Title

Enhanced decomposition of stable soil organic carbon and microbial catabolic potentials by long-term field warming

Journal

Global Change Biology, 23(11)

ISSN

1354-1013

Authors

Feng, Wenting

Liang, Junyi

Hale, Lauren E

et al.

Publication Date

2017-11-01

DOI

10.1111/gcb.13755

Peer reviewed

Enhanced decomposition of stable soil organic carbon and microbial catabolic potentials by long-term field warming

Wenting Feng

Junyi Liang

Lauren E. Hale

Chang Gyo Jung

Ji Chen

Jizhong Zhou

Minggang Xu
Mengting Yuan

Liyou Wu

Rosvel Bracho

Elaine Pegoraro

Edward A. G. Schuur

Yiqi Luo
First published: 09 June 2017

https://doi.org/10.1111/gcb.13755

Cited by: 4

Abstract

Quantifying soil organic carbon (SOC) decomposition under warming is critical to predict carbon–climate feedbacks. According to the substrate regulating principle, SOC decomposition would decrease as labile SOC declines under field warming, but observations of SOC decomposition under warming do not always support this prediction. This discrepancy could result from varying changes in SOC components and soil microbial communities under warming. This study aimed to determine the decomposition of SOC components with different turnover times after subjected to long-term field warming and/or root exclusion to limit C input, and to test whether SOC decomposition is driven by substrate lability under warming. Taking advantage of a 12-year field warming experiment in a prairie, we assessed the decomposition of SOC components by incubating soils from control and warmed plots, with and without root exclusion for 3 years. We assayed SOC decomposition from these incubations by combining inverse modeling and microbial functional genes during decomposition with a metagenomic technique (GeoChip). The decomposition of SOC components with turnover times of years and decades, which contributed to 95% of total cumulative CO_2respiration, was greater in soils from warmed plots. But the decomposition of labile SOC was similar in warmed plots compared to the control. The diversity of C-degradation microbial genes generally declined with time during the incubation in all treatments, suggesting shifts of microbial functional groups as substrate composition was changing. Compared to the control, soils from warmed plots showed significant increase in the signal intensities of microbial genes involved in degrading complex organic compounds, implying enhanced potential abilities of microbial catabolism. These are likely responsible for accelerated decomposition of SOC components with slow turnover rates. Overall, the shifted microbial community induced by long-term warming accelerates the decomposition of SOC components with slow turnover rates and thus amplify the positive feedback to climate change.

1 INTRODUCTION

Quantifying soil organic carbon (SOC) decomposition under warming is critical to predict carbon–climate feedbacks, as soils store four times the amount of carbon (C) as the biotic C pool and its decomposition is a large flux to the atmosphere (IPCC, 2013). SOC decomposition is known to be regulated by substrate quality and accessibility, microbial decomposers, and environmental conditions (Jenkinson & Rayner, 1977; Karhu et al., 2014; Parton, Schimel, Cole, & Ojima, 1987; Xue et al., 2016; Zhou et al., 2012). These regulating factors are simultaneously affected by warming. Therefore, how SOC decomposition responds to warming is not well understood (Bradford et al., 2016). According to the substrate-control principle that has been widely applied in the first-order kinetic soil C models (Jenkinson & Rayner, 1977; Parton et al., 1987), warming decreases soil substrates in quality and quantity (Dalias, Anderson, Bottner, & Coûteaux, 2001; Xu, Sherry, Niu, Zhou, & Luo, 2012), which would theoretically suppress SOC decomposition and lead to less heterotrophic soil respiration (R_h). But some studies show increased soil C decomposition under warming (Hopkins, Torn, & Trumbore, 2012; Li, Schaedel et al., 2013; Lu et al., 2013). Thus, how SOC decomposition will change under planetary warming remains elusive. Current understanding offers inconsistent observations and

predictions (Lu et al., 2013), showing positive (Li, Zhou, Wu, Zhou, & Luo, 2013; Noh et al., 2016; Zhou et al., 2010), negative (Hartley, Heinemeyer, & Ineson, 2007), or neutral (Hartley et al., 2007; Li, Schaedel et al., 2013) responses of SOC decomposition or R_h to warming.

This discrepancy could result from varying SOC properties, associated microbial communities, or environmental factors present in the different assessments. SOC is composed of components with varying pool size and turnover rates (Feng et al., 2016; Sollins et al., 2009), such as labile SOC (LOC) and stable SOC, and their changes regulate soil CO_2efflux (Luo, Keenan, & Smith, 2015). To more accurately predict the magnitude of changes in SOC decomposition under warming, it is necessary to quantify the decomposition of SOC components. Soil LOC has a fast turnover rate and increases in LOC proportions make great contributions to soil CO_2 efflux, especially in the short term. LOC can be increasingly depleted with the duration of field warming, leading to different responses of SOC decomposition to warming across studies (Lu et al., 2013; Xu, Sherry et al., 2012). Soil LOC is also found to decline in treatments with the exclusion of belowground root or aboveground plant litter (Leff et al., 2012; Ross, Scott, Tate, Rodda, & Townsend, 2001; Xu, Sherry et al., 2012), probably resulting in reduced SOC decomposition. Meanwhile, although stable SOC has a slow turnover rate, its decomposition can also make a substantial contribution to soil CO_2 efflux, because it makes up the majority of the total SOC stock (Cai, Feng, Zhang, & Xu, 2016) and is more temperature sensitive (Davidson & Janssens, 2006; Lefevre et al., 2014; Liang et al., 2015).

In addition, divergent responses of SOC decomposition to warming could result from shifts in soil microbial communities so as to change decomposition strategies in response to altered C substrates. As soil substrate lability alters under warming, microbial communities could change their functional potential or structure. Microbes with the catabolic potential to decompose the low-quality substrates may become favored (Dijkstra et al., 2011; Frey, Drijber, Smith, & Melillo, 2008), or the community composition may shift to one with low energy demands or with fast turnover rates (Billings & Ballantyne, 2013; Dijkstra et al., 2011). Warming and its duration are thought to change enzyme activity and carbon use efficiency and consequently alter SOC decomposition (Sinsabaugh, Hill, & Follstad Shah, 2009; Sinsabaugh, Manzoni, Moorhead, & Richter, 2013; Sinsabaugh et al., 2016). Meanwhile, the uncertainties of how SOC decomposition responds to warming could also be due to the confounding changes in environmental conditions. For example, warming has been found to decrease soil moisture (Li, Schaedel et al., 2013; Zhou et al., 2010) and thus reduces SOC decomposition. This could offset the increase in SOC decomposition induced by intensified microbial catabolic abilities.

