Plant Soil (2018) 428:279–290
https://doi.org/10.1007/s11104-018-3644-3

REGULAR ARTICLE

Important interaction of chemicals, microbial biomass and dissolved substrates in the diel hysteresis loop of soil heterotrophic respiration

Qing Wang · Nianpeng He · Yuan Liu · Meiling Li · Li Xu · Xuhui Zhou

Received: 23 November 2017 / Accepted: 5 April 2018 / Published online: 16 May 2018

Abstract

Background and aims Increasing the emission of carbon dioxide by heterotrophic respiration (R_h) might lead to global warming. However, issues remain on how R_h responds to changing temperatures, especially with respect to the hysteresis loop in the relationship between R_h and temperature at the daily scale, along with elucidating the underlying mechanisms.

Method We investigated hysteresis loop by measuring R_h in subtropical forest soil at the daily scale (12 h for warm-up (6–30 °C) and cool-down processes (30–6 °C), respectively) using continuous temperature variation and high resolution of measurements over a 56-day incubation period. The ratios of R_{20} and Q_{10} between warm-up and cool-down were calculated as the characteristics of diel hysteresis. We measured chemical (pH, conductivity, oxidation-reduction potential), microbial biomass and dissolved substrate (carbon and nitrogen) parameters to explain variation of diel hysteresis.

Results R_h was strongly dependent on temperature, with a clockwise hysteresis loop of R_h between the warm-up and cool-down daily processes. The average value of R_{20} [at a reference temperature of 20 °C] during the whole incubation period under the warm-up process was significantly higher (46.05 ± 0.96 μgC g^{-1} d^{-1}) than that under the cool-down process (14.74 ± 0.03 μgC g^{-1} d^{-1}). In comparison, the average value of Q_{10} under the cool-down process (5.27 ± 0.2) was significantly higher than that under the warm-up process (1.66 ± 0.02). Redundancy analysis showed that the interaction effects of soil chemical, microbial biomass, and dissolved substrate parameters explain most variation of diel hysteresis: 98% variation in R_{20} and 93.5% variation in Q_{10}. Compared with the weak effect of chemistry parameters on the diel hysteresis, the sole and interactive effects of microbial biomass and substrate were more important, especially their interaction.

Conclusions Interactions of chemical, microbial biomass, and dissolved substrate parameters dominated the variation in diel hysteresis of R_h with temperature, especially the interaction of microbial biomass and dissolved substrate. Of note, Q_{10} during the warm-up process might be overestimated when using the highly

Responsible Editor: Feike A. Dijkstra.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-018-3644-3) contains supplementary material, which is available to authorized users.

Q. Wang · X. Zhou
School of Ecological and Environmental Science, East China Normal University, Shanghai 200241, China

Q. Wang · N. He (✉) · Y. Liu · M. Li · L. Xu
Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China
e-mail: henp@igsnrr.ac.cn

N. He
College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China

N. He
Institute of Grassland Science, Northeast Normal University, and Key Laboratory of Vegetation Ecology, Ministry of Education, Changchun 130024, China

fitted temperature-dependent function of cool-down period. Furthermore, using a constant value of Q_{10} (Q_{10} = 2) in carbon cycle models might be an important source of uncertainty.

Keywords Warm-up · Cool-down · Substrate · Microbial biomass · Heterotrophic respiration

Introduction

Temperature is one of the most important factors influencing the decomposition of soil organic matter (SOM) caused by microbes (heterotrophic respiration, R_h), which has been widely reported to increase with increasing temperature (Fang and Moncrieff 2001; Davidson and Janssens 2006; Ise and Moorcroft 2006). Given the vast quantity of SOM in terrestrial ecosystems, varied responses of R_h to temperature might produce major fluctuations to the balance of the soil organic carbon (C) pool, leading to uncertainty when predicting the feedback between R_h and global warming (Shibata et al. 2005; Jiang et al. 2013).

Therefore, it is important to understand how R_h changed with changes to daily temperature dynamics. To date, advances have been made to improve the accuracy of predictions through increasing availability of high time resolutions of CO_2 flux and temperature measurements during the diel cycle. However, in contrast to the common assumption that soil respiration varies with soil temperature synchronously, diel variation in soil respiration has been documented to be independent of temperature in a temperate deciduous forest (Liu et al. 2006), demonstrating a hysteresis loop in the relationship between soil respiration and soil temperature (Riveros-Iregui et al. 2007; Ruehr et al. 2010; Barron-Gafford et al. 2011; Phillips et al. 2011). Such diel hysteresis might result from: 1) diurnal changes in soil moisture; 2) changes to the microbial community or activity; 3) changes to the quality of SOM and its accessibility to microbes or enzymes, and 4) the effects of fresh photosynthetic product on root and rhizosphere respiration (Subke and Bahn 2010).

Hysteresis in soil respiration with temperature occurs at a series of scales, from small spatial scales (soil layer) (Subke and Bahn 2010) to large temporal scales (seasonal or annual) (Moren and Lindroth 2000; Gaumont-Guay et al. 2006). If soil respiration responded differently to temperature between the daytime and nighttime periods of these hysteresis, utilizing the same response of respiration to changing temperature to assess the diurnal respiration might underestimate or overestimate how global warming affects the C pool. In particular, Niu et al. (2011) suggested that the hysteresis in net ecosystem exchange at the annual scale explains 8% of variation in ecosystem respiration. While soil respiration is an important component of ecosystem respiration, it might influence the net ecosystem exchange, with the potential mechanisms regulating how soil respiration varies with diel temperature remaining uncertain. Therefore, it might be possible to assess C dynamics accurately by exploring the relationship between R_h and temperature, as well as elucidating the actual mechanism regulating hysteresis.

In additon to the variation in respiration rate, another characterestic of diel hysteresis is how the respiration rate responds to changing temperature, which is defined as temperature sensitivity (Q_{10}) as the reaction rate increases with a 10 °C rise in temperature (Fang and Moncrieff 2001). Similar to variation in R_h during diel hysteresis, Q_{10} does not always exhibit a symmetrical trend with the increasing and decreasing process of soil temperature at the diel scale. For example, Liu et al. (2006) found the sensitivity of soil respiration to temperature was distinctly higher during the daytime than that at nighttime in a temperate deciduous forest.

