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Soil Organic Carbon Mineralization as Affected by Cyclical Temperature Fluctuations in a Karst Region of Southwestern China

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ABSTRACT

The diurnal fluctuation in soil temperature may influence soil organic carbon (SOC) mineralization, but there is no consensus on SOC mineralization response to the cyclical fluctuation in soil temperature. A 56-d incubation experiment was conducted to investigate the effects of constant and variable temperatures on SOC mineralization. Three soils were collected from the karst region in western Guizhou Province, southwestern China, including a limestone soil under forest, a limestone soil under crops and a yellow soil under crops. According to the World Reference Base (WRB) classification, the two limestone soils were classified as Haplic Luvisols and the yellow soil as a Dystric Luvisol. These soils were incubated at three constant temperatures (15, 20 and 25 °C) and cyclically fluctuating temperatures (diurnal cycle between 15 and 25 °C). The results showed that the 56-d cumulative SOC mineralized (C_{56}) at the fluctuating temperatures was between those at constant 15 and 25 °C, suggesting that the cumulative SOC mineralization was restricted by temperature range. The SOC mineralization responses to the fluctuating temperatures were different among the three soils, especially in contrast to those at constant 20 °C. Compared with constant 20 °C, significant ($P < 0.05$) decreases and increases in C_{56} value were found in the limestone soil under forest and yellow soil under crops at the fluctuating temperatures, respectively. At the fluctuating temperatures, the forest soil with lower temperature coefficient Q_{10} (the relative change in SOC mineralization rate as a result of increasing the temperature by 10 °C) had a significantly ($P < 0.05$) lower SOC mineralization intensity than the two cropland soils. These indicated that differences in temperature pattern (constant or fluctuating) could significantly influence SOC mineralization, and SOC mineralization responses to the fluctuating temperatures might be affected by soil characteristics. Moreover, the warmer temperatures might improve the ability of soil microbes to decompose the recalcitrant SOC fraction, and cyclical fluctuations in temperature could influence SOC mineralization through changing the labile SOC pool size and the mineralization rate of the recalcitrant SOC in soils.

Key Words: labile C, limestone soil, recalcitrant C, temperature coefficient (Q_{10}), yellow soil

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INTRODUCTION

Soil organic carbon (SOC) is the largest component of the global terrestrial carbon (C) pool, and plays an important role in the global C cycle (Lal, 2004). The SOC mineralization processes are central to the functions of soils in relation to feedbacks to atmospheric CO_2 concentration, sustainable nutrient supply, soil structural stability and supporting biodiversity (Paterson and Sim, 2013). The SOC mineralization depends on the nature and abundance of SOC and climatic factors, in particular temperature and moisture, which influence SOC mineralization processes through their effects on microbial activity (Leirós *et al.*, 1999).

Temperature is a major controller of SOC mineralization (Conant *et al.*, 2011). A significant positive correlation between soil temperature and SOC mineralization rate has been well established (Kirschbaum, 1995; Gudasz *et al.*, 2010). Most of the recent studies investigating the response of SOC mineralization rate to temperature were based on laboratory incubations, where the incubation temperature was maintained at a constant level throughout the entire experiment duration (Reichstein *et al.*, 2000; Conant *et al.*, 2008; Schütt *et al.*, 2014). However, soil temperature under field conditions is not constant and continuously changing as influenced by climate and other environmental conditions (*e.g.*, diurnal temperature variation). In order to accurately predict and estimate the SOC mineralization potential in the field, it is necessary to understand the response of SOC mineralization to the variation in soil temperature. However, there is no gene-

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ral consensus on the relationship between cyclical temperature variation and SOC mineralization process (Lomander *et al.*, 1998; Zhu and Cheng, 2011).

Soil organic C can be divided into labile and recalcitrant SOC fractions based on the relative susceptibility to biological decomposition (McLauchlan and Hobbie, 2004). The SOC mineralization is significantly affected by changes in SOC fractions (Fang *et al.*, 2005). Modeling the mineralization kinetics based on laboratory incubation showed that the ratio of labile SOC to recalcitrant SOC is significantly affected by temperature and increases with increasing incubation temperature (Haddix *et al.*, 2011). The ratio between the labile and recalcitrant SOC fractions is also affected by changes in land use, with an obvious increase of recalcitrant SOC found during 77 years after land use change from cropland to permanent grassland (Lopes de Gerenyu *et al.*, 2008).

The SOC mineralization sensitivity to temperature change is commonly evaluated by the temperature coefficient Q_{10}, which represents the relative change in SOC mineralization rate as a result of increasing the temperature by 10 °C (Vanhala *et al.*, 2008). An incubation experiment showed that the Q_{10} values for SOC mineralization were not affected by changing temperature and incubation time (Fang *et al.*, 2005). However, some recent studies demonstrated that the Q_{10} values for SOC mineralization were influenced by land use types, soil moisture, vegetation and climate zones (Wang *et al.*, 2008; Kurganova *et al.*, 2012; Li *et al.*, 2014). Thus, the response of SOC mineralization to temperature change is certainly associated with other surrounding environmental conditions controlling SOC stability (Schmidt *et al.*, 2011).

There is an obvious day-night temperature fluctuation in the karst plateau region in western Guizhou Province, southwestern China (Chi *et al.*, 2012). Moreover, the soils in the karst region are characterized by exposure of significant amounts of rock at the soil surface, thin soil layer, soil disturbance, and low soil water-holding capacity. These soil conditions can reduce soil buffering capacity against changes in climate conditions (*e.g.*, temperature change) (Smart *et al.*, 1986; Li *et al.*, 2005). Therefore, quantifying SOC mineralization in response to changes in soil temperature in the karst area can contribute to accurately estimating CO_2 emission from soils under different land use and management practices, and determining which soil is a C sink or source. The study hypothesis is that differences in temperature pattern (constant or fluctuating) could influence SOC mineralization even if the cumulative temperatures of fluctuating and constant temperatures were the same. The objectives of this study were to quantify the responses of SOC mineralization to constant and fluctuating temperatures in three typical karst soils from western Guizhou and examine the effects of diurnal temperature variations on SOC mineralization.

