Soil Respiration Variations in Temperate Rhododendron (*Rhododendronarboreum*) Forest of Annapurna Conservation Area (ACA) in Nepal

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Research

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Abstract

Background: Photosynthetic carbon released into the atmosphere in the form of carbon dioxide (CO_2) which represented by soil respiration (SR), is considered the largest carbon (C) efflux of terrestrial ecosystem. Understanding the dynamics of SR is critical to coping with prevailing climate change from regional to global scale. Temperate forests are considered as most fragile hence need to recognize their vulnerability owing to continuous climatic changes and anthropogenic activities. Predicting the response of SR is essential, owing to the varying environmental factors that are most dominantly effective to become common determinants of forest carbon variability. This study aimed to assess SR by using closed chamber method in the natural *Rhododendron arboretum* forest in Annapurna Conservation Area (ACA) which is recognized as the world's largest forest type located in a temperate region of Nepal. This research additionally aimed to evaluate the consequences of multiple ecological parameters mainly climatic and biotic factors on SR variations in consecutive two years measurement period in October 2016 and 2017.

Results: Overall, SR in the forest well corresponded with the soil temperature (ST) variables. Within a short-range (2-3°C) of ST difference the variation in SR was recognized as highly significant ($p<0.05$) exponential curve ($y=1.049e^{0.529x}$, 2016 and $y = 26.34e^{0.284x}$, 2017). However, the effect of soil water content (SWC) on SR was scattered and clear effects of photosynthetic photon flux density (PPFD) were also not detected. Contrary to ST and SWC, seasonal trend of SR was compatible with the PPFD and litter input. The temporal, diurnal, and inter-annual variations of SR, ST, SWC and litter fall were accountable.

Conclusions: Temperate forest could store the maximum amount of soil C with limited C emission through SR and become a larger sink of atmospheric CO_2. SR is very sensitive to environmental changes and interactively affected by multiple ecological factors, even though it is often difficult to separate their interactions. This founding research is adequate measure in temperate Rhododendron forest; further study seeks understanding on how C emission responds to the regional climate warming, through changing precipitation and landuse, and integrates these feedbacks into global climate models and carbon budget.

Background

Ecosystem carbon (C) assessment is so intricate to understand the global C balance depending on the major appearance of forests (IPCC Climate Change 2013). Forests accomplished to segregate C to become a sink and majority of that C is ultimately stored in soils (Pan et al. 2011; Ballantyne et al. 2012). Evaluation of C in the soil through the estimation of C budget, storages, and fluxes is hence decisive for recognizing the ecosystem C capacity (Dhital et al. 2010b; Wu et al. 2013; Fei et al. 2018; Liang et al. 2020; Ciais et al. 2021). Soil release C in the form of carbon dioxide (CO_2) and is represented as soil respiration (SR). The SR is considered the largest C efflux from the terrestrial ecosystem back to the atmosphere (Wu et al. 2013; Zhao et al. 2017). To deal with prevailing climate change, understanding the dynamics of SR is becoming critical (Meena et al. 2020) from regional to global scale (Valentini 2000). The belowground C should also be taken into account (Brunner et al. 2013) which alters SR in response to predicted climate change scenarios as it is hypothesized that the climatic warming increase the rates of SR potentially fueling further increases in global temperatures (IPCC 2013). Thus, accumulating records of SR from multiple eco-regions of the earth is essential for a reliable estimate of soil C emission on a global scale (Xu and Shang 2016).

Anticipating the response of SR is crucial owing to varying environmental circumstances, exclusively as SR is the integrate flux of respiration of the plant roots known as autotrophic respiration and the respiration of micro-organisms in the decomposition of organic matters in soil known as heterotrophic respiration (Hogberg and Read 2006). Global climate data manifested that forest ecosystems predominantly sequester a large quantity of anthropogenic CO_2 emissions of terrestrial ecosystems (Pan et al. 2011). Warming due to the increase in air temperature and changing precipitation or soil water dynamics (IPCC 2007; Concilio et al. 2009; Kim et al. 2020) is most dominantly effective to become common determinants of variability in the forest C balance (Jassal et al. 2009; Sun et al. 2011). However, litter decomposition in the C cycle, emission and respiration have a different appearance in the temperate forest than that of the tropics (Pan et al. 2013; Leff et al. 2012). Although, soil temperature (ST), soil water content (SWC) and the light activity (photosynthetic photon flux density, PPFD) are considered major environmental criterion in influencing the SR, seasonal fluctuations alter its variability in temporal scale (Ngao et al. 2012) is different in temperate ecosystems (Dhital et al. 2014; Yan et al. 2019; Kim et al. 2020; Klimek et al. 2021) than that of the tropical (Dhital et al. 2020; Yu et al. 2020). The temperate forests sequestrate and emit huge volume of atmospheric CO_2, and that amount could literally contribute a significant role in the global C cycle (Barford et al. 2001; Pan et al. 2011). The recently published data however indicated that temperate forests are an important sink of atmospheric C (Jílková et al. 2019; Ma et al. 2020). Thus, the research interest in a temperate forest is growing under the context of climatic warming (Han and Jin 2018; Huang et al. 2020; Klimek et al. 2021).

About 23% of Nepal's land area has been designated under various categories of protected area which includes national parks, reserves, conservation areas and buffer-zones (LRMP 1986). The middle mountains forest including temperate forest cover representing 124.26 m^3/ha out of the total coverage of forested area (6.4 million ha) that representing 44.7% across all regions. The mean C stock of the forests of Nepal including above- and below-ground biomass and soil C is 176.9 t/ha with 61.5% of this in the tree component and 37.8% in forest soils (MoFSC 2017). Temperate regions represent the most common eco-regions of Asia. Nepal occupies 12% of the total land coverage comprises of 2,000 to 3,000m altitude in which the temperate forest is distributed from east to the larger area in the west mostly dominated by the broad leaved trees such as *Quercus lamellosa*, *Q. semecarpifolia*, *Tsuga dumosa* and *Rhododendron spp*. in pure or mixed stands. The common forest types of the temperate zone include *Rododendraon arboreum*, *Rohododendron barbatum*, *Lyonia spp*., *Pieris formosa*, *Tsuga dumosa*, etc forest. Rhododendron is the largest genus ranges from the tiny structure to the largest evergreen and semi-, deciduous trees distributed in different soil composition (de Milleville 2002; Mao et al. 2017) throughout Asia, Europe, North America and Australia (Chamberlain et al. 1996; Gibbs et al. 2011). Due to the consequences of current issues of climate change, this keystone Rhododendron forest is under the vulnerable condition due to lack of proper conservation, management practices with imbalance carbon, hence need urgent protection in their habitat especially in the temperate region (Paul et al. 2019).

