Seasonality in soil temperature may drive the seasonal dynamics of carbonate-derived CO₂ efflux in a calcareous soil

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Abstract. Carbonates in soils may act as a potential source of CO₂ efflux from calcareous soils to the atmosphere. However, its seasonal dynamics and relationships with soil temperature or soil moisture are not well-understood. A 5-yr warming experiment was performed to evaluate the effects of symmetric (all season) and asymmetric (seasonal) warming on soil organic fraction (SOC)-derived efflux and carbonate-derived CO₂ efflux (partitioned by δ¹³C signature) from a calcareous soil in the karst region of southwest China. In region where precipitation mostly falls during summer, an average of 2.0°C warming under both symmetric and asymmetric warming scenarios produced 6–34% and 11–14% increases in the SOC-derived efflux and carbonate-derived efflux, respectively. The SOC-derived efflux in the symmetric warming scenario was 1.6 times that of the asymmetric warming scenarios, but no changes were found for the carbonate-derived efflux. The SOC-derived efflux was positively correlated with both soil temperature and soil moisture, suggesting an entailment with biotic processes. In contrast, the carbonate-derived efflux was positively correlated with soil temperature, but was poorly correlated with soil moisture. Thus, the seasonal dynamics of the carbonate-derived efflux in calcareous soils were likely driven by abiotic processes.

Key words: carbonate; global warming; soil CO₂ efflux; soil moisture; soil temperature.

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INTRODUCTION

The Earth temperature is rising, and it is expected to rise at accelerated rates in the near future (IPCC 2013). While over the period of 1880–2012 the average global temperature has increased by 0.85°C, this number is largely across large spatial and temporal scales. Easterling et al. (1997) found that warming rate is faster during winter–spring than during summer–autumn. These differences, namely seasonal asymmetric warming, can vary between 0.65°C and 1.06°C (IPCC 2013). Yet, they are often neglected when examining the effects of climate change on terrestrial ecosystems (IPCC 2014).

Warmer temperatures are expected to stimulate the emission of soil CO₂, by accelerating both autotrophic respiration and heterotrophic respiration (Rustad and Fernandez 1998, Zhou et al. 2009, Bond-Lamberty and Thomson 2010, IPCC 2014, Zhou et al. 2018). However, the warming-induced stimulation of soil CO₂ efflux varies substantially with biomes or ecosystem types due to differences in soil resources (moisture, nutrients, and decomposable substrates such as soil organic carbon; Chen and Tian 2005, Ball et al. 2009,
Giardina et al. 2014, IPCC 2014, Soper et al. 2017). In the dry forests of southern Ethiopia, Zeleke (2015) found that 27–38% of the variations in soil CO2 efflux can be explained by soil moisture and 21–53% by soil temperature. In the deserts, SOC breakdown is usually attributed to pulses of rainfall and corresponds to microbial decomposition (Inglima et al. 2009). Although in general increase in soil moisture gradient increases soil respiration (Inglima et al. 2009), Schwendenmann et al. (2003) suggested that lower diffusion rates can be attributed to a decrease in soil CO2 release during periods of high soil moisture in an old-growth Neotropical rainforest, Costa Rica. Thus, there are complex relationships between soil respiration and environmental factors. The effects of warming on calcareous systems, however, are far less known, as the habitat is uncommon and they can be more complex due to a rich deposit of inorganic C, in addition to SOC.

In calcareous soils, inorganic C occurs in the form of soil carbonates, which amounts to 950 Pg C globally (Lal 2004). Such significant amount of C may be an important source of CO2 efflux from the dissolution of carbonates (Gislason et al. 2009, Inglima et al. 2009, Tamir et al. 2011, Ramnarine et al. 2012), described by the following equations:

\[ \text{CaCO}_3 + \text{H}^+ \leftrightarrow \text{Ca}^{2+} + \text{HCO}_3^- \]  \hspace{1cm} (1)

\[ \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CO}_2 + \text{H}_2\text{O} \]  \hspace{1cm} (2)

Soil properties, such as soil pH, soil CO2 partial pressure, and soil moisture and temperature, can shift the reactions represented in Eqs. (1) and (2) toward either weathering of carbonates or absorption of CO2 in the soil solution (Gislason et al. 2009, Cao et al. 2012). Inglima et al. (2009) observed that a rain event would slightly but not significantly increase the carbonate-derived efflux after a prolonged period of drought in Mediterranean ecosystems. With increasing atmospheric acid deposition, however, we can expect that there will be an increasing amount of H+ ions into soils (Gislason et al. 2009). In calcareous soils, H+ ions can also naturally source from (a) organic acids from soil respiration and products of plant root (Inglima et al. 2009, Tamir et al. 2011, Cao et al. 2012), (b) carbonic acid (H2CO3) caused by high soil CO2 partial pressure due to soil respiration (Inglima et al. 2009, Ramnarine et al. 2012), (c) nitric acid (HNO3) produced by mineralization and nitrification of soil organic nitrogen (Tamir et al. 2011, Zhu et al. 2016), and (d) sulfuric acid (H2SO4) formed by oxidation of reduced sulfur (Tamir et al. 2011). Considering the numerous responses of carbonate-derived efflux in calcareous soils to environmental factors (e.g., soil temperature and soil moisture), understanding the seasonal dynamics of the soil CO2 efflux due to warming in calcareous soils and their responses is fundamental to improve the prediction of ecosystem C cycling. Ignoring the contribution of carbonate-derived CO2 may lead to an overestimation of soil respiration in calcareous soils (Tamir et al. 2011, Ramnarine et al. 2012).

