Tables 2 and 3; Figures 2 and 3). Specifically, soils with Japanese cedar root litter were higher in soil CO₂ emission rates when compared with that with moso bamboo in control treatment without N addition (Figure 2). However, when N was added, the soils with mixed fine root litter were significantly higher in soil CO₂ emission rates than that with Japanese cedar, but not significantly different from that with moso bamboo fine root litter (Figure 2).

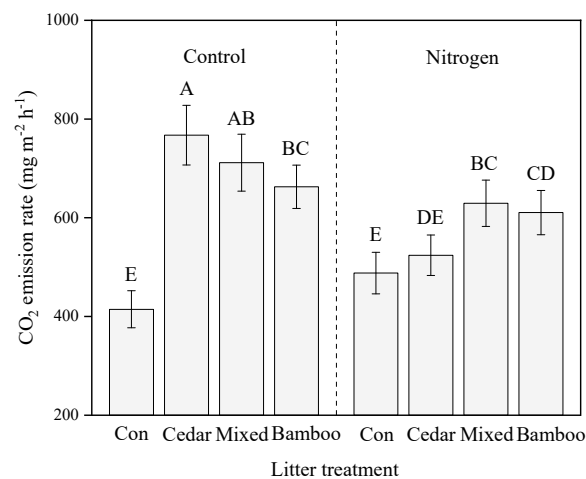


Figure 2. Soil CO₂ emission rates (mg m⁻² h⁻¹, means ± se), as affected by nitrogen (control vs. nitrogen) and fine root litter (con, cedar, bamboo, or mixed litter by cedar and bamboo fine root) treatments. Means with different letters indicate significantly different in post-hoc tests.

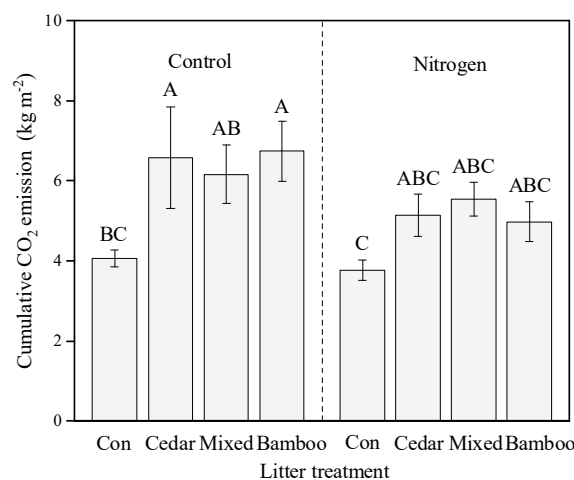


Figure 3. Cumulative soil CO₂ emissions (kg m⁻²), as affected by nitrogen (control vs. nitrogen) and fine root litter (con, cedar, bamboo, or mixed litter by cedar and bamboo fine root) treatments.

3.2. Correlations between Soil CO₂ Emission Rates and Soil Temperature and Moisture

Correlations between soil CO₂ emission rates and soil temperature were well-fitted by single positive exponential growth model (Figure 4). The litter addition treatment decreased the growth rate without N (Figure 4a–d). However, under N deposition treatment, there was no substantial change in growth rate (Figure 4e–h). When compared with litter mixing treatment, moso bamboo fine root litter treatment slightly decreased the growth rate as compared with both Japanese cedar and mixed fine root litter treatment (Figure 4).