Two potential explanations exist for the documented difference in Q_{10} during hysteresis. First, variation in the quantity and quality of the substrate (Liski et al. 1999; Gershenson et al. 2009; Craine et al. 2010; Sierra et al. 2012; Lefevre et al. 2014) might affect on the substrate available for the metabolism of microbes, and is termed the "Carbon quality –temperature sensitivity" hypothesis (Bosatta and Ågren 1999). A substrate of low quality requires higher energy for microbes to degrade, leading to higher sensitivity to warming than the high-quality substrate (Gershenson et al. 2009; He et al. 2013; Wang et al. 2016b). Second, changes to the soil microbial community (Malcolm et al. 2008; de Bruijn and Butterbach-Bahl 2010; Rousk et al. 2012) might affect heterotrophic respiration, which could be associated with the hot topic on respiration acclimation (Bradford et al. 2008; Bradford 2013). For example, microbes might acclimate to the changed diel temperatures, with the acclimation to warming generating an elliptic loop of R_h with temperature in parallel to lower R_h at intermediate temperatures (Hall et al. 2008). These two factors

might interact with one another. Such as, the growth of microorganisms could be accelerated significantly when available substrate is abundant, whereas an increased growth of microbes might cause the amount of available substrate to decrease. Other examples of interaction might be reflected in the different preferences of microbial community for decomposing SOM. For instance, bacteria prefer labile SOM, while fungi prefer stable SOM (Lehmann and Kleber 2015). In conclusion, changes to microbial and substrate properties might explain differences in R_h and Q_{10} between the increasing and decreasing temperature processes of hysteresis.

Although many incubation experiments have reported the response of R_h to changing temperatures (Fang and Moncrieff 2001; Fierer et al. 2005; Gershenson et al. 2009; Lefevre et al. 2014; Wang et al. 2015), evidence of hysteresis in R_h with temperature during diel cycles have seldom been reported (Eberwein et al. 2015). This gap might be attributed to two reasons. First, traditional incubation experiments are conducted with several constant incubation temperatures (Fierer et al. 2005; Gershenson et al. 2009; Malcolm et al. 2008; Wetterstedt et al. 2010; Rousk et al. 2012; Wagai et al. 2013; Wei et al. 2014), and primary focus on the response during the increasing temperature period, but not the decreasing temperature period. Second, the capacity to measure R_h with continuously changing temperature over a high time resolution of measurment has been limited by the instrument technical conditions. These two issues have restricted our ability to investigate the mechanism of hysteresis of R_h and temperature using incubation experiments.

Here, we designed a novel incubation experiment using subtropical forest soil under increasing (from 6 °C to 30 °C over 12 h; warm-up process) and decreasing (from 30 °C to 6 °C over 12 h; cool-down process) temperature regimes to simulate variations in daily temperature (24 h) under the field conditions. Using continuous and high time resolution measurement equipment, we recorded the values of R_h (approximately every 20 min) with soil temperature over a 56-day incubation period. To assess the hysteresis at a diel scale, we calculated the ratio of R_{20} (heterotrophic respiration at a reference temperature of 20 °C) and Q_{10} between the warm-up and cool-down processes, respectively. Furthermore, we measured the potential influence of chemical, microbial, and substrate properties, to explain the variation with incubation time. This study aimed to test whether: 1) variation in R_h was dependent on temperature, shaping the diel hysteresis between the warm-up and cool-down processes, and 2) the interaction effects of microbial and substrates properties or the independent effect of single factor control how R_h varied in the hysteresis loop of with temperature.

Materials and methods

Site description

Soil samples were collected from a primary forest located in Shennongjia, Hubei Province, China (109° 56′–110° 58′ E, 31° 15′–31° 75′ N). This region has a north subtropical monsoon climate, with mean annual temperature of 8.5 °C and mean annual precipitation of 1147 mm (mean of 1962–2012). The climate at the research site tends to be hot and rainy in summer and cool and dry in winter. The dominant tree species of the primary forest are *Fagus engleriana* Seemen and *Cyclobala nopsismultinervis* W. C. Cheng et T. Hong (Chi et al. 2015). Soil pH is approximately 6.93, soil organic carbon (SOC) is 4.19%, and total nitrogen is 0.38% in the 0–10 cm soil layer. The clay, silt, and sand contents are 13.21, 60.59, and 26.20%, respectively. Content of bacteria and fungi are 5.96 and 0.25 nmol g^{-1}, respectively. More detailed information about this sample site is provided in Xu et al. (2017) and He et al. (2018).

Field soil sampling and previous treatment

Field sampling was conducted in August 2013. Three experimental plots (40 × 40 m) were established in the primary forest. Soil samples were collected from the soil surface (0–10 cm depth) and were sieved through a 2-mm mesh (Xu et al. 2017). The roots and visible organic debris in the soil samples were removed manually. Soil were stored at 4 °C before beginning the incubation experiment.

Soil water holding capacity was determined by wetting the soil for 12 h, followed by draining it through filter paper for 12 h. Then, the soil water content was calculated by the weight measured before and after drying the sample at 105 °C for 24 h (Wang et al. 2016ab). The contents of SOC and total nitrogen were measured using an elemental analyzer (Vario Max, Elementar, Langensel bold, Germany).

Design of incubation experiment

In total, there were 18 replicates (3 replicates × 6 groups), with one group for the repeated measurements of R_h throughout the incubation period (0, 7, 14, 21, 28, 35, 42, and 56 days) and five groups (3 replicates ×5 destruction times) for separate destructive sampling (at 0, 14, 28, 42, and 56 days) to measure the factors that potentially regulate variation in diel hysteresis. Each replicate contained 40 g fresh soil mixed with 10 g quartz sand (preventing the soil from hardening), and was placed in a 150 mL polyethylene plastic bottle, and adjusted to 55% water hold capacity by weight. All soil samples were first pre-incubated at 20 °C for 1 week (to make the microbes active and avoid the disturbance caused by the experimental treatment), and were then placed in an incubator that automatically regulated temperature to increase from 6 to 30 °C (6 °C ca. 6 h, 14 °C ca. 6 h, 21 °C ca.6 h, 30 °C ca. 6 h) and then decrease from 30 to 6 °C (Fig. S1) (Li et al. 2017). Although this daily incubation process could not entirely simulate the daily soil temperature in the field, it simulated the daily minimum, middle, and the maximum soil temperature. The incubation temperature was adjusted over several phases in a day, due to the limitation of incubation system, which could still prevent the microbes from adapting to a specific temperature (Rousk and Bååth 2011). Furthermore, to maintain constant soil moisture levels, soil water content was adjusted based on weight at intervals of 3–4 days.

Data availability

Data share can be contacted with Dr. N. P. He (E-mail: henp@igsnrr.ac.cn).