In Nepal, the exploration of soil C emission via SR and its regulating factors in association with forest C cycle research in the temperate region is yet to be focused on. And this vulnerable region is even not included in the global data set of the SR measurements (Bond-Lamberty and Thomson 2014). The temperate regions of Hiamalaya are subsidized as the most fragile and prioritized regions recognizing their vulnerability due to natural factors such as increasing levels of atmospheric CO_2, continuous warming by climatic change, and posing of pressure over the resources through anthropogenic activities. Only measurable researches are being approached limiting the restricted boundaries to focus on the soil C emission and its influencing parameters (Dhital et al. 2019). The research initiation is most urgent to understand the C cycle in the forests of temperate region that contribute the considerable C addition to the global C budget and play a significant role (Heath et al. 1993; Martin et al. 2001). Hence, this study thus aimed to assess the soil C efflux via SR in a natural temperate *Rhododendron arboreum* forest located in Annapurna Conservation Area (ACA) in Nepal. Further, the study specifically desired to evaluate the associated multiple ecological/environmental components of the forest such as climatic and biotic factors that could firmly regulate the SR variations, and compute its influences and sensitivity towards SR with the measurements of closed chamber technique by using an infrared gas analyzer.

Methods

Study area description

The study was conducted in a temperate forest (N 28˚23'40.7", E 083˚46' 07.0", altitude 2675 m a.s.l.) dominated by *Rhododendron arboreum* trees, which is the world's largest densely populated Rhododendron forest distributed in the Annapurna Conservation Area (ACA) of Central region in Nepal (Fig. 1). This is the natural and primary forest. The ACA is the first largest protected area covering 7629 km^2 of Nepal with the forested area of 1029.76 m^2). This is constructed with the major purpose of natural resources and biodiversity conservation in management principles under the national trust for nature conservation (NTNC) and the Annapurna Conservation Area (ACA) project (Bajracharya et al. 2007; Thakali 2012). It is situated at the Annapurna range of Himalayas across Manang, Mustang, Kaski, Myagdi and Lamjung districts. The area ranges in altitude from 790 m a.s.l. to the peak of Annapurna I at 8091 m a.s.l. The study area has a warm-temperate climate i.e. hot and wet summer season, and cold and dry winter season. This area has typically higher rainfall than the eastern region (DHM). This receives most (about 80%) of the annual precipitation during the summer/monsoon season (June-September), and most rain events occurred in July and August. The winter (December-February) is dry and cold with very rare rain events. In October the area receives little autumn/post monsoon rain similar in character to the spring/pre-monsoon but the frequency is low and rapidly decreases and increases with the progress of the autumn and spring season. Similarly, highest temperatures were recorded during summer mostly from July to August and the lowest in winter from December to January (Fig. 2).

Green vegetation of the study area consisted of dominated tall *Rhododendron arboreum (Nepali name: Laligurans, Family: Ericaceae)* trees from small (>1 m) to large (<5 m) diameter at breast height forming a dense Rhododendron forest. The associated other plant species occupies sparsely at the plot were *Acer laevigatum*, *A. carpinifolia*, *Quercus semecarpifolia*, *Q. lamellosa*, *Lyonia spp*, *Pieris formosa*, *Daphne bholua*, etc.

Environmental parameters measurements

Air temperature (°C) and precipitation (mm) of the study area for thirteen years (2005 to 2017) were generated from the data recorded in meteorological station (Lumle) of the Department of Hydrology and Meteorology (DHM), Government of Nepal. The soil temperature (ST, °C) at 5 cm soil depth and the air temperature (°C) at 1.5m height from the forest floor within the study site were measured by a digital lab stem thermometer (AD-5622, A&D, Japan). The soil water content (SWC, Vol %) at 5 cm soil depth was measured by a time-domain-reflectometry (TDR) soil moisture probe (TRIME-PICO64) and recorded with the HD2-TRIME-FM (Imko, Germany) mobile data logger. The ST, air temperature and SWC of the forest were recorded in each measurement of soil respiration (SR) at three different points near and around the soil chamber. The PPFD (light) was measured by using a LI-190SA quantum sensor and LI-1400 data logger of LI-COR, Inc. Lincoln, Nebraska, USA. During measurements the light sensor was placed on top of the soil chamber at three different points and recorded data for each point during the SR measurements. The multiple of three measurements (n=3) of PPFD was recorded in each chamber.

Soil respiration (SR) measurements

In October 2016, the study plot area of size 100 m × 70 m was established for measurement that was located at the Northern slope (about 15°) of the forested area. A multiple of three different sets of SR measurements were conducted each day started from the morning at 7:00 am to the evening at 5:00 pm. The measurements were carried out in October during the morning (7:00 am-9:00 am), afternoon (11:00 am-1:00 pm) and evening (3:00 pm-5:00 pm) with the time interval of 2 hours in each set of measurements between the date 16th and 18th in 2016, and 27th and 30th in 2017 for 2-years. Chambers (n=10) made of polyvinyl chloride with diameter of 18 cm and height of 16 cm were arranged randomly in the experimental area. The chambers were installed below the ground surface at 2 cm into the soil taking care that they were perfectly air tight without any leakage between the soil and the edge of the chambers. This chamber is composed of two parts a lid and a body. The lid was equipped with an IRGA for the measurement of CO_2 and gas temperature of the chamber (body). Vaisala CARBOCAP CO_2 probe GMP343 (Helsinki, *Finland*) was used for the measurement of CO_2 concentration and gas temperature inside the chamber. This method involves placing a chamber over the soil surface and increase in concentration of CO_2 within the chamber is measured as a function of time and data logger (VAISALA humicap hand held device, Helsinki, *Finland*) was used to record the measured data. The density of CO_2 was calculated through recording the air temperature of the chamber. The first day of the measurements of SR was operated one day after placement of the chamber on the forest floor and continued for the rest of the days to avoid the variability of data due to the installation effects.