To fill the knowledge gap, we conducted a manipulative symmetric (equal warming across seasons) and asymmetric warming (different warming across seasons) experiment in a calcareous ecosystem to disentangle the effects of soil moisture and soil temperature on soil respiration. We hypothesize that (a) responses of soil CO2 efflux to symmetric and asymmetric warming in calcareous soils may be different and (b) soil CO2 efflux from carbonates may be influenced by seasonality of soil temperature and soil moisture in calcareous soils. The aim of this study was to evaluate (1) the effects of 5-year symmetric and seasonal asymmetric warming in a calcareous soil on CO2 efflux from two sources: soil organic fraction and carbonate and (2) the driving factors of the seasonal dynamics of carbonate-derived efflux in the karst region, southwest China.

**Materials and Methods**

**Site descriptions**

The study site is situated at Shilin County of Yunnan Province, southwest China (24°40'N, 103°22'E, 1750–1800 m a.s.l.). The climate pattern in the study region is characterized by high temperature with concentrated rainfall during May–October (Fig. 1). During the study period (2013–2017), the annual temperature varied between 16.9° and 18.1°C with an average of 17.5°C and the annual rainfall ranged from 850 to 1368 mm with an average of 1037 mm (Fig. 1). Mean annual temperature increased by 0.19°C.
per decade from 1961 to 2016, with asymmetric seasonal increase of 0.09°C during summer–autumn and 0.28°C during winter–spring, respectively (data are from the Meteorological Station of Shilin County, approximately 0.8 km to the study site).

In 2012, a 1-ha area of abandoned cropland was selected for this study. The surface soil (0-15 cm) is classified as Typic Paleudalfs (Zhang et al. 2019), developed from red limestone, and rich in rock fragments (10.2%). The soil has a pH that varies between 7.5 in July and 7.1 in December. The texture is silt loam with approximately 26% sand, 59% silt, and 15% clay. The soil contains 1.48% organic C and 0.82% inorganic C in the form of carbonates. Throughout the region, historical grazing and mowing for livestock severely influenced the vegetation community. Currently, the native vegetation was dominated by herbaceous species, mainly *Bidens pilosa*, *Spiraea salicifolia*, and *Sophora viciifolia*. Further details about the study region were described by Zhang et al. (2019).

**Experimental details**

In 2012, eighteen experimental plots (2 × 2 m in size, each between 3 and 6 m apart) were established. Two infrared radiators (LPC4030, LP, Guangzhou, China) with adjustable power (0–3000 W/m², 2 m in length, each was placed ~1.0 m distance to the other) were suspended 1.2–1.3 m above the ground of each plot (Inglima et al. 2009, Shaw et al. 2014, Wang et al. 2014, Zhang et al. 2019) to generate six temperature treatments. These treatments are (1) no-warming (ambient temperature, control), (2) symmetric warming (2.0°C above ambient temperature year-round, SW), (3) slightly asymmetric warming (2.5°C/1.5°C above ambient temperature during winter–spring/summer–autumn, SAW), (4) moderately asymmetric warming (3.0°C/1.0°C above ambient temperature during winter–spring/summer–autumn, MAW), (5) highly asymmetric warming (3.5°C/0.5°C above ambient temperature during winter–spring/summer–autumn, HAW), and (6) extremely asymmetric warming (4.0°C above ambient temperature during winter–spring only, EAW) (Table 1). The power of the infrared radiators in each warming plot was automatically adjusted by the integrated temperature and moisture regulators (ITMR, SMUL4, QD, Beijing, China) at 2-minute intervals to steadily maintain a constant temperature (± 0.02°C) according to the experimental design (Zhang et al. 2019). The infrared radiators were operated year-round in the field, and the temperatures were recorded automatically by the ITMR since December 2012.

The average annual warming rate in each warming treatment was kept at 2.0°C above the ambient temperature. The ratios of asymmetric

![Fig. 1. Air temperature and precipitation at the experimental site from 2013 to 2017.](image-url)
Temperature monitoring

Five stations were installed in an approximate X pattern in each plot for monitoring temperature and soil moisture. Air temperatures at 5 cm above the ground were monitored at each of the five stations using thermometers (precision = 0.01°C; ZK-ZD10A, QD, Beijing, China) recorded automatically in real time by the ITMR. The average air temperature at 5 cm above the ground in the no-warming plots was regarded as ambient temperature.

