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# Legacy Effect of Long-Term Elevated $CO_2$ and Warming on Soil Properties Controls Soil Organic Matter Decomposition

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**Highlights:**

- Litter quality change does not affect SOM decomposition under elevated $CO_2$ and warming.
- The legacy effect of elevated $CO_2$ and warming on soil properties controls SOM decomposition.
- Elevated $CO_2$ may promote SOC sequestration by suppressing SOM decomposition.

**Abstract:** Plant litter quality is one of the key factors that control soil organic matter (SOM) decomposition. Under climate change, although significant change in litter quality has been intensively reported, the effect of litter quality change on SOM decomposition is poorly understood. This limits our ability to model the dynamics of soil carbon under climate change. To determine the effect of litter quality and soil property change on SOM decomposition, we performed a controlled, reciprocal transplant and litter decomposition experiments. The soils and plant litters were collected from a long-term field experiment, where four treatments were designed, including: (1) the control without warming at ambient $CO_2$; (2) elevated atmospheric $CO_2$ up to 500 ppm (C); (3) warming plant canopy by 2 °C (T); (4) elevated $CO_2$ plus warming (CT). We found that elevated $CO_2$ and warming altered the litter quality significantly in terms of macronutrients' content and their stoichiometry. Elevated $CO_2$ decreased the concentration of N in rice and wheat straw, while warming decreased the concentration of N and K in wheat straw. However, the change in plant litter quality did not lead to a shift in SOM decomposition. On the contrary, the legacy effect of long-term elevated $CO_2$ and warming on soil properties dominated the decomposition rate of SOM. Elevated atmospheric $CO_2$ suppressed SOM decomposition mainly by increasing phosphorous availability and lowering the soil C/N, fungi/bacteria ratio, and N-acetyl-glucosaminidase activity, while warming or elevated $CO_2$ plus warming had no effect on SOM decomposition. Our results demonstrated that the changes in soil property other than litter quality control the decomposition of SOM under climate change, and soil property change in respond to climate change should be considered in model developing to predict terrestrial soil carbon dynamics under elevated atmospheric $CO_2$ and warming.

**Keywords:** atmosphere $CO_2$ enrichment; plant canopy warming; free air $CO_2$ enrichment; soil organic matter mineralization; plant litter; climate change



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## 1. Introduction

Climate change, mainly characterized by the rapid increase in the atmospheric $CO_2$ concentration and the elevation of global surface temperature, is challenging the sustainable development of global agriculture. The concentration of $CO_2$ in the atmosphere has been increasing since the 1840s, and it has exceeded 400 ppm in 2013 [1]. In the meantime, the global temperature is continuously rising. It is predicted that the atmospheric $CO_2$ concentration

will exceed 700 ppm [2], and the global temperature will increase by 1.1–6.4 °C by the end of this century [3].

Soil organic matter (SOM) in terrestrial ecosystems plays an important role in the global carbon cycle. Approximately 2000 petagrams of carbon are stored in the top two meters of global soils as SOM, and more than twice as much carbon is stored in the soil as in the world's vegetation and atmosphere combined. Therefore, a slight change in SOM will have a profound impact on atmospheric $CO_2$ concentration, which in turn influences the global climate. Although a great number of research studies have been conducted to investigate the effect of climate change on soil carbon cycling, it remains an open question whether elevated $CO_2$ and global warming will promote soil carbon sequestration [4]. Several studies reported that elevated atmospheric $CO_2$ could increase soil organic carbon (SOC) storage by increasing net $CO_2$ uptake [5,6]. Liu et al. (2018) and Luo et al. (2006) predicted that the SOC stock would increase by approximately 5%, although it is quite small compared to the increase in the rate of plant biomass carbon under elevated $CO_2$ [7,8]. However, Koyama et al. (2018) found that elevated atmospheric $CO_2$ did not affect the SOC pool in a Mojave Desert ecosystem [9]. Similar findings were reported in cropland and temperate grassland ecosystems [10,11]. Furthermore, increased soil $CO_2$ flux under elevated $CO_2$ has been frequently reported [7]. Kuzyakov et al. (2019) argued that elevated atmospheric $CO_2$ has no (or little) effect on the soil carbon pool but strongly increases the $CO_2$ fluxes and accelerates carbon cycles [12]. Similarly to elevated $CO_2$, recent meta-analyses have shown that global warming generally has no [13–17] or negative [13,15] effects on the SOC pool. Long-term warming decreased the SOC pool by stimulating microbial utilization of the recalcitrant C pool [13]. However, most of the studies involved in these meta-analyses were conducted in forest or grassland ecosystems. It remains unclear whether global warming will affect the pools and fluxes of SOC in cropland ecosystems. This uncertainty limits our accurate prediction of soil carbon stock change under elevated $CO_2$ and warming.

