Does Litter Quality Change Affect the Decomposition of Soil Organic Matter Under Elevated Atmospheric CO2 and Warming?

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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 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.

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11 Key Points:

- Litter quality change has no effect on soil organic matter decomposition under elevated
 CO₂ and warming.
- The legacy effect of elevated CO₂ and warming on soil property controls the decomposition of soil organic.
- Elevated CO₂ may promote soil carbon sequestration by suppressing soil organic matter
 decomposition.

19 Abstract

Soil property and litter quality are two key factors that control soil organic matter decomposition. 20 Under climate change, it remains unclear how the changes of soil microbial community and litter 21 quality affect soil organic carbon decomposition, although significant changes of these two factors 22 have been reported intensively. This limits our ability to model the dynamics of terrestrial soil 23 24 carbon in a changing climate. Using a long-term Free Air CO₂ Enrichment facility equipped with warming, we investigated the effect of soil property and litter quality change on the decomposition 25 rate of soil organic matter. Results showed that significant change of litter quality was observed 26 under elevated CO₂ and warming. Elevated CO₂ decreased the concentration of N of rice and wheat 27 straw, while warming decreased the concentration of N and K in wheat straw. However, these 28 29 changes in plant litter quality did not lead to a shift in soil organic matter decomposition. The legacy effect of long-term elevated CO₂ and warming on soil properties dominated the 30 31 decomposition rate of soil organic matter. Elevated CO₂ suppressed soil organic matter decomposition mainly by increasing phosphorous availability and lowering soil C/N, 32 fungi/bacteria ratio, and N-acetyl-glucosaminidase activity; while warming or elevated CO₂ plus 33 warming had no effect on soil organic matter decomposition. Our results demonstrated that the 34 change of soil properties other than litter quality control the decomposition of soil organic carbon; 35 and soil property change should be taken into consideration in model developing when predicting 36 37 terrestrial soil carbon dynamics under elevated atmospheric CO₂ and warming.

38 Plain Language Summary

39 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 40 41 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 CO₂ Enrichment facility equipped 42 with warming was used to study the effect of elevated atmospheric CO₂ and warming on soil 43 organic carbon decomposition. We found that soil microbial food source change had no effect on 44 soil organic carbon decomposition, on the contrary soil microbial community and the soil 45 environment condition dominated the carbon cycling under elevated CO₂ and warming. Our results 46 47 demonstrated that food source cannot be considered a key factor in modeling parameterization.

48 **1 Introduction**

Climate change, mainly characterized by the rapid increase in the atmospheric CO₂ concentration and the elevation of global surface temperature, is challenging the sustainable development of global agriculture. The concentration of CO₂ 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 CO₂ 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).

56 Soil organic carbon in terrestrial ecosystem plays an important role in the global carbon cycle.

57 About 2000 Pg of organic carbon are stored in the top two meters of global soils. The forest

58 ecosystem accounts for approximately 73% of the terrestrial soil carbon pool (Six et al., 2002).

59 The carbon pool in the farmland ecosystem is small but it can be managed by human being.

- 60 Therefore, farmland ecosystem has a huge potential of soil organic carbon sequestration (Lal,
- 61 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). 62 Several studies reported that elevated atmospheric CO₂ could increase soil organic carbon storage 63 by increasing net CO₂ uptake (Hyvönen et al., 2007; Jastrow et al., 2005; Luo et al., 2006). Liu et 64 al. (2018) and Luo et al. (2006) predicted that soil organic carbon stock would increase by around 65 5%, although it is quite small compared the increase rate of plant biomass carbon under elevated 66 CO₂. However, Koyama et al. (2018) found that elevated atmospheric CO₂ did not affect the soil 67 organic carbon pool in a Mojave Desert ecosystem. Similar findings were reported in cropland and 68 temperate grassland ecosystems (Keidel et al., 2018; Van Kessel et al., 2000). Furthermore, 69 increased soil CO₂ flux under elevated CO₂ was frequently reported (Liu et al., 2018). Kuzyakov 70 et al. (2019) argued that elevated atmospheric CO₂ has no (or litter) effect on the soil carbon pool, 71 72 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, 73 2019; Lu et al., 2013; Xu & Yuan, 2017; Zhang et al., 2015) or negative (Chen et al., 2020; Lu et 74 al., 2013) effects on soil organic carbon pool. Long-term warming decreased soil organic carbon 75 pool by stimulating microbial utilization of the recalcitrant C pool (Chen et al., 2020). However, 76 most of the studies involved in these meta-analyses were conducted in forest or grassland 77 78 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 79 change under climate change of elevated CO₂ and warming. 80