Soil temperature and moisture were monitored at 15 cm deep using thermometers and soil moisture probes (ZK-ZD10AC, QD, Beijing, China). These probes were connected to the ITMR, which can automatically measure both soil temperature and volumetric soil water content on a daily basis throughout the entire experimental period.

**Soil CO₂ efflux measurement**

To obtain a representative sample of the 2 × 2 m plots, we measured three subsamples of soil CO₂ efflux within each experimental plot (Zhang et al. 2019). Three of the five stations in each plot were randomly selected for soil CO₂ efflux measurement. A stoppered, airtight PVC collar (h = 15 cm, Ø = 20 cm) was inserted 3–4 cm into the soil at each position adjacent to the selected station (three collars for each plot). A 1-L flask containing 500 mL 2.5 mol/L NaOH solution was placed in the PVC collar to trap CO₂ released from the underlying soil (Bertolini et al. 2006, Inglima et al. 2009, Zhang et al. 2019). The collar was stoppered, ensuring an airtight seal. The flask with NaOH solution was removed and replaced by a new flask three times per month (i.e., at roughly 10-d intervals). Triplicate blank samples (PVC collar with closed bottom) were settled to correct the ambient CO₂ trapped by NaOH solution. The NaOH solution, which had trapped soil CO₂ in the three collars in each plot, was mixed at each measurement to produce one composite solution for each plot. Thus, we collected a total of 18 composite samples for each sampling date, from January to December in 2017.

**Sampling and laboratory analysis**

In order not to damage the integrity of experimental plots and not to disturb the warming experiment due to frequent sampling, soil samples were regularly collected from the separation zones between experimental plots, rather than from experimental plots. Twelve undisturbed soil cores were randomly taken using a soil auger from separation zones between experimental plots at the same time as the flasks with NaOH solution were replaced. Each soil core was 6 cm in diameter and, in most cases, 60–75 cm in depth. The soil cores were divided into 0–15, 15–35, 35–55, and 55–80 cm depths. Soil was air-dried and analyzed for the contents of soil carbon fractions and its stable C isotope composition (δ¹³C).

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**Table 1. Soil bulk density, soil pH, and soil organic carbon in the surface soil (0–15 cm) before the start of the experiment (2012) and air temperature in 2017.**

| Treatment                  | Soil bulk density (g cm⁻³) | Soil pH  | Soil organic carbon (g kg⁻¹) | Air temperature (°C) in 2017 |
|----------------------------|-----------------------------|----------|-----------------------------|------------------------------|
|                            | Full year                   | Summer–autumn | Winter–spring |                               |
| No-warming                 | 1.20 ± 0.02 a               | 7.52 ± 0.01 a | 14.77 ± 0.07 a | 15.4 a                       |
| Symmetric warming          | 1.22 ± 0.02 a               | 7.54 ± 0.01 a | 14.69 ± 0.06 a | 17.4 b                       |
| Slightly asymmetric warming| 1.22 ± 0.02 a               | 7.51 ± 0.02 a | 14.70 ± 0.06 a | 17.4 b                       |
| Moderately asymmetric warming| 1.21 ± 0.01 a              | 7.52 ± 0.02 a | 14.77 ± 0.07 a | 17.4 b                       |
| Highly asymmetric warming  | 1.21 ± 0.02 a               | 7.52 ± 0.02 a | 14.75 ± 0.04 a | 17.4 b                       |
| Extremely asymmetric warming| 1.20 ± 0.02 a              | 7.52 ± 0.01 a | 14.73 ± 0.03 a | 17.4 b                       |

Notes: Data are the means of three replicates with standard deviations (SDs) of means. Common letters followed the SD within a column indicate no statistical difference at α = 0.05.
Soil samples in the surface layer (0–15 cm) from each treatment were collected on 30 April and 31 October 2017 to test the warming effects of $^{13}C$ of soil carbon fractions. Eight to nine undisturbed soil cores were collected and then mixed to produce a composite soil sample in each plot (Zhang et al. 2019), resulting in a total of 18 composites for each sampling date.