Data analysis

To prevent any systematic errors, a multiple of three cycles of SR measurements in the forest were made on each soil chamber and the average of three measurements value was used for each chamber. The mean value of SR from all chambers measurements (n=10) recorded in each date was considered as the representative of daily SR and compared integrate the diurnal variations. Similar measurements of ST, SWC and PPFD were operated for the analysis and comparison. Throughout field measurements, few SWC data could not obtained due to some technical glitches caused by the instrument malfunction and unexpected rainfall. The SR rate of the forest was calculated using equation 1as follows:

$$F= (V/A)\ (\Delta c/\Delta t) \qquad (1)$$

Where F is soil respiration (SR, mg CO_2 m^{-2} h^{-1}), V is volume of air within the chamber (m^3), A is area of the soil surface within the chamber (m^2) and $\Delta c/\Delta t$ is the time rate of change of the CO_2 concentration in the air within the chamber (mg CO_2 m^{-2} h^{-1}).

The model to fit for the SR rate and ST was developed by an exponential regression. The dependence of the SR rate on ST was modeled using the following regression equation 2. The fit of models were estimated by using the coefficient of determination (R^2) and residual analysis.

F = α * exp (β *Ts) (2)

Where, F is the predicted SR (mg CO_2 m^{-2} h^{-1}), Ts is the ST (°C) at a depth of 5 cm, and α and β represent the regression coefficients.

The statistical analysis was made by using R software. GIS software of 10.4.1 versions used for mapping of sampling site and the study area arc. Statistical analysis such as correlation, significance of correlations was used to examine the relationship between the variables.

Litter biomass measurements

The five random sub-plots (n = 5) within the study area in SR measurements plot were selected for litter biomass sampling and collected the sample at the ground level of the forest floor. The sampling of litter was made once in each period of SR measurements considering that the amount of litter remains constant between the days of measurements in same season of a year. The litter samples were collected inside the SR chamber and the area was calculated for the square meter. Initially, the samples were oven dried at 70●C for 48 hours and weighed the dried samples with an electronic balance. The dry weight of litter biomass was calculated using following formula.

Biomass = Dry weight (g) / Area (m^2) (3)

Results

Air temperature and precipitation

Over the thirteen years (2005-2017) of the recorded meteorological data (Source: Department of hydrology and meteorology, DHM, Lumle) representing the air temperature and precipitation of the *R. arboreum* forest area (Fig. 2) showed that the area mostly acquired higher air temperature in July and August and the lower during winter in December and January which was reached the highest at 22.3˚C in August and lowest at 7.9˚C in January 2012. The forest area received maximum precipitation as rainfall during the month of summer in July and August however some of the years June and September as well received the higher amount of precipitation due to early and delay rain events. The winter season received the least amount of rain and sometimes no precipitation occurred during that period so-called dry season. The highest rainfall was recorded at 1818.1mm in July 2010. The mean (2005-2017) annual-average air temperature of the forest area was recorded at 16.5˚C with the highest in July and August at 21.0 °C and the lowest was recorded in January at 9.9°C (Table 1). Similarly, the mean (thirteen years) annual-average precipitation of the forest area recorded was 434.7mm with the maximum and minimum monthly mean precipitations were 1523.9mm in July and 8.1mm in December, respectively. The mean (2005-2017) annual precipitation of *R. arboreum* forest was recorded at 5216.1mm y^{-1}. ACA is located in central part of Nepal receives the highest amount of rainfall compared to all regions of Nepal.

Soil respiration (SR) and soil temperature (ST)

The temperature dependency of SR variations of this *R. arboreum* forest was assessed from the ST measured at 5 cm soil depth that was plotted against the SR (Eq. 1) with optimal regression using the exponential form of Equation (2) over the different periods and dates in October 2016 and 2017 for the entire experimental period (Fig. 3). The result exhibited increasing ST boosting the SR exponentially was explained positive statistically significant ($p<0.05$) curve (Day1: D1; R^2=0.21, $y=0.572e^{0.580x}$, Day2: D2; R^2=0.13, $y=0.567e^{0.585x}$, Day3: D3; R^2=0.23, $y=1.248e^{0.525x}$). Similar, exponential statistically significant ($p<0.05$; Day1: D1; R^2=0.20, $y=12.12e^{0.379x}$ and Day2: D2; R^2=0.24, $y=3.789e^{0.500x}$), and statistically insignificant ($p>0.05$; Day3: D3; R^2=0.19, $y=6.688e^{0.448x}$ and Day4: D4; R^2=0.18, $y=14.86e^{0.379x}$) curves were detected in 2017. The highly significant ($p<0.05$) exponential relation with the ST were derived when different dates in a season were integrated (R^2=0.18, $y=1.049e^{0.529x}$) in 2016 and (R^2=0.17, $y = 26.34e^{0.284x}$) in 2017 (Fig. 4 a1, b1). Within a short-range even at 2-3°C of the ST difference i.e. from 9.2°C to 11.3 °C in 2016 and 6.4 °C to 9.3 °C in 2017, the variations of the SR in this forest were well recognized to explain the ST effect on SR of the forest.

Soil respiration (SR) and soil water content (SWC)

The scattered relation was found for SWC dependence on SR variations in the *R. arboretum* forest in an experiment while assessing them in different periods of two consecutive years (Fig. 4 a2, b2) in experiment.

The SWC was ranged between 27.0% and 40.6% in 2016, and 22.3% and 44.8% in 2017. The peak values increase in SWC after the unexpected rain events that were very common in the study area. A comparatively much higher range of SWC was noticed in 2017 than the year 2016 as the measurements dates in 2016 were half a month earlier to the summer season i.e. much near to the rainy season than the year 2017.