The concentration of $CO_2$ in soil is much higher than that in the atmosphere (10–50 times), and elevated atmospheric $CO_2$ (+200 ppm) will probably not affect soil carbon cycling directly [18]. Its effect on soil carbon cycling is indirect, through the plant growth changes. Elevated $CO_2$ and warming affect plant growth by altering leaf stomatal conductance and the photosynthesis rate [19]. Elevated $CO_2$ can increase crop yield by photosynthesis rate and soil nutrients use efficiency increase [5]. As the atmospheric $CO_2$ concentration increases, the nutrient conditions of grains and the shoot biomass will change accordingly. Therefore, some studies have predicted that plants would be exposed to a global nutrient imbalance with lower N contents or higher ratios of C:N and C:P in plant litters under elevated $CO_2$ [20,21]. In addition to macronutrients, the micronutrients in plant litter will also decrease under elevated $CO_2$ [22]. He et al. (2015) even found that elevated $CO_2$ and warming reduced the content of crude protein and the in vitro digestibility of wheat straw [23]. Plant litter with different chemical properties would likely affect the decomposition rate of SOM. For example, Elias et al. (2020) found that plant litter with higher P content and lower lignin to N ratios decompose faster in a forest soil [24]. Fanin et al. (2011) also found that the content of C, N and P and their stoichiometry in plant litters were important factors that regulate soil microbial respiration [25]. However, under elevated $CO_2$ and warming, the effect of litter quality change on SOM decomposition has never been tested.

In addition to plant litter quality, the soil microbial community also regulates the decomposition of SOM. Under elevated $CO_2$ or warming, significant changes in soil microbial communities have been reported intensively [26–28]. Several studies found that elevated $CO_2$ altered the soil microbial composition [29–36]. Soils exposed to elevated $CO_2$ had higher relative abundances of fungi and higher enzyme activity [29,37], which led to more soil carbon loss [30,31,36,38]. Lipson et al. (2005) observed that elevated $CO_2$ had no effect on bacterial diversity but increased fungal biomass in a Chaparral Ecosystem [33]. Sun et al. (2021) found that the soil microbial community evolves from dominating K-

strategists to r-strategists under elevated $CO_2$, with decreasing ratios of fungi to bacteria, Gram-positive to Gram-negative bacteria and Acidobacteria to Proteobacteria [28]. Warming generally increases the abundance of microorganisms related to soil carbon and nitrogen cycling, leading to soil carbon loss and greater $N_2O$ emissions [39,40]. Some studies showed that warming reduced bacterial and fungal abundance in forest ecosystems [41,42]. The soil microbial community structure was also altered by warming [43]. Deslippe et al. (2012) found that warming decreased bacterial communities evenness while it increased fungal communities evenness [44]. Cheng et al. (2017) showed that warming increased the relative abundance of key functional genes involved in soil carbon degradation [39]. Sheik et al. (2011) found that warming increased the soil microbial population size but decreased diversity under wet conditions, whereas it reduced the microbial population size under drought conditions [45]. Under elevated atmospheric $CO_2$ and warming, the abundance of some dominant phyla was significantly increased, and the effect of combined elevated $CO_2$ and warming on soil functional processes was similar to that of only elevated $CO_2$ [46].

Under elevated $CO_2$ or warming, the changes in the soil microbial community and plant litter quality have been observed as mentioned above. Understanding the effect of plant litter quality and the soil microbial community on soil organic carbon decomposition can help us to model soil carbon dynamics under elevated $CO_2$ and warming. To our knowledge, there was no report investigating the effect of plant litter quality and soil microbial community change on soil organic carbon mineralization under these elevated conditions. Three manipulated incubation experiments were conducted to answer the following questions: (1) Does plant litter quality (C:N and nutrient content) change affect SOM decomposition under elevated $CO_2$ and warming? (2) Does soil microbial community change affect SOM decomposition under elevated $CO_2$ and warming? (3) Does plant litter have a greater effect on SOM decomposition than soil microbial community? We hypothesized that plant litter with decreased quality under elevated $CO_2$ and warming would suppress SOM decomposition, whereas the change in the soil microbial community would promote SOM decomposition. The results of this study can be used in soil carbon cycling model developing to predict terrestrial carbon dynamics under future climate change of elevated $CO_2$ and warming more precisely.