Total soil C content ($C_{TC}$) and its stable C isotope composition ($^{13}C_{TC}$) were analyzed using elemental analyzer (VarioMAX C/N, Elemental, Langenselbold, Germany) and mass spectrometer (Delta V plus, Thermo Fisher, Waltham, USA), respectively. The $^{13}C$ of inorganic carbon ($^{13}C_{Ca}$) was analyzed by carbonate rock fragments using mass spectrometer. After the elimination of carbonates in soil samples with HCl, soil organic C ($C_{OC}$) and its isotope composition ($^{13}C_{OC}$) were analyzed as described above. Soil inorganic C ($C_{IC}$) was calculated as the difference between $C_{TC}$ and $C_{OC}$. The $^{13}C$ of the inorganic C fraction ($^{13}C_{IC}$) was estimated by the following mass balance equation (Inglima et al. 2009, Tamir et al. 2011):

$$^{13}C_{IC} = \left( ^{13}C_{TC} \times C_{TC} - ^{13}C_{OC} \times C_{OC}\right)/C_{IC}.$$  

(3)

The values of $^{13}C_{IC}$ should be, at least theoretically, equivalent to the $^{13}C_{Ca}$, owing to the fact that almost all inorganic carbon is in the form of carbonate in the study region. However, the mean determined $^{13}C_{Ca}$ was 7.2‰ (range, 6.3–7.7‰) lower than the $^{13}C_{IC}$ in Eq. (3). This finding might result from (1) the isotopic fractionation in the processes of carbonate weathering and biogenic formation of carbonates in soil solutions (Tamir et al. 2011), (2) the contribution of autotrophic respiration (Zhou et al. 2007), and/or (3) a difference in $^{13}C$ among soil organic fractions with different recalcitrance, such as soil microbial biomass carbon and carbon in low-density fraction (Ramnarine et al. 2012). Except for the seasonal variations of the mean $^{13}C_{OC}$ of the 12 soil cores at each sampling time, there was no significantly spatial (i.e., horizontal or vertical directions in soils) or seasonal variation in the $^{13}C_{OC}$ and $^{13}C_{Ca}$, irrespective of sampling time, soil depth, or sampling core (data not shown). Thus, the reconstruction of $^{13}C_{OC}$ or $^{13}C_{Ca}$ isotopic fractionation in vertical profile was not required. Due to the differences in soil $^{13}C_{OC}$ collected inside and outside experimental plots, it is necessary to correct the warming effects on $^{13}C_{OC}$ (Appendix S1: Table S1). We assumed a similar diffusion coefficient of the CO$_2$ in the soil pores with that in the air (Inglima et al. 2009, Tamir et al. 2011). To measure the CO$_2$-C trapped in the NaOH solution (ECO$_2$), an aliquot of the solution was diluted with distilled water without CO$_2$, and the CO$_2$-C was determined using a C-AutoAnalyzer (Phoenix 8000; Teledyne Tekmar, Mason, Ohio, USA). The $^{13}C$ of CO$_2$-C ($^{13}C_{CO2}$) trapped in NaOH solution was analyzed (Ramnarine et al. 2012) with mass spectrometer. The CO$_2$ efflux from soil organic fraction (EOC, SOC-derived efflux) and carbonates (ECa, carbonate-derived efflux) was separated and calculated according to the protocols reported by Inglima et al. (2009). Briefly, to quantify the proportion of SOC-derived efflux (fOC) and carbonate-derived efflux (fCa), the following isotopic balance model was applied (Inglima et al. 2009, Tamir et al. 2011, Ramnarine et al. 2012):

$$^{13}C_{CO2} = f_{Ca} \times ^{13}C_{Ca} + f_{OC} \times ^{13}C_{OC},$$  

(4)

$$f_{OC} + f_{Ca} = 1$$  

(5)

For each sampling time, the $^{13}C_{CO2}$, $^{13}C_{OC}$, and $^{13}C_{Ca}$ in Eq. (4) were averaged over the 12 soil cores. The isotopic balance model (Eqs. 4, 5) assumes that the SOC-derived CO$_2$ matches the signature of the total SOC. However, the soil autotrophic respiration with large seasonality always accounts for an important contribution to soil CO$_2$ efflux (Zhou et al. 2007). In this study, the CO$_2$ efflux from autotrophic respiration could not be separated and quantified. The $^{13}C_{Ca}$ had a stable spatiotemporal consistency and was 6.3–7.7‰ lower than the $^{13}C_{IC}$. Therefore, the $^{13}C_{OC}$ in Eq. (4) needed to be corrected by adding the difference between $^{13}C_{Ca}$ and $^{13}C_{OC}$ below were already corrected. Since the $^{13}C_{CO2}$ is heavier and diffuses at a lower rate in soil than the $^{13}C_{Ca}$, the isotopic fractionation in soils may briefly affect the $^{13}C_{CO2}$ (Tamir et al. 2011, Ramnarine et al. 2012). However, such event is unlikely to impact our study because of the long sampling interval (i.e., approximately 10 d) that we used. Because
there is a steady-state equilibrium between soil $^{13}$CO$_2$ production and soil $^{13}$CO$_2$ efflux for long sampling interval (Inglima et al. 2009), it is not necessary to correct the isotopic fractionation at vertical direction in this study. However, when the soil releases CO$_2$ into the atmosphere, atmospheric CO$_2$ also diffuses into the soil (Bertolini et al. 2005). Due to the differences in $\delta^{13}$C between atmospheric CO$_2$ and soil CO$_2$, we corrected the diffusion contribution of atmospheric CO$_2$ into the soil according to the methodology described by Bertolini et al. (2005).