In this study, we aimed to determine the responses of the decomposition of SOC and its constituents to long-term field warming. We particularly examined whether substrate quality or microbial community functional potential was more important in regulating the decomposition of existing SOC. We hypothesized that (1) SOC decomposition will be suppressed after soils were subjected to 12 years of field warming, considering the decreases in LOC content and SOC lability (i.e., the ratio of LOC to total SOC) (Xu, Sherry et al., 2012), (2) SOC decomposition in deep collar will decline as plant C inputs to soils are excluded and LOC is depleted after the decadal application, and (3) soil microbial communities under warming will shift to increase the relative abundance of genes that are involved in C-degradation of recalcitrant substrates in order to adapt to reduced SOC lability. A long-term warming study, which has been in place since 1999, offers an opportunity to address our hypotheses. We assessed the decomposition of different SOC components in intact and deep collar soils subjected to 12 years of field warming. The soil samples went through a 3-year laboratory incubation, and the laboratory incubation results were analyzed with a multiple-pool inverse modeling approach to quantify decomposition rates of different SOC components. Meanwhile, we assayed soil microbial functional potentials using a metagenomic technique (GeoChip) to explain warming-induced changes in the decomposition. Laboratory incubations offer the potential to isolate field warming effects on SOC decomposition from confounding effects, such as moisture limitation and interference of fresh plant C inputs to old existing soil C. Our long-term incubation enables us to estimate the decomposition of stable SOC, which is typically difficult with short-term incubations. In addition, the GeoChip analysis can provide information on the abundance of thousands of environmentally relevant microbial genes that are linked with C-degradation and enable profiling of microbial community functional potentials (Bracho et al., 2016; Wu, Liu, Schadt, & Zhou, 2006; Xue et al., 2016; Zhou et al., 2012). The latest version, GEOCHIP 5.0, contains over 60,000 probes covering hundreds of gene families, including over 16,500 probes targeting genes involved in C-degradation of various C substrates (Bracho et al., 2016; Wu et al., 2006; Xue et al., 2016; Zhou et al., 2012). This method has been used to characterize soil microbial communities in many environments and provides benefits over other metagenomics techniques (Liebich et al., 2006; He et al., 2007; Zhou et al., 2010). Notably, information is provided on gene abundance based on probe signal intensities, it allows for the identification of rare genes without deep sequencing, and data analyses are faster than those of metagenome assemblies.

2 MATERIALS AND METHODS

2.1 Study site and warming experiment

The warming experiment was set up in November 1999 in the Kessler Farm Field laboratory in Central Oklahoma, USA (34°59′ N, 97°31′ W). The study site is a typical prairie dominated by C_4 grasses (*Schizachyrium scoparium* and *Sorghastrum nutans*) and C_3 forbs (*Ambrosia psilostachyia*, *Solidago rigida*, and *Solidago nemoralis*), and has not been disturbed by cultivation and grazing in the past 40 years. Mean annual temperature is 16.3°C, and mean annual precipitation is 914 mm. The soil is identified as an Udic Haplustol in the Nash-Lucien soil series, coarse-silty and well drained with neutral pH (USDA, 1979).

A split-plot paired factorial design was used in the long-term experiment to investigate the effects of field warming, human activities, and plant C input exclusion on C cycling. Clipping was used to mimic the grazing in this grassland, while deep collars (PVC tubes of 10 cm in diameter, and 70 cm in depth) were installed to effectively exclude old roots and block new roots and plants from establishing within the deep collars. There are six pairs of warm versus control plots (2 m × 2 m). For each pair, one has been subjected to continuous warming using infrared heaters 1.5 m above the ground since November 1999, while the other serves as the control. Within each plot, four 1 × 1 m subplots were randomly assigned as control, clipping, control + deep collar, and clipping + deep collar. Detailed information about the warming set-up and experimental plots were published previously (Luo, Wan, Hui, & Wallace, 2001; Wan, Luo, & Wallace, 2002).

As this study aimed to test hypotheses pertaining to the effects of field warming and plant C inputs exclusion on SOC decomposition, we collected the top 0–20 cm soils from unclipped subplots within each of the six paired warmed and the control plots in August 2011. Within each subplot, we collected two samples, one from within the deep collar and the other from the intact soil outside of the deep collar tubes. There were 24 samples in total, which were from the four treatments: control + intact soil (CI), warm + intact soil (WI), control + collar soil (CC), and warm + collar soil (WC). The fresh samples were put on ice and transported to the laboratory at the University of Oklahoma on the day of sampling. The fresh soil samples were passed through 2-mm sieve, and visible plant roots were picked out using tweezers and then transported on ice to the University of Florida for the 3-year laboratory incubation.

2.2 Laboratory incubation

From each of the 24 soil samples, eight subsamples of ~10 g were taken and placed over a perforated foil cup, each subsample was then located inside a 30-cm^3 vial on top of a bed of 3-mm glass beads to allow for drainage and keep the soils at field capacity. Subsamples were located in 1-L mason jars and incubated for 3 years at 15°C. For flux measurements, all 24 jars

were located in a water bath and connected to an automatic soil incubation system (ASIS), see Bracho et al. (2016) for more details. CO_2 respiration rates were calculated as the rate of CO_2 increase in the jars' head space with time and were expressed as microgram CO_2-C of initial soil C per day (μg CO_2-C g^{-1} sample-C day^{-1}). The first measure of CO_2respiration rate was conducted approximately one week after the disturbance such as field sampling, sieving, and changes in temperature and moisture; thus, we considered that soil samples have been pre-incubated. Respiration rates were measured daily for the first week of incubation, twice in the second week, once every week in the following 14 weeks, and once every month up to 1 year of incubation and every 60–90 days to the 3 years of incubation.

2.3 Decomposition of individual SOC components

Soil organic carbon is composed of components with varying turnover times and usually represented by multiple C pools in models (Jenkinson & Rayner, 1977; Parton et al., 1987). We used a three-pool C decomposition model to estimate the proportion and decomposition rate constant of different SOC fractions using the following equation:

$$R = C_0 \times \left(f_L \times k_L \times e^{k_L \times t} + f_I \times k_I \times e^{k_I \times t} + f_R \times k_R \times e^{k_R \times t}\right), \quad (1)$$

where R is CO_2 respiration rate (mg CO_2-C g^{-1} soil day^{-1}) at time *t*, C_0 is initial SOC content (mg C g^{-1} soil), f_L, f_I, f_R, k_L, k_I, and k_R are the initial proportions and decomposition rate constants of the LOC, intermediate SOC (IOC), and resistant SOC (ROC) components, and the sum of f_L, f_I, and f_R is 1. The cumulative CO_2 respiration (CR) of the decomposition of SOC components and total SOC can be calculated using

$$CR = C_0 \times ((f_L \times (1 - e^{k_L \times t}) + f_I \times (1 - e^{k_I \times t}) + f_R \times (1 - e^{k_R \times t})).$$

The parameters in this model were estimated using Bayesian probabilistic inversion and the Metropolis–Hastings (M-H) algorithm (Xu, White, Hui, & Luo, 2006). Details about this inversion method can be found in published literature (Li, Zhou et al., 2013; Liang et al., 2015; Xu et al., 2006).

To assess the influences of the proportion and decomposition rate constant (i.e., f_L, f_I, f_R, k_L, k_I, and k_R) on SOC decomposition, we conducted sensitivity analysis of these parameters to total cumulative CO_2 emission in the four treatments (i.e., CI, WI, CC, and WC). We decreased each of the six parameters by 30% and calculated total SOC losses in 3, 10, and 50 years, respectively, then estimated the ratio of the SOC losses based on changed decomposition parameters to the SOC losses based on unchanged parameters.