Measurements of R_h, soil substrate, microbial biomass, and chemical properties

Measurements of R_h

On the days scheduled for measuring R_h, CO_2 concentration released by microbes and soil temperature were continuously measured over a 24-h period, based on the designated program shown in Fig. S1 (Wang et al. 2016b). Specifically, the temperature in the water bath for incubating soil samples steadily and gradually increased from 6 to 30 °C over 12 h, and then decreased from 30 to 6 °C over 12 h. The CO_2 concentration of soil samples was measured at intervals of 20 min using continuous measurement apparatus (PRI-8800; Pri-Eco, Beijing, China), which was newly developed as a modification of He et al. (2013). This method for measuring R_h has its intrinsic advantages and has already been applied in some studies (Wang et al. 2016a; Liu et al. 2017, 2018; Song et al. 2017). At the same time, the actual soil temperatures of the incubating samples were measured by a temperature logger (iButton® DS1922L, Maxim Integrated, San Jose, CA, USA), which was embedded in the surface of the soil sample (to reduce disturbing the soil samples) the day before measuring R_h. This mode of measuring allows the relationship between R_h and temperature to be elucidated in more detail, and provides a more accurate calculation of Q_{10} than that calculated by less frequent measurements (Wang et al. 2016b; Li et al. 2017; Liu et al. 2017).

R_h was first calculated from the slope of the change in CO_2 concentration released by microbes and conversion factors as (Eq. 1) (He et al. 2013):

$$R_h = \frac{C \times V \times \alpha \times \beta}{m} \quad (1)$$

where R_h is the soil heterotrophic respiration rate ($\mu gC\ g^{-1}d^{-1}$); C is the slope of change in CO_2 concentration with measured time; V is the volume of the incubation bottle and gas tube (ml); m is the soil weight (g); α is the conversion coefficient for CO_2 mass (from CO_2 to C); and β is a conversion coefficient for time (from seconds to days).

To compare the difference in R_h at different stages in the diel hysteresis, we calculated the R_h at the reference temperature of 20 °C as R_{20} (Craine et al. 2010) between warm-up and cool-down processes, respectively. In this experiment, it was possible to calculate the relationship between R_h and temperature accurately through continuously measuring changes to temperature automatically and through frequently measuring the R_h (at intervals of 20 min) with a button thermometer (iButton® DS1922L, Maxim Integrated, San Jose, CA, USA). Thus, we assumed that taking the independent data to represent the R_{20} would decrease variation in R_{20} when using regression analysis, because there are different exponential relationships between R_h and soil temperature in the warm-up compared to the cool-down process (Table S1). In practice, R_{20} was calculated as the average of R_h ranging from 18 °C to 22 °C. Furthermore, autocorrelation between R_{20} and temperature sensitivity or

substrate quality using the following equations (Eqs. 2, 3) was avoided.

Q_{10} values were calculated using the following exponential equations (Eqs. 2 and 3) (Lloyd and Taylor 1994):

$$R_h = A \times e^{b \times T} \quad (2)$$

$$Q_{10} = e^{10 \times b} \quad (3)$$

where R_h is the heterotrophic respiration rate (μgC $g^{-1}d^{-1}$), T is the temperature (°C), and A and b are the exponential parameters that represent the intercept and slope of the line, respectively. Because R_h and soil temperature were measured extensively with strongly matching exponential functions (Table S1), the Q_{10} values used in the current study were considered accurate and credible (Wang et al. 2016ab, Li et al. 2017). Furthermore, we equated substrate C quality with parameter A in Eq. 2 to investigate how soil C quality affects the diel hysteresis of R_h, which had also been applied to other studies (Fierer et al. 2005; Xu et al. 2012). Furthermore, Ding et al. (2016) synthesized 386 paired data sets of Q_{10} and A from 55 independent papers, and demonstrated that A could serve as an effective indicator of soil C decomposability. Therefore, parameter A in the Eq. 2 was considered a credible measure of carbon quality.

To explore the daily hysteresis of R_h with temperature between warm-up and cool-down processes, we calculated R_{20} and Q_{10} in the two phases over the whole experiment period (56 d), respectively. Furthermore, we measured all parameters of dissolved substrate, soil chemistry and microbial biomass five times during the whole incubation.

Measurements of soil substrates, microbial biomass and chemical properties

Because this study was conducted with one soil layer under laboratory conditions, we did not discuss certain field factors, such as soil water content (Riveros-Iregui et al. 2007; Ruehr et al. 2010), soil layers (Subke and Bahn 2010; Phillips et al. 2011), and photosynthetic active radiation (Liu et al. 2006). The effect of changes to soil water content during the measuring period was not considered in the current study because soil water content was controlled before every measurement. That is, variation to soil water content in the diel measurement period was same across eight independent measurements.

It is an advantage for us to achieve diel dynamic of R_h with high intensity using the PRI-8800 (Pri-Eco, Beijing, China). However, it is a challenge to simultaneously measure the underlying influencing factors of chemical, substrate and microbial properties at the warm-up and cool-down processes, respectively, although chemical, substrate and microbial properties in soil may change as incubation time lengthened to some extent. Thus, we mainly assessed the effects of initial soil chemistry (pH, oxidation reduction potential [ORP], and conductivity [COND]), microbial biomass (microbial biomass carbon [MBC], microbial biomass nitrogen [MBN]), and dissolved substrate properties (dissolved total nitrogen [DTN] and dissolved organic carbon [DOC]) on how the characteristics of diel hysteresis vary with short-time incubation time (Iqbal et al. 2010).

After 0, 14, 28, 42, and 56 days of incubation, respectively, every three replicates of soil samples were destructed to measure soil dissolved substrate (DTN, DOC), microbial biomass (MBC, MBN), and soil chemical properties (pH, ORP, and COND). On the day that the soil samples were destroyed, measurement of R_h started. Specifically, the chloroform-fumigation method with 50 mL 0.5 M K_2SO_4 solution was used to estimate MBN (1/Kn = 1.85) (Brookes et al. 1985), MBC (1/Kc = 2.22) (Baumann et al. 1996) and soil DTN and DOC. The supernatants of C and N extracted by K_2SO_4 solution were measured using a total organic carbon instrument (Liquid TOC II; Elementar, Germany) and continuous flow analyzer (Futura; AMS Alliance, Frépillon, France), respectively. The supernatants for pH, ORP, and COND were extracted with 25 mL ultrapure water (slurry of soil and ultrapure water, 1:2.5), and were measured by an Ultrameter II™ (Myron L Company, Carlsbad, CA, USA) (Wang et al. 2016b; Li et al. 2017).

Statistical analysis

A repeated-measures analysis of variance was used to test the effects of treatment (warm-up vs. cool-down) and incubation time (8 times during 56 days incubation) on R_{20} and Q_{10}. An independent-samples t-test was used to investigate whether warm-up and cool-down processes resulted in significant differences to the means of R_{20} and Q_{10} after 56 days of incubation. The effects of C quality (parameter A in the Eq. 2), soil chemical, microbial biomass and substrate parameters on R_{20} and Q_{10}

between warm-up-and cool-down processes were assessed using regression analyses. Statistical analysis was conducted using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance level was set at $P = 0.05$.