The $E_{OC}$ and $E_{Ca}$ in each individual measurement were calculated by multiplying the $E_{CO2}$ by the proportion of the source in each individual measurement:

$$E_{OC} = E_{CO2} \times f_{OC},$$

$$E_{Ca} = E_{CO2} \times f_{Ca}.$$  

The monthly or annual soil CO$_2$ efflux, SOC-derived efflux, and carbonate-derived efflux were averaged over each individual efflux type.

Statistical analysis

Plot composite soil CO$_2$ efflux and its sources (SOC-derived and carbonate-derived efflux) were subject to two-way ANOVA (with Tukey’s HSD post hoc tests) with two factors (i.e., temperature treatment and sampling month). Bivariate correlation and partial correlation (with Pearson’s tests) were used to measure the degree of association between CO$_2$ efflux and environmental factors (i.e., soil temperature and soil moisture), with or without the effect of a set of controlling random variables (i.e., soil moisture or soil temperature). All statistical analysis was performed in SPSS 16.0 (SPSS, Chicago, Illinois, USA).

RESULTS

Microclimate

Although the monthly average of soil temperature at 15 cm deep in the six treatments was similar and generally resembled the changes in ambient air temperature in 2017 (Figs. 1, 2a), the temperature fluctuated greatly, ranging from 0° to 5.0°C (Fig. 2a). The annual soil temperature in the five warming treatments increased by 2.2°–2.5°C relative to the control (no-warming treatment). This monthly soil temperature in the warming treatment was greater than the control by 0°–2.4°C during summer–autumn and by 2.5°–4.0°C during winter–spring.

The monthly averages of volumetric soil moisture at a depth of 15 cm were similar in the six treatments in 2017 (Fig. 2b). In general, volumetric soil moisture was low (~10–20%) from January to May, doubled in June and remained at 50–60% until August, and then declined rapidly afterward. There were no significant differences in soil moisture among the six treatments from June to November. Soil moisture in the warming treatments was significantly lower than the control during winter–spring ($P < 0.05$) and was 0.67–1.00 times the soil moisture in the control treatment. Usually, the annual mean soil moisture
In general, smaller amounts of soil CO₂ were emitted with increasing $R_{AW}$ in the warming treatments.

Soil CO₂ efflux and its sources

Soil CO₂ efflux from the calcareous soil ranged from 1.47 to 1.86 µmol CO₂·m⁻²·s⁻¹ for all treatments after 5 yr of continuous warming (Fig. 3). Experimental warming produced an 18% increase in soil CO₂ efflux, from 1.47 µmol CO₂·m⁻²·s⁻¹ in the control to 1.73 µmol CO₂·m⁻²·s⁻¹ across all the warming treatments ($P < 0.05$). In general, smaller amounts of soil CO₂ were emitted with increasing $R_{AW}$ from 1.60 µmol CO₂·m⁻²·s⁻¹ in the EAW treatment to 1.86 µmol CO₂·m⁻²·s⁻¹ in the SW treatment (14% increase). Soil CO₂ efflux in the SW treatment was significantly larger than that in the MAW, HAW, and EAW treatments ($P < 0.05$).

The SOC-derived efflux and carbonate-derived efflux across all warming plots were 1.19 and 0.53 µmol CO₂·m⁻²·s⁻¹ after five years of warming, respectively. They accounted for approximately 70% and 30% of total efflux. These values were 1.19 and 1.12 times larger than those in the control, respectively (Fig. 3). On average, experimental warming produced an extra release of SOC-derived efflux and carbonate-derived efflux of 0.19 and 0.06 µmol CO₂·m⁻²·s⁻¹, respectively, which contribute to approximately three quarters and one quarter of the total warming-induced soil CO₂ efflux in the warming treatments, respectively. There were significant ($P < 0.05$) differences in the SOC-derived efflux among warming treatments, with the largest efflux was observed in SW treatment (1.34 µmol CO₂·m⁻²·s⁻¹) and the lowest in EAW (1.06 µmol CO₂·m⁻²·s⁻¹). The carbonate-derived efflux in the warming treatments was approximately 0.54 µmol CO₂·m⁻²·s⁻¹. The contribution of carbonate-derived efflux to total efflux was 32% in the control. In the warming treatments, this contribution tended to increase with increasing $R_{AW}$ from 28% in the SW to 34% in the EAW treatment.