## 2. Materials and Methods

### *2.1. Soils and Plants Litter*

The soils and plants litter used in this study were collected from the long-term field experiment of Nanjing Agricultural University, which was located in Kangbo Village (31°30′48″ N, 120°33′36″ E), Changshu City, Jiangsu Province of China. The field experiment facility was constructed in 2010, and the objective of this facility was to simulate Free Air $CO_2$ Enrichment and plant canopy warming in an open field (Figure 1). The soil is a Gleyic Stagnic Anthrosol (WRB-FAO) derived from clayey lacustrine deposit and cultivated with summer rice-winter wheat rotation dating back hundreds of years. The basic properties of the topsoil before the experiment onset were: pH ($H_2O$) 7.0, bulk density of 1.2 g $cm^{-3}$, and concentration of organic C and total N of 16.0 g $kg^{-1}$ and 1.9 g $kg^{-1}$, respectively. There were four treatments, including elevated $CO_2$ up to 500 ppm (C), warming plant canopy by 2 °C (T), elevated $CO_2$ plus plant canopy warming (CT), and ambient $CO_2$ without warming as the control (Control). The soils were collected from the top 15 cm in June 2018, after 7 years of treatment. The plant litters (rice and wheat straw) were collected at harvest. Rice straw (Cultivar: Changyou 5) was collected in October 2017, and wheat straw (Cultivar: Yangmai 16) was collected in June 2018.

**Figure 1.** A photograph of the experimental set-up from the air.

### *2.2. Experimental Design*

Three incubation experiments were designed (Table 1). In the first experiment (Experiment I), the soils from the control, C, T and CT treatments were incubated with the addition of crop straw from the control, C, T and CT treatments, respectively. In the second experiment (Experiment II), the soils from the control were incubated with the addition of crop straw from the control, C, T and CT treatments. In the third experiment (Experiment III), the soils from the control, C, T and CT treatments were incubated with the addition of crop straw from the control. All the treatments were replicated three times.

**Table 1.** Experimental design. Control represents the soils or litter that were collected from the ambient atmospheric $CO_2$ without warming; C represents the soils or litter that were collected from elevated $CO_2$; T represents the soils or litter that were collected from plant canopy warming; CT represents the soils or litter that were collected from $CO_2$ plus warming.

| | **Soils** | **Litters** | **Abbreviation** |
|---|---|---|---|
| Experiment I | Control | Control | S + L |
| | C | C | SC + LC |
| | T | T | ST + LT |
| | CT | CT | SCT + LCT |
| Experiment II | Control | Control | S + L |
| | Control | C | S + LC |
| | Control | T | S + LT |
| | Control | CT | S + LCT |
| Experiment III | Control | Control | S + L |
| | C | Control | SC + L |
| | T | Control | ST + L |
| | CT | Control | SCT + L |

Fifty grams of air-dried soils were mixed with 0.06 g of rice straw, and the mixture was placed in a 500 mL flask. All flasks were incubated at 25 °C in the dark. The bottle was sealed with a cap, and two rubber tubes (16 cm and 7 cm in length) were inserted into the bottle cap. A three-way valve was sleeved above the rubber tube for fresh air and gas sample collection. To simulate the soil respiration process during the whole crop growing season in the study area, two soil water conditions were designed. The soils mixed with rice straw were incubated first at aerobic conditions with soil water content maintained at

80% of the soil water holding capacity. Then, they were mixed with wheat straw (0.06 g) and incubated under flooded conditions. During aerobic incubation, gas sampling was performed on Days 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 11, 13, 15, 17, 19, 23, 28, 33, 43 and 64. During anaerobic incubation, gas sampling was performed on Days 65, 65.5, 66, 66.5, 67.5, 69, 71, 73, 82, 89, 98, 115, 123, 131, 139 and 147. Gas samples were collected with a syringe 2 h after ventilation.