MATERIALS AND METHODS

Site description and soil sampling

Soil samples for this study were collected from the Tianlong Mountain area (26°14′ N, 105°45′ E) in Puding County of western Guizhou Province, a typical karst mountainous region of the Yungui Plateau, southwestern China (Zhang *et al.*, 2010). The study area has a northern subtropical wet monsoon climate. The mean annual temperature is 15.1 °C. The highest average monthly temperature is 23 °C in July, and the lowest is 5.2 °C in January. The mean annual precipitation is 1 378.2 mm, mean annual evaporation is 900 mm, mean annual sunshine is 1 164.9 h, frost-free season lasts about 300 d, and effective mean annual accumulated temperature (> 10 °C) is 4 258.2 °C. The altitude ranges from 1 100 to 1 600 m, and the average altitude is 1 350 m. The main types of land use in this area are forest and cropland. Evergreen broad-leaf forest and deciduous broad-leaf forest are the dominant natural vegetation in the forest. Corn is the main crop grown in the cropland. The main soil types in this area are limestone soil and yellow soil formed on the same parent material (limestone). The yellow soil formation is characterized by strong desilicification and allitization, which can lead to the formation of acid soil on limestone parent material. Limestone and yellow soils are also the main soil types in the karst region of Guizhou Province, China (Piao *et al.*, 2000). In the study area, the limestone soil is the main soil type of forest and cropland, but the yellow soil is mainly in row crop production.

Three sites of various land use and soil classification, limestone soils under forest and crops and yellow soil under crops, were selected to collect topsoil (0–10 cm layer) (Table I), and the area selected for soil sampling at each site was approximately 400 m^2. The current cropping system on the cropland was continuous corn planted in late May every year. Traditional plow tillage with hand tools or tools pulled by animals and nitrogen fertilizer in the range of 225 to 375 kg N ha^{-1} were applied to corn on the cropland. A composite soil sample (about 10 kg) was prepared for each site from

21 topsoil samples that were collected with a spade in an S-shaped pattern across the sampling area at each site and were uniformly mixed on May 10, 2013. After removal of roots and plant debris, approximately 500 g of fresh moist soil was taken from each composite soil sample, sieved through a 2-mm mesh sieve and stored at 4 °C until laboratory incubation. The remaining of the soil samples were air-dried and ground to pass through a 2-mm mesh sieve before chemical analyses. The basic soil properties are summarized in Table II.

Soil chemical analyses

The air-dried and sieved (2 mm) soil samples were used for determination of pH, available N, available P, available K and clay content. Soil pH was measured with a Metter-Toledo pH meter at a soil:water ratio of 1:2.5 (weight:volume) (Lu, 1999). Available N (alkaline-hydrolyzable N) in soil was determined using a micro-diffusion technique after alkaline hydrolysis (ISSAS, 1978). Available P in soil was extracted with 0.5 mol L^{-1} $NaHCO_3$ solution (pH 8.5) and determined by the Olsen method (Olsen *et al.*, 1954). Available K was extracted with 1 mol L^{-1} NH_4Ac and measured with a flame photometer (AP1200, Shanghai Precision Instrument, Shanghai, China) (Lu, 1999). Clay content was determined using a standard pipette method (Lu, 1999).

For determination of SOC, total N, total P and total K concentrations in soil, the air-dried soil samples sieved to < 2 mm were ground to pass through a 150-μm (100-mesh) plastic sieve. The SOC was measured using the dichromate oxidation method (Nelson and Sommers, 1996). Soil total N (TN) was determined using the semi-micro Kjeldahl method (Bremner and Mulvaney, 1982). Soil total P (TP) was determined colorimetrically after wet digestion with sulphuric and perchloric acids (Parkinson and Allen, 1975). Soil total K (TK) was determined after digestion with hydrofluoric and perchloric acids (Lu, 1999). Microbial biomass C (MBC) in the fresh moist soils was estimated using the fumigation-extraction (FE) method (Vance *et al.*, 1987).

Laboratory incubation

During the incubation of the soil samples in laboratory, four temperature regimes were established: 1) constant temperature of 25 °C (CT-25); 2) cyclically fluctuating temperatures from 15 to 25 °C (VT-15/25); 3) constant temperature of 20 °C (CT-20); and 4) constant temperature of 15 °C (CT-15). For the fluctuating temperature regime, the temperature cycle was that in one day (24 h), soil samples were incubated for 12 h at 15 °C and then the temperature was automatically switched to 25 °C for another 12 h. The automatic and cyclical change control of the incubation temperature was achieved with a digital biochemical incubator (GXZ-280B, Ningbo Apparatus Manufactory, Ningbo, China). There are a total of 12 incubation treatments (three soil types × four temperature regimes) in the

TABLE I

Site information and classification of the soils used in this study

Soil[a)]	Land use	Genetic soil classification of China[b)]	World Reference Base (WRB) soil classification[c)]	Latitude	Longitude	Altitude
						m
FL	Forest	Limestone soil	Haplic Luvisol	26°14′34″ N	105°45′58″ E	1 474
CL	Cropland	Limestone soil	Haplic Luvisol	26°14′31″ N	105°45′44″ E	1 365
CY	Cropland	Yellow soil	Dystric Luvisol	26°14′25″ N	105°45′13″ E	1 307

[a)]FL = limestone soil under forest; CL = limestone soil under crops; CY = yellow soil under crops.
[b)]State Soil Survey Service of China, 1998.
[c)]Shi *et al.*, 2010.

