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Soil respiration and its environmental response varies by day/night and by growing/dormant season in a subalpine forest

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Zongda Hu^{1,2,3}, Shirong Liu^{2,3}, Xingliang Liu⁴, Liyong Fu⁵, Jingxin Wang⁶, Kuan Liu⁷, Xueman Huang^{2,3}, Yuandong Zhang^{2,3} & Fei He⁸

Comparisons of soil respiration (R_s) and its components of heterotrophic (R_H) and rhizospheric (R_R) respiration during daytime and nighttime, growing (GS) and dormant season (DS), have not been well studied and documented. In this study, we compared R_s , R_H , R_R and their responses to soil temperature (T_s) and moisture (θ_s) in daytime vs. nighttime and GS vs. DS in a subalpine forest in 2011. In GS, nighttime R_s and R_H rates were $30.5 \pm 4.4\%$ (mean \pm SE) and $30.2 \pm 6.5\%$ lower than in daytime, while in DS, they were $35.5 \pm 5.5\%$ and $37.3 \pm 8.5\%$ lower, respectively. DS R_s and R_H accounted for $27.3 \pm 2.5\%$ and $27.6 \pm 2.6\%$ of GS R_s and R_H , respectively. The temperature sensitivities (Q_{10}) of R_s and R_H were higher in nighttime than daytime, and in DS than GS, while they all decreased with increase of T_s . Soil C fluxes were more responsive to θ_s in nighttime than daytime, and in DS than GS. Our results suggest that the DS and nighttime R_s play an important role in regulating carbon cycle and its response to climate change in alpine forests, and therefore, they should be taken into consideration in order to make accurate predictions of R_s and ecosystem carbon cycle under climate change scenarios.

As an important component of the terrestrial C cycle, soil respiration (R_s) in forest ecosystems accounts for 30–90% of the total ecosystem respiration^{1,2}. Thus, forest R_s and its components, including heterotrophic respiration and autotrophic respiration, have been extensively studied over the past decades^{3,4}. However, most previous studies on R_s , heterotrophic respiration, autotrophic respiration and their responses to environmental changes were conducted during the growing season (GS) and during daytime, and few studies were executed at nighttime and during the dormant season (DS), due mainly to difficulty in field measurement at nighttime and during the DS, in particular, very cold and wet conditions at night time in winter season. Considering greater warming effects (temperature differences) at nighttime than in the daytime, and in winter season than in summer season in Tibetan Plateau^{5,6}, it is essential to quantify soil CO₂ fluxes and their temperature responses at nighttime and during the DS.

Soil CO₂ efflux in the DS plays an important role in the regional, national, and the global carbon balance^{7,8}, which accounts for 3–50% of annual soil CO₂ efflux in forests^{8–10}. Annual carbon sequestration would be largely overestimated if soil CO₂ fluxes in the DS were not included¹⁰. Moreover, several studies by using different approaches including stable isotope methods¹¹, modeling methods verified by eddy covariance system¹², or by using soil respiration monitoring system^{13,14} indicated that soil CO₂ fluxes differed largely between daytime and nighttime. Nevertheless, we have still known little about the proportions and variations of nighttime and the DS R_s in annual soil C fluxes in specific ecosystems.

¹College of Resources, Sichuan Agricultural University, 211 Huiming Road, Wenjiang District, Chengdu 611130, Sichuan, China. ²The Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Dongxiaofu No.2, Haidian District, Beijing 100091, China. ³Key Laboratory of Forest Ecology and Environment, China's State Forestry Administration, Dongxiaofu No.2, Haidian District, Beijing 100091, China. ⁴Sichuan Academy of Forestry, 18 Xinghui West Road, Chengdu, 610081 Sichuan, China. ⁵Research Institute of Forest Resource Information Techniques, Chinese Academy of Forestry, Dongxiaofu No.1, Haidian District, Beijing 100091, China. ⁶Division of Forestry and Natural Resources, West Virginia University, P.O. Box 6215, Morgantown, WV, 26506-6125, USA. ⁷Dalla Lana School of Public Health, University of Toronto, 155 College Street, Toronto, Ontario M5T 3M7, Canada. ⁸Sichuan Engineering Consulting and Research Institute, 201 Yu Sha Road Xinhua Avenue, Chengdu, 610016, Sichuan, China. Correspondence and requests for materials should be addressed to S.L. (email: liusr@caf.ac.cn)

The temperature sensitivity (Q_{10}) of R_S is an important fundamental parameter in soil carbon cycle models¹⁵. It varies with environmental factors and with different components of R_S , largely due to the fact that Q_{10} is regulated by various biotic and abiotic factors, such as soil temperature and soil water content, microbial biomass, substrate quality, and plant physiological activity^{13,15,16}. However, estimates of Q_{10} are mostly based on measurements in daytime and during the GS, and few measurements were conducted in winter and at nighttime. It was reported that Q_{10} varied from 1.16 to 24.30 in growing season in sub-alpine forest ecosystem¹⁷. However, Wang *et al.* found that annual Q_{10} ranged from 3.10 to 4.69, indicating that Q_{10} varies between the GS and the DS⁸. Additionally, some studies reported that Q_{10} value of heterotrophic respiration was lower than that of autotrophic respiration¹⁸, while other studies showed the opposite result^{4,13}. Because a slight deviation of Q_{10} may cause a huge bias in the estimate of R_S ¹⁵, a better understanding of Q_{10} of different R_S components in different times can improve our understanding of the roles of forests in regulating carbon cycle and shaping its response to climate change.

The subalpine ecosystems in Tibetan Plateau are considered to be highly sensitive and prone to global warming impacts¹⁹. The changes in R_S and its temperature sensitivity will have a significant influence on regional and global carbon cycle and their feedback on climate change²⁰. Some studies on R_S and heterotrophic respiration have been conducted in subalpine forest ecosystems^{9,21}, but most of them were based on short term measurements in daytime and during the GS. The CO_2 fluxes in the DS and at nighttime in the subalpine forests in Eastern Tibetan Plateau have not been fully determined, and the factors controlling the temperature sensitivity of the R_S are even less understood. In the context of global climate change, the rising temperatures may result in greater CO_2 emissions to the atmosphere from soils in these areas^{22,23}. Therefore, it is very necessary to accurately estimate R_S and to explore its controlling factors, especially in DS and at nighttime.