The concentration of CO_2 in soil is much higher than that in the atmosphere (10- 50 times), and 81 elevated atmospheric CO₂ (+ 200pm) will probably not affect soil organic carbon cycling directly. 82 Its effect on soil carbon cycling is through the changes of plant growth indirectly. Elevated 83 atmospheric CO₂ and warming affect plant growth by altering leaf stomatal conductance and the 84 photosynthesis rate (Long et al., 2004). Elevated CO₂ can increase crop yield via increasing the 85 photosynthesis rate and soil nutrients use efficiency (Hyvönen et al., 2007). As the atmospheric 86 CO₂ concentration increases, the nutrients condition of grains and the shoot biomass will change 87 accordingly. Therefore, some studies predicted that the plants would be exposed to a global 88 89 nutrient imbalance with lower N concentration or higher ratios of C: N and C: P in plant litters under elevated CO₂ (Sardans, 2012; Wang et al., 2019). In addition to macronutrients, the 90 micronutrients in plant litter will also decrease under elevated CO₂ (Wang et al., 2020). He et al. 91 (2015) even found that elevated CO₂ and warming reduced the content of crude protein and the in 92 vitro digestibility of wheat straw. Plant litter with different chemical properties would probably 93 affect the decomposition rate of soil organic carbon. However, this conjecture has never been 94 95 tested although the changes in plant litter quality have been observed under elevated CO₂ and warming. 96

97 In addition to plant litter quality, soil organic carbon mineralization is also regulated by soil microbial community. Under elevated CO₂ or warming, significant change of soil microbial 98 communities has been reported intensively (Butterly et al., 2016; He et al., 2014; Sun et al., 2021). 99 Several studies found that elevated CO₂ altered soil microbial composition (Carney et al., 2007; 100 Chung et al., 2007; He et al., 2010; Jin et al., 2020; Lipson et al., 2005; Yang et al., 2019; Yu et 101 al., 2021; Zhou et al., 2011). Soils exposed to elevated CO₂ had higher relative abundances of 102 fungi and higher enzyme activity (Carney et al., 2007; Drigo et al., 2010), which led to more soil 103 carbon loss (Chung et al., 2007; Cotton et al., 2015; He et al., 2010; Zhou et al., 2011). Lipson et 104 al. (2005) observed that elevated CO_2 had no effect on bacterial diversity, but it increased fungal 105 biomass in a Chaparral Ecosystem. Sun et al. (2021) found that soil microbial community evolves 106 from K-strategists dominated to r-strategists dominated community under elevated CO₂, with 107

decreasing ratios of fungi to bacterial, Gram positive to Gram negative and Acidobacteria to 108 Proteobacteria. Warming generally had negative effect on soil microbial community, which led to 109 soil carbon loss and greater N₂O emission (Cheng et al., 2017; Dai et al., 2020). Some studies 110 observed that warming reduced bacterial and fungal abundance in forest ecosystem (Allison & 111 Treseder, 2008; Frey et al., 2008). The soil microbial community structure was also altered by 112 warming (Guo et al., 2018). Deslippe et al. (2012) found that warming decreased evenness of 113 bacterial communities while increased evenness of fungal communities. Cheng et al. (2017) 114 showed that warming increased the relative abundance of key functional genes involved soil 115 carbon degradation. Sheik et al. (2011) found that warming increased soil microbial population 116 size but decreased diversity under wet conditions; whereas it reduced microbial population size 117 under drought condition. Under elevated CO₂ plus warming, the abundance of some dominant 118 phyla was significantly increased, and the effect of combined elevated CO₂ and warming on soil 119 functional processes was similar to elevated CO₂ alone (Yu et al., 2018). 120