The important parameters of soil chemical, microbial and substrate properties for the variation of ratios of R_{20} and Q_{10} between the warm-up-and cool-down phases were selected by regress relationships and were took into the redundancy analysis (RDA) with Canoco software version 5.0 (ter Braak and Smilauer 2012). Before RDA, we used the general addition model to assess whether the relationships between R_{20}, Q_{10} and these factors had linear effects. We transferred those factors existed nonlinear relationships with the variation of ratios of R_{20} and Q_{10} between the warm-up-and cool-down phases using the results of general addition model. Judging and transferring analyses were conducted with R version 3.4.3 for Windows.

Results

Changes to R_h with temperature at the daily scale

The diel pattern of R_h was synchronized with that of soil temperature (Fig. 1). The relationship between R_h and soil temperature during the warm-up and the cool-down processes formed a clear clockwise pattern of the hysteresis loop (Fig. S2), which matched with exponential functions better in the cool-down process than in the warm-up processes (Table. S1).

Changes to R_{20} and Q_{10} during warm-up and cool-down processes

R_{20} increased slightly, then decreased, as incubation time lengthened under the warm-up process, and exhibited a significant linear decrease with incubation time under the cool-down process, except at 35 d of incubation (Fig. 2a, b). The average R_{20} at 56 days of incubation under the warm-up process (46.05 ± 0.96 μgC g^{-1} d^{-1}) was significantly higher than that under the cool-down process (14.74 ± 0.03 μgC g^{-1} d^{-1}) (Fig. 2c). Q_{10} decreased first, then increased slightly, as incubation time lengthened under the warm-up process, and exhibited a significant linear increase with incubation time under the cool-down process (Fig. 2d, e). Importantly, the average Q_{10} value at 56 days of incubation during the cool-down process (5.27 ± 0.20) was significantly higher than that during in the warm-up process (1.66 ± 0.02) (Fig. 2f).

Factors affecting R_{20} and Q_{10} during warm-up and cool-down processes

The characteristics of the diel hysteresis (R_{20} and Q_{10}) were mainly influenced by MBC, MBN, DOC, DTN, pH, and COND (Fig. S3). Especially, the ratios of R_{20} between the warm-up and cool-down processes significantly increased exponentially with those of C quality ($R^2 = 0.87$, $P < 0.001$, Fig. 3a). In comparison, the ratios of Q_{10} between the warm-up and cool-down processes exponentially decreased with those of C quality ($R^2 = 0.78$, $P < 0.001$) (Fig. 3b). C quality during the warm-up process (2.65 ± 1.13 μgC g^{-1} d^{-1}) was significantly higher than that during the cool-down process (0.08 ± 0.05 μgC g^{-1} d^{-1}) ($P < 0.001$, Fig. S4). Furthermore, the ratios of C quality between the warm-up and cool-down processes significantly increased with DOC and decreased with MBC (Fig. 4).

The RDA results showed that microbes, chemicals, and substrates explained most variation for the two characteristics of the diel hysteresis: 98% of variation in R_{20} ($P = 0.002$, Fig. 5a) and 93.5% of variation in Q_{10} ($P = 0.002$, Fig. 5b). The joint effect of microbes, chemicals, and substrates explained 60.2% of variation in R_{20} and 64.9% of variation in Q_{10}, respectively. Compared with the weak effect of soil chemical property on diel hysteresis, the sole and interactive effects of soil microbes and substrate were more important, especially their interaction.

Discussion

Using the technology of continuous altering temperature and high-frequency measurement, this study clearly showed that R_h was significantly dependent on soil temperature with a clockwise hysteresis loop between warm-up and cool-down processes, which was dominated by the interactions of soil substrate, and microbial properties mainly.

Distinct differences between warm-up and cool-down processes in the hysteresis loop

The hysteresis loop reported here showed a synchronized relationship between R_h and temperature, with

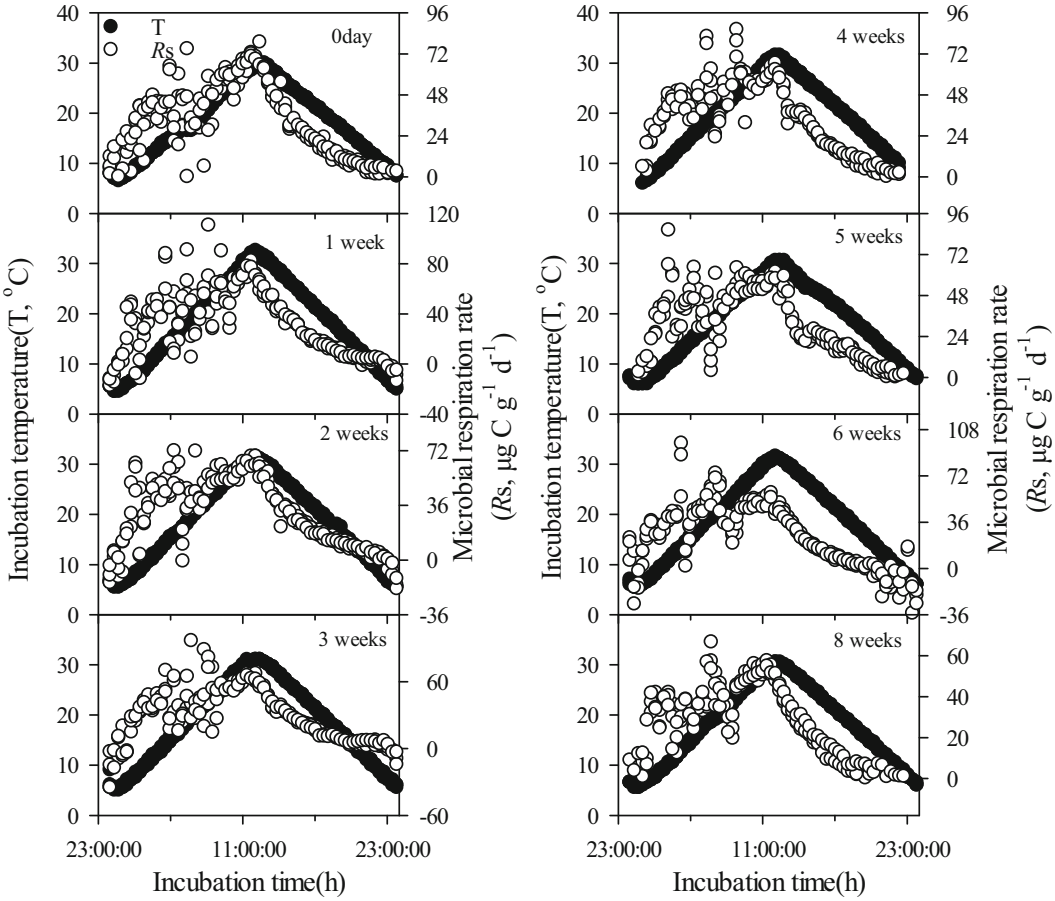

Fig. 1 Changes in heterotrophic respiration (R_h, μgC g^{-1} d^{-1}) and measure temperature (°C) throughout the day (24 h) over an 8-week period