Soil respiration (SR) and photosynthetic photon flux density (PPFD)

To elucidate the dependence of SR on the variability of light in the forest, SR was plotted against the PPFD in different periods, dates and years of the measurements showed that the light parameter, PPFD was not able to establish the relationship with SR variations to define independently the PPFD effect on SR in the *R. arboreum* forest (Fig. 43 a3, b3). The values obtained in measurements of PPFD were assessed much random in different chamber and even constitute in the same chamber at different points. The range of the PPFD observed was between 2.7 μ mol m^{-2} s^{-1} and 96.2 μ mol m^{-2} s^{-1} in 2016, and 1.3 μ mol m^{-2} s^{-1} and 761.0 μ mol m^{-2} s^{-1} in 2017. In two years, the values of the PPFD were most likely concentrated within 100 μ mol m^{-2} s^{-1} that were correctly measured in a different time period of a day for the consecutive 3-4 days in a year. The high and low values of the PPFD were most likely recorded in a different period sunny to the cloudiest weather.

Comparisons of soil respiration (SR) and soil temperature (ST), soil water content (SWC), photosynthetic photon flux density (PPFD) and litter biomass variations

The temporal, diurnal and inter-annual variations of the SR, ST, SWC and PPFD of the forest represented the degree of correlations between the SR and the climatic parameters. Comparatively higher SR (n=10) was observed in the afternoon (11:00 am – 1:00 pm) and the maximum value was recorded at 306.3 mg CO_2 m^{-2} h^{-1} on 18th October 2016. But, the values became random in 2017 and the maximum value was observed during evening at 348.9 mg CO_2 m^{-2} h^{-1} on 27th October (Fig. 5). The minimum SR (n=10) was observed in the morning on 17th October at 201.6 mg CO_2 m^{-2} h^{-1} in 2016 and on 29th October at 232.5mg CO_2 m^{-2} h^{-1} in 2017. With acquiring the temporal variations of the SR in each day of measurements, the values of SR were not compatible with each other. However, the difference value between the maximum and minimum SR was most likely compatible in both years. The ST (n=10) was recorded maximum at 10.7 °C on 16th October 2016 and 8.9 °C on 27th October 2017 during evening between 3:00 pm – 5:00 pm among different days of the measurements. The minimum ST was observed during morning at 9.6°C on 17th October 2016 and 7.1°C on 27th October 2017. The ST followed the similar trend of the SR and it was always lower during morning than that of the afternoon and evening.

The maximum SWC (n=10) was recorded during the evening at 36.7% on 16th October 2016 and 36.9% on 27th October 2017. The minimum SWC was observed during the afternoon at 31.8% and 31.4% on 16th October 2016 and 30th October 2017 respectively. The maximum and minimum SWC of the forest was very close and compatible for both years but it varied among the dates and the time of measurements in each year. The temporal and diurnal variations of the SWC were not compatible to the SR of the forest. The SR was increased during the afternoon when the SWC decreased and higher was observed while the SWC was decreased in the evening (Fig. 6).

Correlating to other climatic parameters, the PPFD of the forest was noticed much fluctuating in different dates and years in measurements. The maximum PPFD (n=10) were recorded at 41.0 μ mol m^{-2} s^{-1} during the morning on 18th October 2016 and 253.2 μ mol m^{-2} s^{-1} during the afternoon on 30th October 2017. Where, the minimum values of PPFD were recorded at 9.0 μ mol m^{-2} s^{-1} on 18th October 2016 and 7.3 μ mol m^{-2} s^{-1} on 27th October 2017 during the evening (Figure 7). The range of maximum and minimum values of the PPFD of this forest was found much higher than that of the other related parameters. Comparing the temporal variations of PPFD to the SR it was much similar to each other in the days of sunny weather; however the trend of diurnal changes in PPFD was not visible in cloudy day and they were not directly effects on the temporal changes in SR of the forest.

The diurnal variations of the SR, ST, SWC and PPFD were detected in both years (Table 2). The daily average SR was 278.2, 242.0 and 289.5 mg CO_2 m^{-2} h^{-1} on 16th, 17th and 18th October in 2016 and it was 332.1, 301.6, 273.3 and 273.5 mg CO_2 m^{-2} h^{-1} on 27th, 28th, 29th and 30th October in 2017. The rates of SR were varied among the days of measurements however the values were compatible with each other and not unexpectedly different. Similarly, the ST was 10.4, 10.0 and 10.1°C in 2016 and 8.5, 8.6, 8.0 and 7.4°C in 2017. The minimal diurnal variations of the ST were detected in both years (upto 0.4°C in 2016 and 1.2°C in 2017), to detect its effect. The SWC was 34.2, 34.2 and 34.5% in 2016 and 35.7, 32.8, 33.6 and 33.4% in 2017. The diurnal variations of SWC were minor (0.3%) in 2016 however it was higher (2.9%) in 2017. Much more variations between the days were observed for PPFD were 17.1, 14.1 and 23.7 μ mol m^{-2} s^{-1} in 2016 and 12.3, 39.0, 20.0 and 90.4 μ mol m^{-2} s^{-1} in 2017. Compared to the rest of climatic parameters, the PPFD varied more to the diurnal changes of season.