In this study, we measured spatial and temporal variations of R_S in a typical oak forest in subalpine mountains and further examined their soil temperature and moisture sensitivity in daytime and at nighttime, and during the GS vs. the DS. We conducted a trenching experiment to partition soil respiration (R_S) into rhizospheric (R_R) and heterotrophic respiration (R_H). The specific objectives of this study were to: (1) quantify the differences of R_S , R_H , R_R and their responses to environmental factors between daytime and nighttime, and the GS vs. the DS; (2) examine the temperature sensitivity of R_H and R_S and their controlling factors during daytime vs. nighttime, and GS vs. DS.

Results

Variations of soil surface CO_2 fluxes in different time and seasons. Seasonal dynamics of R_H and R_S showed the similar patterns with that of soil temperature (Fig. 1a and b). The maximum R_S (daytime $4.15 \pm 0.20 \mu\text{mol m}^{-2}\text{s}^{-1}$; nighttime $3.04 \pm 0.13 \mu\text{mol m}^{-2}\text{s}^{-1}$) and R_H (daytime $3.05 \pm 0.16 \mu\text{mol m}^{-2}\text{s}^{-1}$; nighttime $2.33 \pm 0.11 \mu\text{mol m}^{-2}\text{s}^{-1}$) occurred in August, while the minimum R_S (daytime $0.47 \pm 0.02 \mu\text{mol m}^{-2}\text{s}^{-1}$; nighttime $0.30 \pm 0.02 \mu\text{mol m}^{-2}\text{s}^{-1}$) and R_H (daytime $0.35 \pm 0.02 \mu\text{mol m}^{-2}\text{s}^{-1}$; nighttime $0.18 \pm 0.01 \mu\text{mol m}^{-2}\text{s}^{-1}$) occurred in January. Additionally, soil CO_2 fluxes was obviously lower in trenched plots than in untrenched plots in the first 6 months after trenching (before December 2010), in order to minimize influence of decomposing roots on R_S , we used the data (R_S , R_H , T_5 and θ_5) from 2011 to analyze the changes in soil surface CO_2 fluxes.

Both R_S and R_H were significantly higher in daytime than at nighttime (all $P < 0.05$) in all seasons (Table 1). On an average, nighttime R_S accounted for $69.5 \pm 4.4\%$ (mean \pm std err) and $64.5 \pm 5.5\%$ of daytime values in the GS and DS, respectively, while nighttime R_H made up $69.8 \pm 6.5\%$ and $62.3 \pm 8.5\%$ of the daytime values, respectively. Although T_5 and θ_5 were higher in trenched than in untrenched plots throughout 2011 (Fig. 1), they were not significantly different ($P > 0.05$). Soils generally were drier in DS and wetter in GS (Fig. 1c). Moreover, either T_5 or θ_5 was not significantly different between daytime and nighttime (all $P > 0.05$, Table 2).

The mean R_S rates and R_H rates were significantly higher in the GS than in the DS ($P < 0.05$, Table 1). Overall, DS total mean R_S and R_H (daytime plus nighttime data, respectively, Table 1) account for $27.3 \pm 2.5\%$ and $27.6 \pm 2.6\%$ of GS total mean R_S and R_H , respectively. θ_5 and T_5 were also higher in the GS than in the DS in both daytime and nighttime (Table 2). On average, total mean T_{T5} (trenched)/ T_{UT5} (untrenched) and θ_{T5} (trenched)/ θ_{UT5} (untrenched) were 7.4/8.3 and 1.8/1.6 times larger in the GS than in the DS, respectively.

Factors controlling daytime and nighttime R_S and its components. Both daytime and nighttime R_S and its components followed exponential regression relationships with T_5 (all $P < 0.001$, Fig. 2) and a power model relationship with θ_5 across the seasons (Fig. 3). T_5 alone explained approximately 42.8–90.8% and 49.5–86.7% of the variations in the daytime and nighttime soil C fluxes, respectively. Across all seasons, θ_5 alone explained approximately 61.9–70.4% and 16.2–37.2% of the variations in the daytime and nighttime soil C fluxes, respectively. In DS, the variability in R_S and its components can be explained alone by θ_5 more in daytime than in nighttime. In the GS, we found that only nighttime R_S had significant relationship with θ_5 (Fig. 3d), suggesting that θ_5 was not key factor controlling diurnal dynamics of soil respiration components during the GS.

The combined functions of T_5 and θ_5 (T - θ model, equation 4) can better explain the variability of R_S , R_H , and R_R , indicating that soil respiration and its components were dominated by the interaction of T_5 and θ_5 rather than a single factor.

Variations in Q_{10} and its relationships with soil properties. The mean Q_{10} values of R_S and R_H were significantly lower in daytime than in nighttime either in the GS or in the DS (all $P < 0.05$, Table 3). Either in daytime or nighttime, we found that Q_{10} values of R_S and R_H were significantly higher in the DS than in the GS (all $P < 0.05$).

Q_{10} values of R_S and R_H in daytime and nighttime showed a significantly negative correlation with T_5 (Fig. 4). θ_5 had no significant correlation with Q_{10} of either R_S ($P = 0.077$) or R_H ($P = 0.663$) in daytime, while a strong positive/negative correlation was observed between nighttime Q_{10} of R_S / R_H and θ_5 . Either in daytime or nighttime, Q_{10} of R_S and R_H had positive correlation with TOC and MBC. TN had no effect on the Q_{10} of R_S or R_H in the daytime, but significant effect occurred at nighttime (Fig. 4).