2.4 Soil microbial community analyses

Field warming in our studied site led to decrease in SOC lability (Xu, Sherry et al., 2012) and may alter microbial community functions as well. We aimed to identify the links between

microbial community functional potentials and changes in SOC properties over incubation time and to test whether the links enhance or inhibit SOC decomposition. The characterization of SOC components during the incubations provided an opportunity to profile the microbial community in soils wherein C utilization and substrate quality were modeled. We assessed microbial communities at multiple times during decomposition to reveal temporal dynamics of their functional potentials. We used the GEOCHIP 5.0 to profile microbial functional genes to reflect a community's functional potential, particularly focusing on genes involved in C-degradation. One vial of soil was removed from each jar of the 24 treatments after 14, 90, and 270 days of incubation for microbial analysis. A PowerSoil® DNA isolation kit was used to extract microbial community DNA from soil subsamples (1–5 g) following standard procedures of the PowerSoil® DNA extraction kit (MoBio Laboratories, Inc, Carlsbad, CA, USA). In some samples, DNA of high purity (Nanodrop 260/280 and 260/230 absorbance ratios above 1.70) could not be obtained via the kit alone so a freeze-grind method was used to obtain raw DNA (Zhou, Bruns, & Tiedje, 1996), which was subsequently purified with the PowerSoil® kit. For this, 500 ng of soil community DNA was labeled and hybridized to GEOCHIP 5.0 60K microarrays, and scanned with a NimbleGen MS200 Microarray Scanner using techniques described previously (Cong et al., 2015). The image data were processed using the Agilent Feature Extraction program that designates values for probe signal intensities and background (noise) signal intensities based on the scanned images. Extracted data were then loaded onto an in-house GeoChip data analysis pipeline (ieg.ou.edu/microarray/). Data normalization and quality filtering were performed with multiple standard steps (Liang et al., 2010; Tu et al., 2014).

As our objective was to examine SOC decomposition, we focused on the data from probes targeting genes involved in C degradation (roughly 16,500 probes per sample). The signal intensities of only probes with positive signals were used to assess the abundance of C-degradation genes present in each sample. Then, we calculated the response ratio of signal (RR) intensity to warming using the equation: $R = \ln(I_{warm}/I_{ck})$, where I_{warm} and I_{ck} are the signal intensity of C-degradation probes shared in both warming and control plots. Lastly, we categorized probes based on the C-substrates utilized by the enzymes they were designed to target (e.g., starch, cellulose, and lignin) and calculated the mean of the response ratios of probes for each substrate type in each sample. The number of functional genes and their abundances (signal intensities) detected were used to calculate the Shannon–Weiner index (H'), Simpson's index (1/D), Simpson evenness index (E), and Pielou evenness (J') for the genes that are involved in C-degradation. Simpson's and Shannon–Weiner's indices can be used to estimate functional gene diversity (Liang et al., 2011; Xie et al., 2011) and characterize communities by accounting for abundance and evenness of functional genes present in each sample. Additionally, Simpson evenness and

Pielou evenness consider the ratio of observed diversity to maximum diversity. Indices for diversity and evenness were calculated in the R package, VEGAN, and equations are provided in Methods S1.

2.5 Statistical analyses

Before statistical analyses, the data were subjected to the Shapiro–Wilk test for normality and to the Levene test for homogeneity of variance. We used logarithmic transformation when data were not normally distributed. Two-way ANOVA were used to examine the effects of field warming and plant root C exclusion (collar) on the cumulative CO_2 respiration from the decomposition of total SOC and the three SOC components (i.e., LOC, IOC, and ROC), the proportion (i.e., f), decomposition rate constant (i.e., k), and turnover time (i.e., τ) of the three SOC components (SPSS 18.0, SPSS Inc., Chicago, IL, USA). We also conducted the Turkey multiple comparison to examine whether cumulative CO_2 respiration, the proportion of cumulative CO_2 respiration, and the f, k, and τ values of LOC, IOC, and ROC were statistically different among the four treatments (i.e., CI, WI, CC, and WC). One of our goals was to assess whether SOC decomposers from the field warmed plots were different from those from the control during decomposition, but microbial decomposers may vary over decomposition time. We conducted the three-way ANOVA to test whether field warming, plant C exclusion (collar), and incubation time affected the biodiversity of C-degradation microbial gene intensities. We found that incubation time significantly affected the signal intensity of C-degradation microbial genes, then performed the pairwise comparisons to test whether field warming significantly affected the signal intensity of microbial genes that degrade substrates from labile to recalcitrant C after 2 weeks, 3 months, and 9 months of incubation (SPSS 18.0; SPSS Inc.). To find the potential links between microbial community and SOC decomposition, we examined the correlations between CO_2 respiration rate and cumulative CO_2 respiration from the decomposition of SOC components and the diversity indexes of C-degradation microbial genes and the average intensity of microbial genes that degrade nine categories of organic compounds. The significance levels of all the statistical analyses were determined based on values of $p \leq .05$.

3 RESULTS

3.1 Decomposition of total SOC and SOC components

After a decade of field warming, soil LOC measured by the $KMnO_4$ oxidation method and the ratio of LOC to total SOC reduced significantly according to previous studies on the same site (Xu, Luo, & Zhou, 2012; Xu, Sherry et al., 2012) (Table 1). The 3-year incubation showed that cumulative CO_2 emission from the decomposition of total SOC was larger from the field

warming plots than from the control plots for both the intact and collar soils (Figure 1). The cumulative CO_2 emission from the decomposition of total SOC was slightly smaller for the intact soil than for the collar soils under the unwarmed condition (Figure 1). The inverse modeling results show that cumulative CO_2 emissions from the decomposition of LOC, IOC, and ROC made up 5.2 ± 1.0%, 54.0 ± 5.1%, and 40.8 ± 5.2% of total cumulative CO_2 emission in all the treatments (Figure 2). Field warming significantly accelerated cumulative CO_2emission from the decomposition of IOC, ROC, and total SOC, especially for the deep collar soils (Figure 3), while deep collar only markedly intensified cumulative CO_2 respiration from the IOC decomposition (Table 2). ROC with turnover times of hundreds of years made up of the vast majority of total SOC (Table 3). Field warming and deep collar demonstrated different impacts on the proportion (f) and decomposition rate constant (k) of the three SOC components (Table 3). Field warming had no impact on LOC proportions and significantly increased IOC proportions in the intact soils by 100% and in the deep collar soils by 240%, but significantly reduced ROC proportions in both the intact and deep collar soils (Table 3). Plant C exclusion (collar) did not significantly influence LOC proportions in soils from both the field warming and the control treatments, while it significantly increased ROC proportions in soils from the field warming treatment (Table 3). Comparing the responses of the decomposition three SOC components to field warming and plant C exclusion (collar), field warming significantly intensified the ROC decomposition by reducing its proportion and enhancing the decomposition rate constant. In contrast, plant C exclusion (collar) retarded the IOC decomposition in soils from the field warming treatment, by reducing the IOC proportion and increasing its decomposition rate constant (Table 3). The sensitivity analysis showed that among the six parameters (f_L, f_I, f_R, k_L, k_I, and k_R) in determining the decomposition of SOC from the four field treatments, the decomposition of total soil C is most sensitive to changes in the ROC proportion (f_R), followed by the ROC decomposition rate (k_R) (Fig. S1). We only observed the impacts of the IOC proportion (f_I) and decomposition rate (k_I) on C decomposition of bulk soil in the 3-year projection (Fig. S1). These results suggest that changes in the pool size and turnover time of ROC drove the amount of total heterotrophic respiration, while the changes in the pool size and turnover time of LOC and IOC were less important than ROC.

Table 1. The characteristics of soil C and N after subjected to field warming and plant C exclusion for 12 years. Four treatments: control + intact soil (CI), warm + intact soil (WI), control + collar soil (CC), and warm + collar soil (WC)

Treatment	TOC[a] (mg C g^{-1} soil)	Total N[a] (mg/g)	C:N[a]	LOC[b] (kg C m^{-2})	LOC/TOC[b]
CI	12.42 ± 0.17^{a}	1.13 ± 0.14^{a}	10.95 ± 0.47^{a}	58.6 ± 1.0^{a}	3.69 ± 0.18^{a}
WI	9.72 ± 0.11^{a}	0.90 ± 0.10^{ab}	10.84 ± 0.52^{a}	69.4 ± 1.0^{b}	4.42 ± 0.09^{b}
CC	10.50 ± 0.21^{a}	0.98 ± 0.16^{ab}	10.61 ± 1.27^{a}		
WC	7.47 ± 0.09^{b}	0.72 ± 0.09^{b}	10.54 ± 0.70^{a}		

- TOC, total soil organic carbon; LOC, labile soil organic carbon.
- Different superscript letters mean values are statistically different at the level of 0.05.
- a Data are from Xu, Luo et al. (2012).
- b Data are from Xu, Sherry et al. (2012).

