Does Litter Quality Change Affect the Decomposition of Soil Organic Matter Under Elevated Atmospheric CO_2 and Warming

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Key Points:

- Litter quality change has no effect on soil organic matter decomposition under elevated CO_2 and warming.
- The legacy effect of elevated CO_2 and warming on soil property controls the decomposition of soil organic.
- Elevated CO_2 may promote soil carbon sequestration by suppressing soil organic matter decomposition.

Abstract

Soil property and litter quality are two key factors that control soil organic matter decomposition. Under climate change, it remains unclear how the changes of soil microbial community and litter quality affect soil organic carbon decomposition, although significant changes of these two factors have been reported intensively. This limits our ability to model the dynamics of terrestrial soil carbon in a changing climate. Using a long-term Free Air CO_2 Enrichment facility equipped with warming, we investigated the effect of soil property and litter quality change on the decomposition rate of soil organic matter. Results showed that significant change of litter quality was observed under elevated CO_2 and warming. Elevated CO_2 decreased the concentration of N of rice and wheat straw, while warming decreased the concentration of N and K in wheat straw. However, these changes in plant litter quality did not lead to a shift in soil organic matter decomposition. The legacy effect of long-term elevated CO_2 and warming on soil properties dominated the decomposition rate of soil organic matter. Elevated CO_2 suppressed soil organic matter decomposition mainly by increasing phosphorous availability and lowering soil C/N, fungi/bacteria ratio, and N-acetyl-glucosaminidase activity; while warming or elevated CO_2 plus warming had no effect on soil organic matter decomposition. Our results demonstrated that the change of soil properties other than litter quality control the decomposition of soil organic carbon; and soil property change should be taken into consideration in model developing when predicting terrestrial soil carbon dynamics under elevated atmospheric CO_2 and warming.

Plain Language Summary

Soil microbes are the key players in soil organic carbon cycling in terrestrial ecosystem. Under future climate change, it is critical to understand the effect of soil microbial community and their food source change on soil organic carbon decomposition before modeling the dynamics of soil organic carbon in the ecosystem level. A long-term Free Air CO_2 Enrichment facility equipped with warming was used to study the effect of elevated atmospheric CO_2 and warming on soil organic carbon decomposition. We found that soil microbial food source change had no effect on soil organic carbon decomposition, on the contrary soil microbial community and the soil environment condition dominated the carbon cycling under elevated CO_2 and warming. Our results demonstrated that food source cannot be considered a key factor in modeling parameterization.

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 410 ppm (Pachauri et al., 2015). In the meantime, the global temperature is continuous to rise. It is predicted that the atmospheric CO_2 concentration will exceed 700 ppm (Prentice et al., 2001) and the global temperature will increase by 1.1- 6.4 °C by the end of this century (IPCC, 2007).

Soil organic carbon in terrestrial ecosystem plays an important role in the global carbon cycle. About 2000 Pg of organic carbon are stored in the top two meters of global soils. The forest ecosystem accounts for approximately 73% of the terrestrial soil carbon pool (Six et al., 2002). The carbon pool in the farmland ecosystem is small but it can be managed by human being. Therefore, farmland ecosystem has a huge potential of soil organic carbon sequestration (Lal, 2004). However, it remains an open question whether soil organic carbon stock will increase under

future climate change of elevated atmospheric CO_2 and global warming (Terrer et al., 2021). Several studies reported that elevated atmospheric CO_2 could increase soil organic carbon storage by increasing net CO_2 uptake (Hyvönen et al., 2007; Jastrow et al., 2005; Luo et al., 2006). Liu et al. (2018) anf Luo et al. (2006) predicted that soil organic carbon stock would increase by around 5%, although it is quite small compared the increase rate of plant biomass carbon under elevated CO_2. However, Koyama et al. (2018) found that elevated atmospheric CO_2 did not affect the soil organic carbon pool in a Mojave Desert ecosystem. Similar findings were reported in cropland and temperate grassland ecosystems (Keidel et al., 2018; Van Kessel et al., 2000). Furthermore, increased soil CO_2 flux under elevated CO_2 was frequently reported (Liu et al., 2018). Kuzyakov et al. (2019) argued that elevated atmospheric CO_2 has no (or litter) effect on the soil carbon pool, but it strongly increases the CO_2 fluxes and accelerates carbon cycles. Similar to elevated CO_2, recent meta-analyses showed that global warming generally has no (Chen et al., 2020; Gao & Yan, 2019; Lu et al., 2013; Xu & Yuan, 2017; Zhang et al., 2015) or negative (Chen et al., 2020; Lu et al., 2013) effects on soil organic carbon pool. Long-term warming decreased soil organic carbon pool by stimulating microbial utilization of the recalcitrant C pool (Chen et al., 2020). However, most of the studies involved in these meta-analyses were conducted in forest or grassland ecosystem. It remains unclear whether warming would affect the pools and the fluxes of soil organic carbon in cropland ecosystem. This limits our accurate prediction of soil carbon stock change under climate change of 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 (+ 200pm) will probably not affect soil organic carbon cycling directly. Its effect on soil carbon cycling is through the changes of plant growth indirectly. Elevated atmospheric CO_2 and warming affect plant growth by altering leaf stomatal conductance and the photosynthesis rate (Long et al., 2004). Elevated CO_2 can increase crop yield via increasing the photosynthesis rate and soil nutrients use efficiency (Hyvönen et al., 2007). As the atmospheric CO_2 concentration increases, the nutrients condition of grains and the shoot biomass will change accordingly. Therefore, some studies predicted that the plants would be exposed to a global nutrient imbalance with lower N concentration or higher ratios of C: N and C: P in plant litters under elevated CO_2 (Sardans, 2012; Wang et al., 2019). In addition to macronutrients, the micronutrients in plant litter will also decrease under elevated CO_2 (Wang et al., 2020). 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. Plant litter with different chemical properties would probably affect the decomposition rate of soil organic carbon. However, this conjecture has never been tested although the changes in plant litter quality have been observed under elevated CO_2 and warming.