The measurements of SR, ST, SWC, PPFD, litter biomass, and air temperature in 2-years determined that the inter-annual variations of the soil C emission via. SR and the ecological parameters were much common in the *R. arboreum* forest; however the intensity of variance were incompatible for each component (Fig. 8). The SR over the entire measurements period each year averaged at 269.9 mg CO_2 m^{-2} h^{-1} in 2016 and 295.1 mg CO_2 m^{-2} h^{-1} in 2017, and lower the SR rate in 2016 than the year 2017 were even comparable. The 2-years averaged SR represented the seasonal SR of the *R. arboreum* forest was 282.5 mg CO_2 m^{-2} h^{-1}. The variations from seasons to the years in carbon sequestration and emission were adopted in multiple years of estimation were observed in a temperate ecosystem (Dhital et al. 2014). Similar variations of ST were observed in 2-years averaged throughout the measurements period was higher at 10.2°C in 2016 than at 8.1°C in 2017. The seasonal ST of the forest was calculated from two years records averaged were at 9.1°C. Comparing to the other climatic parameter of the forest, less variation was detected for SWC records between the years at 34.3% in 2016 and 33.9% in 2017, and slightly higher SWC was observed in 2016 than the year 2017, but the values were compatible to each other. The seasonal SWC value estimated from two years' data of SWC averaged was 34.1%. Similarly, the PPFD of the forest in 2016 was 18.3 μ mol m^{-2} s^{-1} and in 2017 was 41.4 μ mol m^{-2} s^{-1} and the variance was noticeable between the years. The represented seasonal PPFD averaged both years' measurement was 29.9 μ mol m^{-2} s^{-1}. The dry weight of litter biomass collected from the forest floor was observed at 8.9 g d. w. m^{-2} in 2016 and 15.3 g d. w. m^{-2} in 2017 (Eq. 3). The higher litter was recorded in 2017 than the year 2016 as the collected litter in 2017 was from the late October and it was the season to leaf abscission of the seasonal trend of the forest. The amount of the seasonal litter of *R. arboreum* forest averaged 2-years was calculated at 12.1 g d. w. m^{-2}. The air temperature of the forest was noticeably varied between the years and it was higher at 7.1 °C in 2016 than the year at 5.6 °C in 2017, and the average of 2-years was at 6.4 °C. Overall this study showed that, comparatively higher SR, PPFD and litter was detected in 2017 than the year 2016 that was across from the ST, SWC and the air temperature of the forest owed to the measurements in 2016 were carried half a month earlier in the season than the year 2017 that was not much far from the wet summer season with high temperature and precipitation.

Discussion

Soil respiration (SR) is one of the major pathways of the carbon dioxide efflux from the soil, thus determining SR is the most effective measure to elucidate the carbon emission that could be made accurate in estimation of the global carbon budget (Trumbore 2006). Given their critical role in the global carbon SR measurement and modeling issues have been kept in priority and well studied (Xu and Shang 2016; Ciais et al. 2021). Many studies have focused on investigating the effects of environmental variables driving the SR rate in the forests of temperate regions in different climatic conditions (Heskel and Tang 2018; Yan et al. 2019; Kim et al. 2020; Klimek et al. 2021). In this study, the major environmental factor, the ST showed a statistically significant exponential effect on SR variations in 2-years (Fig. 2, Fig. 3a1 and b1) of observations illustrating that the temperate forest soil could be more proactive in C emission while temperature rises and warming could be alarming more in emission from the forest floor in future. These results comply with the previous studies focused on temperate regions (Han and Jin 2018; Dhital et al. 2019; Yan et al. 2019). It was more appreciable that the ST effect on SR was even detected within the narrow range (2-3°C) of the ST difference. However, the effect of ST on SR in two days of 2017 was found statistically insignificant ($p>0.05$, Fig. 2b, Fig. D3 and D4) because the difference between the maximum and minimum ST was very narrow range normally within 2°C. Comparing to this study, no significant variations of SR due to narrow variation range of the ST was also detected in the tropical lowland rainforest of China during the spring season by Cui et al. (2020) when the variations of ST were very less as we observed in the autumn season. The compatible trend of SR and ST with significant exponential relation of this study was most likely noticed previously while observing the cutting effect on SR in Rhododendron forest in the National Park area in the UK (Jones et al. 2019). The result hence revealed that the factor for determining SR might be primarily the ST in the temperate forest, however, the angle of possession could be altered in different ecosystems (Dhital et al. 2019; Tiwari et al. 2021) and the exponential effect of ST on SR was the most quoted (Davidson et al. 2006; Dhital et al. 2010b; Kim et al. 2020; Wang et al. 2020).

The SWC dependency of SR of this *R. arboretum* forest was not satisfied to define the effect and variations of SR under different soil moisture conditions (Fig. 3a2 and b2). Higher range of the SWC (27–40.6% in 2016, 22.3–44.8% in 2017) was noticed in both years during the entire measurements period which was not harmonized to the SR variations that might be the cause of undetectable relationship between the SR and SWC. The higher range of SWC measured in a single month of a season was owed to the effect of monsoon (June-September) and post monsoon (October-November) rain which was the common phenomenon of this region which was also observed in the nearby grassland (23.3–59.6% and 15.6–42.2%) of the same region (Dhital et al. 2019). The nature of unpredictable and fluctuated climate of this region with having instant and short period of seasonal rain is the reason for lower and higher SWC before and after rain events. The instant events of rainfall in the forest might cause increasing the surface soil water level and the effect couldn't reach up to the deep soil tree rooting system due to the thick layer of the litter fall to vary the SR and the ST which indicated that increased precipitation may not always respond immediately to the increase in SR. Similar to our results, no SWC effects on variations of SR due to fluctuated climate were observed in the temperate forests in China (Han and Jin 2018). The SWC effect on SR and its variations are not simply understood due to the intricacy of soil on climate and its limitations in the temperate regions under the influence of monsoon climate with exceptionally getting short period of saturation and drought of these regions (Mo et al. 2005; Kim et al. 2020). However, the SWC effect of SR is the effective measure to evaluate the SR in the semi-arid ecosystems (Bao et al. 2019; Meena et al. 2020), tropical region (Yu et al. 2020; Cui et al. 2020) and the soil with having different texture (Lei and Han 2020), where precipitation enhance the most accepted driver of the ecosystem function. The knowledge of soil water effect on SR and the breach between them could be filled with the extended research for years with continuous measurements.