TABLE II

Basic properties[a)] of the limestone soil under forest (FL), limestone soil under crops (CL) and yellow soil under crops (CY) in this study

Soil	Depth	pH	SOC	TN	TP	TK	Clay	MBC	AN	AP	AK
	cm		g kg^{-1}					mg kg^{-1}			
FL	0–10	7.4	109.2	10.2	3.3	18.9	146	1 361	677	34	195
CL	0–10	7.4	31.0	3.1	2.0	60.0	409	102	168	24	233
CY	0–10	4.8	17.6	2.0	3.1	32.5	683	127	123	53	127

[a)]SOC = soil organic carbon; TN = total nitrogen; TP = total phosphorus; TK = total potassium; MBC = microbial biomass carbon; AN = available nitrogen; AP = available phosphorus; AK = available potassium.

whole incubation experiment, and each treatment had 20 replicates, where 4 replicates were used for investigating SOC mineralization and 16 replicates were used for measuring MBC of the soils.

Twenty grams of the soil samples (oven-dry basis) were transferred into each pre-weighed 250-mL bottle with a rubber stopper except the bottle used for the blank control. The soils in the incubation bottles were adjusted to 60% of water-holding capacity (WHC), which is commonly considered optimum for microbial respiration (Rey *et al.*, 2005; Vanhala *et al.*, 2008), and pre-incubated for 5 d at 25 °C to stabilize the mineralization rate in the incubators. After that, all the replicates were continually incubated for 56 d under the sealed condition. Carbon dioxide (CO_2) was extracted from the headspace of the 250-mL incubation bottles with a syringe after 1, 3, 5, 7, 14, 21, 28, 35, 42, 49 and 56 d, respectively. The CO_2 concentration in each syringe was determined immediately using gas chromatography (7820A, Agilent Technologies, Santa Clara, USA) with pure N_2 as a carrier gas. The measured CO_2 concentration (μL L^{-1}) for each sampling period was used to calculate the amount of evolved CO_2-C (mg CO_2-C kg^{-1} soil). After each sampling period, the bottles were opened and purged with fresh air for 20 min to allow replenishment of O_2, and deionized water was added to maintain gravimetric soil moisture at 60% of WHC in the bottles. In addition, MBC was determined for the soils by taking soil samples out of the incubation bottles and measured using the FE method (Vance *et al.*, 1987) after 1, 7, 21 and 56 d. All laboratory measurements were performed in 4 duplicates. All calculations for evolved CO_2-C are expressed on an oven-dry (105 °C) basis.

SOC mineralization intensity and Q_{10} calculation

The mineralization intensity of total C can be represented by a ratio of mineralized C to total C (Alvarez *et al.*, 1995; Ma *et al.*, 2009). In this study, the mineralization intensity of SOC in an incubated soil was represented by a ratio of C_{56} to initial SOC concentration in the incubated soil (C_{56}/SOC), where C_{56} is the cumulative SOC mineralized in 56 d of incubation (mg C kg^{-1} soil).

The temperature coefficient Q_{10} is a widely used index of temperature dependence, which describes the proportional change in carbon mineralization rate at a 10 °C increment change in temperature (Kirschbaum, 1995), and can be calculated by the following equation (Reichstein *et al.*, 2000):

$$Q_{10} = R_{(t,T+10)}/R_{(t,T)} \quad (1)$$

where $R_{(t,T)}$ and $R_{(t,T+10)}$ are soil C mineralization rates at time t at the incubation temperatures of T and T + 10 °C, respectively. In this study, Q_{10} was used to describe the proportional change in average SOC mineralization rate with a 10 °C increase in temperature and T + 10 °C and T were 25 and 15 °C, respectively, throughout the entire incubation period.

SOC mineralization kinetic model

The SOC mineralization kinetics was determined with a two-component exponential model (Reichstein *et al.*, 2000; Ouyang *et al.*, 2008):

$$C_t = C_o \times (1 - e^{-kt}) + (\mathrm{TOC} - C_o) \times (1 - e^{-ht}) \quad (2)$$

where C_t (mg C kg^{-1} soil) is the cumulative amount of SOC mineralized until time t (d); C_o (mg C kg^{-1} soil) is the amount of the labile SOC fraction; TOC (mg C kg^{-1} soil) is the total organic C in soil and the product of subtracting C_o from it (TOC – C_o) is the amount of the recalcitrant SOC fraction; and k and h are the mineralization rate constants (d^{-1}) of the labile and recalcitrant SOC fractions, respectively. All calculations for soil C mineralization are expressed on an oven-dry (105 °C) basis.

As k and h represent the mineralization rate constants of the labile and recalcitrant SOC fractions, respectively, the mean residence time (MRT) for the labile and recalcitrant SOC fractions can be determined as $1/k$ and $1/h$, respectively (Fazle Rabbi *et al.*, 2014).

Statistical analyses

All the statistical analyses were performed using SPSS19.0 (SPSS, Chicago, USA), and the graphs were created using SigmaPlot 9.0 (SSI, San Jose, USA). Equality of variances was tested using Levene's test. Two-way analysis of variance (ANOVA) was used to determine the effects of temperature and soil type on SOC mineralization (*e.g.*, accumulative mineralization and mineralization intensity), and one-way ANOVA was used to compare Q_{10} values among the three soils. Mean separations were obtained using Fisher's least significant difference (LSD) test at the 95% probability ($P < 0.05$).

The correlation between two variables was determined using the bivariate model of SPSS. The parameters C_o, k and h in the two-component exponential model (Eq. 2) were estimated using nonlinear regression and the fits of the different regression equations to these data were evaluated by calculating the coefficient of determination (R^2), and statistical significance was evaluated at $P < 0.05$ and $P < 0.01$.