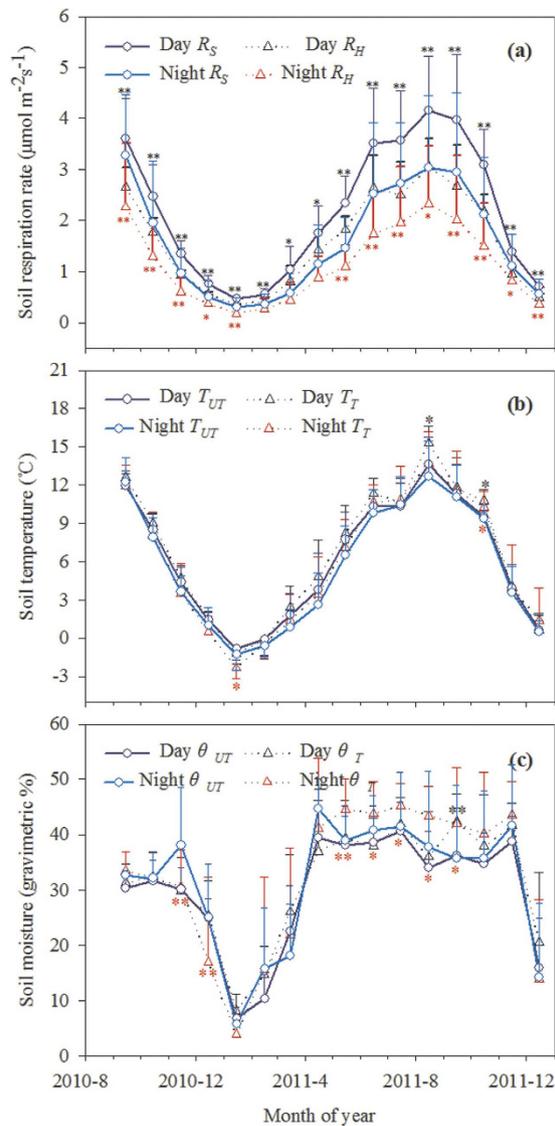


Figure 1. Seasonal dynamics of the mean soil surface CO_2 fluxes from the daytime (Day R_S and R_H) and nighttime (Night R_S and R_H), soil temperature (Day T_{UT} and Night T_T) and soil moisture (Day θ_{UT} and Night θ_T) from untilled and tilled plots in 2010–2011. Vertical bars represent the standard deviation of the mean ($n = 9$ for untilled and tilled, respectively). * and ** denote significant difference at $P = 0.05$, $P = 0.01$, respectively. Red and black asterisk represent the significant difference of the day R_S vs. day R_H and night R_S vs. night R_H , respectively.

Components	Diel	GS	DS	All seasons
R_S	Daytime	3.44 (0.09)aA	0.98 (0.05)aB	2.21 (0.09)a
	Nighttime	2.47 (0.05)bA	0.67 (0.02)bB	1.57 (0.04)b
R_H	Daytime	2.47 (0.07)aA	0.73 (0.04)aB	1.60 (0.06)a
	Nighttime	1.77 (0.04)bA	0.49 (0.02)bB	1.13 (0.03)b

Table 1. The differences of R_H and R_S ($\mu\text{mol m}^{-2}\text{s}^{-1}$) between daytime and nighttime in the growing season and dormant seasons. The different lowercase letters in a column represent significant difference between daytime and nighttime. The different uppercase letters represent significant difference between the GS and the DS at $P = 0.05$ level. Values in parentheses are standard errors of means for $N = 162$.

Q_{10} of R_S in the GS and the DS had significant correlations with T_5 (Fig. 5). Q_{10} of R_H had negative correlations with T_5 in the DS but not in the GS. In contrast, θ_5 showed significant relationship with Q_{10} of R_S in the GS, but not in the DS. Additionally, in the GS, Q_{10} of R_S had positive correlation with TOC and TN, but Q_{10} of R_H was not affected by TOC and TN. In the DS, however, Q_{10} of both R_S and R_H was positively influenced by TOC and MBC (Fig. 5).

Plots	T_5/θ_5	Diel	GS	DS	All seasons
Untrenched	T_5	Daytime	10.46 (0.20)aA	1.53 (0.21)aB	5.99 (0.29)a
		Nighttime	9.97 (0.12) aA	0.93 (0.10)bB	5.94 (0.72)a
Trenched		Daytime	11.33 (0.20)aA	1.74 (0.21)aB	6.53 (0.29)a
		Nighttime	10.66 (0.12)bA	1.22 (0.10)aB	5.45 (0.72)a
Untrenched	θ_5	Daytime	37.04 (1.23)aA	22.26 (2.60)aB	29.65 (1.67)a
		Nighttime	38.35 (0.41)aA	23.32 (0.75)aB	30.84 (0.49)a
Trenched		Daytime	39.19 (0.63)aA	24.62 (1.21)aB	31.91 (0.83)a
		Nighttime	43.14 (0.29)bA	23.29 (0.80)aB	33.21 (0.53)a

Table 2. The statistics of soil temperature and moisture between daytime and nighttime in the trenched and untrenched plots in different seasons of the *Q. aquifolioides* forest during 2011. The different lowercase letters in a column represent significant difference between daytime and nighttime in the GS, the DS and all seasons, respectively. The different uppercase letters represent significant difference between the GS vs. the DS in the daytime or at nighttime at $P = 0.05$ level. Values in parentheses are standard errors of means for $N = 162$.