significantly higher R_h under the warm-up process and significantly higher Q_{10} under the cool-down process. Previous studies found that R_h was independent of temperature in the hysteresis loop (Liu et al. 2006; Subke and Bahn 2010; Phillips et al. 2011). For instance, Liu et al. (2006) demonstrated that diel variation in soil respiration was independent of temperature in a temperate deciduous forest with lower Q_{10} at night compared to daytime. This result could be explained by the diel variation in photosynthetic active radiation associated with phenological stages of the forest stand. In contrast to Liu et al. (2006), significant higher Q_{10} was observed during the cool-down process in this study. According to the "carbon quality- temperature sensitivity" hypothesis, recalcitrant SOM had higher Q_{10} than liable SOM (Bosatta and Ågren 1999). Thus, we assumed that the difference detected between the two studies might be due to different amounts of available SOM at the two stages. In the field experiment of Liu et al. (2006), SOM input were continuous, with more liable SOM being available for microbial metabolism at night. In comparison, in the current incubation experiment, more liable SOM were decomposed in the warm-up period, which led to more recalcitrant SOM but less liable SOM being available for the cool-down period.

Of note, differences in Q_{10} between daytime and nighttime decreased with increasing time of the phenological stage during the treatment of high-CO_2 in the study of Liu et al. (2006). However, even this pattern was reversed during the winter (higher Q_{10} at night). These phenomena implied that the different response of respiration to temperature during the day and night should be emphasized, especially in consideration of global warming. Furthermore, the higher daily Q_{10} value (3.47 [mean value of warm-up and cool-down processes in this study] and 2.93 [mean value of daytime and night-time in the study of Liu et al. (2006)]) was far higher than the common value ($Q_{10} = 2$) used in C models. The hysteresis of soil respiration with temperature is common

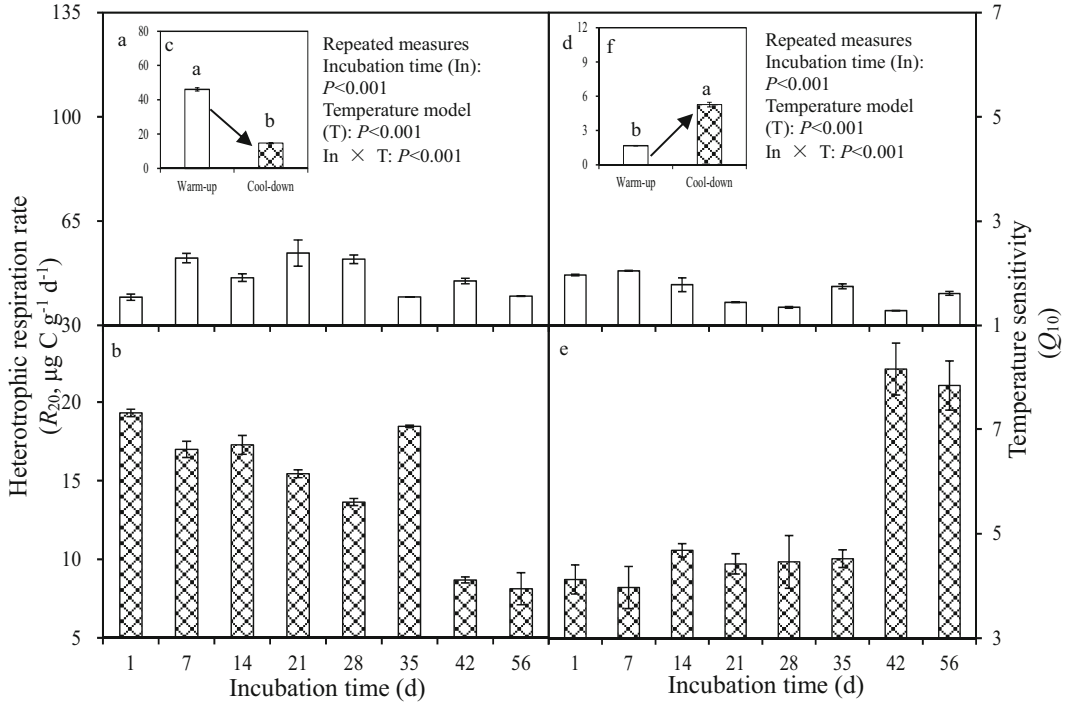

Fig. 2 Changes in heterotrophic respiration at a reference temperature of 20 °C (R_{20}, μgC g^{-1} d^{-1}, panels a, b, c) and temperature sensitivity (Q_{10}, panels d, e, f) during warm-up and cool-down periods over 8 weeks. Data in c and f are the mean values of R_{20} and Q_{10} over 8 weeks of incubation, respectively. Error bars represent SD ($n = 3$). Different lowercase letters indicate significant differences at $P = 0.05$

in the natural world, ranged from daily to season, even, annual scale (Moren and Lindroth 2000; Nakai et al. 2003; Gaumont-Guay et al. 2006). Thus it is important to incorporate the distinctly different periods of daily hysteresis of soil respiration in relation to soil temperature in the C cycle model, which might weaken the deviation between actual flux data and the predicted values.

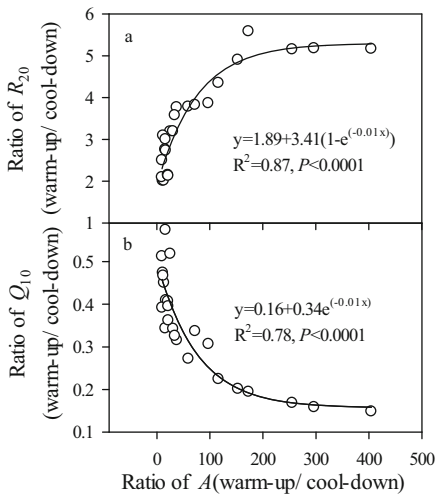

Fig. 3 Changes to the ratios of heterotrophic respiration rates at a reference temperature of 20 °C (R_{20}) and temperature sensitivity (Q_{10}) between warm-up and cool-down processes with that of the carbon quality index (parameter A in Eq. 2) over 8 weeks

In contrast to the common assumption that the relationship between soil respiration and temperature during the increasing and decreasing temperature stages have similar variation, higher variation was observed during the warm-up process in this study. The better exponential relationships between soil respiration and soil temperature during the cool-down versus warm-up stages were observed here, and also occurred during the daytime and nighttime periods at a seasonal scale (Liu et al. 2006). This distinct variation of different periods needs further investigated to pinpoint the response of soil respiration to temperature.