In addition to plant litter quality, soil organic carbon mineralization is also regulated by soil microbial community. Under elevated CO_2 or warming, significant change of soil microbial communities has been reported intensively (Butterly et al., 2016; He et al., 2014; Sun et al., 2021). Several studies found that elevated CO_2 altered soil microbial composition (Carney et al., 2007; Chung et al., 2007; He et al., 2010; Jin et al., 2020; Lipson et al., 2005; Yang et al., 2019; Yu et al., 2021; Zhou et al., 2011). Soils exposed to elevated CO_2 had higher relative abundances of fungi and higher enzyme activity (Carney et al., 2007; Drigo et al., 2010), which led to more soil carbon loss (Chung et al., 2007; Cotton et al., 2015; He et al., 2010; Zhou et al., 2011). Lipson et al. (2005) observed that elevated CO_2 had no effect on bacterial diversity, but it increased fungal biomass in a Chaparral Ecosystem. Sun et al. (2021) found that soil microbial community evolves from K-strategists dominated to r-strategists dominated community under elevated CO_2, with

decreasing ratios of fungi to bacterial, Gram positive to Gram negative and Acidobacteria to Proteobacteria. Warming generally had negative effect on soil microbial community, which led to soil carbon loss and greater N_2O emission (Cheng et al., 2017; Dai et al., 2020). Some studies observed that warming reduced bacterial and fungal abundance in forest ecosystem (Allison & Treseder, 2008; Frey et al., 2008). The soil microbial community structure was also altered by warming (Guo et al., 2018). Deslippe et al. (2012) found that warming decreased evenness of bacterial communities while increased evenness of fungal communities. Cheng et al. (2017) showed that warming increased the relative abundance of key functional genes involved soil carbon degradation. Sheik et al. (2011) found that warming increased soil microbial population size but decreased diversity under wet conditions; whereas it reduced microbial population size under drought condition. Under elevated CO_2 plus 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 elevated CO_2 alone (Yu et al., 2018).

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

2 Materials and Methods

2.1 Soil and plant litter

The soils and plant litters used in this study were taken 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 the open field. There are 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 the 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) were collected in October 2017, and wheat straw (Cultivar: Yangmai 16) were collected in June 2018.

2.2 Experiment design

Three incubation experiments were designed (Table 1). In the first experiment (Experiment I), the soils from the treatment of Control, C, T and CT were incubated with the addition of crop straw from Control, C, T and CT, respectively. In the second experiment (Experiment II), the soils from the Control were incubated with the addition of crop straw from Control, C, T and CT. In the third experiment (Experiment III), the soils from the treatment of Control, C, T and CT 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 litters that collected from the ambient atmospheric CO_2 without warming; C represents the soils or litters that collected from elevated CO_2; T represents the soils or litters that collected from plant canopy warming; CT represents the soils or litters that 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 0.06g of rice straw and the mixture was placed in a 500 mL flask. All the flasks were incubated at 25 °C in dark. The bottle is sealed with a cap, and two rubber tubes (16 cm and 7 cm in length) are inserted into the bottle

cap. A three-way valve is sleeved above the rubber tube for fresh air and gas sample collection. To simulate soil respiration process during the whole crop growing season in the studied area, two soil water condition was designed. The soil mixed with rice straw were incubated first at aerobic with soil water content maintained at 80% of the soil water holding capacity. Then the soils were mixed with wheat straw (0.06 g) and incubated at flooded condition. During the aerobic incubation, gas sampling was performed at day 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 11, 13, 15, 17, 19, 23, 28, 33, 43, 64. During the anaerobic incubation, gas sampling was performed at day 65, 65.5, 66, 66.5, 67.5, 69, 71, 73, 82, 89, 98, 115, 123, 131, 139, 147. Gas samples were collected with a syringe 2 hours after ventilation.