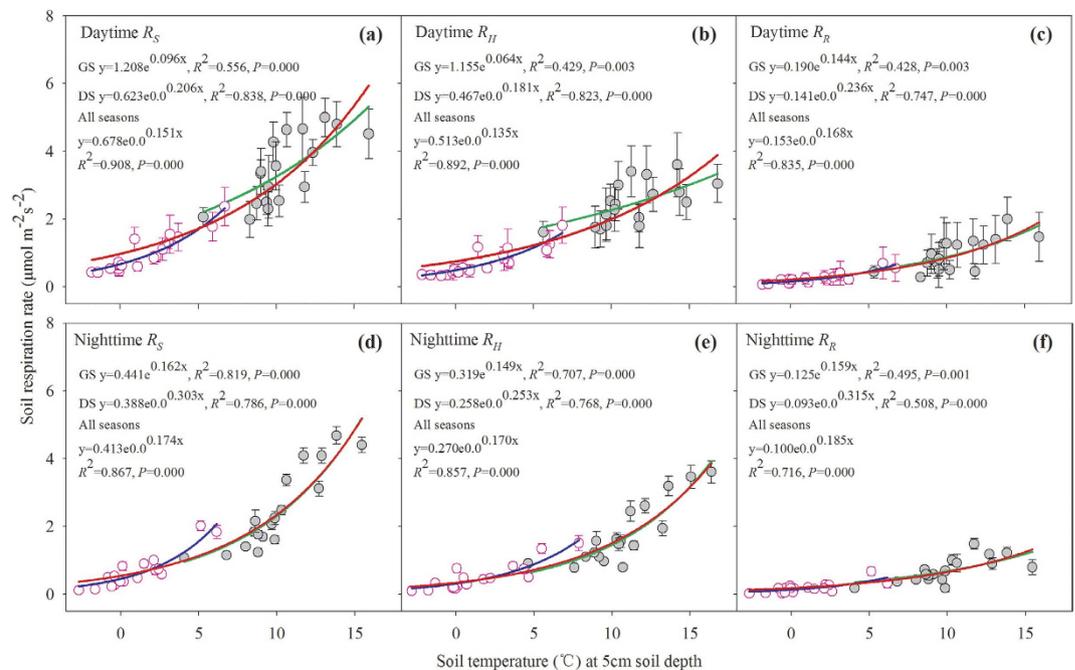


Figure 2. Relationship of soil respiration and its components with soil temperature at 5 cm soil depth in the daytime (a–c) and nighttime (d–f). Each value represents the monthly mean of soil CO₂ flux from the subplot collars measurements for three altitudinal gradients in different seasons (DS pink and GS grey, $n = 18$. All seasons, $n = 36$) during 2011. The solid line represents the respiration–temperature relationship estimated according to the model of Eqn (1). All bars indicate mean \pm Std Dev (standard deviation). Pink blank circles and grey solid circles represent the monthly mean of soil CO₂ flux rate in the DS and the GS, respectively.

Discussion

Comparisons of soil respirations in daytime vs. nighttime and the GS vs. the DS. In this study, we found that the measured R_S and R_H was $30.5 \pm 4.4\%$ (mean \pm SE) and $30.2 \pm 6.5\%$ lower at nighttime than in daytime during the GS, while they were $35.5 \pm 5.5\%$ and $37.3 \pm 8.5\%$ lower during the DS, respectively. The changes in soil C fluxes in daytime and at nighttime were due mainly to variations of soil temperature and moisture. Additionally, previous studies reported that the diurnal variability of soil CO₂ efflux was affected by the turnover of recent photosynthate¹¹, microbial growth²⁴, plant biological activities²⁵, and allocation of photosynthetic C^{11,26}. In the daytime, favorable soil temperature and light condition can promote the enhancement of microbial metabolism. Moreover, higher daytime R_S rates may result from greater translocation of high photosynthesis from the plant shoots to the roots during the daytime relative to at nighttime. The differences in the content and quality of SOC were also reported to determine high and low R_S in previous studies^{27–29}.

Previous studies shows that R_H for a certain time after trenching treatment may be increased in trenched plots³⁰, or kept almost unchanged, or decreased³¹. In this study, we observed that R_H rates in trenched plots significantly lower than in untrenched plots for over 2 months after trenching (September 2010; Fig. 1). The results are consistent with that previously reported in different forest types^{21,32}. In our study, we found that R_H was the

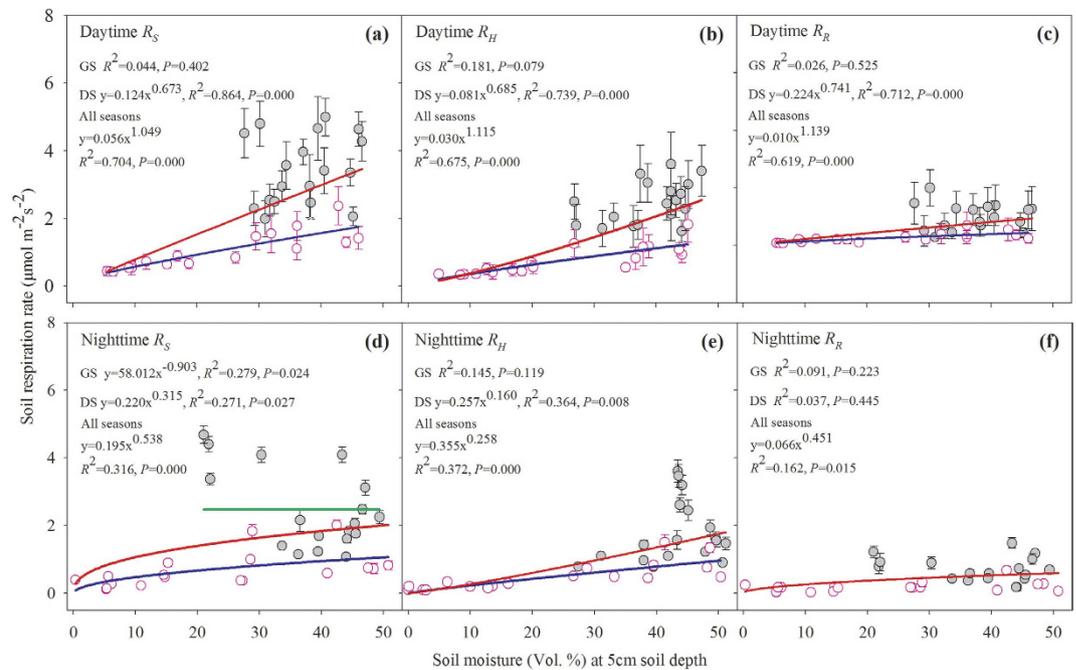


Figure 3. Relationship of soil respiration and its components with soil moisture (volumetric, %) at 5 cm soil depth in the daytime (a–c) and nighttime (d–f); Each value represents the monthly mean of soil CO₂ flux from the subplot collars measurements for three altitudinal gradients in different seasons (DS and GS, n = 18. All seasons, n = 36) during 2011. The solid line represents the respiration–moisture relationship estimated according to the model of Eqn (4). All bars indicate mean ± Std Dev (standard deviation). Pink blank circles and grey solid circles represent the monthly mean of soil CO₂ flux rate in the DS and the GS, respectively.