Seasonal dynamics of soil CO₂ efflux and its sources

Soil CO₂ efflux exhibited seasonal variations in all treatments after 5 yr of warming (Fig. 4a), which differed significantly ($P < 0.001$) among treatments (Fig. 4a, Table 2). With increasing $R_{AW}$, the efflux in the warming treatments tended to increase in winter–spring, but decrease in summer–autumn (Fig. 4a).

The seasonal curves of the SOC-derived efflux in the six treatments resembled those of the total efflux with a peak during June to September (Fig. 4b). The SOC-derived CO₂ released during this peak contributed to 59–66% of the whole SOC-derived efflux in 2017. Our monthly largest SOC-derived CO₂ emission across all plots occurred in June (2.40 µmol CO₂·m⁻²·s⁻¹), 16.0 times higher than the lowest value in December (0.15 µmol CO₂·m⁻²·s⁻¹). Higher temperature increased SOC-derived efflux ($P < 0.001$), though the effect varied significantly with sampling months (Fig. 4b, Table 2). On monthly average basis, the SOC-derived efflux among the five warming treatments differed from April to November 2017 ($P < 0.05$), but there were no significant differences in other months.

The seasonal patterns of carbonate-derived efflux in the six treatments, however, were substantially different with those of the total efflux or the SOC-derived efflux (Fig. 4). In general, the monthly averages of carbonate-derived efflux in the warming treatments increased rapidly from January (0.69 µmol CO₂·m⁻²·s⁻¹) to May (0.82 µmol CO₂·m⁻²·s⁻¹) and then fluctuated afterward (Fig. 4c). Meanwhile, the monthly averages of carbonate-derived efflux in the...
control treatment increased from January to July (0.63 μmol CO$_2$·m$^{-2}$·s$^{-1}$) and decreased slightly. Although the largest emission of carbonate-derived CO$_2$ across all plots was observed in May (0.72 μmol CO$_2$·m$^{-2}$·s$^{-1}$) or 3.6 times higher than the lowest emission in December (0.20 μmol CO$_2$·m$^{-2}$·s$^{-1}$), the monthly contribution of carbonate-derived efflux to total efflux across all treatments was larger in December (57%) compared with that in June (20%; Fig. 4).

**Relationships between environmental factors and soil CO$_2$ efflux**

In each individual treatment or across all treatments, soil CO$_2$ efflux and its sources correlated ($P < 0.001$) more strongly with soil temperature than with soil moisture (Table 3). A weak correlation was found between the carbonate-derived efflux and soil moisture in the HAW and EAW treatment.

The partial correlation was used to assess the degree of association between CO$_2$ efflux and environmental factors without the effect of a set of controlling random variables. After removing the effect of soil moisture, partial correlations ($P < 0.05$) between soil efflux (i.e., soil CO$_2$ efflux, SOC-derived efflux, and carbonate-derived efflux) and soil temperature were observed in each individual treatment or across all treatments (Table 3). When the effect of soil temperature was removed, soil moisture was positively correlated ($P < 0.05$) with soil CO$_2$ efflux and with SOC-derived efflux in each individual treatment or across all treatments. However, the partial correlation of carbonate-derived efflux with soil moisture was only significant ($P < 0.05$) when all treatments were considered, but not significant in each individual treatment.

![Fig. 4. Seasonal dynamics of soil CO$_2$ efflux (a), soil organic fraction (SOC)-derived efflux, (b) and carbonate-derived efflux (c) in the six treatments in 2017. Vertical bars indicate standard error, $n = 3$. See Fig. 2 for abbreviations.](image)

| Table 2. ANOVA table of $F$-values on the effects of temperature treatment and sampling month on soil CO$_2$ efflux, soil organic fraction (SOC)-derived efflux, and carbonate-derived efflux in 2017. |
| --- |
| **Factor** | Soil CO$_2$ efflux | SOC-derived efflux | Carbonate-derived efflux |
| | df | $F$ | $P$ | df | $F$ | $P$ | df | $F$ | $P$ |
| Treatment | 5 | 188.26 | *** | 310.03 | *** | 21.82 | *** |
| Month | 11 | 4625.42 | *** | 6845.95 | *** | 474.00 | *** |
| Treatment × month | 55 | 23.61 | *** | 35.41 | *** | 4.14 | *** |

*** $P < 0.001$. 

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Effects of warming on soil CO$_2$ efflux

Our finding is consistent with many other studies suggesting the stimulation of soil CO$_2$ efflux with warming (Chen and Tian 2005, Uvarov et al. 2006, Zhou et al. 2007, 2009, 2018, Bond-Lamberty and Thomson 2010, Giardina et al. 2009, Inglima et al. 2009, Tamir et al. 2011, Ramnarine et al. 2012, Roland et al. 2013). In this study, the 5-year warming of 2°C increased soil CO$_2$ efflux by 17%, although the increments of soil CO$_2$ efflux varied substantially (9–27%) with warming scenarios (Fig. 3). Our results also suggest that symmetric warming potentially stimulate more CO$_2$ efflux from calcareous soils, compared with asymmetric warming scenarios. Soil CO$_2$ efflux in the symmetric warming treatment was as 1.1 times that of the average of the four asymmetric warming treatments.