The effect of solar radiation/sunlight is the effective measure to increasing respiration rate of the plants importantly above-ground vegetation by enhancing the temperature and photosynthesis equally in high altitude regions (Tiwari et al. 2021). In this study, the variations of PPFD of the forest floor were different among time and date, and even between the chambers, and that was caused due to the close canopy cover of the trees in forest. The canopy allows partial penetration of the sunlight that depends on the position and angle of the sun, and the forest floor receives sunlight in patches. The undefined scattered effect of PPFD on SR of this study established (Fig. 3a3 and b3) in two years also owed the unpredictable seasonal and diurnal variations from clear and sunny to the cloudy and rainy weather within a single day faced during measurements, and particularly that phenomena was very common in the area. However, the open grassland of the same region has observed the visible linear relation with defined PPFD effect on SR variation at 37.6% in October. The minimum (2.7 μ mol m^{-2} s^{-1} in 2016 and 1.3 μ mol m^{-2} s^{-1} in 2017) and maximum (96.2 μ mol m^{-2} s^{-1} in 2016 and 761.0 μ mol m^{-2} s^{-1} in 2017) PPFD values of PPFD of this study were comparably lower than the PPFD (51.68 and 1526.0 μ mol m^{-2} s^{-1}) obtained in the nearby grassland (Dhital et al. 2019). However, not only the ecologically adopted environmental parameters but the land use is also considered as one of the major factors affecting soil carbon emissions via SR (Li et al., 2013). As compared to the grassland, the forest floor received only about 50% of the total PPFD to utilize. In two years of this study observed that most of the PPFD values were within 100 μ mol m^{-2} s^{-1} in autumn season, and this revealed that only that much of the photosynthetic rays could able to utilize the autotrophic and heterotrophic activities within the forest.

The seasonal variations of SR and its corresponding climatic factors are very common in the temperate forests of Asian monsoon climate (Kim et al. 2020). The temporal and diurnal variations in SR of this natural *R. arboreum* forest were very noticeable (Figure 5). The maximum values of SR (306.3 mg CO_2 m^{-2} h^{-1} in 2016 and 348.9 mg CO_2 m^{-2} h^{-1} in 2017) of this forest were recorded during afternoon from 11:00 am to 1:00 pm and the evening from 3:00 pm to 5:00 pm that was much consistent to the nearby temperate grassland of the region (Dhital et al. 2019). However, the values of maximum SR of this forest were much less than (963.42 mg CO_2 m^{-2} h^{-1} in October 2015 and 1132.6 mg CO_2 m^{-2} h^{-1} in April 2016) that of the grassland site. This explained that the temperate *R. arboreum* forest could store the maximum amount of C in the soil by the storage of soil C with limiting C emission through SR and could be the more sink of atmospheric CO_2. As compared to the values of the maximum SR, the minimum SR values between the years (201.6 mg CO_2 m^{-2} h^{-1} in 2016 and 232.5 mg CO_2 m^{-2} h^{-1} in 2017) of this forest were not much lowered than (45.3 mg CO_2 m^{-2} h^{-1} in October and 172.5 mg CO_2 m^{-2} h^{-1} in April) the minimum SR of the grassland. The minimum SR of this *R. arboreum* forest was higher than the nearby grassland which showed that the temperate forest possesses less difference between the maximum and minimum SR than the grassland. This proved that the forest of the temperate region is less sensitive to the increasing temperature than the grassland hence forest deserves more sink capacity of the atmospheric carbon than the open grassland which is considered as the major part for the land-use change conversion (Ma et al. 2020; Kim et al 2020). Compared to our observations, the daily mean SR of this forest varied among the days of measurements in 2016 (278.2, 242.0 and 289.4 mg CO_2

m^{-2} h^{-1}) and 2017 (332.1, 301.6, 273.3 and 273.5 mg CO_2 m^{-2} h^{-1}) (Table 2) that were consistent to the diurnal variations of SR observed previously (517.0, 430.5, 123.4, 357.0 mg CO_2 m^{-2} h^{-1}) in the nearby temperate open grassland of ACA during October (Dhital et al. 2019). The diurnal trend of the ST concerning the SR of the forest (Figure 5, Table 2) was also comparable to the nearby temperate grassland where the lowest SR and ST were detected during morning whereas they were very near in the afternoon and evening Similarly, the difference (1.1-1.7°C) between the maximum and minimum ST of the forest was very less as compared (9.2-20.3°C) to the grassland. This showed the higher temperature sensitivity of the SR in this *R. arboreum* forest which might be vulnerable to the warming climate.

The diurnal fluctuations of the SWC of the forest were very low (Table 2) and there were not much difference of the maximum (36.7% on 16th October 2016 and 36.9% on 27th October 2017) and minimum (31.8% on 16th 2016 and 31.4% on 30th 2017) soil water values (Fig. 6) while averaging all the point; however the rain was frequent and the weather was unpredictable. The coverage of litter on forest floor in October is high due to the seasonal litter fall preventing the soil surface from getting wet with a lowest SWC value and the SWC value was higher where the where the litter coverage was less. The maximum SWC values were measured during evening and minimum were during the afternoon because, in most of the days in our measurements, the short and frequent rain events were detected between the late afternoon and early evening measurements. The occurrence of both varying precipitation with the lower and higher rate than the usual has significant impacts on SR with the association of ecosystem components in the seasonally dry tropical forest (Yu et al. 2020). However, the tropical forests are more sensitive to the changing rainfall than that of the forests in temperate region where the temperature becomes the most prominent in controlling the SR (Rubio and Detto 2017; Yu et al. 2020; Klimek et al. 2021).

Canopy coverage plays a major role to prevent the light from the sun to the under vegetation and suffers from inadequate light intensity influencing their adaptability and optimum growth by the limitations of nutrient use efficiency. The research revealed that the light intensity (PPFD) enhanced the soil water use efficiency by increasing nutrient uptake significantly (Baligar et al. 2020) and hence the net ecosystem production (Dhital 2010a). In this study, the PPFD values randomly fluctuated throughout the day and only partial light could reach the forest floor due to the close canopy cover of the *R. arboreum* forest structure. The diurnal variations of the PPFD within our entire study period were not comparable to the days of the same season (Table 2) and even between the years (Fig. 7). The higher PPFD recorded during the afternoon in sunny weather of this forest was better compared to the grassland of the region (Dhital et al. 2019), however the forest is different from the grassland under the coverage of the leafy canopy with differences in exposure to the sunlight. The higher PPFD in the afternoon and evening than the morning during clear sunny weather of this forest much followed the trend of higher SR during afternoon and evening than the morning showed that PPFD could be the major variable of SR when the forest is not dense and has open canopy. This could be better understood while the measurements of SR and PPFD were carried out in selected sunny weather days in the different seasons and days of a year.