Daily	GS		DS	
	R _S	R _H	R _S	R _H
Daytime	2.84 (0.26)aA	2.20 (0.13)aA	7.42 (0.83)aB	6.41 (0.61)aB
Nighttime	3.94 (0.28)bA	2.72 (0.08)bA	20.02 (3.92)bB	18.99 (3.48)bB

Table 3. Mean Q₁₀ in the daytime and at nighttime during the GS and the DS. The different lowercase letters in a column represent significant difference between the daytime and nighttime in the GS and the DS, respectively. The different uppercase letters represent significant difference between the GS vs. the DS at P = 0.05 level. Values in parentheses are standard errors of means for N = 27.

dominant component of R_S during the DS and the GS in 2011. The R_H accounted for 72.2 ± 1.2% (mean ± SE) of the whole year R_S, with 73.9 ± 2.0% of R_S in the DS and 71.7 ± 1.3% of R_S in the GS, respectively (Table 1). Our findings are within the range of 16–80% of the contribution of R_H to R_S reported previously in temperate coniferous forests^{7,33}. However, there were no significant differences in proportion of R_H to R_S between DS and GS. Higher rates of R_H in DS were likely due to relatively high metabolic reaction of roots and soil microbial activity for maintaining respirations in the study stands in the winter. Our results suggest that R_H was the dominant component of R_S, indicating a dominant control of microorganism-associated respiration on R_S in the subalpine forest. In the DS, we supposed that soil microbial activity is still functioning to generate CO₂ fluxes by decomposing soil organic matters, while the root activity of plants is inhibited in winter because plants stop growing in cold temperature⁹, and consequently, ending up with a high proportion of R_H to total R_S (Table 1). Therefore, soil C fluxes in the DS must be taken into consideration when assessing the carbon sink/source patterns of the subalpine forests.

Environmental factors influencing soil CO₂ flux. In this study, the daytime and nighttime R_S and its components were significantly influenced by the interactions of T₅ and θ₅. Our results are similar to the findings observed in other forests^{32,34}. In this study, however, a new finding is that T–θ model fits the observations better in the DS than the GS for the all components of R_S, indicating that R_S and its components can be better predicted through T₅ and θ₅ in the DS than in the GS. This provides a simple but effective basis for estimating soil respirations in winter.

We also found that θ₅ alone didn't impact either the daytime or the nighttime R_H and R_R in the GS but T₅ did so, suggesting that T₅ rather than θ₅ is a main factor controlling R_H and R_R in the GS. Daytime R_S had no relationship with θ₅, but nighttime R_S decreased linearly with increasing θ₅ in the GS (Fig. 3d). This is because a

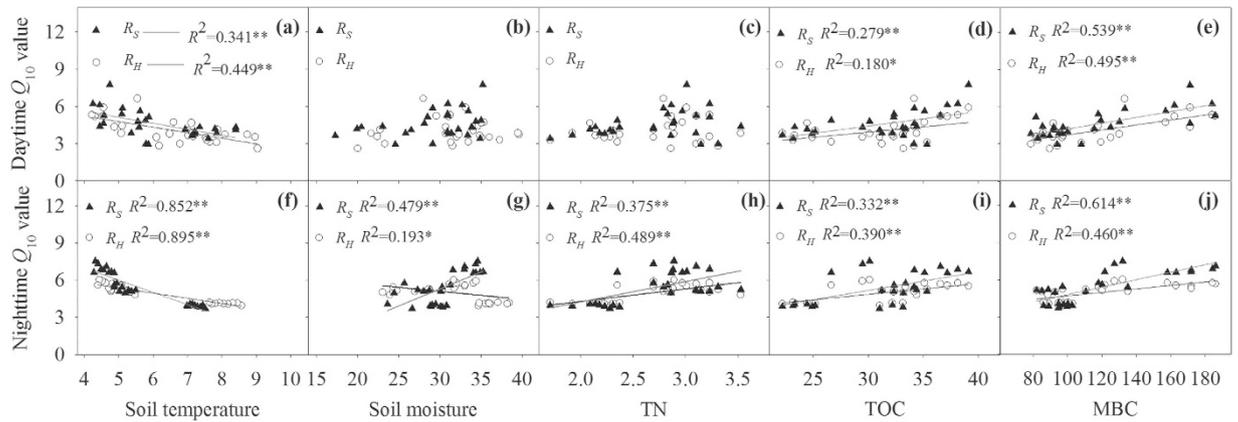


Figure 4. The relationship of Q_{10} of R_S and R_H in the daytime (upper panels) and nighttime (lower panels) with soil temperature (a,f) and soil moisture (b,g), soil total nitrogen (c,h), soil total organic carbon (d,i), soil microbial biomass carbon and nitrogen stocks (e,j) ($n = 27$). Each dot represents a Q_{10} value that was calculated across seasons for each subplot during the daytime and at nighttime. *and **Denote significant difference at $P = 0.05$, $P = 0.01$, respectively.

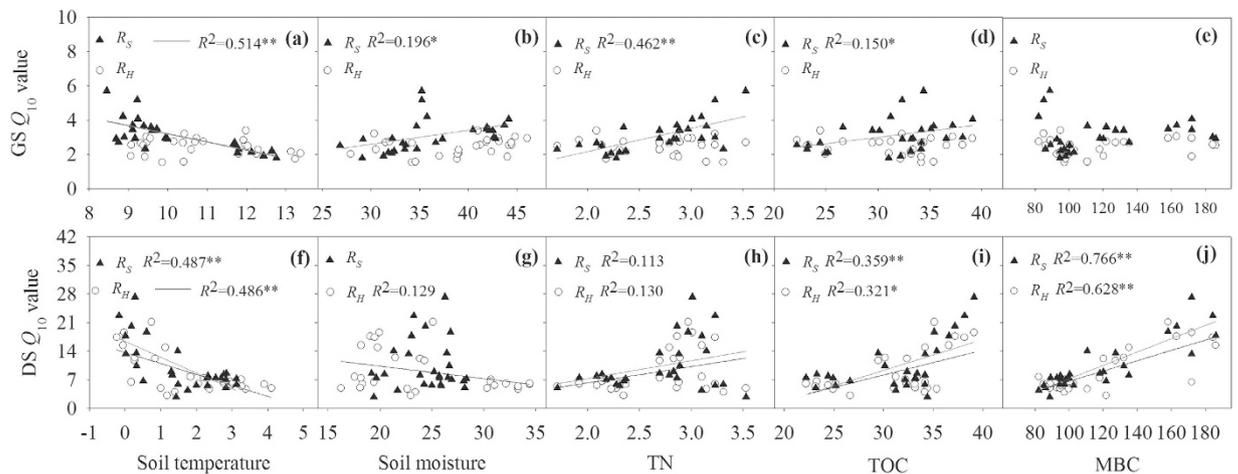


Figure 5. The relationship of Q_{10} of R_S and R_H in the GS (upper panels) and the DS (lower panels) with soil temperature (a,f) and soil moisture (b,g), soil total nitrogen (c,h), soil total organic carbon (d,i), soil microbial biomass carbon and nitrogen stocks (e,j) ($n = 27$). Each dot represents a Q_{10} value that was calculated across daytime and nighttime observations for each subplot in the GS and the DS. *and **Denote significant difference at $P = 0.05$, $P = 0.01$, respectively.