The concentration of $CO_2$ in the gas samples was detected in a gas chromatograph (Agilent 7890A). The emission rate of $CO_2$ was calculated using the following equation:

$$F = \rho \times \frac{V}{m} \times \frac{\Delta C}{\Delta t} \times \frac{273}{273 + T} \times \alpha$$

where F represents the $CO_2$ emission rate (mg $C \cdot kg^{-1} \cdot d^{-1}$); ρ represents the density of $CO_2$, which is 1.997 $g \cdot L^{-1}$; V represents the volume of air above the flask (L); m represents the mass of soil (g); ΔC represents the change in $CO_2$ concentration in the gas sample ($\mu mol \cdot mol^{-1}$); Δt represents the sampling time (d) of the closed flask; and T is the temperature of the incubation (25 °C). α represents the conversion coefficient, 12/44 ($C/CO_2$).

### *2.3. Plant and Soil Sample Analysis*

Plant and soil samples were analyzed following the protocol described by Lu (2000) [47]. The plant samples were digested with sulfuric acid and hydrogen peroxide. The contents of nitrogen, phosphorus and potassium in the digestion were determined by the micro-Kjeldahl determination method, colorimetric method and flame photometer method, respectively. Total organic carbon and total nitrogen were measured by a CNS Macro Elemental Analyzer (Elementar, Germany). Dissolved organic carbon (DOC) was extracted with 0.05 $mol \cdot L^{-1}$ $K_2SO_4$ solution. The mixture was shaken at 180 $r \cdot min^{-1}$ for 30 min and then passed through a 0.45 μm filter. The concentration of DOC in the liquid was measured in a TOC analyzer. Soil pH was measured in distilled water (soil/water ratio of 1/2.5 *w*/*w*) with a pH meter (Seven Easy Mettler Toledo, China, 2008). Soil available K was extracted with 1.0 mol $L^{-1}$ ammonium acetate (pH 7.0) and determined with a flame photometer (FP6410, Company of Shanghai Jingke, China). Soil available P was extracted with 1.0 mol $L^{-1}$ sodium bicarbonate and determined using colorimetric method.

Soil microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method. Fresh soils were fumigated at 25 °C for 24 h. The fumigated soils were extracted with 0.5 $mol \cdot L^{-1}$ $K_2SO_4$ solution for 30 min in a shaker (180 $r \cdot min^{-1}$). Then, the mixture was filtered through a 0.45 μm water-based filter membrane. The concentration of carbon in the extract was measured with a TOC analyzer (Multi N/C 3100). MBC = (fumigated C-unfumigated C)/0.45

Microbial metabolic quotient is the ratio of carbon emitted by soil respiration to soil microbial biomass during incubation time. Soil PLFA was determined according to the method of Frostegård and Bååth (1996) [48]. PLFAs were extracted from freeze-dried soil samples (2 g) with a single-phase chloroform/methanol/citric acid buffer (15 mL at a 1:2:0.8 vol ratio). Total concentration of PLFAs ($nmol \cdot g^{-1}$) was set to account for total microbial biomass. Bacterial/fungal ratio (B/F) was calculated by dividing the bacterial biomass by the fungal biomass. Soil enzyme activity was determined by fluorescence microplate method with MUB (4-methylumbelliferone) and L-DOPa (L-3,4-dihydroxyphenylalanine) substrates. Fresh soil samples equivalent to 2.0 g of dry soil were weighed into a glass beaker, 300 mL buffer was added to make soil suspension, and this was homogenized thoroughly on a magnetic stirrer. Then, 200 μL soil suspension and 50 μL of 200 $\mu mol \cdot L^{-1}$ MUB substrate were siphoned off with a pipette gun into a 96-well black polystyrene microplate. At the same time, MUB standard solution was used to make the standard curve of each soil sample to be tested. The fluorescence values were measured by Perkinelmer EnSight (Perkinelmer, MA, USA) with excitation and absorption wavelengths of 365 nm and 450 nm after 3 h of culture at 25 °C under dark conditions.

### 2.4. Statistical Analysis

Data are expressed as the mean plus/minus one standard deviation of three replicates. One-way ANOVA followed by the least significant difference (LSD) was used to test the difference among the various treatments. Statistical significance was set at $p < 0.05$. All statistical analyses were carried out in SPSS 20.0, and figures were made by Origin 2021.