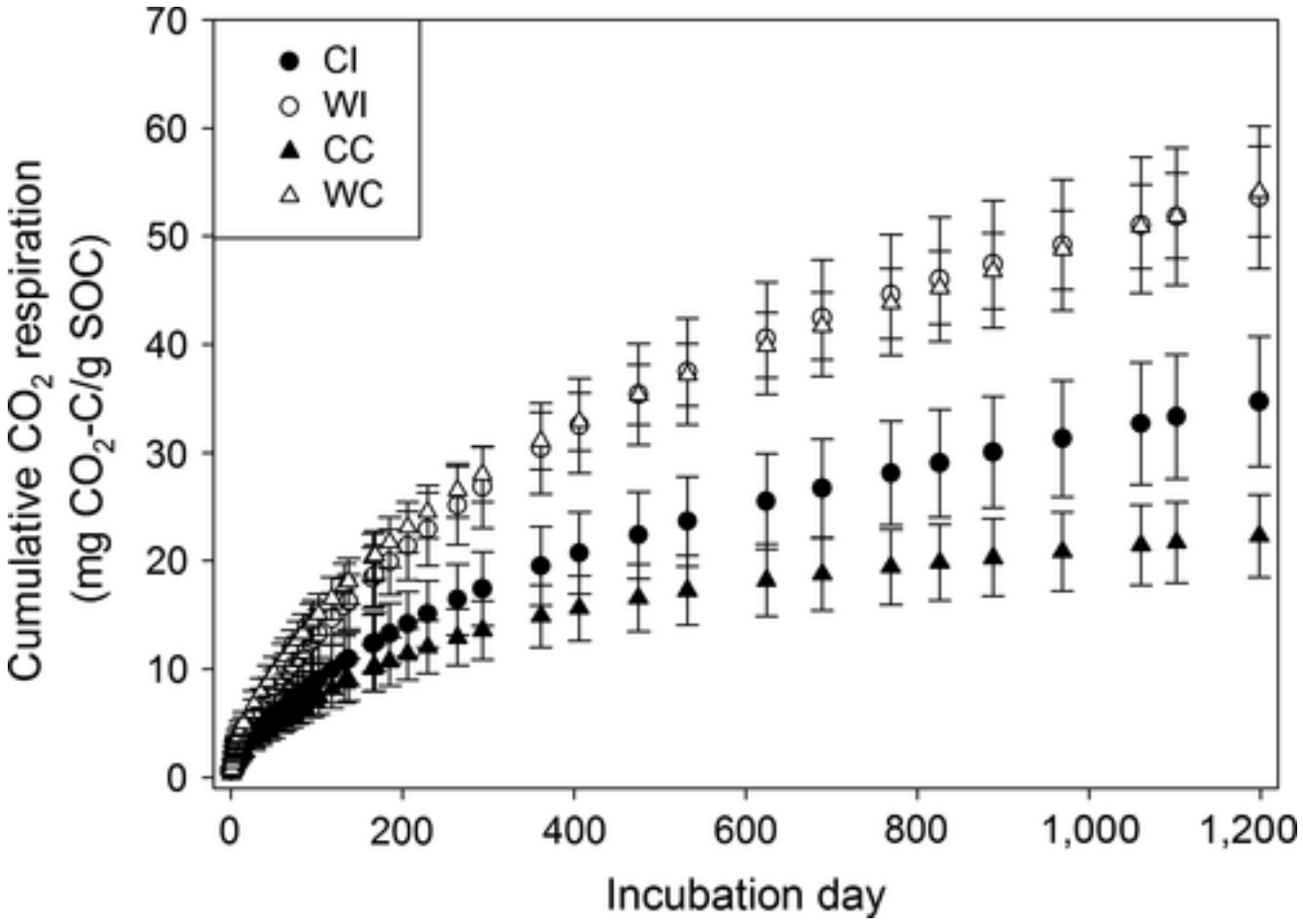

Figure 1

Open in figure viewerPowerPoint

Cumulative CO_2 respiration of soils from the four field treatments over the 3-year laboratory incubation. CI: control + intact soil; WI: warm + intact soil; CC: control + collar soil; WC: warm + collar soil. Data are mean ± *SE* (*n* = 6)