Interactive effects of chemical, substrate and microbes on the hysteresis loop

Both R_{20} and Q_{10} (characteristics of hysteresis loop) were influenced by the interaction of soil chemical, microbial and substrate properties. The significant

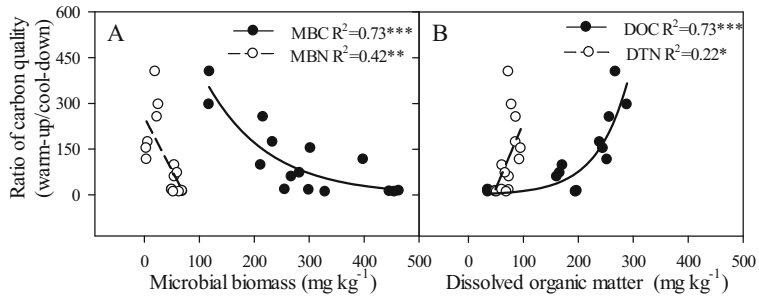

Fig. 4 Changes to the ratios of carbon quality (parameter *A* in Eq. 2) between warm-up and cool-down processes with respect to soil substrate and microbial properties. Microbial properties included: MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; substrate properties included: DOC: dissolved organic carbon; DTN: dissolved total nitrogen

interaction between microbes and substrate on the variation in diel hysteresis is reflected by their effects on the index of carbon quality, which might influence R_h and Q_{10} (Briones et al. 2014; Davidson and Janssens 2006; Ding et al. 2016). This phenomenon is consistent with our results in Fig. 4. Furthermore, we put forward a precondition that the microbial community might evolve selective expression under the long-term diel dynamics of soil temperature because the microbial community exhibits different preferences in SOM (Lehmann and Kleber 2015). In other words, the bacteria community is more active than the fungi community at decomposing SOM during the daytime, and vice versa at night. This preference of the microbial community could help to explain our results, to some extent.

When substrate supply was enough for microbial metabolism, the differences in microbial activity (fungi $_{VS.}$ bacteria) might alter the availability of substrate (liable and recalcitrant SOM) between warm-up and cool-down processes. However, soil microbial activity was finally limited by the availability of substrate over long-term incubation (due to the lack of exogenous SOM input). The relationships between R_{20} and Q_{10} with C quality (based on the ratios between warm-up and cool-down processes, Fig. 3) might support the proposed hypothesis of microbial preferences for SOM. Theoretically, shifts in the structure of microbial communities might be observed, due differences in their temperature-dependency among species (Frey et al. 2008; Malcolm et al. 2008; Balser and Wixon 2009). Previous studies have demonstrated that warming resulted into shifts to the microbial community: an increase in the abundance of gram-positive bacteria and a decrease in the abundance of gram-negative bacteria and fungi (Wei et al. 2014).

Although data on the microbial community and substrate quantity and quality during warm-up and

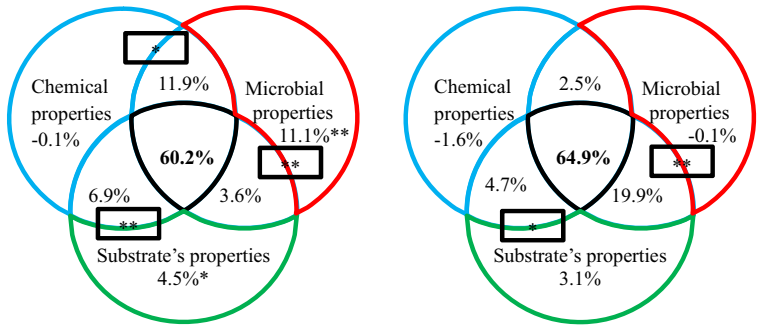

Fig. 5 Results of the redundancy analysis on the effects of chemical, microbial and substrate properties on the ratios of heterotrophic respiration rates a reference temperature of 20 °C (R_{20}, a) and temperature sensitivity (Q_{10}, b) between warm-up and cool-down processes based on the data of five destructive samplings. Substrate properties included dissolved organic carbon, dissolved total nitrogen; microbial properties included microbial biomass carbon, microbial biomass nitrogen; chemical properties included pH, conductivity. Two adjacent parts outlined by a black rectangle indicate that the combined effect on R_{20} or Q_{10} was significant. *, ** represent significance levels of $P < 0.05$, $P < 0.01$

cool-down processes have not been measured, the important interaction of microbe and substrate could be reflected in the parameter A to some extent, derived from the Eq. 2. It was heterotrophic respiration rate in essence, which not only related to C quality but also with microbial biomass and activities. Thus, it is plausible for the observed significant higher parameter A in the warm-up process, compared to that in the cool-down. First, the background values of bacteria were significant higher than fungi (ca. 20 times). Second, the content of liable SOM was quite much than recalcitrant SOM at the original stage. Based on the hypothesis of different preferences in microbes to SOM in diel scale, these two parts made a beneficial condition to shape the high R_h and low Q_{10} in the warm-up process.

Although soil chemical properties (such as pH and COND) were not the most important factors for diel hysteresis in this study, they still had vital interactions with substrate and microbial properties (Briones et al. 2014). For example, ratios of R_{20} (warm-up $_{VS}$. cool-down) decreased with increasing pH and increased with increasing COND (Fig. S3). Besides, pH exerted opposite effects on C quality under the warm-up process versus the cool-down process (Fig. 4). These effects could be supported by the fact that pH induces different effects on the reaction rates and Q_{10} of SOM decomposition, leading to changes in the relative availability of SOM for microbial assimilation (Min et al. 2014) and the microbial community composition (Shen et al. 2013).

Improvements in the future

Although our measurement system recorded R_h and soil temperature with a high resolution in the diel scale, there is still room for improvement. For example, the daily incubation system could be adjusted to synchronize with measured temperatures that match with field condition. The apparatus used to measure soil temperature could be connected with a moisture sensor to investigate the effect of soil water content on diel hysteresis based on one circulation of increasing and decreasing temperature. Microbial and substrate properties need to be measured at a high frequency during the warm-up and cool-down processes, to provide more direct and powerful evidence supporting our hypotheses. Furthermore, the effects of microbial property on diel hysteresis in this study were only assessed at the level of microbial biomass, which limited our ability to explore the effects of acclimation (individual physiological), adaptation (genetic changes within species), and the ecological responses (competition altering species composition) of microbes on diel hysteresis. In the future, the effect of the microbial community could be quantified by using stable isotope probing (Cheng et al. 2017), as well as testing substrate quality and quantity with physical (mass-spectrometer, differential thermo-gravimetric analysis) (von Lutzow et al. 2006) and chemical (acid or alkali extraction) methods (Rovira et al. 2010).