RESULTS

SOC mineralization rate

Generally, the daily SOC mineralization rates for all temperature regimes during the incubation followed the same pattern (Fig. 1). They dropped considerably from days 1 to 14 and remained low and relatively constant after that. Under the same incubation temperature regime, the daily SOC mineralization rates of the three soils on the same day were in the order as follows: FL > CL > CY ($P < 0.05$). The highest and lowest average SOC mineralization rates of each soil during the first 14 d and the last 42 d were associated with CT-25 and CT-15, respectively (Table III). The avera-

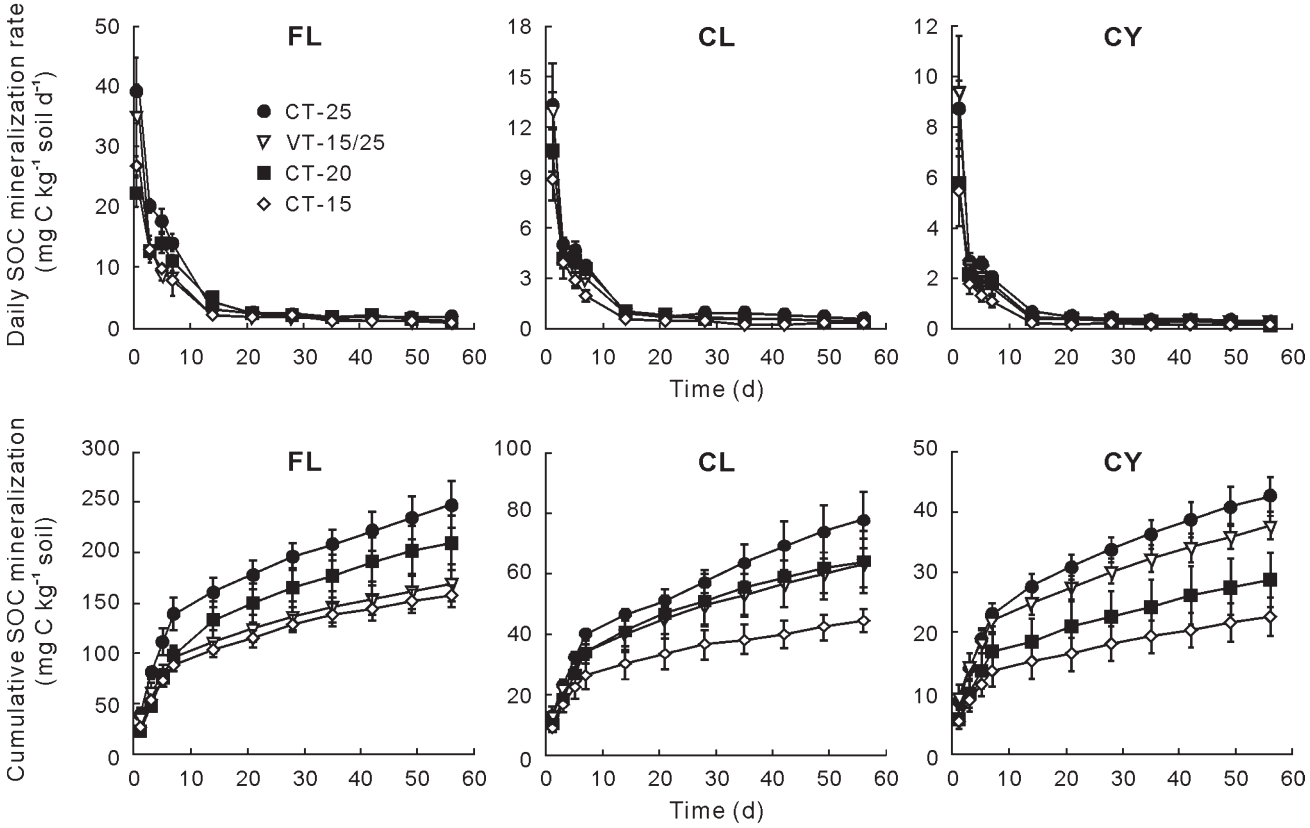

Fig. 1 Daily and cumulative mineralization of soil organic carbon (SOC) in the limestone soil under forest (FL), limestone soil under crops (CL) and yellow soil under crops (CY) during the 56-d laboratory incubation under different temperature regimes. Error bars represent standard deviations of the means ($n = 4$). CT-25 = constant temperature of 25 °C; VT-15/25 = cyclically fluctuating temperatures from 15 to 25 °C; CT-20 = constant temperature of 20 °C; CT-15 = constant temperature of 15 °C.

TABLE III

Average mineralization rates of soil organic carbon (SOC) in the limestone soil under forest (FL), limestone soil under crops (CL) and yellow soil under crops (CY) during the first 14 d and the last 42 d of the 56-d laboratory incubation under different temperature regimes[a)]

Temperature regime	FL		CL		CY	
	First 14 d	Last 42 d	First 14 d	Last 42 d	First 14 d	Last 42 d
	mg C kg^{-1} soil d^{-1}					
CT-25	11.45 ± 0.98[b)]a[c)]	2.07 ± 0.45a	3.32 ± 0.12a	0.74 ± 0.20a	1.98 ± 0.16a	0.36 ± 0.04a
VT-15/25	7.93 ± 0.83c	1.39 ± 0.21bc	2.86 ± 0.30a	0.55 ± 0.09b	1.80 ± 0.15a	0.30 ± 0.03b
CT-20	9.49 ± 1.18b	1.82 ± 0.30ab	2.92 ± 0.47a	0.55 ± 0.09b	1.33 ± 0.26b	0.24 ± 0.03c
CT-15	7.35 ± 0.55c	1.29 ± 0.11c	2.15 ± 0.35b	0.34 ± 0.03c	1.09 ± 0.19b	0.18 ± 0.03d

[a)] CT-25 = constant temperature of 25 °C; VT-15/25 = cyclically fluctuating temperatures from 15 to 25 °C; CT-20 = constant temperature of 20 °C; CT-15 = constant temperature of 15 °C.
[b)] Means ± standard deviations ($n = 4$).
[c)] Means followed by the same letter(s) in a column are not significantly different at $P < 0.05$.

ge SOC mineralization rates of the FL soil during the first 14 d and the last 42 d deceased in the following order: CT-25 > CT-20 > VT-15/25 > CT-15 (Table III). The average SOC mineralization rate of the FL soil during the first 14 d was significantly lower at VT-15/25 than that at CT-20 ($P < 0.05$). The average SOC mineralization rates of the CL soil during the first 14 d and the last 42 d at CT-20 were not significantly different from those at VT-15/25. However, the average SOC mineralization rates of the CY soil during the first 14 d and the last 42 d were significantly higher at VT-15/25 than those at CT-20 ($P < 0.05$).