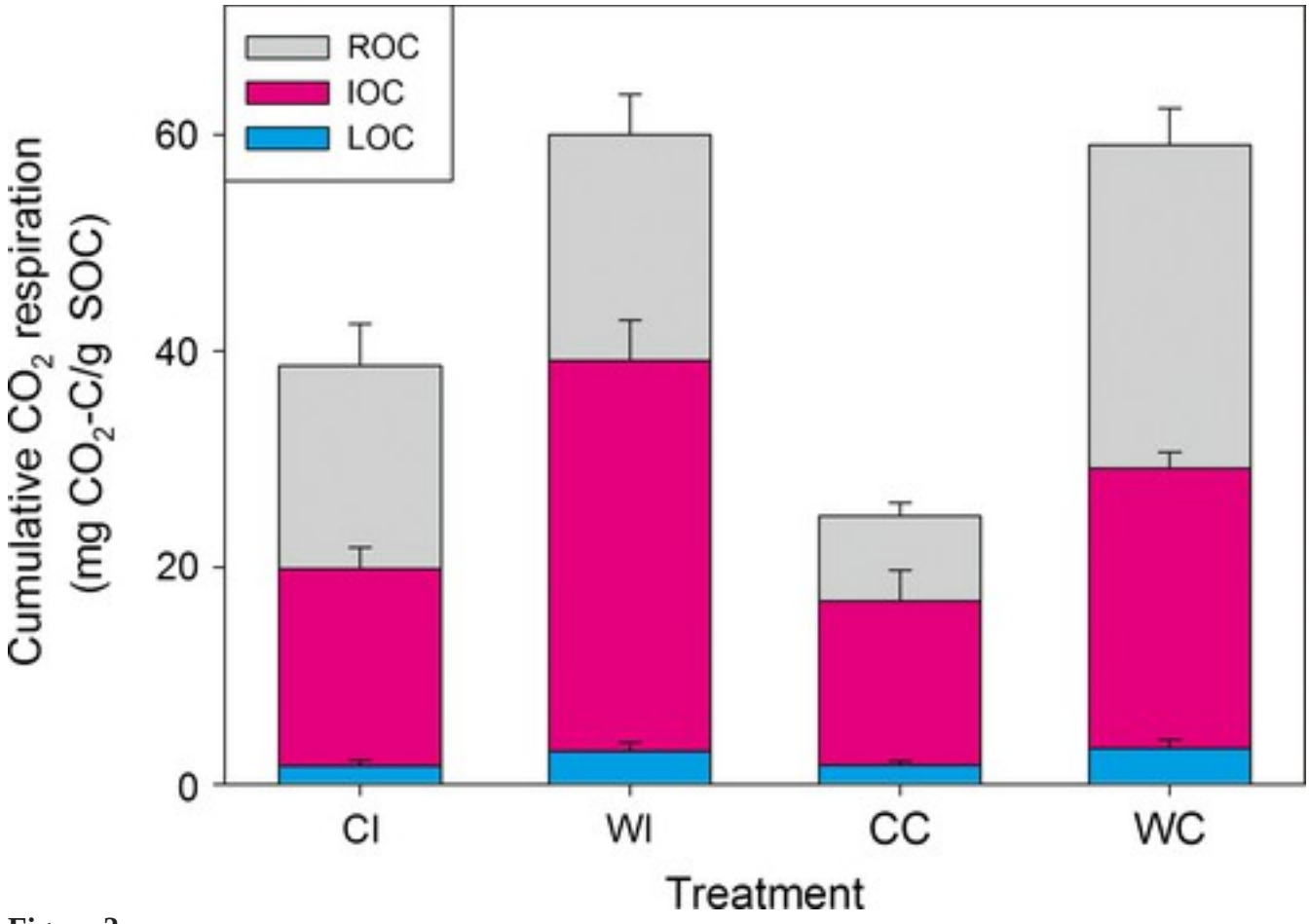

Figure 2

Open in figure viewerPowerPoint

Cumulative CO_2 respiration from the decomposition of SOC components in the four treatments after the 3-year incubation. LOC: labile SOC; IOC: intermediate SOC; ROC: resistant SOC. CI: control + intact soil; WI: warm + intact soil; CC: control + collar soil; WC: warm + collar soil. Bars are mean ± *SE* (*n* = 6) [Colour figure can be viewed at wileyonlinelibrary.com]

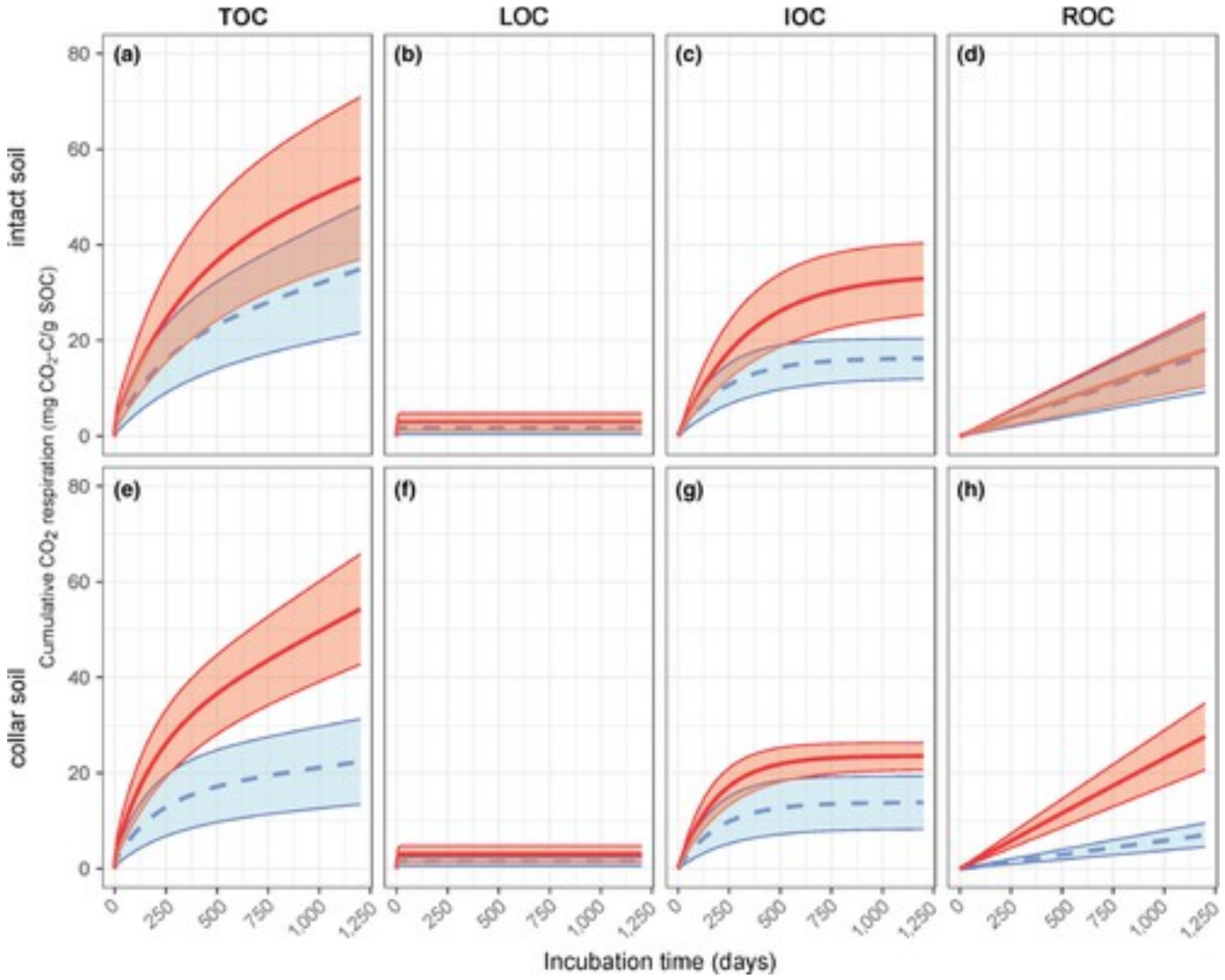

Figure 3

Open in figure viewerPowerPoint

Modeled cumulative CO_2 respiration from the decomposition of SOC components of the intact (a–d) and the deep collar soils (e–h) over the 3-year incubation. LOC: labile SOC; IOC: intermediate SOC; ROC: resistant SOC; TOC: total SOC. Red lines refer to field warming, and blue lines refer to the control. Shade areas refer to 95% confidence interval

Table 2. Multiple comparison and two-way ANOVA results of cumulative CO_2 respiration and their proportions from the decomposition of SOC components after the 3-year incubation

Treatment	Cumulative CO_2 respiration (mg CO_2-g g^{-1} SOC)				Proportion of cumulative CO_2respiration		
	Labile SOC	Intermediate SOC	Resistant SOC	Total SOC	Labile SOC	Intermediate SOC	Resistant SOC
Control + intact	1.6 ± 0.6[a]	16.1 ± 2.1[b]	17.0 ± 3.9[ab]	34.7 ± 6.0[ab]	4.1 ± 0.7%[a]	48.1 ± 3.5%[a]	47.8 ± 3.0
Warm + intact	2.9 ± 0.9[a]	32.7 ± 3.6[a]	18.0 ± 3.8[ab]	53.6 ± 6.6[a]	5.1 ± 0.9%[a]	62.3 ± 4.8%[a]	32.6 ± 4.6
Control + collar	1.5 ± 0.5[a]	13.8 ± 2.8[b]	7.0 ± 1.2[b]	22.3 ± 3.8[b]	6.1 ± 1.0%[a]	59.5 ± 5.2%[a]	34.4 ± 5.6
Warm + collar	3.1 ± 0.9[a]	23.5 ± 1.4[ab]	27.5 ± 3.5[a]	54.1 ± 4.2[a]	5.4 ± 1.0%[a]	44.4 ± 3.7%[a]	50.2 ± 3.6
ANOVA factor	*p*-Values						
Warm	.067	**<.001***	**.005***	**<.001***	.873	.924	.950
Collar	.969	**.046***	.952	.289	.233	.479	.640
Warm × collar	.870	.223	**.009***	.252	.372	**.004***	**.002***

- Different lower case letters mean that values are statistically different among treatments.
- * and bold represent $p < .05$. Data are mean ± *SE* ($n = 6$).