In this study, experimental warming produced a 6–34% and 11–14% increase in the SOC-derived efflux and carbonate-derived efflux, respectively (Fig. 3), showing that warming could promote both SOC-derived efflux and carbonate-derived efflux in calcareous soils. The SOC-derived efflux in the symmetric warming treatment was 1.6 times that of the average of four asymmetric warming treatments. In contrast, the carbonate-derived efflux was similar between symmetric warming and asymmetric warming. As microbial growth and root growth are promoted with constantly warm temperature, it is unsurprising that symmetric warming scenario was primarily attributed to the enhancement of the SOC-derived CO$_2$ efflux. Meanwhile, the increasing carbonate-derived efflux might be attributed to ventilation and weathering process (Gislason et al. 2009, Inglima et al. 2009, Tamir et al. 2011, Ramnarine et al. 2012, Roland et al. 2013). The process can be likened to the phenomenon of excessive release of CO$_2$ during the day and absorption of CO$_2$ at night in semi-arid and arid karst region (Roland et al. 2013).

**Effects of soil temperature and soil moisture on soil CO$_2$ efflux**

In this study, the seasonal dynamics of the SOC-derived efflux were substantially different from those of the carbonate-derived efflux, irrespective of warming scenario (Fig. 4). These results suggested that CO$_2$ efflux from the two sources was controlled by different environmental processes, as also suggested by the partial correlation results (Table 3). SOC-derived efflux was affected by both soil temperature and soil moisture, while carbonate-derived efflux largely depended on soil temperature.

Concentrated rainfall in summer–autumn effectively maintains high soil moisture. But since calcareous soils are porous (Cao et al. 2012), they remain well-aerated. Warm temperature that also occurs during this period likely improves the activity of soil microbes and enzymes, promoting SOC mineralization and

Table 3. Bivariate correlation and partial correlation of soil CO$_2$ efflux, soil organic fraction (SOC)-derived efflux, and carbonate-derived efflux with soil temperature and with soil moisture in 2017.

| Treatment | $R_{SW,T}$ | $R_{RW,T}$ | $R_{REW,T}$ | $R_{REW,T}$ |
|-----------|------------|------------|-------------|-------------|
| Soil CO$_2$ efflux† | 0.974*** 0.898*** 0.893*** 0.808*** | 0.968*** 0.962*** 0.782*** 0.732* | 0.967*** 0.970*** 0.789*** 0.807*** | 0.969*** 0.956*** 0.875*** 0.814*** |
| No-warming | 0.969*** 0.953*** 0.928*** 0.852*** | 0.957*** 0.911*** 0.959*** 0.916*** | 0.925*** 0.883*** 0.958*** 0.935*** | 0.969*** 0.937*** 0.928*** 0.852*** |
| SW | 0.913*** 0.921*** 0.820*** 0.838*** | 0.956*** 0.965*** 0.665* 0.750** | 0.947*** 0.968*** 0.729* 0.848** | 0.941*** 0.957*** 0.819*** 0.871*** |
| SAW | 0.949*** 0.976*** 0.604* 0.839** | 0.947*** 0.968*** 0.729* 0.848** | 0.917*** 0.943*** 0.881*** 0.919*** | 0.874*** 0.928*** 0.913*** 0.950*** |
| MAW | 0.947*** 0.968*** 0.729* 0.848** | 0.917*** 0.943*** 0.881*** 0.919*** | 0.874*** 0.928*** 0.913*** 0.950*** | 0.949*** 0.976*** 0.604* 0.839** |
| HAW | 0.913*** 0.921*** 0.820*** 0.838*** | 0.956*** 0.965*** 0.665* 0.750** | 0.947*** 0.968*** 0.729* 0.848** | 0.941*** 0.957*** 0.819*** 0.871*** |
| EAW | 0.913*** 0.921*** 0.820*** 0.838*** | 0.956*** 0.965*** 0.665* 0.750** | 0.947*** 0.968*** 0.729* 0.848** | 0.941*** 0.957*** 0.819*** 0.871*** |
| SOC-derived efflux† | 0.855*** 0.599*** 0.830*** −0.277*** | 0.918*** 0.835*** 0.694* −0.054 | 0.909*** 0.758*** 0.826** −0.470 | 0.896*** 0.691* 0.856* −0.542 |
| Carbonate-derived efflux† | 0.896*** 0.691* 0.856* −0.542 | 0.919*** 0.675* 0.892*** −0.534 | 0.919*** 0.675* 0.892*** −0.534 | 0.919*** 0.675* 0.892*** −0.534 |
| All plots | 0.882*** 0.551 0.883*** −0.499 | 0.882*** 0.551 0.883*** −0.499 | 0.882*** 0.551 0.883*** −0.499 | 0.882*** 0.551 0.883*** −0.499 |
| No-warming | 0.887*** 0.494 0.861*** −0.289 | 0.887*** 0.494 0.861*** −0.289 | 0.887*** 0.494 0.861*** −0.289 | 0.887*** 0.494 0.861*** −0.289 |

Notes: SW, symmetric warming; SAW, slightly asymmetric warming; MAW, moderately asymmetric warming; HAW, highly asymmetric warming; EAW, extremely asymmetric warming.