Under elevated CO₂ or warming, significant changes of soil microbial community and plant litter 121 quality have been observed. Understanding the effect of plant litter quality and soil microbial 122 community on soil organic carbon decomposition can help us model soil carbon dynamics under 123 elevated CO₂ and warming. To our knowledge, there was no report that investigating the effect of 124 plant litter quality and soil microbial community change on soil organic carbon mineralization 125 under elevated CO₂ and warming. Three manipulated incubation experiments were conducted to 126 answer the fowling questions: 1) Does plant litter quality change (C: N and nutrients content) affect 127 soil organic carbon decomposition under elevated CO₂ and warming; 2) Does soil property change 128 (soil microbial community) affect soil organic carbon decomposition under elevated CO₂ and 129 warming; 3) Does plant litter have greater effect on soil organic carbon decomposition than soil 130 microbial community. We hypothesized that plant litter with decreased quality under elevated CO₂ 131 and warming would suppress soil organic carbon decomposition; whereas the change of soil 132 microbial community would promote soil organic carbon decomposition. The results of this study 133 can be used in soil carbon cycling model developing to predict terrestrial carbon dynamics 134 135 precisely under future climate change of elevated CO₂ and warming.

136 2 Materials and Methods

137 2.1 Soil and plant litter

The soils and plant litters used in this study were taken from the long-term field experiment 138 of Nanjing Agricultural University, which was located in Kangbo Village (31°30'48"N, 139 120°33'36"E), Changshu City, Jiangsu Province of China. The field experiment facility 140 was constructed in 2010 and the objective of this facility was to simulate Free Air CO₂ 141 Enrichment and plant canopy warming in the open field. There are four treatments 142 including elevated CO₂ up to 500 ppm (C), warming plant canopy by 2 °C (T), elevated 143 CO₂ plus plant canopy warming (CT), and the ambient CO₂ without warming as the control 144 (Control). The soils were collected from the top 15 cm in June 2018 after 7 years of 145 treatment. The plant litters (rice and wheat straw) were collected at harvest. Rice straw 146

147 (Cultivar: Changyou 5) were collected in October 2017, and wheat straw (Cultivar:
148 Yangmai 16) were collected in June 2018.

149 2.2 Experiment design

Three incubation experiments were designed (Table 1). In the first experiment (Experiment 151 I), the soils from the treatment of Control, C, T and CT were incubated with the addition 152 of crop straw from Control, C, T and CT, respectively. In the second experiment 153 (Experiment II), the soils from the Control were incubated with the addition of crop straw 154 from Control, C, T and CT. In the third experiment (Experiment III), the soils from the 155 treatment of Control, C, T and CT were incubated with the addition of crop straw from the 156 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 CO₂; T represents the soils or litters that collected from plant canopy warming; CT represents the
- 159 CO₂; T represents the soils or litters that collected from plant canopy warming; CT repr 160 soils or litters that collected from CO₂ plus warming.

	Soils	Litters	Abbreviation
Experiment I	Control	Control	S+L
	С	С	SC+LC
	Т	Т	ST+LT
	СТ	СТ	SCT+LCT
Experiment II	Control	Control	S+L
	Control	С	S+LC
	Control	Т	S+LT
	Control	СТ	S+LCT
Experiment III	Control	Control	S+L
	С	Control	SC+L
	Т	Control	ST+L
	СТ	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

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cap. A three-way valve is sleeved above the rubber tube for fresh air and gas sample 164 collection. To simulate soil respiration process during the whole crop growing season in 165 the studied area, two soil water condition was designed. The soil mixed with rice straw 166 were incubated first at aerobic with soil water content maintained at 80% of the soil water 167 holding capacity. Then the soils were mixed with wheat straw (0.06 g) and incubated at 168 flooded condition. During the aerobic incubation, gas sampling was performed at day 1, 169 1.5, 2, 3, 4, 5, 6, 8, 9, 11, 13, 15, 17, 19, 23, 28, 33, 43, 64. During the anaerobic incubation, 170 gas sampling was performed at day 65, 65.5, 66, 66.5, 67.5, 69, 71, 73, 82, 89, 98, 115, 171 123, 131, 139, 147. Gas samples were collected with a syringe 2 hours after ventilation. 172