The concentration of CO_2 in gas samples was detected in a gas chromatogragh (Agilent 7890A). The emission rate of CO_2 was calculated with 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 CO2 emission rate (mg $C \cdot kg^{-1} \cdot d^{-1}$); ρ represents the density of CO_2, which is 1.997 $g \cdot m^{-2}$; V represents the volume of air above the flask (L); m represents the mass of soil (g); ΔC represents the concentration of CO_2 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).

2.3 Soil physical-chemical analysis

Plant and soil samples were analyzed following the protocol described by Lu (2000). The plant samples were digested with sulfuric acid and hydrogen peroxide. The concentrations of nitrogen, phosphorus and potassium in the digestion were determined by the micro-Kjeldahl Determination method, colorimetric method and flame photometer method, respectively. Dissolved organic carbon (DOC) was extracted with 0.05 mol Plant and soil samples were analyzed following the protocol described by Lu (2000). The plant samples were digested with sulfuric acid and hydrogen peroxide. The concentrations of nitrogen, phosphorus and potassium in the digestion were determined by the micro-Kjeldahl Determination method, colorimetric method and flame photometer method, respectively. 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 minutes, and then pass through a 0.45 μm filter. The concentration of DOC in the liquid was measured in a TOC analyzer. Soil microbial biomass carbon (MBC) was determined using chloroform fumigation-extraction method. Fresh soils were fumigated at 25 °C for 24 hours. The fumigated soils were extracted with 0.05$mol \cdot L^{-1}$ K_2SO_4 solution for 30 minutes 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).

2.4 Statistic analysis

Data were expressed as 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 different treatments. Statistical significance was set at $P < 0.05$. All the 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 following one crop growth season treatment of elevated CO_2 and warming. Elevated CO_2 decreased the concentration of N of rice and wheat straw by 1.75% and 3.68%, respectively. Under elevated CO_2, the concentration of K in wheat also decreased significantly. Warming decreased the concentration of N and K in wheat straw by 3.19% and 8.71% respectively. Under elevated CO_2 plus warming, the concentration of N and P in rice straw, and the concentration of N and K in wheat straw decreased significantly compared to the control.

Table 2. Nutrients concentration of plant litter under elevated CO2 and warming

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

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 that during anaerobic stage (Fig. 1 a). During the aerobic stage, the emission peak occurred in the first day of incubation and from then on it decreased dramatically until day 2. From day 4 to day 64, soil CO_2 emission rate decreased gradually. During the anaerobic stage, soil CO_2 rate increased dramatically in the first 15 days and then declined gradually. The emission peak was observed at day 82.

The cumulative release of CO_2 (Soil respiration hereafter) from the soil is shown in Fig. 2 a. Much more CO_2 was released during the aerobic stage, which accounted for about 90% of the overall released 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.