high precipitation frequency at nighttime in the GS decreases T_5 and consequently suppresses soil CO_2 efflux at nighttime. The different relationships of R_S and its components with θ_5 between the GS and the DS (Fig. 3) suggest that the different roles of T_5 and θ_5 as independent environmental variables in GS vs. the DS should be specifically taken into consideration when we predict soil respirations.

Constraints on Q_{10} . There have been inconsistent conclusions on Q_{10} values of R_S and its components in previous studies³⁵. Moreover, few studies have compared Q_{10} of R_S and its components in the daytime vs. the nighttime and the GS vs. the DS, especially at the high altitude in subalpine regions. In this study, the R_S and R_H at nighttime and during the DS are apparently more sensitive to temperature than that during daytime and the GS (Table 3). Our result is in line with some of previous studies that Q_{10} values were higher at lower temperature^{17,36}, but conflicts with other reports that Q_{10} was not characterized by a reflection of temperature change³⁷. The high temperature sensitivity of R_S and R_H at low temperature environments may be due to a high rate of substrate utilization and changes in microbial populations and microbial activity aiming to maintain high decomposition rates^{37,38}. This indicates that R_S or R_H at nighttime and during the DS might be more sensitive to temperature change than during daytime and the GS. In the GS, other parameters, such as substrate supply by roots and leaf area index in relation to phenology, may be more important than temperature in controlling the carbon flux rates^{14,39}.

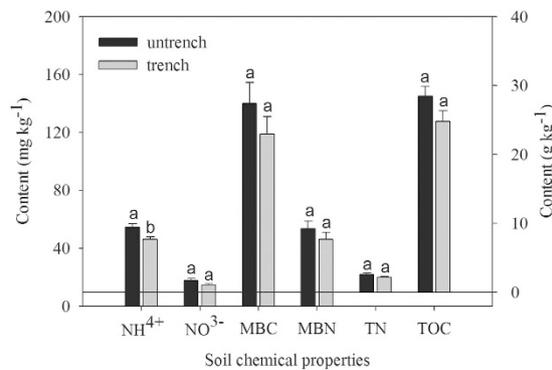


Figure 6. Mean contents of inorganic nitrogen (IN, $\text{NH}_4^+ + \text{NO}_3^-$), soil microbial biomass carbon (MBC) and nitrogen (MBN), total nitrogen (TN), soil total organic carbon (TOC) in the soil of trenched and untrenched plots ($n = 27$), respectively. Bars with the different lower-case letters indicate mean \pm SE (standard error) within trenched and untrenched subplots that are significantly different ($P < 0.05$).

In this study, soil moisture did not influence Q_{10} values of R_S and R_H in the daytime and the DS, but we found that Q_{10} values of R_S increased with increasing θ_5 at nighttime and the GS, while Q_{10} of R_H decreased with increasing θ_5 at nighttime (Figs 4 and 5). The similar results were also reported in the sub-alpine forests of the Eastern Qinghai-Tibet Plateau¹⁷. Soil moisture may cause changes in microbial community structure and soil mineralization rate^{40,41}, and furthermore affect sensitivities of their biotic and physicochemical processes to temperature⁴². In addition, plant-microbe-soil interactions and plant metabolism could contribute to Q_{10} values of R_S and its components⁴³.

The availability of soil substrates can influence microbial metabolism⁴⁴. Therefore, increased availability of either soil carbon or nitrogen is expected to stimulate microbial growth and activity in soils^{45,46}, leading to the subsequent changes in Q_{10} of R_S and R_H ⁴⁷. In this study, we found that TOC and MBC were key factors affecting the temperature sensitivities of both daytime R_S and R_H and nighttime R_S and R_H during DS (Figs 4 and 5). Therefore, in addition to soil temperature and moisture, the changes in TOC and MBC additionally explain the temperature sensitivity of soil C flux⁴⁷. Our results suggest that if a constant seasonal Q_{10} is used in the models for estimating soil CO_2 efflux, we cannot make accurate prediction on future soil CO_2 losses¹⁵.

Uncertainty of trenched effects on soil respirations. The trenching method for root exclusion is generally used to estimate R_H , especially in forest ecosystems although some uncertainties exist on its accuracy and interpretation of the results^{48,49}. One possible uncertainty may originate from the change in soil environmental conditions caused by trenching⁵⁰. In this study, we found a higher level of T_5 and θ_5 through 2011 (daytime: 9.0% and 7.6%, nighttime: 8.6% and 7.7%, respectively, absolute difference) in the trenched plots compared with the untrenched plots, which may lead to an overestimation of R_H ⁵¹. However, we did not find any significant trenching effect on T_5 or θ_5 in either the GS or the DS (Table 2). Therefore, the trench-induced changes in soil microclimate can be neglected in this study. Another possible impact of trenching on soil chemical parameters is that trenching can change the nutrient conditions and thus alter microbial activities⁵². In the present study, the soil carbon and nitrogen components were different degrees higher in untrenched plots than in trenched plots (Fig. 6), and suggested that the difference could be attributed to the suppression of photosynthetic products supply to the rhizosphere. Additionally, we used a linear regression model to determine this estimation error⁴. Our results showed that the trenched respiration contribution decreased by 4.3% and 3.9% after this correction in daytime and nighttime. We believe this process is unlikely to affect our estimation of R_H due to its minor contribution (5–8%) to soil respirations in temperate forests^{4,18,53}. Although some under- or over-estimation may be inevitable, we feel that trenching method is reasonable in this study.