173The concentration of CO2 in gas samples was detected in a gas chromatogragh (Agilent1747890A). The emission rate of CO2 was calculated with the following equation:

175
$$\mathbf{F} = \rho \times \frac{V}{m} \times \frac{\Delta C}{\Delta t} \times \frac{273}{273 + T} \times \alpha$$

176 Where F represents CO2 emission rate (mg C·kg⁻¹·d⁻¹); ρ represents the density of CO₂, 177 which is 1.997 g·m⁻²; V represents the volume of air above the flask (L); m represents the 178 mass of soil (g); Δ C represents the concentration of CO₂ in the gas sample (µmol·mol⁻¹); 179 Δ t represents the sampling time (d) of the closed flask, and T is the temperature of the 180 incubation (25 °C).

181 2.3 Soil physical-chemical analysis

Plant and soil samples were analyzed following the protocol described by Lu (2000). The 182 plant samples were digested with sulfuric acid and hydrogen peroxide. The concentrations 183 of nitrogen, phosphorus and potassium in the digestion were determined by the micro-184 Kjeldahl Determination method, colorimetric method and flame photometer method, 185 respectively. Dissolved organic carbon (DOC) was extracted with 0.05 mol Plant and soil 186 samples were analyzed following the protocol described by Lu (2000). The plant samples 187 were digested with sulfuric acid and hydrogen peroxide. The concentrations of nitrogen, 188 phosphorus and potassium in the digestion were determined by the micro-Kjeldahl 189 Determination method, colorimetric method and flame photometer method, respectively. 190 Dissolved organic carbon (DOC) was extracted with 0.05 mol \cdot L⁻¹ K₂SO₄ solution. The 191 mixture was shaken at 180 r \cdot min⁻¹ for 30 minutes, and then pass through a 0.45 μ m filter. 192 The concentration of DOC in the liquid was measured in a TOC analyzer. Soil microbial 193 194 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 195 0.05 mol·L⁻¹ K₂SO₄ solution for 30 minutes in a shaker (180 r·min⁻¹). Then the mixture was 196 filtered through a 0.45 µm water-based filter membrane. The concentration of carbon in 197 198 the extract was measured with a TOC analyzer (Multi N/ C 3100).

- 199 2.4 Statistic analysis
- Data were expressed as mean plus/minus one standard deviation of three replicates. Oneway 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.

204 **3 Results**

3.1 Changes in litter quality under elevated CO₂ and warming

Table 2 shows the nutrient concentration of rice and wheat straw following one crop growth season treatment of elevated CO₂ and warming. Elevated CO₂ decreased the concentration of N of rice and wheat straw by 1.75% and 3.68%, respectively. Under elevated CO₂, 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 CO₂ 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		
	Ν	Р	K	Ν	Р	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
С	8.84±0.50b	0.90±0.11a	14.90±0.31a	5.60±0.85b	0.67±0.16a	11.56±1.65b
Т	11.42±0.17a	0.97±0.08a	16.69±1.44a	6.94±0.78b	0.89±0.06a	7.47±2.52c
СТ	8.05±0.71b	0.66±0.03b	16.48±0.54a	6.09±0.65b	1.09±0.29a	7.16±1.98c