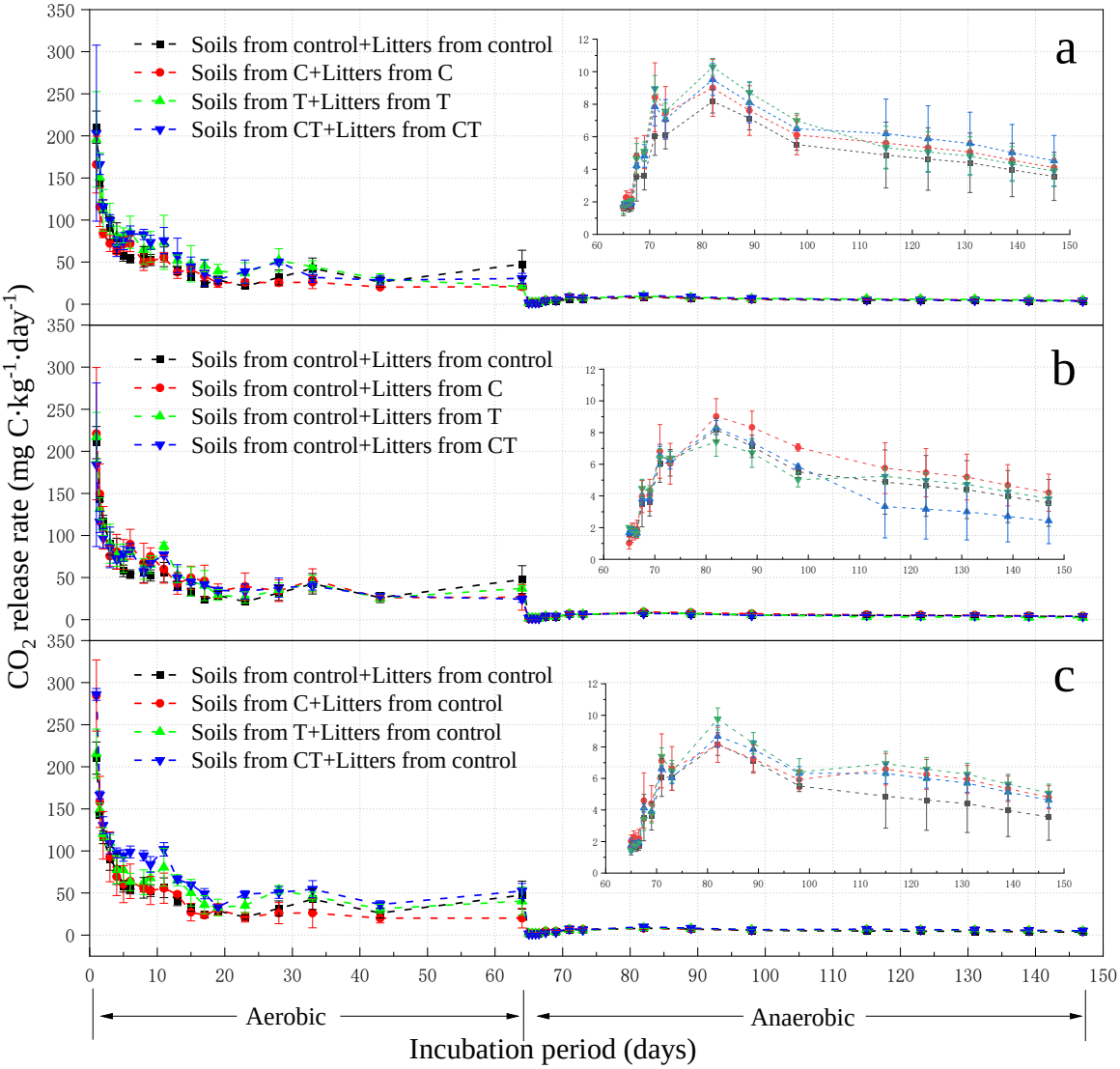

Fig. 1 CO_2 released rate during the aerobic and anaerobic stage. Control represents the soils or litters that collected from the ambient atmospheric CO_2 without warming; C represents the soils or litters that collected from elevated CO_2; T represents the soils or litters that collected from plant

canopy warming; CT represents the soils or litters that collected from CO_2 plus warming. In Fig. 1, a, b and c represent Experiment I, Experiment II and Experiment III respectively.

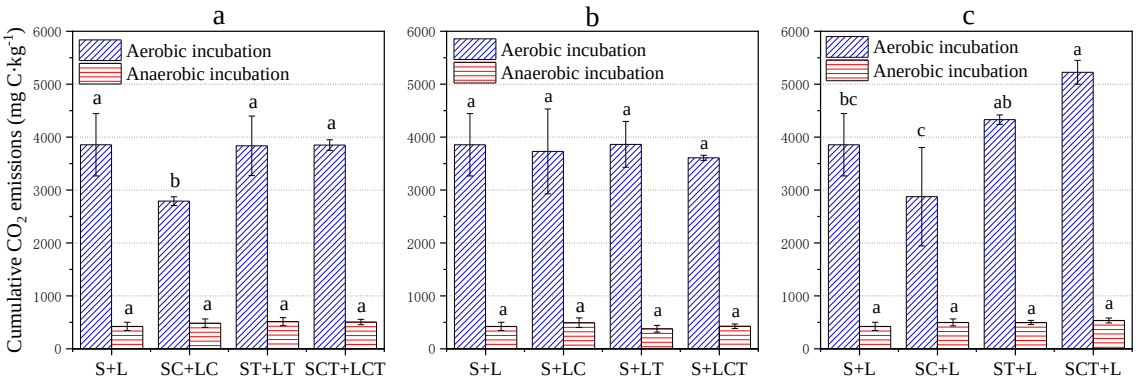

Fig. 2 The cumulative CO_2 emission during aerobic and anaerobic stage. Please refer to Table 1 for the treatment abbreviations. In Fig. 2, a, b and c represent Experiment I, Experiment II and Experiment III respectively.

3.3 The effect of litter quality change on soil respiration (Experiment II)

As shown in Fig. 1 b, the CO_2 release dynamics across treatments was very similar to Experiment I. During the anaerobic stage, the CO_2 release rate increased dramatically in the first 15 days and then declined gradually. 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 (Fig. 2 b).

3.4 The effect of soil property change on soil respiration (Experiment III)

As shown in Fig. 1 c, the CO_2 release dynamics across treatments was 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 much more 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 was no significant treatment effects (Fig. 2 c).

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, the ratio of fungi to bacteria and the enzyme activity of N-acetyl-glucosaminidase, but negatively correlated with soil available P (Table 3). In Experiment II, soil respiration rate was positively correlated with soil organic carbon, dissolved organic carbon, microbial metabolic quotient, soil available K, and the enzyme activity of β-

Glucosidase, but negatively correlated with soil microbial biomass carbon and available P content.

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 significant at 0.05; ** indicates significant at 0.01.