Table 3. Statistics of model fit to CO_2 respiration during incubation in the four treatments and their two-way ANOVA results. f_L, f_I, f_R, k_L, k_I, k_R, τ_L, τ_I, and τ_R are the proportion, decomposition rate constant, and turnover time of labile, intermediate, and resistant SOC components

Treatment	f_L	f_I	f_R	k_L (day^{-1})	$k_I \times 10^{-3}$(day^{-1})	$k_R \times 10^{-5}$(day^{-1})
Control + intact	0.10 ± 0.06%^{a}	1.63 ± 0.21%^{b}	98.21 ± 0.25%^{ab}	0.31 ± 0.02^{a}	4.29 ± 0.57^{ab}	1.46 ± 0.34^{b}
Warm + intact	0.29 ± 0.09%^{a}	3.39 ± 0.36%^{a}	96.31 ± 0.43%^{c}	0.25 ± 0.03^{ab}	2.95 ± 0.29^{b}	1.58 ± 0.34^{ab}
Control + collar	0.15 ± 0.05%^{a}	1.39 ± 0.28%^{c}	98.46 ± 0.32%^{a}	0.20 ± 0.02^{b}	4.99 ± 0.52^{a}	0.60 ± 0.11^{c}
Warm + collar	0.31 ± 0.09%^{a}	2.36 ± 0.14%^{b}	97.33 ± 0.19%^{b}	0.20 ± 0.03^{b}	5.24 ± 0.60^{a}	2.40 ± 0.31^{a}
ANOVA factor	*p*-Values					
Warm	.067	**<.001***	**<.001***	.325	.309	**.004***
Collar	.969	**.029***	.063	**.010***	**.010***	.941
Warm × collar	.870	.163	.253	.251	.142	**.010***

- Different lower case letters mean that values are statistically different among treatments.
- * and bold represent $p < .05$. Data are mean ± *SE* ($n = 6$).

3.2 Soil microbial community under warming and plant C exclusion

Neither warming nor deep collar significantly affected the diversity of C-degradation microbial genes, but incubation time substantially impacted the diversity (Figure 4). The diversity indexes of C-degradation microbial genes were smaller after 9 months of the incubation compared to those after 2 weeks or 3 months of the incubation (Figure 4), suggesting that the microbial

community shifted in response to changing C substrate quality over time. Meanwhile, the response ratios (warm/control) of the signal intensity of C-degradation microbial genes also changed temporally in both the intact and collar soils (Figure 5), implying that the variance in microbial community C-degradation functional potentials became clearer as C substrate quality lowered. In addition, we observed the positive response ratio values (warm/control) of the signal intensity of C-degradation microbial genes in the intact soils after 9 months of the incubation (Figure 5a), suggesting that the depletion of LOC over time probably stimulated the portion of the microbial community containing genes that are responsible for the degradation of complex SOC, such as cellulose, hemicellulose, lignin, chitin, and terpenes. Differently, in the deep collar soils there was no significant difference of the signal intensity of C-degradation microbial genes from warmed plots compared to the control in any time during the incubation (Figure 5).

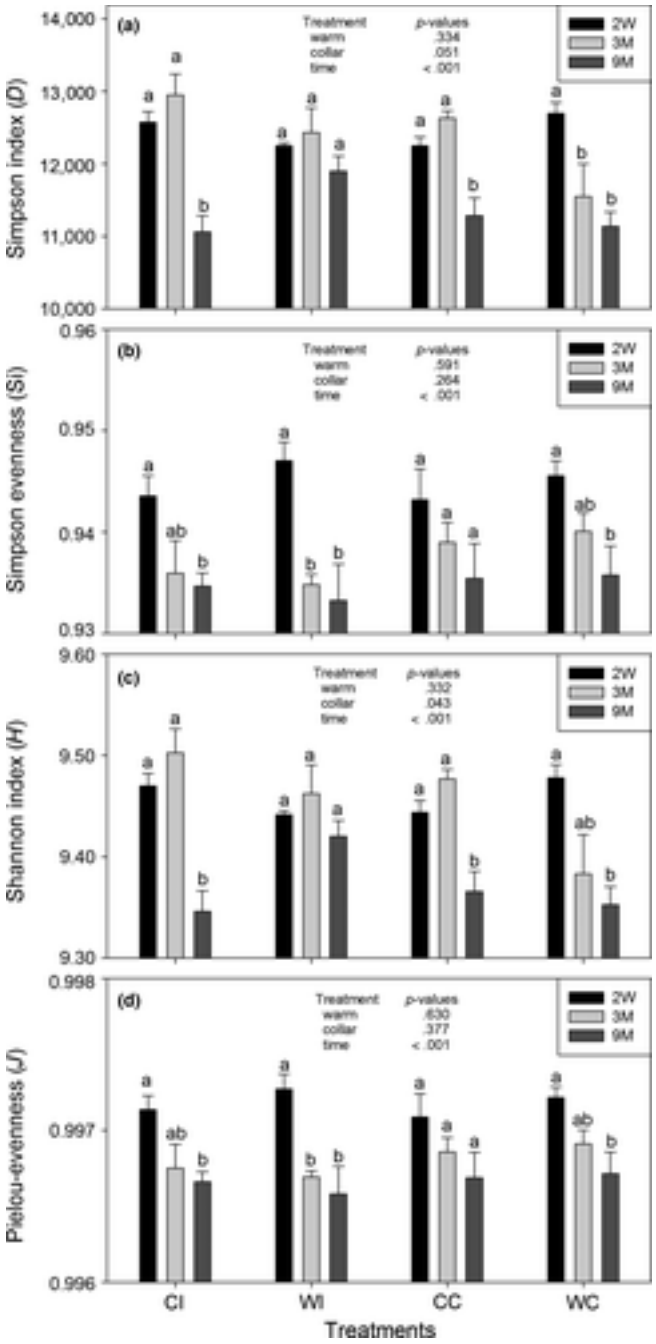

Figure 4

Open in figure viewerPowerPoint

Diversity (a–d) of C-degradation microbial genes estimated two weeks (2W), three months (3M), and nine months (9M) after incubation in the four treatments. CI: control + intact soil; WI: warm + intact soil; CC: control + collar soil; WC: warm + collar soil. Different letters mean statistical difference among the three times in each treatment