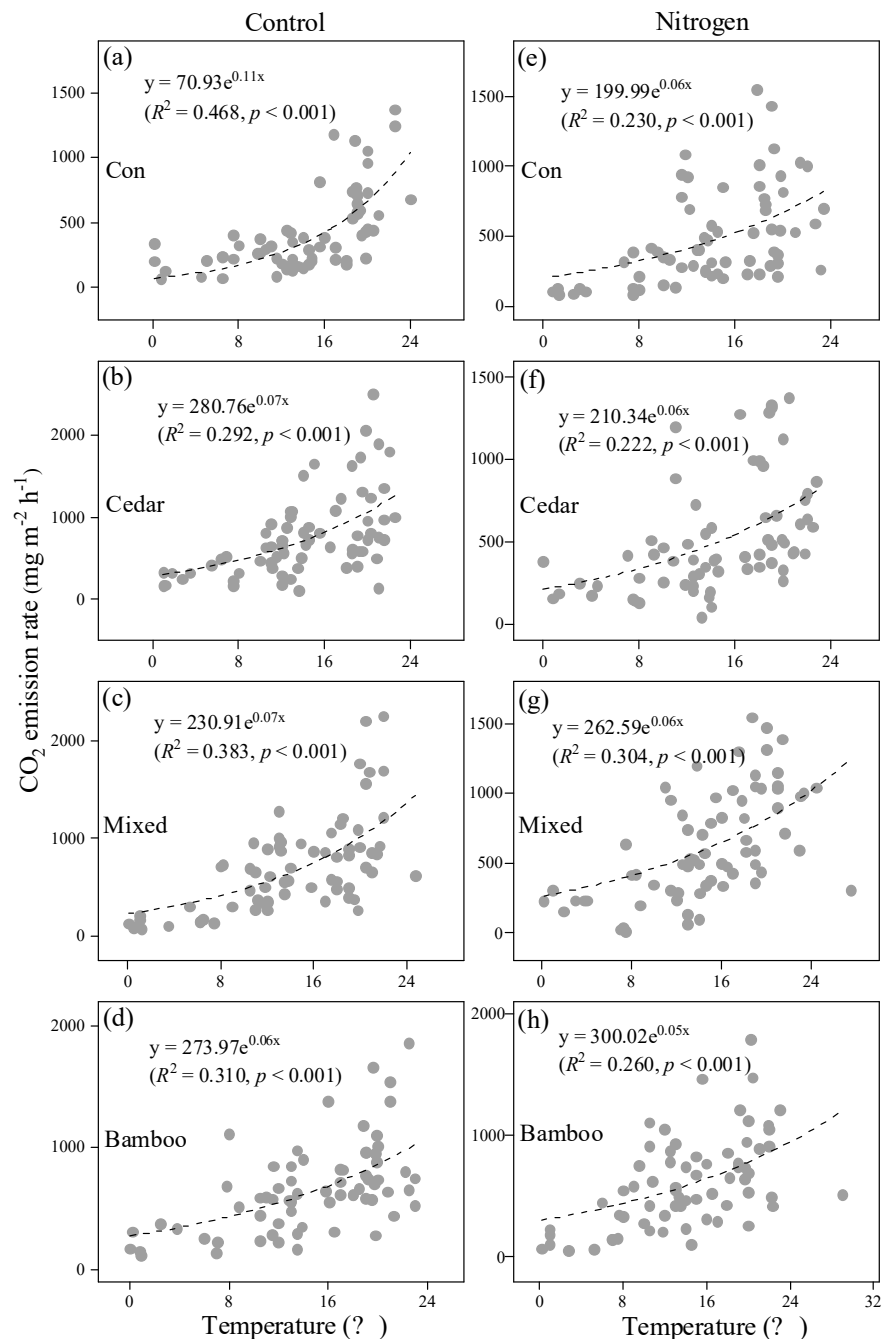


Figure 4. Correlations between soil CO₂ emission rate (mg m⁻² h⁻¹) and soil temperature (°C) with different nitrogen (control vs. nitrogen) and fine root litter (con, a and e; cedar fine root, b and f; mixed fine root litter, c and g; bamboo fine root, d and h) treatments. Goodness of fit of the data to the single positive exponential model (R²) and p values are shown.

Correlations between the soil CO₂ emission rates and soil moisture could be fitted by quadratic functions in the control and mixed root litter treatment with N deposition treatment, while other treatment showed no significant results (Figure 5).