4 Discussion

The environmental conditions in the soils and the quality of the added residues as a food sources for soil organisms are two key factors that control rates of residue decomposition and mineralization of soil organic carbon(Brady & Weil, 2016). Soil condition refers to soil moisture, aeration, temperature, pH and most importantly the microbial community composition. Litter quality is described as the physical particle size, water content, nutrient condition, C: N, lignin and polyphenol content. Under future climate change of elevated

CO_2 and warming, the changes of soil condition and litter quality were supposed to alter the mineralization of soil organic carbon. A new balance between organic carbon input and soil carbon loss might be reached, which can be used to predict the dynamics of soil organic carbon in a changing climate. 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 condition rather than plant litter quality change dominated the decomposition rate of soil organic carbon. Plant litter quality change had no effect on soil organic carbon mineralization, although significant changes of plant litter quality had been observed in this study and others (Lieffering et al., 2004; Wang et al., 2019).

We were surprised to found that elevated CO_2 suppressed soil respiration compared with the ambient control. While most FACE experiments have shown that elevated CO_2 increased soil respiration by 25% on average (King et al., 2004; Liu et al., 2018), although neutral or negative effects were also reported (Bader & Körner, 2010; Clark et al., 2010; Keidel et al., 2015). Two reasons account for the higher soil respiration rate under elevated CO_2. Firstly, elevated CO_2 stimulates soil respiration by increasing the labile carbon pools. These carbons are mainly derived from fine roots development and their exudates; and most of them are decomposed by soil microbe and released to the atmosphere directly without forming soil aggregates with soil minerals (Andrews & Schlesinger, 2001; Lagomarsino et al., 2013). Therefore, no net carbon gains were observed in soils under elevated CO_2. Secondly, elevated CO_2 stimulates soil respiration via water saving effect. Under elevated CO_2, leaf stoma closure reduces plant transpiration and more water can be stored in soil, which facilitates soil microbial respiration (Bader & Körner, 2010). 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. Furthermore, the magnitude of soil respiration stimulating effect do not persist forever, and it will decline over the years of atmospheric CO_2 enrichment (Bernhardt et al., 2006). This suggests that soil microbial community can adapt to long-term elevated CO_2 and a new balance between carbon input and output is 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 was no continues carbon input via root exudates. Therefore, no stimulation effect was observed in this study. The soils under long-term elevated CO_2 had higher phosphorous availability and lower soil C: N, ratio of fungi to bacteria, and N-acetyl-glucosaminidase activity, which collectively led to the lower soil respiration rate (Table 3). Further study is needed to explore the direct link between soil respiration and these factors.

Though significant changes in litter quality were observed, they had no effect on soil carbon decomposition under elevated CO_2 and warming in this study. Hillstrom et al. (2010) found that elevated CO_2 had minimal effect on microbial respiration although it affected litter quality. Cornwell et al. (2008) found that the decomposition rate of litter caused by litter quality is three times that of climate factors. This may be true for large scale of ecosystem level, but for small areas of field level, like the current study, this might be not true. This study also demonstrated that the soil under elevated CO_2 plus warming responded differently to litter addition in terms of respiration rate (Fig. 2 Experiment I,

Experiment III). The soil incorporated with litter from the control had significantly higher CO_2 emission rate than the soil with litter from the treatment of elevated CO_2 plus warming. In experiment III, the respiration rate of soil under elevated CO_2 plus warming is 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 soil microbial community to long-term elevated CO_2 and warming (Bradford, 2013). The soil microbes under 7 years of elevated CO_2, warming or both in this study have got used to obtaining nutrients and energy from soil organic matter and litters in a more efficient way, and under this condition less CO_2 was emitted. Whereas, a sudden change of food resources (adding litter from other environment, 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. In other words, the soil microbes need to decompose more organic matter to get similar amounts of nutrients after food change.

5 Conclusions

The study showed that, under future climate change of elevated CO_2 and warming, the change of plant litter has no effect on the decomposition of soil organic matter though significant change of litter quality have been observed. The decomposition of soil organic matter is controlled by the legacy effect of soil property change under long-term elevated CO_2 and warming. Elevated atmospheric CO_2 may promote soil carbon sequestration by suppressing soil microbial respiration under no warmed condition.

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References

Allison, S. D., & Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils: Warming suppresses microbial activity. *Global Change Biology*, *14*(12), 2898–2909. https://doi.org/10.1111/j.1365-2486.2008.01716.x

Andrews, J. A., & Schlesinger, W. H. (2001). Soil CO_2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO_2 enrichment. *Global Biogeochemical Cycles*, *15*(1), 149–162. https://doi.org/10.1029/2000GB001278

Bader, M. K.-F., & Körner, C. (2010). No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment: Forest soil respiration under elevated CO_2. *Global Change Biology*, *16*(10), 2830–2843. https://doi.org/10.1111/j.1365-2486.2010.02159.x

Bernhardt, E. S., Barber, J. J., Pippen, J. S., Taneva, L., Andrews, J. A., & Schlesinger, W. H. (2006). Long-term effects of Free Air CO_2 Enrichment (FACE) on soil respiration. *Biogeochemistry*, *77*(1), 91–116. https://doi.org/10.1007/s10533-005-1062-0

Bradford, M. A. (2013). Thermal adaptation of decomposer communities in warming soils. *Frontiers in Microbiology*, *4*. https://doi.org/10.3389/fmicb.2013.00333

Brady, N. C., & Weil, R. R. (2016). The nature and properties of soils. *Soil Science Society of America Journal*, *80*(5), 1086.