† $R_{SW,T}$ and $R_{REW,T}$ bivariate correlation coefficient of soil efflux with soil temperature and with soil moisture, respectively. $R_{SW,T}$ partial correlation coefficient between soil efflux and soil temperature, without the effect of soil moisture. $R_{REW,T}$ partial correlation coefficient between soil efflux and soil moisture, without the effect of soil temperature.

*** $P < 0.001$, **$P < 0.01$, *$P < 0.05$. 

**DISCUSSION**

**Effects of warming on soil CO$_2$ efflux**

Our finding is consistent with many other studies suggesting the stimulation of soil CO$_2$ efflux with warming (Chen and Tian 2005, Uvarov et al. 2006, Zhou et al. 2007, 2009, 2018, Bond-Lamberty and Thomson 2010, Giardina et al. 2014, Zeleke 2015). In this study, the 5-year warming of 2°C increased soil CO$_2$ efflux by 17%, although the increments of soil CO$_2$ efflux varied substantially (9–27%) with warming scenarios (Fig. 3). Our results also suggest that...
root respiration (Zhou et al. 2007, Wang et al. 2014). This mechanism may explain why a large amount of SOC-derived CO₂ was released during periods of high temperature and high rainfall, that is, from June to September (Figs. 2, 4), but not during cold and dry season (November–May).

In contrast, the carbonate-derived efflux was positively correlated with soil temperature regardless of soil moisture (Table 3). As soil ecological processes (e.g., soil respiration, nitrification of soil organic N, oxidation of reduced S) are promoted by higher temperature, they also produce more H⁺ ions in soil, due to the formation of soil organic acids, H₂CO₃, HNO₃, and H₂SO₄ (Inglima et al. 2009, Tamir et al. 2011, Ramnarine et al. 2012, Zhu et al. 2016). High H⁺ concentration can move Eqs. 1, 2 toward the weathering of carbonates and the subsequent concentration can move Eqs. 1, 2 toward the weathering of carbonates and the subsequent release of CO₂. In addition, a high temperature may, theoretically, improve the thermodynamic reaction rates of Eqs. 1, 2, and disperse CO₂ in soil solution to soil pores or to the atmosphere (Gislason et al. 2009). Carbonate-derived efflux was only minimally influenced by soil moisture without the effect of soil temperature (Table 3). Inglima et al. (2009) observed an abrupt decrease in the contribution of carbonate-derived efflux after a rain event and attributed this outcome to the leaching of dissolved inorganic C into deeper soils and not to the lower dissolution of carbonate minerals. Immediately after rain events, ecosystems with a deep vadose zone could temporarily store CO₂ in soil via the process of soil CO₂ dissolution and subsequent infiltration (Serrano-Ortiza et al. 2010). Roland et al. (2013) considered that ventilation can explain the atypical CO₂ exchange between the carbonaceous system and the atmosphere. Given a high water permeability of calcareous soils, rainwater quickly leaches via subsurface seepage flows and carries with a large amount of dissolved inorganic C (Cao et al. 2012). But lower diffusion rates of CO₂ from water-filled pores during periods of high soil moisture result in low soil CO₂ emission (Schwendenmann et al. 2003, Serrano-Ortiza et al. 2010). On the other hand, when drought occurs, it limits both the aqueous chemistry and carbonate-derived CO₂ release. Therefore, the period of sparse rainfall and relatively high temperature (April–May) could lead to high carbonate-derived efflux compared with periods when rainfall and temperature were both high (June–September; Figs. 1, 2, 4).

CONCLUSIONS

The 5-year warming of a calcareous soil produced a 6–34% and 11–14% increase in the SOC-derived and carbonate-derived CO₂ efflux, respectively. Symmetric warming has a potential to stimulate more soil CO₂ efflux compared with asymmetric warming scenarios, primarily owing to the SOC-derived efflux. The seasonal patterns of the carbonate-derived efflux were substantially different from those of total efflux or the SOC-derived efflux. When seasonal dynamics of the SOC-derived efflux was driven by both the seasonality of soil temperature and soil moisture, the carbonate-derived efflux was largely driven by the seasonality of soil temperature, rather than soil moisture.

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**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3281/full