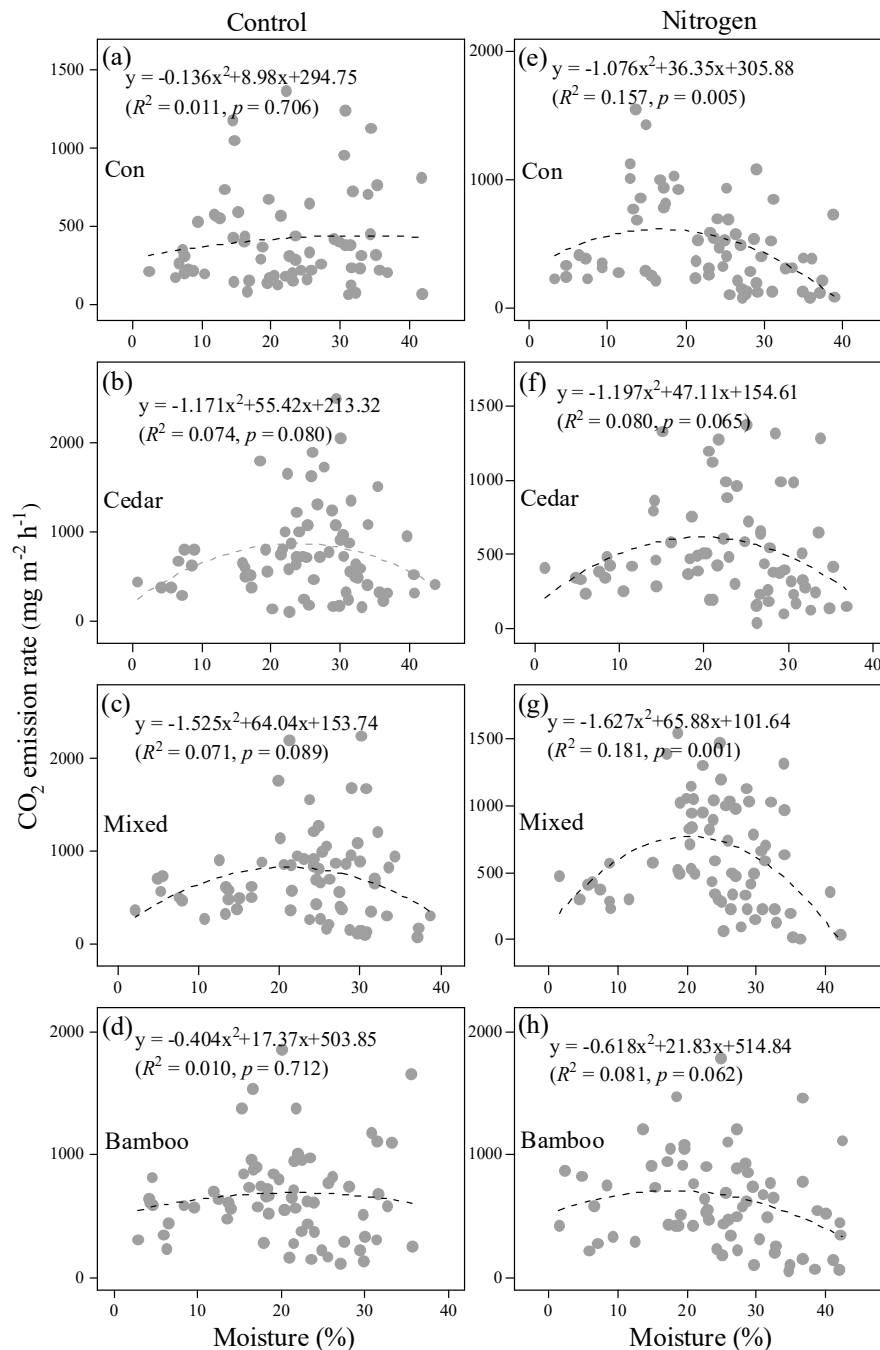


Figure 5. Correlations between soil CO₂ emission rate (mg m⁻² h⁻¹) and soil moisture (%) with different nitrogen (control vs. nitrogen) and fine root litter (con, a and e; cedar fine root, b and f; mixed fine root litter, c and g; bamboo fine root, d and h) treatments. Goodness of fit of the data to the quadratic equation (R^2) and p values are shown.

4. Discussion

Japanese cedar and moso bamboo fine root litter both increased soil CO₂ emission rates. However, moso bamboo consistently increased soil CO₂ emission, despite N deposition treatment. In addition,

soils with N deposition and mixed litter treatment were higher in soil CO₂ emission rates when compared with that with Japanese cedar fine root litter. All litter treatment decreased the increase rate in soil CO₂ emission rates with soil temperature when N was not added, which indicated that changes in soil CO₂ emission rates are multiple factors dependent in mixed forests with Japanese cedar and moso bamboo.