Butterly, C. R., Phillips, L. A., Wiltshire, J. L., Franks, A. E., Armstrong, R. D., Chen, D., et al. (2016). Long-term effects of elevated CO_2 on carbon and nitrogen functional capacity of microbial communities in three contrasting soils. *Soil Biology and Biochemistry*, *97*, 157–167. https://doi.org/10.1016/j.soilbio.2016.03.010

Carney, K. M., Hungate, B. A., Drake, B. G., & Megonigal, J. P. (2007). Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. *Proceedings of the National Academy of Sciences*, *104*(12), 4990–4995. https://doi.org/10.1073/pnas.0610045104

Chen, J., Elsgaard, L., Groenigen, K. J., Olesen, J. E., Liang, Z., Jiang, Y., et al. (2020). Soil carbon loss with warming: New evidence from carbon-degrading enzymes. *Global Change Biology*, *26*(4), 1944–1952. https://doi.org/10.1111/gcb.14986

Cheng, L., Zhang, N., Yuan, M., Xiao, J., Qin, Y., Deng, Y., et al. (2017). Warming enhances old organic carbon decomposition through altering functional microbial communities. *The ISME Journal*, *11*(8), 1825–1835. https://doi.org/10.1038/ismej.2017.48

Chung, H., Zak, D. R., Reich, P. B., & Ellsworth, D. S. (2007). Plant species richness, elevated CO_2 , and atmospheric nitrogen deposition alter soil microbial community composition and function. *Global Change Biology*, *13*(5), 980–989. https://doi.org/10.1111/j.1365-2486.2007.01313.x

Clark, K. L., Skowronski, N., & Hom, J. (2010). Invasive insects impact forest carbon dynamics: Defoliation and forest carbon dynamics. *Global Change Biology*, *16*(1), 88–101. https://doi.org/10.1111/j.1365-2486.2009.01983.x

Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., et al. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, *11*(10), 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

Cotton, T. E. A., Fitter, A. H., Miller, R. M., Dumbrell, A. J., & Helgason, T. (2015). Fungi in the future: interannual variation and effects of atmospheric change on arbuscular mycorrhizal fungal communities. *New Phytologist*, *205*(4), 1598–1607. https://doi.org/10.1111/nph.13224

Dai, Z., Yu, M., Chen, H., Zhao, H., Huang, Y., Su, W., et al. (2020). Elevated temperature shifts soil N cycling from microbial immobilization to enhanced mineralization, nitrification and denitrification across global terrestrial ecosystems. *Global Change Biology*, *26*(9), 5267–5276. https://doi.org/10.1111/gcb.15211

Deslippe, J. R., Hartmann, M., Simard, S. W., & Mohn, W. W. (2012). Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiology Ecology*, *82*(2), 303–315. https://doi.org/10.1111/j.1574-6941.2012.01350.x

Drigo, B., Pijl, A. S., Duyts, H., Kielak, A. M., Gamper, H. A., Houtekamer, M. J., et al. (2010). Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proceedings of the National Academy of Sciences*, *107*(24), 10938–10942. https://doi.org/10.1073/pnas.0912421107

Frey, S. D., Drijber, R., Smith, H., & Melillo, J. (2008). Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biology*, 4.

Gao, W., & Yan, D. (2019). Warming suppresses microbial biomass but enhances N recycling. *Soil Biology and Biochemistry*, *131*, 111–118. https://doi.org/10.1016/j.soilbio.2019.01.002

Guo, X., Feng, J., Shi, Z., Zhou, X., Yuan, M., Tao, X., et al. (2018). Climate warming leads to divergent succession of grassland microbial communities. *Nature Climate Change*, *8*(9), 813–818. https://doi.org/10.1038/s41558-018-0254-2

He, X., Wu, Y., Cai, M., Mu, C., Luo, W., Cheng, Y., & Zhu, W. (2015). The effect of increased atmospheric temperature and CO_2 concentration during crop growth on the chemical composition and in vitro rumen fermentation characteristics of wheat straw. *Journal of Animal Science and Biotechnology*, *6*(1), 46. https://doi.org/10.1186/s40104-015-0045-9

He, Z., Xu, M., Deng, Y., Kang, S., Kellogg, L., Wu, L., et al. (2010). Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2: Changes in the soil microbial community at elevated CO_2. *Ecology Letters*, *13*(5), 564–575. https://doi.org/10.1111/j.1461-0248.2010.01453.x