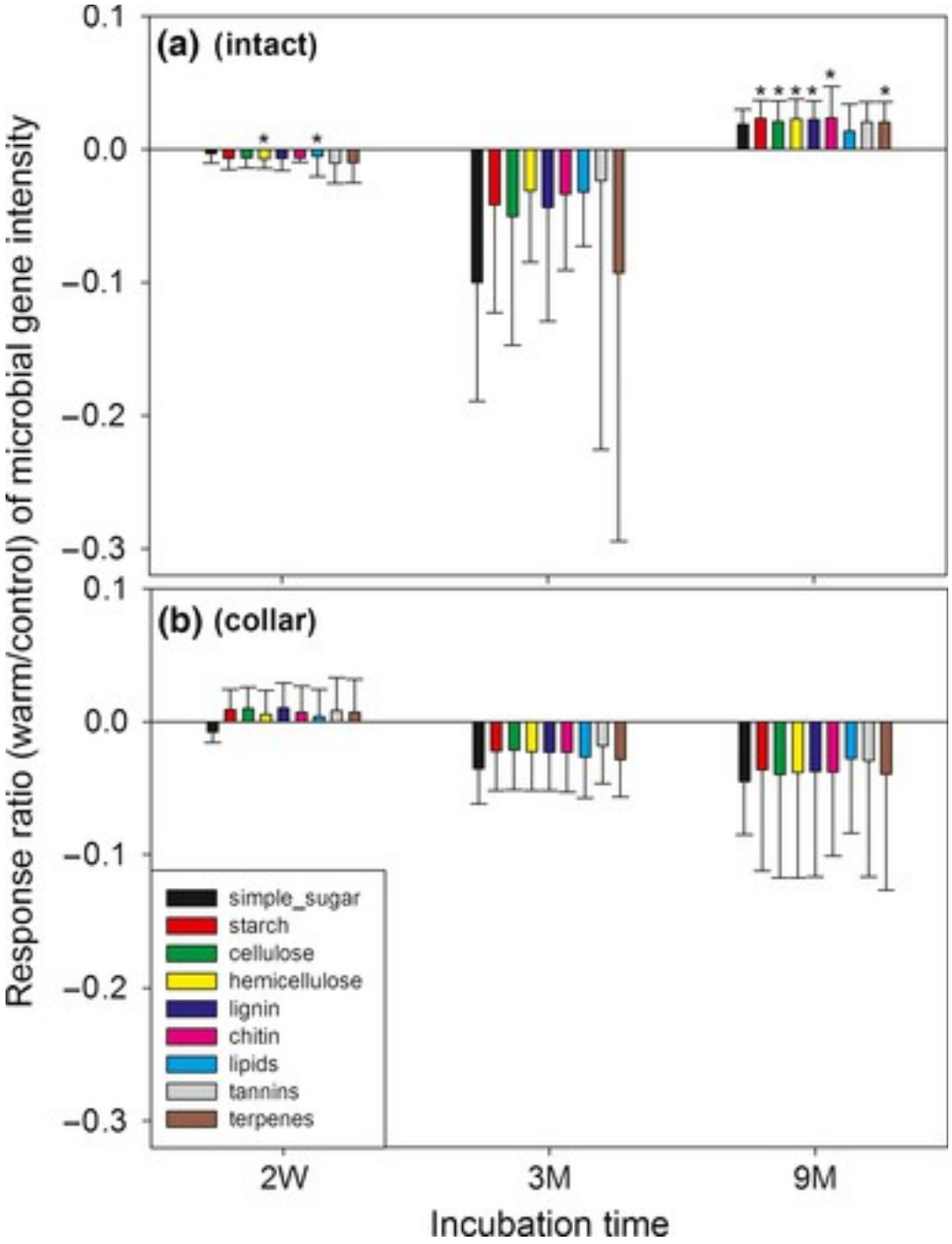

Figure 5

Open in figure viewerPowerPoint

The response ratio of signal intensities of microbial genes for the degradation of various organic compounds to warming in intact soils (a) and deep collar (b) soils over the 3-year incubation. 2W, 3M, and 9M are two weeks, three months, and nine months after the laboratory incubation. *represents $p < .05$

The diversity indexes of C-degradation microbial genes were negatively correlated with cumulative CO_2 respiration of the ROC decomposition and positively correlated with CO_2respiration rate of the LOC decomposition over the incubation time (Table 4). These

correlations suggest C-degradation microbial genes became more heterogeneous when soil C quality reduced. Meanwhile, the microbial genes that degrade complex organic compounds (e.g., lignin and terpenes) were positively with cumulative CO_2 respiration of the ROC decomposition, and negatively correlated with CO_2 respiration rate of the LOC decomposition over incubation time (Table 4). The results suggest reduced soil C quality could stimulate microbial potentials to degrade complex SOC.

Table 4. Correlations between CO_2 respiration of the decomposition of SOC components and the diversity and intensity of C-degradation microbial genes during the laboratory incubation

Microbial community	**Cumulative CO_2 respiration**				**CO_2 respiration rate**			
	LOC	**IOC**	**ROC**	**TOC**	**LOC**	**IOC**	**ROC**	**TOC**
Biodiversity indexes								
Shannon H	−0.08	**−0.40***	**−0.59***	**−0.44***	0.22	**0.30***	−0.17	**0.24***
Simpson evenness D	−0.08	**−0.42***	**−0.59***	**−0.46***	**0.24***	**0.31***	−0.16	**0.26***
Pielou evenness J	−0.05	**−0.47***	**−0.55***	**−0.48***	**0.47***	**0.35***	−0.07	**0.36***
Simpson Si	−0.06	**−0.46***	**−0.55***	**−0.47***	**0.46***	**0.34***	−0.08	**0.35***
Nine categories of C-degradation microbial genes								
Simple sugar	−0.07	0.08	0.18	0.09	−0.12	−0.22	−0.01	−0.20
Starch	−0.04	**0.32***	**0.39***	**0.32***	**−0.45***	**−0.27***	−0.06	**−0.29***

Microbial community	Cumulative CO_2 respiration				CO_2 respiration rate			
	LOC	IOC	ROC	TOC	LOC	IOC	ROC	TOC
Cellulose	0.14	**0.24***	0.21	**0.24***	−0.20	0.03	0.19	0.04
Hemi-cellulose	−0.05	0.12	0.19	0.13	−0.18	−0.15	0.03	−0.15
Lignin	0.10	**0.36***	**0.31***	**0.35***	**−0.39***	−0.08	−0.01	−0.09
Chitin	0.04	**0.34***	**0.41***	**0.35***	**−0.46***	**−0.28***	−0.01	**−0.29***
Lipids	−0.12	−0.07	−0.14	−0.10	0.11	0.08	−0.03	0.02
Tannin	0.16	0.18	**0.39***	**0.25***	−0.18	**−0.24***	0.17	−0.15
Terpernes	−0.10	0.19	0.23	0.18	**−0.27***	**−0.27***	−0.09	**−0.29***

- LOC, labile SOC; IOC, intermediate SOC; ROC, resistant SOC; TOC, total SOC.
- * and bold represent $p < .05$.

4 DISCUSSION

4.1 Warming effects on SOC decomposition

We hypothesized that the decomposition of existing SOC would be retarded after 12 years of field warming in the studied temperate grassland as a result of LOC depletion and reduced SOC lability and stock (Xu, Sherry et al., 2012; Table 1). However, our results showed that the C decomposition of soils that were subjected to the long-term field warming became more intensive compared to that from the unwarmed plots, especially for ROC (Figures 2 and 3), which contrasts with our hypothesis. The accelerated SOC decomposition probably occurred because ROC proportions (f_R) became smaller, ROC decomposition rate (k_R) became higher, and IOC proportion (f_I) became larger under warming (Table 3). These changes facilitated the decomposition of total SOC. The changes in these three parameters indeed had great impacts on the decomposition of total SOC, as the sensitivity analyses showed that the decomposition of total SOC was sensitive to the ROC proportion and decomposition rate and the IOC proportion (f_R, k_R, and f_I) (Fig. S1). This finding is not surprising as SOC with long turnover times typically makes up the majority of total SOC (Table 3). Numerous studies have demonstrated that SOC components with long turnover times, such as mineral-associated organic C, aggregate-protected C, or C in the heavy soil fraction, account for at least half of total SOC (Marín-Spiotta, Silver, Swanston, & Ostertag, 2009; Feng et al., 2014; Cai et al., 2016). In spite of long turnover time, ROC can make great contribution to heterotrophic respiration because of the large pool size. We found that CO_2 emission from the decomposition of IOC and ROC made up the vast majority (~95%), while CO_2 emission from the decomposition of LOC only accounted for about 5% of total cumulative CO_2emission during the 3-year incubation (Fig. S2). Other warming studies that have used C isotopes to differentiate relative contributions of SOC sources to soil CO_2 efflux also showed CO_2 efflux from the decomposition of old soil C comprised a large proportion of total soil CO_2 efflux (27.3%–59%; Hopkins et al., 2012; Lin, Zhu, & Cheng, 2015).