Cumulative SOC mineralization

For all temperature regimes, the cumulative SOC mineralization of each soil during the first week increased much greater than that in the last seven weeks (Fig. 1). After one week, the differences between cumulative SOC mineralization of each soil at different constant temperatures gradually became greater as the duration of incubation progressed (Fig. 1). Two-way ANOVA (temperature regime × soil type) indicated that the C_{56} value was significantly ($P < 0.01$) affected by temperature regime, soil type and their interactions. The C_{56} values of the three soils under the same temperature regime were different in the order of FL > CL > CY. The C_{56} values of each soil under the four temperature regimes were different and decreased in the following orders: CT-25 > CT-20 > VT-15/25 > CT-15 for the FL soil; CT-25 > CT-20 > VT-15/25 > CT-15 for the CL soil; CT-25 > VT-15/25 > CT-20 > CT-15 for the CY soil (Fig. 1). At the constant temperatures, the C_{56} value of each soil increased significantly ($P < 0.05$) with the increase in incubation temperature. Differences in C_{56} value between CT-20 and VT-15/25 were significant ($P < 0.05$) for the FL and CY soils, but they were not significant for the CL soil.

The value of C_{56}/SOC, representing the SOC mineralization intensity, of the FL soil was significantly ($P < 0.05$) lower than those of other two soils at VT-15/25 (Fig. 2). The difference in C_{56}/SOC value between the CL and CY soils was obvious ($P < 0.05$) at CT-20. Although the C_{56}/SOC values were significantly affected by soil type (two-way ANOVA, $P = 0.024$), the C_{56}/SOC values of the three soils were not significantly different at CT-25 and CT-15. In addition, the correlation coefficients between the two SOC mineralization parameters (C_{56} and C_{56}/SOC) and SOC concentration at different temperatures were calculated (Table IV).

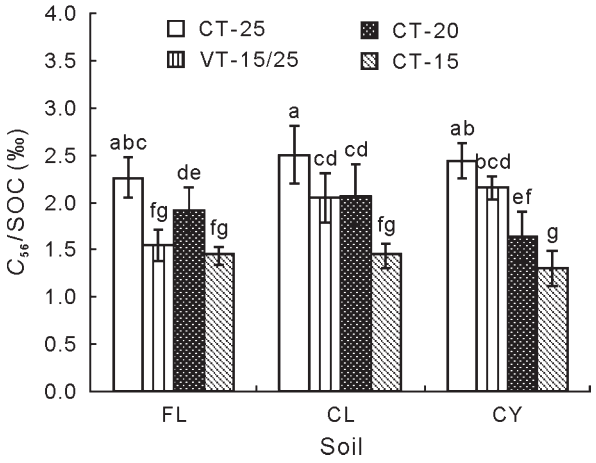

Fig. 2 Mineralization intensities (C_{56}/SOC) of the limestone soil under forest (FL), limestone soil under crops (CL) and yellow soil under crops (CY) during the 56-d laboratory incubation under different temperature regimes. Error bars represent standard deviations of the means ($n = 4$). Bars labeled with the same letter(s) are not significantly different at $P < 0.05$. C_{56}/SOC is the ratio of the cumulative SOC mineralized in the 56-d incubation (C_{56}) to the initial soil organic carbon (SOC) concentration. CT-25 = constant temperature of 25 °C; VT-15/25 = cyclically fluctuating temperatures from 15 to 25 °C; CT-20 = constant temperature of 20 °C; CT-15 = constant temperature of 15 °C.

TABLE IV

Correlation coefficients between soil organic carbon (SOC) concentration and two SOC mineralization parameters[a)] during the 56-d laboratory incubation under different temperature regimes[b)]

Mineralization parameter	SOC concentration			
	CT-25	VT-15/25	CT-20	CT-15
C_{56}	0.9997*	0.9989*	0.9988*	0.9999**
C_{56}/SOC	−0.9098	−0.9994*	0.3054	0.6173

*, **Significant at $P < 0.05$ and $P < 0.01$, respectively.
[a)] C_{56} = cumulative SOC mineralized in the 56-d incubation; C_{56}/SOC = ratio between C_{56} and SOC concentration.
[b)] CT-25 = constant temperature of 25 °C; VT-15/25 = cyclically fluctuating temperatures from 15 to 25 °C; CT-20 = constant temperature of 20 °C; CT-15 = constant temperature of 15 °C.

SOC mineralization kinetics and Q_{10}

The two-component exponential model (Eq. 2) could fit well the data of SOC mineralization of all the treatments ($P < 0.01$) (Table V). The amount of the labile SOC fraction (C_o), which also refers to the labile SOC pool size, and k values of each soil were different under different temperature regimes (Table V). At the constant temperatures, the C_o values of the FL and CY soils increased with the increase in incubation temperature. The C_o value of the CL soil was slightly, but not significantly ($P < 0.05$), lower at CT-25 than that at CT-20. The C_o value of the FL soil was obviously ($P < 0.05$) lower at VT-15/25 than that at CT-20, and

TABLE V

Kinetic parameters[a)] of the two-component model[b)] for soil organic carbon (SOC) mineralization of the limestone soil under forest (FL), limestone soil under crops (CL) and yellow soil under crops (CY) during the 56-d laboratory incubation under different temperature regimes[c)]