## 3. Results

### 3.1. Changes in Litter Quality under Elevated $CO_2$ and Warming

Table 2 shows the nutrient concentration of rice and wheat straw under elevated $CO_2$ and warming. Elevated $CO_2$ decreased the N concentration of rice and wheat straw by 16.5% and 39.7%, respectively. Under elevated $CO_2$, the K concentration of wheat also decreased significantly. Warming decreased the N and K concentration of wheat straw by 25.2% and 52.9%, respectively. Under elevated $CO_2$ plus warming, the N and P concentration of rice straw and the N and K concentration of wheat straw decreased significantly compared to the control.

**Table 2.** Nutrients concentration of plant litter under elevated $CO_2$ and warming.

| Treatment | Rice Straw | | | Wheat Straw | | |
|---|---|---|---|---|---|---|
| | N ($g \cdot kg^{-1}$) | P ($g \cdot kg^{-1}$) | K ($g \cdot kg^{-1}$) | N ($g \cdot kg^{-1}$) | P ($g \cdot kg^{-1}$) | K ($g \cdot kg^{-1}$) |
| Control | 10.59 ± 1.59 a | 1.06 ± 0.18 a | 16.70 ± 2.28 a | 9.28 ± 1.20 a | 1.11 ± 0.30 a | 15.87 ± 0.05 a |
| C | 8.84 ± 0.50 b | 0.90 ± 0.11 a | 14.90 ± 0.31 a | 5.60 ± 0.85 b | 0.67 ± 0.16 a | 11.56 ± 1.65 b |
| T | 11.42 ± 0.17 a | 0.97 ± 0.08 a | 16.69 ± 1.44 a | 6.94 ± 0.78 b | 0.89 ± 0.06 a | 7.47 ± 2.52 c |
| CT | 8.05 ± 0.71 b | 0.66 ± 0.03 b | 16.48 ± 0.54 a | 6.09 ± 0.65 b | 1.09 ± 0.29 a | 7.16 ± 1.98 c |

Different lower-case letters indicate significant differences among treatments ($p < 0.05$).

### 3.2. The Effect of Elevated $CO_2$ and Warming on Soil Respiration (Experiment I)

The average $CO_2$ emission rate during the aerobic stage was 66.39 mg $C \cdot kg^{-1} \cdot d^{-1}$, which was about 13 times higher than the one during the anaerobic stage (Figure 2A). During the aerobic stage, the emission peak occurred on the first day of incubation, and since then, it decreased dramatically until Day 2. From Day 4 to Day 64, soil $CO_2$ emission rate gradually decreased. During the anaerobic stage, soil $CO_2$ emission rate dramatically increased in the first 15 days and then gradually declined. The emission peak was observed at Day 82.

The cumulative release of $CO_2$ (soil respiration hereafter) from the soil is shown in Figure 3A. Much more $CO_2$ was released during the aerobic stage, which accounted for about 90% of the overall release rate. During the aerobic process, elevated $CO_2$ decreased soil respiration by 27.60% compared to the control, while warming or elevated $CO_2$ plus warming had no effect on it. During the anaerobic process, all the treatments had no effect on soil respiration.

### 3.3. The Effect of Litter Quality Change on Soil Respiration (Experiment II)

As shown in Figure 2B, the $CO_2$ released dynamics across treatments were very similar to Experiment I. During the anaerobic stage, the $CO_2$ release rate increased dramatically in the first 15 days and was then gradually declined. The emission peak was observed at Day 82. Adding litters from different climate change treatments to the control soil had no effect on the soil respiration rate (Figure 3B).

### 3.4. The Effect of Soil Property Change on Soil Respiration (Experiment III)

As shown in Figure 2C, the $CO_2$ release dynamics across treatments were very similar to Experiment I and Experiment II. However, soil respiration varied greatly across treatments during the aerobic incubation stage. Compared to the ambient control, soils treated with elevated $CO_2$ plus warming emitted higher amounts of $CO_2$. The accumulated $CO_2$ emission of soils treated with elevated $CO_2$ was 2874 mg $C \cdot kg^{-1}$, which was significantly

lower than the values from soils under warming and elevated $CO_2$ plus warming. During the anaerobic stage, there were no significant treatment effects (Figure 3C).