4.1. Changes in Soil CO₂ Emission Rates as Affected by N and Litter Treatments

Nitrogen deposition is important N input into soil ecosystems [19]. Increased N input would cause imbalance between C and N due to the balance between soil C and N, potentially impacting soil CO₂ emissions (Tables 2 and 3; Figures 1–3). However, soil greenhouse gas emissions, including not only CO₂, but also nitrous oxide. In natural ecosystems with more than one species, N input via deposition or fertilization practice may interact with litter input impacting nitrous oxide emissions, which should be measured in forest ecosystems by future studies [12].

It was also observed that N interacted with root litter treatment impacting soil CO₂ emissions (Table 2; Figure 2), which indicated that N availability could be a limiting factor during litter decomposition and, hence, soil CO₂ emission in the studied forests [20]. Indeed, Japanese cedar root litter was higher in N concentration relative to moso bamboo (Table 1). More N input will generally impose positive effects on decomposition rate of litter with relatively lower N concentration [21]. Without N addition, soils with Japanese cedar fine root litter were higher in soil CO₂ emission rates than that with moso bamboo fine root litter, which could be ascribed to the limited N input via deposition and relatively higher litter quality, represented as a lower C:N ratio [17] (Table 1; Figure 2). Therefore, Japanese cedar forests encroached by moso bamboo might have experienced profound alterations in soil CO₂ emissions, as effected by both fine root litter and N input. Under the context of increased atmospheric greenhouse gas emissions and global climate change, soil CO₂ emission budget should be performed based on a consideration of changes in litter input and N deposition in mixed forests.

The mixing of Japanese cedar and moso bamboo fine root litter increased the soil CO₂ emission rate with N deposition, but no significant difference when compared to each species alone without N addition (Figure 2). Even though not presented here, the litter decomposition rate of mixed fine root might have been altered due to the non-additive mixing effects and, hence, the effects on soil CO₂ emission rate [17]. The mixing of both litter would lead to nutrient transfer between the component litter due to substantial difference in litter quality between two root litter and, hence, the overall decomposition rate [22], which should be examined by future studies. Moreover, the encroachment of moso bamboo into adjacent forests could also generate changes in soil N cycling process that depend on expanding stages [23], which will also need future examination in future studies with more adjacent forest types with moso bamboo encroachment. This study provided a primary investigation of moso bamboo encroachment on soil CO₂ emissions via litter input or alterations in soil abiotic factors [24]. When considering the substantial contribution of soil CO₂ emissions to global atmospheric composition changes, the results could not be ignored in sustainable management of moso bamboo expansion, especially under the context of N deposition [25] and warming [23].

4.2. Correlations between Soil CO₂ Emission Rates and Soil Temperature

The overall increase rate of soil CO₂ emission rate with soil temperature was higher in the soils without litter [26] (Figure 4). Soils with mixed litter showed a similar increase rate when compared with that with root litter of Japanese cedar, indicating no substantial changes in soil CO₂ emission rates under warming environment. However, soils with moso bamboo were slightly lower in the increase rate of soil CO₂ emission rate with soil temperature, which might have implications for the management of moso bamboo forests or Japanese cedar forest thoroughly encroached by moso bamboo in the future. Under the global warming context, these changes should be considered in mitigation of greenhouse gas emissions and climate change.

Among all of the litter treatments, only mixed litter and no litter control treatments with N addition showed significant correlations with soil moisture in quadratic functions (Figure 5), which suggested that soil moisture was not a key factor influencing soil CO₂ emission rate in the studied area (Table 2). However, soil moisture should still be considered in studies on soil CO₂ emissions rate due to its importance in effects on soil C cycling, especially in areas where soil moisture could easily be altered by litter management [27].

5. Conclusions

Fine root litter input of Japanese cedar and moso bamboo both increased soil CO₂ emission rates, with mixed litter positively increasing the soil CO₂ emission rate with N addition treatment. Moso bamboo fine root litter input decreased the sensitivity of the soil CO₂ emission rate to soil temperature. The encroachment of moso bamboo into adjacent forests decreased soil CO₂ emission rates, especially in areas with N input, which might benefit soil C sequestration under warming climate and also the mitigation of global climate change. In future management of forests that were encroached with moso bamboo, both above-ground and below-ground litter input, as well as the mixing effects on soil CO₂ emissions, should be considered with respect to their important role played in forest element cycling and CO₂ emissions.

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Conflicts of Interest: The authors have declared that no competing interests exist.

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