Soil	Temperature regime	C_o	k	h
		mg C kg^{-1} soil	d^{-1}	$\times 10^{-5}$ d^{-1}
FL	CT-25	139.06 ± 17.68[d)]a[e)]	0.280 ± 0.029cd	1.79 ± 0.45bc
	VT-15/25	95.23 ± 9.97b	0.323 ± 0.023bc	1.26 ± 0.22efg
	CT-20	123.03 ± 18.11a	0.167 ± 0.034e	1.44 ± 0.38cde
	CT-15	91.40 ± 10.61b	0.276 ± 0.049cd	1.13 ± 0.12efg
CL	CT-25	36.29 ± 2.43c	0.337 ± 0.049abc	2.44 ± 0.71a
	VT-15/25	33.01 ± 3.65c	0.347 ± 0.051ab	1.79 ± 0.29bc
	CT-20	36.45 ± 6.96c	0.239 ± 0.034d	1.66 ± 0.22bcd
	CT-15	26.65 ± 5.71d	0.325 ± 0.058bc	1.04 ± 0.12fg
CY	CT-25	23.56 ± 1.56de	0.294 ± 0.025bcd	2.02 ± 0.22ab
	VT-15/25	20.88 ± 1.67e	0.408 ± 0.110a	1.79 ± 0.11bc
	CT-20	16.19 ± 3.7f	0.270 ± 0.038cd	1.32 ± 0.23def
	CT-15	12.84 ± 2.39g	0.403 ± 0.052a	1.04 ± 0.18g

Soil	Temperature regime	MRT_k	MRT_h	C_o/C_{56}	C_o/SOC
		d	$\times 10^4$ d	%	‰
FL	CT-25	3.60 ± 0.37bcd	5.88 ± 1.68de	56.55 ± 8.74a	1.27 ± 0.16ab
	VT-15/25	3.11 ± 0.22de	8.14 ± 1.52ab	56.31 ± 4.56a	0.87 ± 0.09cde
	CT-20	6.20 ± 1.30a	7.35 ± 1.93bcd	58.99 ± 8.16a	1.13 ± 0.17ab
	CT-15	3.71 ± 0.70bcd	8.94 ± 1.01ab	58.19 ± 4.93a	0.84 ± 0.10de
CL	CT-25	3.02 ± 0.47def	4.31 ± 0.98f	47.54 ± 7.79b	1.17 ± 0.08ab
	VT-15/25	2.93 ± 0.44def	5.67 ± 0.79de	52.22 ± 2.54ab	1.07 ± 0.12b
	CT-20	4.24 ± 0.60b	6.13 ± 0.93cde	56.79 ± 2.35a	1.18 ± 0.22ab
	CT-15	3.15 ± 0.50cde	9.71 ± 1.26a	59.44 ± 7.22a	0.86 ± 0.18cde
CY	CT-25	3.42 ± 0.28bcd	4.99 ± 0.52ef	55.24 ± 2.13a	1.34 ± 0.09a
	VT-15/25	2.56 ± 0.55ef	5.62 ± 0.35de	55.15 ± 2.26a	1.19 ± 0.09ab
	CT-20	3.76 ± 0.55bc	7.75 ± 1.34bc	55.92 ± 6.44a	0.92 ± 0.21cd
	CT-15	2.52 ± 0.33f	9.85 ± 1.59a	56.07 ± 4.88a	0.73 ± 0.14e

[a)] C_o is the amount of the labile SOC fraction; k and h are the mineralization rate constants of the labile and recalcitrant SOC fractions, respectively; MRT_k and MRT_h are the mean residence time for the labile and recalcitrant SOC fractions, respectively; C_o/C_{56} is the ratio between C_o and C_{56}, where C_{56} is the cumulative SOC mineralized in the 56-d incubation; C_o/SOC is the ratio between C_o and SOC concentration.

[b)] The model fitting for all the treatments is significant at $P < 0.01$ ($R^2 > 0.99$).

[c)] CT-25 = constant temperature of 25 °C; VT-15/25 = cyclically fluctuating temperatures from 15 to 25 °C; CT-20 = constant temperature of 20 °C; CT-15 = constant temperature of 15 °C.

[d)] Means ± standard deviations ($n = 4$).

[e)] Means followed by the same letter(s) in a column are not significantly different at $P < 0.05$.

and the C_o value of the CY soil was obviously ($P < 0.05$) higher at VT-15/25 than that at CT-20. However, the difference in C_o value between VT-15/25 and CT-20 was not significant for the CL soil. The highest k values were observed at VT-15/25 for all three soils, and the k value of each soil was obviously ($P < 0.05$) higher at VT-15/25 than that at CT-20. The highest and lowest h values of each soil were found at CT-25 and CT-15, respectively (Table V). At the constant temperatures, the h values of each soil always increased with the increase in incubation temperature. The h value of the CY soil was significantly ($P < 0.05$) higher at VT-15/25 than that at CT-20. The MRT_k and MRT_h (Table V) represent the mean residence times for the labile and recalcitrant SOC fractions, respectively. The Q_{10} values (mean ± standard deviation) of the three soils for the temperature interval from 15 to 25 °C were calculated as follows: 1.58 ± 0.13 (FL), 1.83 ± 0.22 (CL) and 1.79 ± 0.19 (CY). The Q_{10} value of the FL soil was significantly ($P < 0.05$) lower than those of other two soils, and there was no significant difference in Q_{10} value between the CL and CY soils.

MBC

Under the same incubation temperature regime, the MBC contents of the FL soil were higher than those of the other two soils, but the MBC/SOC ratios of the FL soil were not always higher than those of the other two soils (Table VI). During the entire incubation period, the MBC contents of each soil incubated at the

TABLE VI

Microbial biomass carbon (MBC) and its ratio to soil organic C (MBC/SOC) of the limestone soil under forest (FL), limestone soil under crops (CL) and yellow soil under crops (CY) during the 56-d laboratory incubation period under different temperature regimes[a)]