He, Z., Xiong, J., Kent, A. D., Deng, Y., Xue, K., Wang, G., et al. (2014). Distinct responses of soil microbial communities to elevated CO_2 and O_3 in a soybean agro-ecosystem. *The ISME Journal*, *8*(3), 714–726. https://doi.org/10.1038/ismej.2013.177

Hillstrom, M., Meehan, T. D., Kelly, K., & Lindroth, R. L. (2010). Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO_2 and O_3. *Plant and Soil*, *336*(1–2), 75–85. https://doi.org/10.1007/s11104-010-0449-4

Hyvönen, R., Ågren, G. I., Linder, S., Persson, T., Cotrufo, M. F., Ekblad, A., et al. (2007). The likely impact of elevated CO_2, nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review: *Tansley review*. *New Phytologist*, *173*(3), 463–480. https://doi.org/10.1111/j.1469-8137.2007.01967.x

Intergouvernemental panel on climate change (Ed.). (2007). *Climate change 2007: the physical science basis*. Cambridge: Cambridge university press.

Jastrow, J. D., Michael Miller, R., Matamala, R., Norby, R. J., Boutton, T. W., Rice, C. W., & Owensby, C. E. (2005). Elevated atmospheric carbon dioxide increases soil carbon. *Global Change Biology*, *11*(12), 2057–2064. https://doi.org/10.1111/j.1365-2486.2005.01077.x

Jin, J., Wood, J., Franks, A., Armstrong, R., & Tang, C. (2020). Long-term CO_2 enrichment alters the diversity and function of the microbial community in soils with high organic carbon. *Soil Biology and Biochemistry*, *144*, 107780. https://doi.org/10.1016/j.soilbio.2020.107780

Keidel, L., Kammann, C., Grünhage, L., Moser, G., & Müller, C. (2015). Positive feedback of elevated CO_2 on soil respiration in late autumn and winter. *Biogeosciences*, *12*(4), 1257–1269. https://doi.org/10.5194/bg-12-1257-2015

Keidel, L., Lenhart, K., Moser, G., & Müller, C. (2018). Depth-dependent response of soil aggregates and soil organic carbon content to long-term elevated CO_2 in a temperate grassland soil. *Soil Biology and Biochemistry*, *123*, 145–154. https://doi.org/10.1016/j.soilbio.2018.05.005

King, J. S., Hanson, P. J., Bernhardt, E., DeAngelis, P., Norby, R. J., & Pregitzer, K. S. (2004). A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments: Elevated CO_2 increases forest soil respiration. *Global Change Biology*, *10*(6), 1027–1042. https://doi.org/10.1111/j.1529-8817.2003.00789.x

Koyama, A., Harlow, B., Kuske, C. R., Belnap, J., & Evans, R. D. (2018). Plant and microbial biomarkers suggest mechanisms of soil organic carbon accumulation in a Mojave Desert ecosystem under elevated CO_2. *Soil Biology and Biochemistry*, *120*, 48–57. https://doi.org/10.1016/j.soilbio.2018.01.033

Kuzyakov, Y., Horwath, W. R., Dorodnikov, M., & Blagodatskaya, E. (2019). Review and synthesis of the effects of elevated atmospheric CO_2 on soil processes: No changes in pools, but increased fluxes and accelerated cycles. *Soil Biology and Biochemistry*, *128*, 66–78. https://doi.org/10.1016/j.soilbio.2018.10.005

Lagomarsino, A., Lukac, M., Godbold, D. L., Marinari, S., & De Angelis, P. (2013). Drivers of increased soil respiration in a poplar coppice exposed to elevated CO_2. *Plant and Soil*, *362*(1–2), 93–106. https://doi.org/10.1007/s11104-012-1261-0

Lal, R. (2004). Soil carbon sequestration to mitigate climate change. *Geoderma*, *123*(1–2), 1–22. https://doi.org/10.1016/j.geoderma.2004.01.032

Lieffering, M., Kim, H.-Y., Kobayashi, K., & Okada, M. (2004). The impact of elevated CO_2 on the elemental concentrations of field-grown rice grains. *Field Crops Research*, *88*(2–3), 279–286. https://doi.org/10.1016/j.fcr.2004.01.004

Lipson, D. A., Wilson, R. F., & Oechel, W. C. (2005). Effects of elevated atmospheric CO_2 on soil microbial biomass, activity, and diversity in a Chaparral ecosystem. *Applied and Environmental Microbiology*, *71*(12), 8573–8580. https://doi.org/10.1128/AEM.71.12.8573-8580.2005

Liu, S., Ji, C., Wang, C., Chen, J., Jin, Y., Zou, Z., et al. (2018). Climatic role of terrestrial ecosystem under elevated CO_2: a bottom-up greenhouse gases budget. *Ecology Letters*, *21*(7), 1108–1118. https://doi.org/10.1111/ele.13078

Long, S. P., Ainsworth, E. A., Rogers, A., & Ort, D. R. (2004). Rising atmospheric carbon dioxide: Plants FACE the future. *Annual Review of Plant Biology*, *55*(1), 591–628. https://doi.org/10.1146/annurev.arplant.55.031903.141610

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., et al. (2013). Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology*, *94*(3), 726–738. https://doi.org/10.1890/12-0279.1

Luo, Y., Hui, D., & Zhang, D. (2006). Elevated CO_2 stimulates net accumulations of carbon and nitrogen in Land ecosystems: A meta-analysis. *Ecology*, *87*(1), 53–63.