Furthermore, a clockwise hysteresis loop in surface soils was observed in the forest ecosystem in this study, as well as that in grassland ecosystem soils from the Qinghai-Tibet Plateau (Li et al. 2017). Some studies have reported clockwise, and even anticlockwise, hysteresis loops in either the subsurface (≥5 cm) (Phillips et al. 2011) or surface layers (≤5 cm) (Subke and Bahn 2010; Phillips et al. 2011). Therefore, it is necessary to explore that how daily hysteresis varies spatially with the heterogeneity of temperature, water, oxygen, and other factors.

Conclusions

Our study confirmed that R_h is dependent on temperature in the hysteresis loop at the diel scale. Furthermore, the clock-wise hysteresis loop of R_h with temperature exhibited significantly higher Q_{10} during the cool-down process compared to the warm-up processes. The interactive effects of chemical, microbial, and substrate properties are important to control variation of diel hysteresis, in view of Q_{10} and R_{20}. In conclusion, our findings verified the asymmetrical responses of soil respiration to warm-up and cool-down processes at a daily scale, with it being important for this phenomenon to be incorporated into soil C cycle models in future.

Acknowledgements This work was partially supported by the National Key R&D Program of China (2016YFC0500102) and the National Natural Science Foundation of China (31770655, 41571130043). Nianpeng He contributes the conception and design; Qing Wang conducted the experiment and analyzes the data. All authors contributed to the final version. There are no conflicts of interest to declare. We thank the reviewers to improve the manuscript.

References

Balser TC, Wixon DL (2009) Investigating biological control over soil carbon temperature sensitivity. Glob Chang Biol 15: 2935–2949

Barron-Gafford GA, Scott RL, Jenerette GD, Huxman TE (2011) The relative controls of temperature, soil moisture, and plant functional group on soil CO_2 efflux at diel, seasonal, and annual scales. J Geophys Res Biogeo 116:G010223

Baumann A, Schimmack W, Steindl H, Bunzl K (1996) Association of fallout radiocesium with soil constituents: Effect of sterilization of forest soils by fumigation with chloroform. Radiat Environ Biophys 35:229–233

Bosatta E, Ågren GI (1999) Soil organic matter quality interpreted thermodynamically. Soil Biol Biochem 31:1889–1891

Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils. Front Microbiol 4:333

Bradford MA, Davies CA, Frey SD, Maddox TR, Melillo JM, Mohan JE, Reynolds JF, Treseder KK, Wallenstein MD (2008) Thermal adaptation of soil microbial respiration to elevated temperature. Ecol Lett 11:1316–1327

Briones MJ, McNamara NP, Poskitt J, Crow SE, Ostle NJ (2014) Interactive biotic and abiotic regulators of soil carbon cycling: evidence from controlled climate experiments on peatland and boreal soils. Glob Chang Biol 20:2971–2982

Brookes PC, Kragt JF, Powlson DS, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biol Biochem 17:831–835

Cheng L, Zhang NF, Yuan MT, Xiao J, Qin YJ, Deng Y, Tu QC, Xue K, Van Nostrand JD, Wu LY, He ZL, Zhou XH, Leigh MB, Konstantinidis KT, Schuur EAG, Luo YQ, Tiedje JM, Zhou JZ (2017) Warming enhances old organic carbon decomposition through altering functional microbial communities. ISME J 11:1825–1835

Chi X, Tang Z, Xie Z, Guo Q, Zhang M, Ge J, Xiong G, Fang J (2015) Effects of size, neighbors, and site condition on tree growth in a subtropical evergreen and deciduous broadleaved mixed forest, China. Ecol Evol 5:5149–5161

Craine JM, Fierer N, McLauchlan KK (2010) Widespread coupling between the rate and temperature sensitivity of organic matter decay. Nat Geosci 3:854–857

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173

De Bruijn AMG, Butterbach-Bahl K (2010) Linking carbon and nitrogen mineralization with microbial responses to substrate availability - the DECONIT model. Plant Soil 328:271–290

Ding JZ, Chen LY, Zhang BB, Liu L, Yang GB, Fang K, Chen YL, Li F, Kou D, Ji CJ, Luo YQ, Yang YH (2016) Linking temperature sensitivity of soil CO_2 release to substrate, environmental, and microbial properties across alpine ecosystems. Global Biogeochem Cycles 30:1310–1323

Eberwein JR, Oikawa PY, Allsman LA, Jenerette GD (2015) Carbon availability regulates soil respiration response to nitrogen and temperature. Soil Biol Biochem 88:158–164

Fang C, Moncrieff JB (2001) The dependence of soil CO_2 efflux on temperature. Soil Biol Biochem 33:155–165

Fierer N, Craine JM, McLauchlan K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–326

Frey SD, Drijber R, Smith H, Melillo J (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol Biochem 40:2904–2907

Gaumont-Guay D, Black TA, Griffis TJ, Barr AG, Jassal RS, Nesic Z (2006) Interpreting the dependence of soil respiration on soil temperature and water content in a boreal aspen stand. Agric For Meteorol 140:220–235

Gershenson A, Bader NE, Cheng WX (2009) Effects of substrate availability on the temperature sensitivity of soil organic matter decomposition. Glob Chang Biol 15:176–183

Hall EK, Neuhauser C, Cotner JB (2008) Toward a mechanistic understanding of how natural bacterial communities respond to changes in temperature in aquatic ecosystems. Isme J 2:471–481

He NP, Wang RM, Gao Y, Dai JZ, Wen XF, Yu GR (2013) Changes in the temperature sensitivity of SOM decomposition with grassland succession: Implications for soil C sequestration. Ecol Evol 3:5045–5054

He NP, Liu CC, Tian M, Li ML, Yang H, Yu GR, Guo DL, Smith MD, Yu Q, Hou JH (2018) Variation in leaf anatomical traits from tropical to cold-temperate forests and linkage to ecosystem functions. Funct Ecol 32:10–19

Iqbal J, Hu RG, Feng ML, Lin S, Malghani S, Ali IM (2010) Microbial biomass, and dissolved organic carbon and nitrogen strongly affect soil respiration in different land uses: A case study at Three Gorges Reservoir Area, South China. Agric Ecosyst Environ 137:294–307

Ise T, Moorcroft PR (2006) The global-scale temperature and moisture dependencies of soil organic carbon decomposition: an analysis using a mechanistic decomposition model. Biogeochemistry 80:217–231

Jiang H, Deng Q, Zhou G, Hui D, Zhang D, Liu S, Chu G, Li J (2013) Responses of soil respiration and its temperature/moisture sensitivity to precipitation in three subtropical forests in southern China. Biogeosciences 10:3963–3982