Soil	Temperature regime	MBC content				CV[b)]
		Day 1	Day 7	Day 21	Day 56	
		mg C kg^{-1}				%
FL	CT-25	1 060 ± 120[c)] b[d)]	1 769 ± 264a	1 852 ± 230a	1 305 ± 123a	25.2
	VT-15/25	1 569 ± 261a	1 705 ± 192a	2 016 ± 260a	954 ± 301a	28.6
	CT-20	1 204 ± 63ab	414 ± 232bc	769 ± 101b	502 ± 146b	49.1
	CT-15	219 ± 32cde	232 ± 129bcd	375 ± 89c	651 ± 216b	54.3
CL	CT-25	189 ± 37de	153 ± 26de	448 ± 112c	176 ± 9de	57.3
	VT-15/25	279 ± 83cd	41 ± 13f	423 ± 102c	181 ± 28de	69.7
	CT-20	311 ± 28c	373 ± 13bc	217 ± 14d	171 ± 55de	33.9
	CT-15	109 ± 20f	39 ± 13f	71 ± 40f	313 ± 29c	92.5
CY	CT-25	248 ± 46cd	137 ± 30de	116 ± 4e	145 ± 9e	36.5
	VT-15/25	314 ± 156c	249 ± 124bcd	95 ± 1ef	162 ± 7de	47.0
	CT-20	149 ± 2ef	207 ± 1cd	122 ± 2e	219 ± 25d	26.7
	CT-15	126 ± 25f	90 ± 13e	112 ± 8e	162 ± 31de	24.6

Soil	Temperature regime	MBC/SOC			
		Day 1	Day 7	Day 21	Day 56
		‰			
FL	CT-25	9.71 ± 1.10cde	16.20 ± 2.42a	16.95 ± 2.11ab	11.95 ± 1.13ab
	VT-15/25	14.37 ± 2.39ab	15.61 ± 1.76a	18.46 ± 2.38a	8.74 ± 2.76b
	CT-20	11.03 ± 0.58bcd	3.79 ± 2.12de	7.04 ± 0.92c	4.60 ± 1.34d
	CT-15	2.00 ± 0.29h	2.13 ± 1.18ef	3.43 ± 0.81d	5.96 ± 1.98cd
CL	CT-25	6.10 ± 1.20f	4.96 ± 0.85cd	14.47 ± 3.62ab	5.70 ± 0.28d
	VT-15/25	9.01 ± 2.67def	1.32 ± 0.42f	13.66 ± 3.30b	5.86 ± 0.89cd
	CT-20	10.03 ± 0.91cde	12.04 ± 0.41ab	7.00 ± 0.46c	5.54 ± 1.76d
	CT-15	3.53 ± 0.65g	1.27 ± 0.42f	2.29 ± 1.28d	10.10 ± 0.92ab
CY	CT-25	14.11 ± 2.61abc	7.83 ± 1.70bc	6.59 ± 0.21c	8.25 ± 0.53bc
	VT-15/25	17.90 ± 8.88a	14.18 ± 7.09ab	5.39 ± 0.03c	9.24 ± 0.41ab
	CT-20	8.47 ± 0.10def	11.78 ± 0.04ab	6.95 ± 0.11c	12.50 ± 1.45a
	CT-15	7.19 ± 1.42ef	5.13 ± 0.75cd	6.37 ± 0.46c	9.23 ± 1.77ab

[a)] CT-25 = constant temperature of 25 °C; VT-15/25 = cyclically fluctuating temperatures from 15 to 25 °C; CT-20 = constant temperature of 20 °C; CT-15 = constant temperature of 15 °C.
[b)] The coefficient of variation for the MBC content during the entire incubation period.
[c)] Means ± standard deviations ($n = 4$).
[d)] Means followed by the same letter(s) in a column are not significantly different at $P < 0.05$.

constant temperatures did not always increase with the increasing temperature on the same day (Table VI). The MBC contents of the FL soil at VT-15/25 were significantly ($P < 0.05$) higher than those at CT-20 on day 7 of incubation. However, no significant difference in MBC content between VT-15/25 and CT-20 was found in the CY soil on day 7 of incubation. The MBC contents of the three soils incubated under different temperature regimes did not decrease continually with the increase in the incubation period. Also, no significant correlation between daily SOC mineralization rates and MBC contents was found in all incubation treatments, with an exception for the CY soil that was incubated at 25 °C ($P < 0.05$). The coefficient of variance (CV) for MBC contents in different incubation treatments ranged between 24.6% and 92.5% during the entire incubation period (Table VI). At different temperatures except CT-20, the greater CV values were associated with the CL soil than the other two soils.

DISCUSSION

In this study, cumulative CO_2 evolution from all the soils incubated at the constant temperatures increased significantly with increasing temperatures, which is in agreement with the other studies (Gudasz *et al.*, 2010; Wang *et al.*, 2010). As SOC is the substrate for soil microbial respiration, its concentration may influence its mineralization rate by directly affecting microbial activity and enzymatic reactions in soil (Hopkins *et al.*, 2006; Burns *et al.*, 2013). In this study,

there was a significantly ($P < 0.05$) positive correlation between C_{56} and SOC concentration under the same incubation temperature regime (Table IV), similar to the finding recently reported by Dimassi *et al.* (2014). This showed that the SOC concentration was an important factor controlling soil C mineralization process. In this study, the cumulative temperature over the entire incubation period (56 d) of VT-15/25 was the same as that of CT-20. However, there were obvious differences in SOC mineralization between VT-15/25 and CT-20. At VT-15/25, the C_{56} value of the FL soil reduced and the C_{56} value of the CY soil increased significantly ($P < 0.05$) as compared to those at CT-20. These showed that changes in temperature pattern (constant or fluctuating) could significantly influence the SOC mineralization of the incubated soils differently. Therefore, determining the responses of SOC mineralization to temperature variations is necessary in order to accurately evaluate CO_2 emission from soils in the karst region of western Guizhou that is characterized by drastic weather changes. In addition, SOC mineralization responses at VT-15/25 were different among the three different soils, especially in contrast to those at CT-20 (Fig. 1), indicating that the SOC mineralization responses to the varying temperatures might be influenced by some soil properties. A previous study also has shown that some soil characteristics (*e.g.*, chemical composition of soil organic matter) might influence the temperature sensitivity of soil organic matter (SOM) and total amount of CO_2 respired during a long-term soil incubation (Haddix *et al.*, 2011).