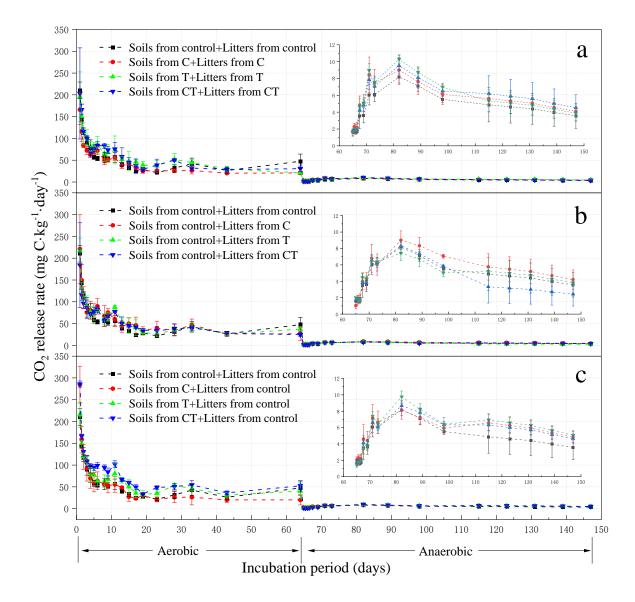
214 Different lower-case letters indicate significant differences among treatments (P < 0.05).

3.2 The effect of elevated CO₂ 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 CO₂ (Soil respiration hereafter) from the soil is shown in Fig. 2 a. Much more CO₂ was released during the aerobic stage, which accounted for about 90% of the overall released rate. During the aerobic process, elevated CO₂ decreased soil respiration by 27.60% compared to the control; while warming or elevated CO₂ plus

warming had no effect on it. During the anaerobic process, all the treatments had no effecton soil respiration.



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Fig. 1 CO₂ released rate during the aerobic and anaerobic stage. Control represents the soils or litters that collected from the ambient atmospheric CO₂ without warming; C represents the soils or litters that collected from elevated CO₂; T represents the soils or litters that collected from plant

canopy warming; CT represents the soils or litters that collected from CO₂ plus warming. In Fig. 1, a, b and c represent Experiment I, Experiment II and Experiment III respectively.

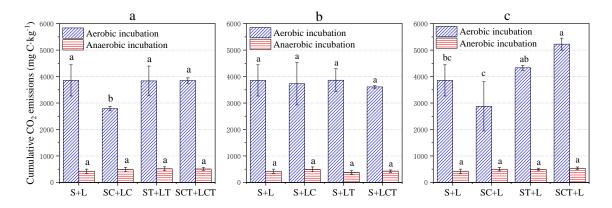


Fig. 2 The cumulative CO₂ emission during aerobic and anaerobic stage. Please refer to Table 1 for the treatment abbreviations. In Fig. 2, a, b and c represent Experiment I, Experiment II and Experiment III respectively.

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

As shown in Fig. 1 b, the CO₂ release dynamics across treatments was very similar to Experiment I. During the anaerobic stage, the CO₂ 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 CO₂ 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 CO₂ plus warming emitted much more CO₂. The accumulated CO₂ emission of soils treated with elevated CO₂ was 2874 mg C·kg⁻¹, which was significantly lower than the values from soils under warming and elevated CO₂ plus warming. During the anaerobic stage, there was no significant treatment effects (Fig. 2 c).

253 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-acetylglucosaminidase, 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 β259 Glucosidase, but negatively correlated with soil microbial biomass carbon and available P 260 content.

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	

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

²⁶³ * indicates significant at 0.05; ** indicates significant at 0.01.

264 **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

CO₂ and warming, the changes of soil condition and litter quality were supposed to alter 271 the mineralization of soil organic carbon. A new balance between organic carbon input and 272 soil carbon loss might be reached, which can be used to predict the dynamics of soil organic 273 carbon in a changing climate. However, this hypothesis was not fully supported by the 274 current study. We found that the legacy effect of long-term elevated CO₂ and warming on 275 soil condition rather than plant litter quality change dominated the decomposition rate of 276 soil organic carbon. Plant litter quality change had no effect on soil organic carbon 277 mineralization, although significant changes of plant litter quality had been observed in 278 this study and others (Lieffering et al., 2004; Wang et al., 2019). 279