Previous studies suggested that the fine roots may quickly decompose after trenching and cause a high CO_2 fluxes^{4,52}. In this study, we found that the following third month after trenching, the R_S was obviously higher in untrenched plots than in trenched plots (Fig. 1, $P = 0.000$). The similar phenomenon were also reported by Wang and Yang³² in the temperate deciduous and evergreen pine forest³². In fact, we used the measurement data after 6 months since the onset of trenching experiment in order to avoid the impact of rapid CO_2 flush caused by trenching. Previous studies indicated that priming effects from dead roots after trenching can be avoided if it is properly managed^{4,54}. The trenching had no significant influence on T_5 and θ_5 in this study, whereas controlling mechanisms on the temporal and spatial variation of R_H were more complex. Advanced techniques (e.g. stable ¹⁴C and ¹³C isotope tracing) and laboratory incubation experiments based on plant physiology are needed to elucidate the variation mechanism of R_H and rhizospheric respirations. Moreover, we observed in the study site that most roots (<5 mm) of *Q. aquifolioides* forest were distributed at the soil depth of 0–30 cm and few roots existed below the depth of 50 cm. Trenching down to the soil depth of 70 cm in this study should be deep enough to exclude the root impacts, and our results justified it to some extent.

Altitude (m)	Density (clumps ha ⁻¹)	TD (cm)	HT (m)	CR (m ²)	Litter layer (cm)	pH	BD (g/cm ³)
3549	3897	3.62 (1.44)a	2.45 (0.82)a	4.47 (3.20)a	1.46 (1.11)a	6.75 (0.75)a	1.46 (0.59)a
3091	2167	4.56 (1.71)b	4.50 (0.90)b	8.69 (4.45)b	2.24 (1.01)b	5.34 (0.09)b	1.24 (0.04)a
2551	2142	6.74 (3.09)c	6.43 (1.48)c	10.99 (7.84)b	2.13 (1.08)b	5.20 (0.21)b	1.47 (0.05)a

Table 4. Site characteristics of the three sites in *Quercus aquifolioides* forest. TD, HT, CR and BD refer to stem diameter at 50 cm from ground, height, crown of the *Q. aquifolioides* and bulk density, respectively. Values in parentheses are standard deviations of means.

Conclusion

In subalpine forests, R_S and its components are obviously greater in the GS than in the DS and are higher during daytime than at nighttime. The temporal variations of daytime and nighttime R_S and its components can be well explained by the interactions of soil temperature and moisture regardless of seasons. R_S and its components are more sensitive to temperature in the DS than in the GS, and at nighttime than in daytime. Additionally, the substrate availabilities affected differently on Q_{10} of R_S and R_H on a daily and seasonal scale. Our findings suggest that soil CO₂ emission throughout the DS and at nighttime in the high-altitude subalpine area plays a vital role in annual soil carbon budgets and their responses to climate change. Temporal variations of R_S and its responses to environmental factors need to be considered in order to accurately predict changes in soil CO₂ fluxes.

Materials and Methods

Site description. The research was conducted in Wolong Nature Reserve in Sichuan Province, China (102°58′–103°06′ E, 30°53′–58′ N), which is located at the Balang Mountain in the east branch of Qionglai Mountains, southeast of Tibetan Plateau. The brown mountainous soil (Chinese classification) is the main soil type across the whole study area of the *Quercus aquifolioides* distribution. The area is characterized by the typical Tibetan Plateau climate with distinct wet and dry seasons. At the 2700 m a.s.l., annual mean air temperature is about 8.4 °C, with the average minimum/maximum (January) and maximum/minimum (July) air temperature being $-1.7/+5.1$ and $+17/6.3$ °C, respectively. The annual precipitation is 861.8 mm, of which 68.1% occurs from May to September. According to the field observation, the average soil temperature at 5 cm soil depth in 2011 was 5.2 ± 4.2 °C (mean \pm std. deviation) at 3549 m a.s.l., 4.8 ± 5.3 °C at 3091 m a.s.l., and 7.4 ± 5.3 °C at 2551 m a.s.l., with corresponding soil moisture values of $29.1 \pm 10.3\%$, $30.4 \pm 15.8\%$ and $34.6 \pm 11.1\%$, respectively. In the study site, *Q. aquifolioides* is the dominant species of this typical oak forest, while other species include *Cotoneaster horizontalis*, *Daphne tangutica*, *Deyeuxia levipes*, *Oryzopsis munroi*, *Pedicularis davidii*, *Athyrium pachyphlebium*.

Experiment design. Three *Q. aquifolioides* forest experimental sites were set up along the altitude gradient within its optimal spatial coverage (2551, 3091 and 3549 m, Table 4), on the southeast slope of Balang Mountain. At each site, three 20 m \times 20 m replicate plots were established with a distance of 10 m between plots. In each plot, three 80 cm \times 80 cm subplots were randomly deployed for trenching experiment and a buffer area was set with 10 m apart from each other. On 18 June 2010, at the outside edges of each subplot, a trench of 0.7 m deep (to the bedrock or below where few roots existed) was dug using a steel knife and shovel. On this study site, we observed that most roots (diameter < 5 mm) were distributed in soil between 0 to 30 cm in depth in *Q. aquifolioides* forest, and few roots existed below the depth of 50 cm. To prevent the trenched plots from root encroachment, we lined the trenches with double-layer plastic sheets, and then refilled them carefully with the same soil. At each trenched subplot, one PVC (20 cm inside diameter \times 8 cm in height) collar was installed into soil to a depth of 5 cm for CO₂ efflux sampling (R_H). Furthermore, all aboveground vegetation was carefully removed with minimal soil disturbance to keep a free of seedlings and herbaceous vegetation in these subplots throughout the course of experiment (from Sept, 2010 to Dec, 2011). Another three PVC collars were randomly inserted into the soil to a depth of 5 cm within 3 m around each trenched subplot (including the litter layer) to measure the R_S , which was considered as the total soil respirations rate. Once inserted, the collars were left unmoved during the entire study period. Our first measurements of R_S started two and half months later after the onset collar installation (mid-September 2010). Site characteristics are listed in Table 4 and Fig. 6.