Lefevre R, Barre P, Moyano FE, Christensen BT, Bardoux G, Eglin T, Girardin C, Houot S, Katterer T, van Oort F, Chenu C (2014) Higher temperature sensitivity for stable than for labile soil organic carbon-Evidence from incubations of long- term bare fallow soils. Glob Chang Biol 20:633–640

Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. Nature 528:60–68

Li J, He NP, Xu L, Chai H, Liu Y, Wang DL, Wang L, Wei XH, Xue JY, Wen XF, Sun XM (2017) Asymmetric responses of soil heterotrophic respiration to rising and decreasing temperatures. Soil Biol Biochem 106:18–27

Liski J, Ilvesniemi H, Mäkelä A, Westman CJ (1999) CO_2 emissions from soil in response to climatic warming are overestimated-The decomposition of old soil organic matter istolerant of temperature. Ambio 28:171–174

Liu Q, Edwards NT, Post WM, Gu L, Ledford J, Lenhart S (2006) Temperature-independent diel variation in soil respiration observed from a temperate deciduous forest. Glob Chang Biol 12:2136–2145

Liu Y, He NP, Zhu JX, Xu L, Yu GR, Niu SL, Sun XM, Wen XF (2017) Regional variation in the temperature sensitivity of soil organic matter decomposition in China's forests and grasslands. Glob Chang Biol 23:3393–3402

Liu Y, He NP, Wen XF, Xu L, Sun XM, Yu GR, Liang LY, Schipper LA (2018) The optimum temperature of soil microbial respiration: Patterns and controls. Soil Biol Biochem 121:35–42

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. Funct Ecol 8:315–323

Malcolm GM, Lopez-Gutierrez JC, Koide RT, Eissenstat DM (2008) Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. Glob Chang Biol 14:1169–1180

Min K, Lehmeier CA, Ballantyne F, Tatarko A, Billings SA (2014) Differential effects of pH on temperature sensitivity of organic carbon and nitrogen decay. Soil Biol Biochem 76:193–200

Moren AS, Lindroth A (2000) CO_2 exchange at the floor of a boreal forest. Agric For Meteorol 101:1–14

Nakai Y, Kitamura K, Suzuki S, Abe S (2003) Year-long carbon dioxide exchange above a broadleaf deciduous forest in Sapporo, Northern Japan. Tellus Ser B Chem Phys Meteorol 55:305–312

Niu SL, Luo YQ, Fei SF, Montagnani L, Bohrer G, Janssens IA, Gielen B, Rambal S, Moors E, Matteucci G (2011) Seasonal hysteresis of net ecosystem exchange in response to temperature change: patterns and causes. Glob Chang Biol 17: 3102–3114

Phillips CL, Nickerson N, Risk D, Bond BJ (2011) Interpreting diel hysteresis between soil respiration and temperature. Glob Chang Biol 17:515–527

Riveros-Iregui DA, Emanuel RE, Muth DJ, McGlynn BL, Epstein HE, Welsch DL, Pacific VJ, Wraith JM (2007) Diurnal hysteresis between soil CO_2 and soil temperature is controlled by soil water content. Geophys Res Lett:34

Rousk J, Baath E (2011) Growth of saprotrophic fungi and bacteria in soil. Fems Microbiol Ecol 78:17–30

Rousk J, Frey SD, Bååth E (2012) Temperature adaptation of bacterial communities in experimentally warmed forest soils. Glob Chang Biol 18:3252–3258

Rovira P, Jorba M, Romanya J (2010) Active and passive organic matter fractions in Mediterranean forest soils. Biol Fert Soils 46:355-369.Rousk J, Bååth E (2011) Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiol Ecol 78:17–30

Ruehr NK, Knohl A, Buchmann N (2010) Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry 98:153–170

Shen C, Xiong J, Zhang H, Feng Y, Lin X, Li X, Liang W, Chu H (2013) Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biol Biochem 57:204–211

Shibata H, Hiura T, Tanaka Y, Takagi K, Koike T (2005) Carbon cycling and budget in a forested basin of southwestern Hokkaido, northern Japan. Ecol Res 20:325–331

Sierra CA, Trumbore SE, Davidson EA, Frey SD, Savage KE, Hopkins FM (2012) Predicting decadal trends and transient responses of radiocarbon storage and fluxes in a temperate forest soil. Biogeosciences 9:3013–3028

Song XL, Zhu JX, He NP, Huang JH, Tian J, Zhao X, Liu Y, Wang CH (2017) Asynchronous pulse responses of soil carbon and nitrogen mineralization to rewetting events at a short-term: Regulation by microbes. Sci Rep 7:7492

Subke JA, Bahn M (2010) On the 'temperature sensitivity' of soil respiration: Can we use the immeasurable to predict the unknown? Soil Biol Biochem 42:1653–1656

ter Braak CJF, Smilauer P (2012) Canoco reference manual and user's guide: software for ordination, version 5.0. Ithaca USA: Microcomputer Power, 2012

von Lutzow M, Kogel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. Eur J Soil Sci 57:426–445

Wagai R, Kishimoto-Mo AW, Yonemura S, Shirato Y, Hiradate S, Yagasaki Y (2013) Linking temperature sensitivity of soil organic matter decomposition to its molecular structure, accessibility, and microbial physiology. Glob Chang Biol 19: 1114–1125

Wang Q, Wang D, Wen XF, Yu GR, He NP, Wang RF (2015) Differences in SOM decomposition and temperature sensitivity among soil aggregate size classes in a temperate grasslands. PLoS One 10:e0117033

Wang Q, He NP, Liu Y, Li ML, Xu L (2016a) Strong pulse effects of precipitation events on soil microbial respiration in temperate forests. Geoderma 275:67–73

Wang Q, He NP, Yu GR, Gao Y, Wen XF, Wang RF, Koerner SE, Yu Q (2016b) Soil microbial respiration rate and temperature sensitivity along a North-South forest transect in eastern China: Patterns and influencing factors. J Geophys Res Biogeo 121:399–410

Wei H, Guenet B, Vicca S, Nunan N, AbdElgawad H, Pouteau V, Shen WJ, Janssens IA (2014) Thermal acclimation of organic matter decomposition in an artificial forest soil is related to shifts in microbial community structure. Soil Biol Biochem 71:1–12

Wetterstedt JAM, Persson T, Ågren GI (2010) Temperature sensitivity and substrate quality in soil organic matter decomposition: results of an incubation study with three substrates. Glob Chang Biol 16:1806–1819

Xu X, Luo YQ, Zhou JZ (2012) Carbon quality and the temperature sensitivity of soil organic carbon decomposition in a tallgrass prairie. Soil Biol Biochem 50:142–148

Xu ZW, Yu GR, Zhang XY, He NP, Wang QF, Wang SZ, Wang RL, Zhao N, Jia YL, Wang CY (2017) Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). Soil Biol Biochem 104:152–163