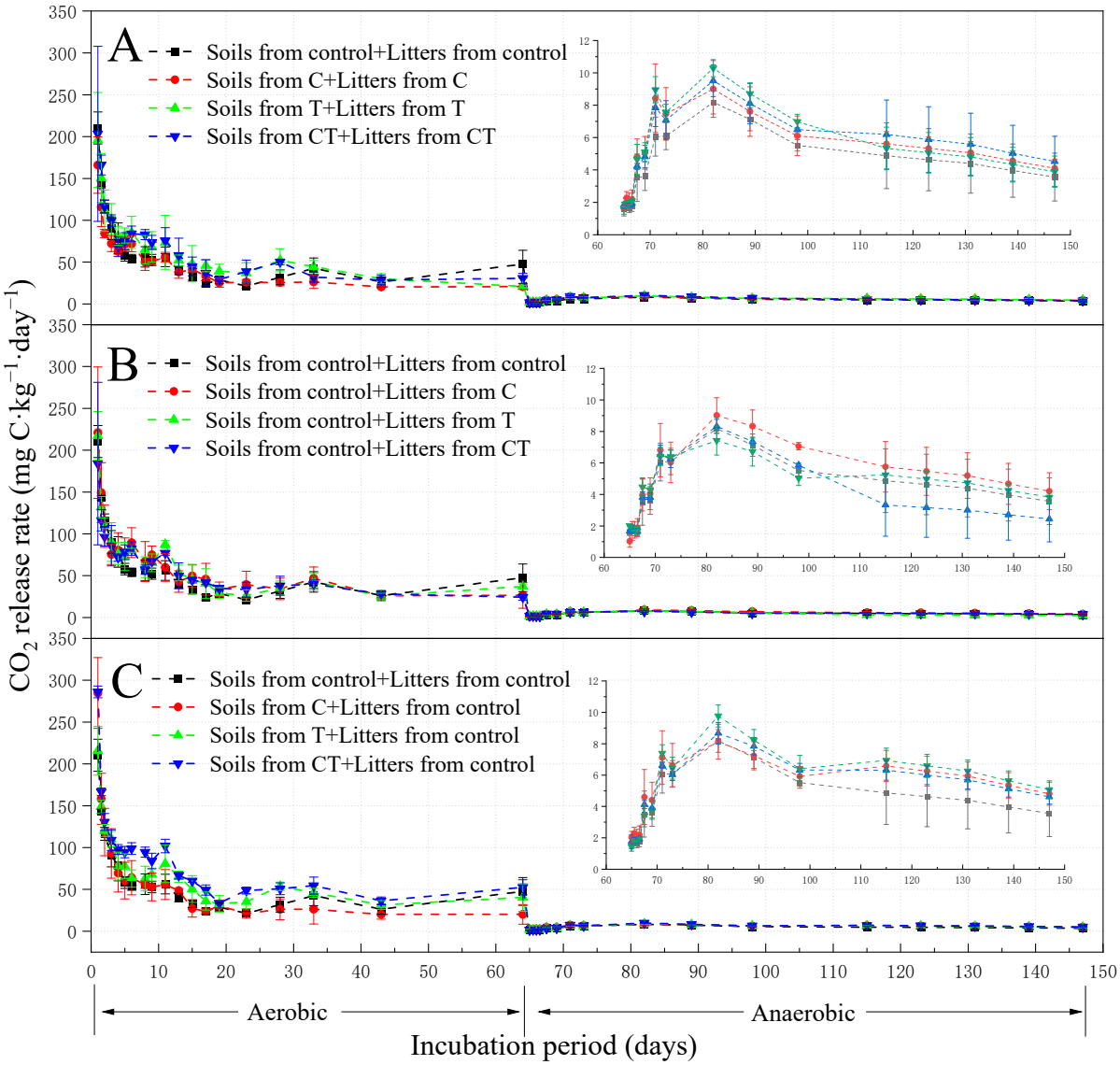


**Figure 2.** $CO_2$ released rate during the aerobic and anaerobic stage. Control represents the soils or litter that were collected from the ambient atmospheric $CO_2$ without warming; C represents the soils or litter that were collected from elevated $CO_2$; T represents the soils or litter that were collected from plant canopy warming; CT represents the soils or litter that were collected from $CO_2$ plus warming. The letters (**A**–**C**) represent Experiment I, Experiment II and Experiment III, respectively. The inset represents the period of flooded incubation from Day 65 to Day 147.