` The inter-annual variations in SR are much common in the forests owing to the common soil factors altered each year known by temperature and precipitation (Irvine et al. 2008). In this study, inter-annual variation of SR was observed however the variation between the years (269.9 mg CO_2 m^{-2} h^{-1} in 2016 and 295.1 mg CO_2 m^{-2} h^{-1} in 2017) was most likely comparable (Fig. 8.) The SR rate in 2016 was comparatively lower than the year 2017 however they were nearly corresponding to each other. The two years average seasonal mean SR (282.5 mg CO_2 m^{-2} h^{-1}) of this temperate *R. arboreum* forest was within the range (90-551 mg CO_2 m^{-2} h^{-1} and 98-305 mg CO_2 m^{-2} h^{-1}) of daily mean SR during autumn season in cool-temperate Mongolian *Quercus* forests in Korea (Kim et al. 2020). Due to the complexity of the components and spatially heterogeneous nature of the forest's soil, it is much difficult to understand the environmental variability (Li et al., 2013), and uniquely coupled with other biotic processes, the most common might be the soil properties in generating a broad spectrum of CO_2 emission rates in the temperate forests (Klimek et al. 2021). The prior observations by Cui et al. (2020) and Kochiieru et al. (2021) suggested that it is not surprising that SR rates vary considerably in different seasons, years and in the different forests, eco-zones, and even in the different soils and land use types.

The inter-annual variations of ST observed (10.2°C in 2016 and 8.1°C in 2017) in this study was similar to those seen for SR and it was surprised to record a low SR rate in 2016 at the time of high ST and the higher SR rate in 2017 at low ST. The cause might be the early measurements in 2016 i.e. mid of October than the year 2017 i.e. late October. The temperature decreases day by day with increase in the autumn season. Slightly higher SWC was detected in 2016 (34.3%) than in 2017 (33.9%) owed to the nearby summer and post monsoon rain. Thus, higher SWC might play the role to suppress the SR in 2016 even though the ST was high. The increasing SR rate might decrease with increasing SWC above its limit in temperate forests was detected in different soils and land use types (Kochiieru et al 2021). The accountability of the soil water effect on SR in temperate region was less (Dhital et al. 2019) to overcome the temperature effect however it was detected while comparing in two years separately. As the variety of biotic and abiotic factors regulates the rate of SR (You et al. 2019), the optimum soil moisture (30-40%, Fig. 4a2 and b2) with maximal water holding capacity allows in achieving the maximal respiration rate (Klimek et al. 2021), and the moisture content in soil may modify the temperature sensitivity of soil microbial respiration and thus activate the temperature sensitivity of the SR (Lellei-Kovács et al. 2011). The previous study also revealed that when both ST and SWC are not at their extremes, these two factors interactively influence the SR and integrated can account for most of its variability and more during active growing season than the dormant seasons (Luo and Zhou 2006).

The PPFD between the years were varied and it was lower (18.3 μ mol m^{-2} s^{-1}) in 2016 than (41.4 μ mol m^{-2} s^{-1}) in 2017 (Fig. 8). The light penetration within the forest floor is determined by the canopy structure. The closed canopy cover of the forest structure was the reason for the low value of the PPFD and the cause for sparsely distributed under-story vegetation of the forest floor. The seasonal two years average PPFD (29.9 μ mol m^{-2} s^{-1}) of this *R. arboreum* forest was visibly much lower than (51.68 μ mol m^{-2} s^{-1}) the minimum PPFD recorded in the nearby grassland (Dhital et al. 2019). However, the study in a similar temperate deciduous forest revealed that the temperature and light both led to the inhibition of leaf-level respiration and photosynthesis; as result carbon-use efficiency declined with increasing the leaf-level temperature and hence the basal respiration rate (Heskel and Jianwu 2018). The overall net ecosystem production is controlled by the sunlight (PPFD), and the increasing rate of the PPFD accelerates the respiration of the vegetation only up to its maximum limit and beyond the limit, the respiration started to decline (Dhital et al. 2010a). The PPFD of this forest could not reach to its maximum to the peak and decline in SR were not visible. Therefore, in this study, a higher seasonal SR was recorded in the year when the PPFD was recorded higher.

The inter-annual variations (8.9 g d. w. m^{-2} in 2016 and 15.3 g d. w. m^{-2} in 2017) of the litter biomass was well observed in this study and it was higher in the year 2017 when the SR, SWC and PPFD was high than the year 2016 (Fig. 8) except the ST which corresponds to the air temperature (7.1°C in 2016 and 5.6°C in 2017) of the forest. The SR visibly reduces (35%) with the reduction of litter input and induces (77%) with the doubled addition of the litter was visibly detected in the forests (Han et al. 2015). The SR, like many other physiological processes of plants and microbes, are very sensitive to environmental changes; often interactively affected by multiple factors, although it is often difficult to separate their interactions.

Conclusions

This study elucidates SR in the natural *R. arboreum* forest located in the temperate region of Nepal and highlights the sensitivity of SR correlated with multiple ecological parameters known as climatic and biotic factors. In the study, ST was found to be the most visible factor to the variations of SR and within a short range (2-3°C) of the ST difference, the variations of SR showed significant exponential relation with the ST. However, the SWC effect on SR variation was scattered and no clear relation between SR and SWC was detected. Similarly, in the study, the effect of PPFD on SR variations was also not correlated. The seasonal SR of the forest was much consistent with the seasonal PPFD and litter input, but it was not consistent with the seasonal ST and SWC. Accountable temporal, diurnal and inter-annual variations of SR with the modification of major climatic and biological factors of the forest were determined. The seasonal SR rate of the forest is observed comparatively low as compared to photosynthetic carbon. The study revealed that the temperate forest could store the maximum amount of C in soil with limiting C emission through SR and could be the more sink of atmospheric CO_2. Thus, the SR is very sensitive to environmental changes and interactively affected by multiple factors, although it is often difficult to separate their interactions. This founding research of the SR is adequate measure in the temperate *Rhododendron arboreum* forest; further study needs to seek how C emission of the temperate region responds to regional climatic warming and land-use, and to incorporate these feedbacks into global climate models and carbon budget.