We were surprised to found that elevated CO₂ suppressed soil respiration compared with 280 the ambient control. While most FACE experiments have shown that elevated CO_2 281 increased soil respiration by 25% on average (King et al., 2004; Liu et al., 2018), although 282 neutral or negative effects were also reported (Bader & Körner, 2010; Clark et al., 2010; 283 Keidel et al., 2015). Two reasons account for the higher soil respiration rate under elevated 284 CO₂. Firstly, elevated CO₂ stimulates soil respiration by increasing the labile carbon pools. 285 These carbons are mainly derived from fine roots development and their exudates; and 286 most of them are decomposed by soil microbe and released to the atmosphere directly 287 without forming soil aggregates with soil minerals (Andrews & Schlesinger, 2001; 288 Lagomarsino et al., 2013). Therefore, no net carbon gains were observed in soils under 289 elevated CO₂. Secondly, elevated CO₂ stimulates soil respiration via water saving effect. 290 291 Under elevated CO₂, leaf stoma closure reduces plant transpiration and more water can be stored in soil, which facilitates soil microbial respiration (Bader & Körner, 2010). 292 However, the water saving effect can only be observed in dry soil conditions; under wet 293 soil conditions, it will decrease soil respiration because of low soil aeration. 294 Therefore, Bader and Körner (2010) argued that there was no overall simulation of soil 295 respiration under elevated CO_2 in a mature deciduous forest ecosystem. Furthermore, the 296 297 magnitude of soil respiration stimulating effect do not persist forever, and it will decline over the years of atmospheric CO₂ enrichment (Bernhardt et al., 2006). This suggests that 298 soil microbial community can adapt to long-term elevated CO₂ and a new balance between 299 carbon input and output is reached. In the current study, there was no water saving effect 300 as described in previous studies, because the soils were incubated at the same water 301 condition. And there was no continues carbon input via root exudates. Therefore, no 302 stimulation effect was observed in this study. The soils under long-term elevated CO₂ had 303 higher phosphorous availability and lower soil C: N, ratio of fungi to bacteria, and N-304 acetyl-glucosaminidase activity, which collectively led to the lower soil respiration rate 305 (Table 3). Further study is needed to explore the direct link between soil respiration and 306 these factors. 307

Though significant changes in litter quality were observed, they had no effect on soil 308 carbon decomposition under elevated CO₂ and warming in this study. Hillstrom et al. 309 (2010) found that elevated CO₂ had minimal effect on microbial respiration although it 310 affected litter quality. Cornwell et al. (2008) found that the decomposition rate of litter 311 caused by litter quality is three times that of climate factors. This may be true for large 312 scale of ecosystem level, but for small areas of field level, like the current study, this might 313 be not true. This study also demonstrated that the soil under elevated CO_2 plus warming 314 responded differently to litter addition in terms of respiration rate (Fig. 2 Experiment I, 315

Experiment III). The soil incorporated with litter from the control had significantly higher 316 CO₂ emission rate than the soil with litter from the treatment of elevated CO₂ plus warming. 317 In experiment III, the respiration rate of soil under elevated CO₂ plus warming is even 318 higher than the rate of soil under the control and elevated CO₂ alone, which was different 319 from the results in experiment I. We attributed this to the adaptation of soil microbial 320 community to long-term elevated CO₂ and warming (Bradford, 2013). The soil microbes 321 under 7 years of elevated CO₂, warming or both in this study have got used to obtaining 322 nutrients and energy from soil organic matter and litters in a more efficient way, and under 323 this condition less CO2 was emitted. Whereas, a sudden change of food resources (adding 324 litter from other environment, such as the litter from the control in this study) led to a lower 325 carbon use efficiency, which caused a high soil respiration rate, especially for the warming 326 treatment soils. In other words, the soil microbes need to decompose more organic matter 327 to get similar amounts of nutrients after food change. 328

329 **5 Conclusions**

The study showed that, under future climate change of elevated CO₂ and warming, the change of

plant litter has no effect on the decomposition of soil organic matter though significant change of

332 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 CO₂ and warming. Elevated

334 atmospheric CO₂ may promote soil carbon sequestration by suppressing soil microbial respiration

under no warmed condition.

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341 reasonable request.

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