### *3.5. Correlation between Soil Respiration and Soil Characteristics*

In Experiment I, soil respiration rate was positively correlated with microbial metabolic quotient, soil C:N, fungi to bacteria ratio and N-acetyl-glucosaminidase activity, but was negatively correlated with soil available P (Table 3). In Experiment III, soil respiration rate was positively correlated with SOC, dissolved organic carbon, microbial metabolic quotient, soil available K and β-Glucosidase activity, but it was negatively correlated with soil microbial biomass carbon and available P content.

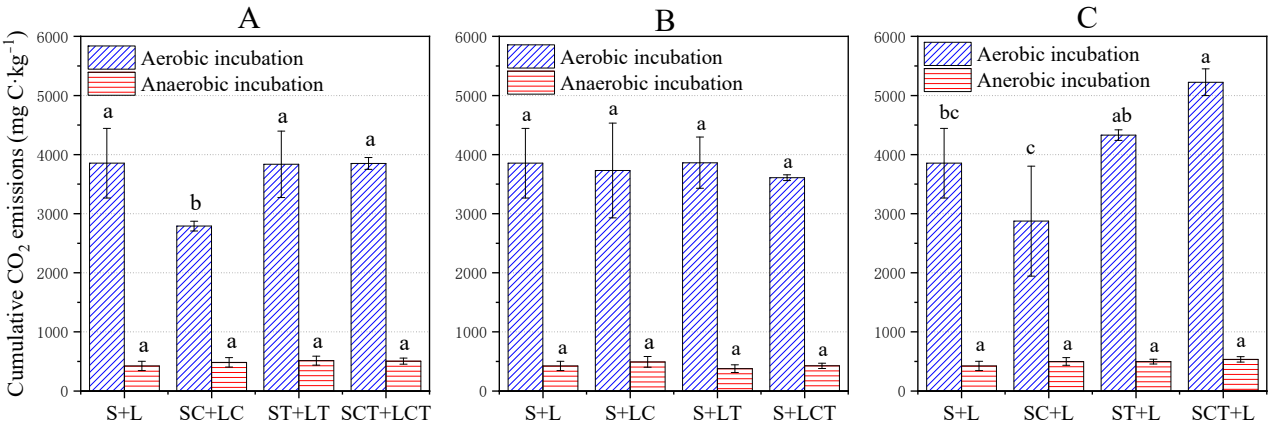


**Figure 3.** The cumulative $CO_2$ emission during aerobic and anaerobic stage. Please refer to Table 1 for the treatment abbreviations. The letters (**A**–**C**) represent Experiment I, Experiment II and Experiment III, respectively. Different lower-case letters indicate significant differences among treatments ($p < 0.05$).

**Table 3.** Person correlation between soil respiration during the aerobic period and soil characteristics.

| Soil Characteristics | Soil Respiration (Experiment I) | Soil Respiration (Experiment III) |
|---|---|---|
| Soil organic carbon | 0.403 | 0.672 * |
| Dissolved organic carbon | 0.259 | 0.586 * |
| Microbial biomass carbon | −0.232 | −0.780 ** |
| Microbial metabolic quotient | 0.831 ** | 0.914 ** |
| Soil pH | 0.175 | −0.284 |
| Soil C/N | 0.676 * | 0.549 |
| Soil available K | 0.413 | 0.674 * |
| Soil available P | −0.601 * | −0.754 ** |
| Total PLFAs | 0.045 | −0.125 |
| Bacterial PLFAs | −0.062 | −0.199 |
| Fungal PLFAs | 0.135 | −0.037 |
| F/B ratio | 0.631 * | 0.429 |
| α-Glucosidase | 0.138 | 0.311 |
| β-Glucosidase | 0.236 | 0.664 * |
| N-acetyl-glucosaminidase | 0.738 ** | 0.426 |
| Cellobiohydrolase | −0.042 | 0.441 |
| β-Xylosidase | −0.163 | −0.016 |

* indicates significance at 0.05; ** indicates significance at 0.01.