For all the soils used in this study, the C_{56} values at VT-15/25 were always between those at CT-25 and CT-15. This suggested that the SOC mineralization under periodical fluctuation of temperatures was restricted by the temperature range. Lomander *et al.* (1998) also showed that the temperature fluctuation between −4 and 5 °C did not significantly enhance CO_2 evolution from the incubated soils compared with constant 5 °C and the CO_2 evolution was greater than that from the soils incubated at −4 °C. Under all different temperature regimes in this study, the average SOC mineralization rates of each soil in the first 14 d were significantly ($P < 0.05$) greater than those during the last 42 d of the incubation. It was found that approximately 60%–68% of total CO_2-C evolution was produced during the first 14 d, which showed that the cumulative SOC mineralization during the entire incubation was mostly dictated by the mineralization rate in the first 14 d. There was no significant correlation between mineralization intensities (C_{56}/SOC) and SOC concentrations under the same temperature regimes except VT-15/25 ($P < 0.05$) (Table IV), and the C_{56}/SOC values were mostly controlled by incubation temperatures (Fig. 2). The fluctuation in temperature (VT-15/25) significantly ($P < 0.05$) reduced the C_{56}/SOC value for the FL soil compared with the other two soils (Fig. 2), which might be closely associated with the lower Q_{10} for the FL soil than the other two soils. Although the changes in MBC could not be used to effectively explain the differences in SOC mineralization between constant and fluctuating temperatures in this study, the SOC mineralization process was driven mainly by soil microbes. Moreover, Bárcenas-Moreno *et al.* (2009) found that different soil temperatures could progressively select microbial communities growing better at specific temperatures in an incubation experiment. So, the adaptation capability of microbial communities to external temperature changes may be weak in the FL soil with low Q_{10} as compared with the CL and CY soils, which suggested that the optimum structure or activity of soil microbial communities for SOC mineralization could not be rapidly achieved when there was a temperature fluctuation in the FL soil (Yang *et al.*, 2011; Birgander *et al.*, 2013). Thus, the C mineralization capability of microbial communities may be restricted, leading to lower mineralization intensity (C_{56}/SOC) as it was found in the FL soil at VT-15/25.

Substrate quality and availability are known to influence the temperature sensitivity of SOC mineralization (Schütt *et al.*, 2014). In this study, the Q_{10} value of the forest soil was significantly ($P < 0.05$) lower than those of the other two cropland soils, which might be attributed to differences in SOM composition. The SOM in the soils under forest and crops was derived from forest vegetation and annual crop plants (*e.g.*, corn), respectively, and possessed different chemical composition influencing its quality and availability as a substrate for microbial mineralization (Stewart *et al.*, 2011; Jagadamma *et al.*, 2014). Moreover, the FL soil under forest had a high SOC concentration with abundant coarse organic fractions of low stability as compared with the other two cropland soils. The temperature sensitivity of decomposition increases with the increasing stability of organic compounds, and stable substrates are more temperature-sensitive than liable substrates (von Lützow and Kögel-Knabner, 2009). Therefore, the less temperature-sensitive C pool was found in the FL soil. These connections between SOM composition, Q_{10} and C_{56}/SOC indicated that the SOC mineralization responses to the fluctuating temperatures might be affected by SOM composition.

The mineralization rate constants (k and h) can reflect the relative mineralization rates of the labile and recalcitrant SOC fractions, respectively (Reichstein *et al.*, 2000; Ouyang *et al.*, 2008). At constant temperatures, the h values increased with the increase in incubation temperature, indicating that the warmer temperature could improve the capability of microbes to decompose the recalcitrant SOC in soils. Although the h values were obviously lower than the k values, the recalcitrant SOC pool size was much greater than the labile SOC pool size. In the 56-d incubation, over 40% of cumulative mineralized SOC came from the recalcitrant SOC pool in the incubated soils, which indicated that the recalcitrant SOC fraction may also play an important role in SOC mineralization as the labile SOC fraction. Through a comparison between VT-15/25 and CT-20 (Table V), it was found that the cyclical fluctuation in temperature influenced the SOC mineralization process mainly through affecting the liable SOC pool size (C_o) and mineralization rate constant of the recalcitrant SOC (h). In addition, the impacts of the fluctuation in temperature on C_o and h were different among the three soils, which might be related to the soil chemical and physical properties (Saviozzi *et al.*, 2014). In this study, the MBC contents were not significantly correlated with the daily SOC mineralization rates during the entire incubation period. This indicated that the SOC mineralization was not regulated by the microbial biomass size or microbial population. Similar results were also reported in a study by Kemmitt *et al.* (2008). However, the composition, structure and activity of microbial communities might be affected by temperature changes (Castro *et al.*, 2010; Yuste *et al.*, 2011), which can result in the change in microbial ability to utilize SOC. Therefore, microbial communities still can play an important role in contributing to SOC mineralization process as induced by temperature changes. The SOC mineralization responses to temperature changes may also be related to some non-biological process (Ågren and Wetterstedt, 2007; Kemmitt *et al.*, 2008; Benbi *et al.*, 2014), which needs to be explored.

CONCLUSIONS

The cumulative SOC mineralization at the cyclically fluctuating temperatures was different from that at the constant temperatures. The C_{56} values at VT-15/25 were always between those at CT-25 and CT-15, suggesting that the cumulative SOC mineralization was restricted by the temperature range. The SOC mineralization responses to the cyclical fluctuation in temperature were different among the three soils tested. At the fluctuating temperatures, the forest soil with lower Q_{10} had lower mineralization intensity than the two cropland soils. These indicated that the difference in temperature pattern (constant or fluctuating) could significantly influence SOC mineralization, and the SOC mineralization responses to the fluctuating temperatures might be affected by some soil characteristics (*e.g.*, SOM composition). The changes in temperature could influence the ability of soil microbes to utilize SOC fractions and the warmer temperature might improve the capability of microbes to decompose the recalcitrant SOC in soils. The cyclical fluctuations in temperature could influence SOC mineralization through changing the labile SOC pool size and mineralization rate of the recalcitrant SOC in soils.

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