Soil respirations, temperature and moisture measurements. In order to minimize the possible influence of live and dead roots in trenched plots decay, soil CO₂ fluxes was measured 75 days later since the onset of trenching experiment³². In this study, we actually used the measurement data 6 months later since the onset of trenching for minimizing the effects of root decomposition in trenched plots. Therefore, soil CO₂ flux in trenched plots can be regarded as the trench respiration (R_H), while R_R was the difference between R_S and R_H ^{18,21}. Soil respirations rates in the untrenched plots (R_S) and the trenched (R_H) were measured monthly from September 2010 to December 2011 (8th–18th per month except rainy days). At the time of R_H and R_S measurements, Soil temperature (T_S) and moisture (volumetric water content, θ_S) at 5 cm depth were measured automatically using the soil temperature probe and an attached Theta probe ML2x (Delta-T Devices, Cambridge, England) equipped with a Li-8100A soil CO₂ flux system (LI-Cor Inc., Lincoln, NE, USA). In the daytime, respiration was measured between 9:00 and 16:00 hours (local time), while at nighttime it was measured between 20:00 and 4:00 hours^{55,56}. The R_S and R_H were measured continuously in two cycles at each collar, 3 minutes per each cycle. The two measurements were averaged to produce the collar's mean R_S and R_H rates and mean T_S or θ_S . The average values of the 9 (3 subplots \times 3 replicates) measurements at each altitude for R_H , T_T and θ_T (trenched), respectively, while the three collars' measurements from the nearby trenched subplots were averaged to produce one mean value of R_S , T_{UT} and θ_{UT} (untrenched), respectively. The average values of the 27 (3 subplots \times 3 replicates \times 3 altitudes)

measurements around each trenched subplot in each month for soil CO₂ rates, T_5 and θ_5 were used for data analysis.

Soil chemical property measurements. Soil was sampled at 0–30 cm depth with a sample ring kit with closed ring holder (Eijkelkamp 07.53.SC, Holland) in mid-April, mid-August and mid-November of 2011, respectively. In each subplot, near each PVC collar (within 80 cm) three soil cores were collected and mixed thoroughly into one sample. Altogether, nine samples from each plot were stored in a cooler before being carried back to the lab. Roots, gravel, and other miscellany of things in the samples were manually removed and the soil was sieved with a 2 mm sieve. Each sample was divided into two parts. One was stored at room temperature and was air-dried for analysis of soil chemical properties, the other was immediately deposited in a refrigerator at 4 °C for soil microbial biomass measurement that was conducted within 3–4 days.

The contents of soil NH⁴⁺ and NO³⁻ were determined by using the indophenol blue colorimetric method and dual-wavelength spectrophotometry, respectively. Soil TOC was determined with the potassium dichromate oxidation heating method, and TN was determined with the semimicro Kjeldahl method using the air-dried soil. The measurements of soil microbial biomass carbon (MBC) and nitrogen (MBN) was performed by using the chloroform fumigation extraction method according to Vance *et al.*⁵⁷.

Calculation of temperature sensitivity. Based on the measured data in 2011, an exponential equation (Van't Hoff model, T-model; Eq. 1) was formulated to interpret the relationship between R_S , R_H or R_R and soil temperature (T_5) at 5 cm soil depth in three altitudinal plots. The temperature response of R_S , R_H or R_R was estimated in this study according to the following equations Eqs 1 and 2, respectively.

$$R_S(R_H, R_R) = a \times e^{(b \times T_5)} \quad (1)$$

$$Q_{10} = e^{10b} \quad (2)$$

Where R_S , R_H are the mean soil surface CO₂ fluxes ($\mu\text{mol m}^{-2}\text{s}^{-1}$), a and b are fitted parameters. The b values were used to calculate the temperature sensitivity (Q_{10}). We calculated daytime and nighttime respiration Q_{10} across seasons for each subplot. Additionally, we calculated the DS (early November through late April) and the GS (early May through late October, the growing season was determined by the phenological events of dominant *Q. aquifolioides*) Q_{10} values across daytime and nighttime measurements.

Relationship between R_S (R_H or R_R) and soil moisture. A power equation (θ -model; Eq. 3) was used to describe the relationship between R_S , R_H or R_R and soil moisture (θ_5) at 5 cm soil depth at three altitudinal plots. A logarithm transformation was performed on R_S and R_H to achieve linearity and homogeneity of variance test, respectively, and was used as a function of T_5 and θ_5^4 (T- θ model; Eq. 4).

$$R_S(R_H, R_R) = a \times \theta_5^b \quad (3)$$

$$\ln(R_S, R_H \text{ or } R_R) = a + b \times T_5 + c \times \theta_5 \quad (4)$$

Where a , b and c are the fitted parameters.

Statistical analyses. A repeated measures analysis of variance (ANOVA) was used to test for differences of treatment (trenched and untrenched) on soil CO₂ rates, T_5 and θ_5 based on the measurement data. A one-way ANOVA was used to compare the differences of soil CO₂ flux, T_5 and θ_5 between daytime and nighttime in different seasons. Based on the monthly mean of soil CO₂ fluxes at each altitudinal gradient, the dependence of R_S , R_H and R_R on T_5 or θ_5 was investigated using nonlinear and multiple linear regression models. A linear regression was used to analyze the relationship of Q_{10} with T_5 , θ_5 , and other soil properties in daytime/nighttime and seasons across 27 subplots (3 subplots \times 3 replicates \times 3 altitudes). The significant differences of Q_{10} values between daytime and nighttime, between the DS and the GS, were tested using the ANOVA at $\alpha = 0.05$, respectively. In order to ensure the comparability of R_H and eliminate the effects of trenching treatment on R_H , measured data were corrected where T_5 and θ_5 diverged on trenched and untrenched plots throughout the measurements in 2011. We used a multiple linear regression function to evaluate the trenching effect on soil C fluxes. All statistical analyses were performed using SPSS (version 19.0 for Windows).

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Author Contributions

Z.H. was the principal investigator for this study. He undertook the main data analyses and wrote the manuscript. S.L. supervised the research project and contributed to layout and design of the experiment, and the manuscript writing and revision of earlier drafts until final approval. J.W. and K.L. contributed largely to data processing and statistical analysis, and X.L., L.F., X.H., Y.Z. and F.H. contributed to the interpretation and discussion of the results.

Additional Information

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