## 4. Discussion

Under future climate change of elevated $CO_2$ and warming, the changes in soil condition and litter quality have been observed, and they were supposed to alter the decomposition of SOM. Then, a new balance between organic carbon inputs and soil carbon losses, which can be used to predict the dynamics of SOC under climate change conditions, might be reached. However, this hypothesis was not fully supported by the current study. We found that the legacy effect of long-term elevated $CO_2$ and warming on soil conditions dominated the decomposition of SOM. Plant litter quality change had no effect on SOM mineralization, although significant changes in plant litter quality were observed in this study and among others [21,49]. Hillstrom et al. (2010) also found that elevated $CO_2$ had a minimal effect on microbial respiration in a forest system, although it affected litter quality significantly [50], whereas Cornwell et al. (2008) found that the decomposition rate of litter caused by litter quality is three times that of climate factors [51]. This may be true for ecosystems on a large scale, but for small areas of field, like that in the current study, this might not be true.

The response of soil respiration to elevated $CO_2$ varied across studies [7,52–55]. A recent study had shown that elevated $CO_2$ increased soil respiration by 25% on average [7]; however, this study showed that elevated $CO_2$ suppressed soil respiration compared with the ambient control, although neutral or negative effects have also been reported. Two reasons accounted for the higher soil respiration rate found under elevated $CO_2$ levels. Firstly, elevated $CO_2$ stimulated soil respiration by increasing the labile carbon pools. This carbon derived mainly from fine roots development and their exudates; most of it was decomposed by soil microbes and released to the atmosphere directly without forming aggregates with soil minerals [56,57]. Therefore, no net carbon gains were observed in soils under elevated $CO_2$. Secondly, elevated $CO_2$ stimulated soil respiration via the water saving effect. Under elevated $CO_2$, leaf stoma closure reduced plant transpiration, and more water could be stored in soil, which facilitated soil microbial respiration [53]. However, the water saving effect can only be observed in dry soil conditions; under wet soil conditions, it will decrease soil respiration because of low soil aeration. Therefore, Bader and Körner (2010) argued that there was no overall simulation of soil respiration under elevated $CO_2$ in a mature deciduous forest ecosystem [53]. Furthermore, the magnitude of the soil respiration stimulating effect does not persist forever, and it will decline over the years with atmospheric $CO_2$ enrichment [58]. This suggests that soil microbial community can adapt to long-term elevated $CO_2$, and a new balance between carbon inputs and outputs can be reached. In the current study, there was no water saving effect as described in previous studies, because the soils were incubated at the same water condition, and there were no carbon additions via root exudates. Therefore, no stimulation effect was observed. The soils under long-term elevated $CO_2$ had higher phosphorous availability and lower soil C:N, fungi-to-bacteria ratio, and N-acetyl-glucosaminidase activity, which collectively led to the lower soil respiration rates (Table 3).

This study also demonstrated that soil respiration under elevated $CO_2$ plus warming responded differently to litter addition (Figure 3; Experiment I, Experiment III). The soil incorporated with litter from the control had a significant higher $CO_2$ emission rate than the soil with litter from the treatment of elevated $CO_2$ plus warming. In experiment III, the soil respiration rate of soil under elevated $CO_2$ plus warming was even higher than the rate of soil under the control and elevated $CO_2$ alone, which was different from the results in experiment I. We attributed this to the adaptation of the soil microbial community to long-term elevated $CO_2$ and warming [59]. The soil microbes in this study under 7 years of elevated $CO_2$, warming or both elevated $CO_2$ and warming have to obtaining nutrients and energy from SOM and litter in a more efficient way, and $CO_2$ was emitted. In contrast, a sudden change in food resource (adding litter from other environments, such as the litter from the control in this study) led to a lower carbon use efficiency, which caused a high soil respiration rate, especially for the warming treatment soils. Therefore, the soil microbes need to decompose more organic matter to obtain similar amounts of nutrients after food change.

## 5. Conclusions

The study showed that under elevated $CO_2$ and warming, the change in plant litter has no effect on the decomposition of soil organic matter, even though significant changes in litter quality have been observed. The decomposition of soil organic matter is controlled by the legacy effect of soil properties changes under climate change conditions. Changes in soil phosphorous availability and C/N, fungi/bacteria ratio and N-acetyl-glucosaminidase activity may be attributed to the alternation of SOM decomposition under elevated $CO_2$. Elevated atmospheric $CO_2$ may promote soil carbon sequestration by suppressing soil microbial respiration under no temperature elevation conditions.

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**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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