Pachauri, R. K., Mayer, L., & Intergovernmental Panel on Climate Change (Eds.). (2015). *Climate change 2014: synthesis report*. Geneva, Switzerland: Intergovernmental Panel on Climate Change.

Prentice, I. C., Farquhar, G. D., Fasham, M. J. R., Goulden, M. L., Heimann, M., Jaramillo, V. J., et al. (2001). The carbon cycle and atmospheric carbon dioxide. *Climate Change 2001: The Scientific Basis*, 183–237.

Sardans, J. (2012). The C: N: P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives, 15.

Sheik, C. S., Beasley, W. H., Elshahed, M. S., Zhou, X., Luo, Y., & Krumholz, L. R. (2011). Effect of warming and drought on grassland microbial communities. *The ISME Journal*, *5*(10), 1692–1700. https://doi.org/10.1038/ismej.2011.32

Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S. J., Gregorich, E. G., et al. (2002). Measuring and understanding carbon storage in Afforested soils by physical fractionation. *Soil Science Society of America Journal*, *66*(6), 1981–1987. https://doi.org/10.2136/sssaj2002.1981

Sun, Y., Wang, C., Yang, J., Liao, J., Chen, H. Y. H., & Ruan, H. (2021). Elevated CO_2 shifts soil microbial communities from K- to r-strategists. *Global Ecology and Biogeography*, *30*(5), 961–972. https://doi.org/10.1111/geb.13281

Terrer, C., Phillips, R. P., Hungate, B. A., Rosende, J., Pett-Ridge, J., Craig, M. E., et al. (2021). A trade-off between plant and soil carbon storage under elevated CO_2. *Nature*, *591*(7851), 599–603. https://doi.org/10.1038/s41586-021-03306-8

Van Kessel, C., Nitschelm, J., Horwath, W. R., Harris, D., Walley, F., Lüscher, A., & Hartwig, U. (2000). Carbon-13 input and turn-over in a pasture soil exposed to long-term elevated atmospheric CO_2: Pasture soil C-cycling. *Global Change Biology*, *6*(1), 123–135. https://doi.org/10.1046/j.1365-2486.2000.00287.x

Wang, J., Liu, X., Zhang, X., Li, L., Lam, S. K., & Pan, G. (2019). Changes in plant C, N and P ratios under elevated CO_2 and canopy warming in a rice-winter wheat rotation system. *Scientific Reports*, *9*(1), 5424. https://doi.org/10.1038/s41598-019-41944-1

Wang, J., Li, L., Lam, S. K., Liu, X., & Pan, G. (2020). Responses of wheat and rice grain mineral quality to elevated carbon dioxide and canopy warming. *Field Crops Research*, *249*, 107753. https://doi.org/10.1016/j.fcr.2020.107753

Xu, W., & Yuan, W. (2017). Responses of microbial biomass carbon and nitrogen to experimental warming: A meta-analysis. *Soil Biology and Biochemistry*, *115*, 265–274. https://doi.org/10.1016/j.soilbio.2017.08.033

Yang, S., Zheng, Q., Yuan, M., Shi, Z., Chiariello, N. R., Docherty, K. M., et al. (2019). Long-term elevated CO_2 shifts composition of soil microbial communities in a Californian annual grassland, reducing growth and N utilization potentials. *Science of The Total Environment*, *652*, 1474–1481. https://doi.org/10.1016/j.scitotenv.2018.10.353

Yu, H., Deng, Y., He, Z., Van Nostrand, J. D., Wang, S., Jin, D., et al. (2018). Elevated CO_2 and warming altered grassland microbial communities in soil top-layers. *Frontiers in Microbiology*, *9*, 1790. https://doi.org/10.3389/fmicb.2018.01790

Yu, H., Deng, Y., He, Z., Pendall, E., Carrillo, Y., Wang, S., et al. (2021). Stimulation of soil microbial functioning by elevated CO_2 may surpass effects mediated by irrigation in a semiarid grassland. *Geoderma*, *401*, 115162. https://doi.org/10.1016/j.geoderma.2021.115162

Zhang, X.-Z., Shen, Z.-X., & Fu, G. (2015). A meta-analysis of the effects of experimental warming on soil carbon and nitrogen dynamics on the Tibetan Plateau. *Applied Soil Ecology*, *87*, 32–38. https://doi.org/10.1016/j.apsoil.2014.11.012

Zhou, J., Deng, Y., Luo, F., He, Z., & Yang, Y. (2011). Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. *MBio*, *2*(4). https://doi.org/10.1128/mBio.00122-11