In this study, after subjected to the long-term field warming how the decomposition of LOC and IOC respond was different from ROC. The IOC decomposition from the warmed plots also accelerated compared to the control, which constituted half of the total cumulative CO_2emission over the three-year incubation (Figure 2). But the sensitivity analysis results demonstrate that changes in IOC only influenced total cumulative CO_2 emission in short term (3 years). Meanwhile, the 12-year warming did not significantly affect the LOC decomposition (Figures 2 and 3; Table 2), implying that changes in the decomposition of SOC components with turnover times of days have marginal impacts on heterotrophic soil respiration. Given different responses of the decomposition of LOC, IOC, and ROC after subjected to the 12-year field warming (Figures 2 and 3), it is necessary to assess changes in the proportion and turnover rate of specific SOC component for the accurate projection of soil C decomposition under planetary warming. Our findings indicate that we should pay more attention to disturbances and

management that could impact the pool size of SOC components with long turnover time rather than those that affect SOC components with fast turnover time, because the former may have greater contribution to the heterotrophic soil respiration than the latter in the long term.

4.2 Effects of plant C exclusion on SOC decomposition

Deep collar installation cut off plant root C inputs. Plus, regular removal of plants that were growing within collar excluded aboveground plant C inputs. Excluding plant C inputs decreased SOC content, as LOC and the ratio of LOC to total SOC have been found to decline under field warming in this study site (Table 1). However, excluding plant C inputs (collar) did not significantly reduce SOC decomposition especially under warming (Figures 1-3; Tables 2and 3). Plant C exclusion tended to reduce the decomposition of all the three SOC components in the control, but it increased the ROC decomposition and decreased the IOC decomposition under warming (Figures 2 and 3; Table 2). The reason could be that reduced C substrate supply due to deep collar installation limited microbial activity, while warming stimulated microbial community to utilize the remaining recalcitrant C in soils. Accelerated ROC decomposition by plant C exclusion was pronounced under warming (Figures 2 and 3; Table 3), supporting the hypothesis that the microbial community changed C-degradation functions or shifted to the species that can more effectively degrade ROC at higher temperature. The results suggest that interactions between warming and plant C exclusion played a role in the ROC decomposition.

The IOC and ROC decomposition with plant C exclusion differ across studies, which might be due to varying experimental lengths. Plant C exclusion is commonly conducted by trenching which generates root debris (Luo et al., 2001; Schaefer, Feng, & Zou, 2009). The decomposition of root debris may replenish the cut-off of C supply to microbes at early stages of an experiment (1–2 years), leading to no significant changes in the heterotrophic soil respiration (Li, Schaedel et al., 2013; Schaefer et al., 2009). As the decomposition of root debris and existing SOC proceeds, the shortage of C supply to microbes may induce reduced soil CO_2 efflux (Eliasson et al., 2005; Hartley et al., 2007; Li, Schaedel et al., 2013). Later on, microbial communities may shift to greater abundances of microorganisms that can fully utilize low-quality C, consequently leading to accelerated SOC decomposition. Altogether, divergence in how SOC decomposition responds to plant C exclusion is related to the length of an experiment, which regulates the quantity and quality of C-substrates and microbial community.

4.3 Soil microbial community to warming and plant C exclusion

Soil microbial communities have been found to change under warming or with altered substrate quality (Billings & Ballantyne, 2013; Xue et al., 2016; Zhou et al., 2012). A functional gene

array, GeoChip, provides information pertaining to microbial genes that are linked to the degradation of organic C compound ranging from labile to recalcitrant (Wu et al., 2006; Zhou et al., 2012). The GeoChip analysis showed that the diversity of C-degradation microbial genes significantly decreased with incubation time (Figure 4), suggesting the shift to favor some microbial functional groups that can utilize complex soil C or a loss in many organisms that thrive in soils with higher SOC availability. The diversity indexes (e.g., Simpson evenness) of C-degradation microbial genes were negatively correlated with cumulative CO_2 respiration of the ROC decomposition over the incubation time (Table 4), suggesting that the utilization of complex soil C was accompanied with C-degradation microbial genes of high heterogeneity. This shift may reflect the adaptation of microbial community to altered substrates over time. The depletion of soil LOC over time may select microbial communities that utilize recalcitrant soil C, so it is not surprising that we observed declined diversity of microbial C-degradation genes. The diversity of soil biota groups typically declines with increasing stress or disturbances (Wardle, 2002).

After 9 months of incubation, the signal intensities of microbial genes that target C-degradation of complex compounds (e.g., lignin, chitin, and terpenes) were greater for the intact soils from field warming plots compared to the unwarmed (Figure 5a); however, the same trend was not observed for soils from the deep collars. A potential explanation for this may be that within the deep collars the microbial microbial communities in both warmed and control treatments were already under selective pressure to favor microbes with ROC degradation potentials. The field warming may also be exerting a similar pressure, but not to a significantly detectable level. However, as the intact soil microbial communities underwent laboratory incubations ROC became a larger component of total SOC, resulting in shifts in the microbial communities. Because microbial communities in the intact, warmed treatment were already slightly primed to degrade ROC, the genetic potential was already higher in these communities, resulting in a more drastic temporal increase of microbial C-degradation genes as compared to those in the intact and control treatment. This suggests that soil microbes have great potentials to decompose complex soil C, ultimately leading to more CO_2 emission in soils that have been subjected to long-term field warming. This could be the reason for the accelerated IOC decomposition under warming, especially enhanced turnover rate of this SOC component (Figure 3; Table 2). Altered microbial community structure and functions have been suggested to change the turnover rate of soil C (Barnard, Osborne, & Firestone, 2015).

Knowledge on whether altered microbial communities impact SOC turnover is critical for how to explicitly integrate microbial community to SOC models. Thus, understanding and quantifying

the relations between microbial functions and SOC turnover could be one way to improve the prediction of SOC dynamics under warming. This study shows that although the diversity of C-degradation microbial genes in soils was reduced as incubation proceeded, microbial functions responded differently to warming, implying that microbial community functional diversity is not always positively correlated microbial functions. Also the intensity of microbial genes that degrade complex C was positively correlated with cumulative CO_2respiration of the ROC decomposition and negatively correlated with CO_2 respiration rate of the LOC decomposition (Table 4), suggesting that there are varying potential links between soil microbial community and the decomposition of SOC constituents. When integrating microbial community data into SOC dynamic models, the complexity of these relationships must be considered. We are aware that although the GeoChip technique can provide us comprehensive information about microbial function potentials in degrading a variety of organic compounds, it cannot inform us of the relative contribution of specific microbial genes in decaying SOC which is composed of various organic substances. More studies are required to link the genomic information of soil microbes with soil C decomposition processes and quantify their relations.

ACKNOWLEDGEMENTS

We thank anonymous reviewers for their insightful comments and suggestions on this article. We also thank Dr. Xin Jing for his help in making figures. This study is financially supported by the United States Department of Energy, Terrestrial Ecosystem Sciences grant DESC0008270, and Biological Systems Research on the Role of Microbial Communities in Carbon Cycling Program grants DESC0004601 and DE-SC0010715.