Abbreviations

C: Carbon; CO_2: Carbon dioxide; SR: Soil respiration; ST: Soil temperature; SWC: Soil water content; PPFD: Photosynthetic photon flux density; ACA: Annapurna conservation area; DHM: Department hydrology and meteorology

Declarations

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Authors' contributions

DD conceived the research idea and designed the study. JS and DD arranged for the field visits, equipments and provided necessary support for the research. DD, PM and BG conducted the field measurements. DD conducted the laboratory work, analyzed the data and led writing and prepared the first draft of the manuscript. DD and JS contributed to revising the final manuscript. All authors gave approval for the publication.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Table 1 The record of thirteen years (2005–2017) means monthly average air temperature and precipitation of the *Rhododendron arboreum* forest area (Source: Department of hydrology and meteorology (DHM), Lumle)

Mean	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
Air temperature (°C)	9.9	11.7	15.0	17.8	19.1	20.6	21.0	21.0	20.2	17.4	13.9	11	16.5
Precipitation (mm)	28.3	43.8	70.5	106.0	269.9	783.0	1523.9	1303.6	851.2	214.6	13.3	8.1	434.7

Table 2 Diurnal variations of soil respiration(SR), soil temperature (ST, 5 cm soil depth), soil water content (SWC, 5 cm soil depth) and photosynthetic photon flux density (PPFD) over the period of experiment (October 2016 and 2017)

Parameters	Measurements date						
	October 2016			October 2017			
	16th	17th	18th	27th	28th	29th	30th
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4
Soil respiration (mg CO_2 m^{-2} h^{-1})	278.2	242.0	289.5	332.1	301.6	273.3	273.5
Soil temperature (°C)	10.4	10.0	10.1	8.5	8.6	8.0	7.4
Soil water content (Vol %)	34.2	34.2	34.5	35.7	32.8	33.6	33.4
Photosynthetic photon flux density (PPFD, μ mol m^{-2} s^{-1})	17.1	14.1	23.7	12.3	39.0	20.0	94.4

Figures

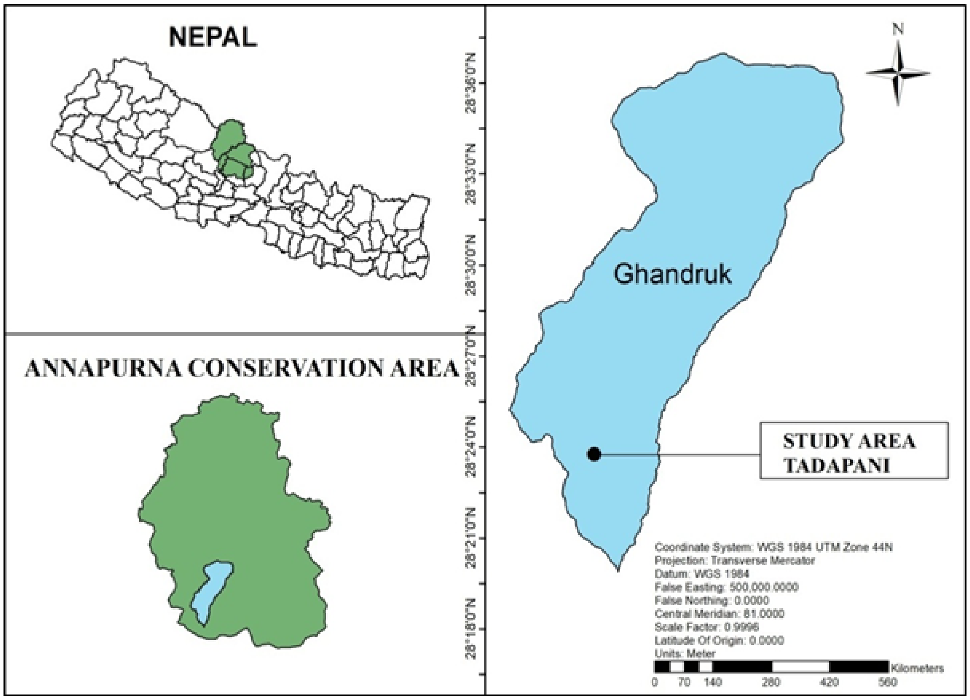

Figure 1

Geographical maps of (a) Nepal showing Annapurna Conservation Area (ACA), (b) study area (Ghandruk) located in ACA and (c) study site (Tadapani) located in Ghandruk

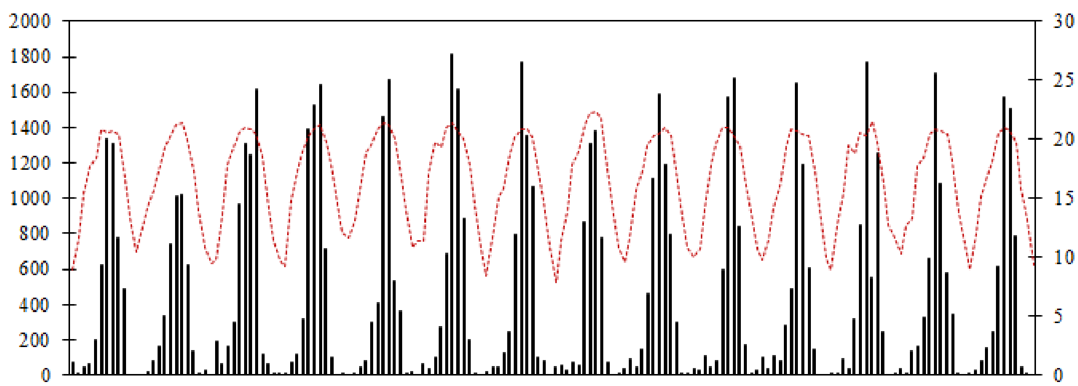

Figure 2

Mean precipitation and air temperature of the study area from 2005 to 2018 (a) monthly (b) ten years average (Source: Lumle station, Department of hydrology and meteorology, DHM, Kathmandu). Bar, precipitation; line, air temperature

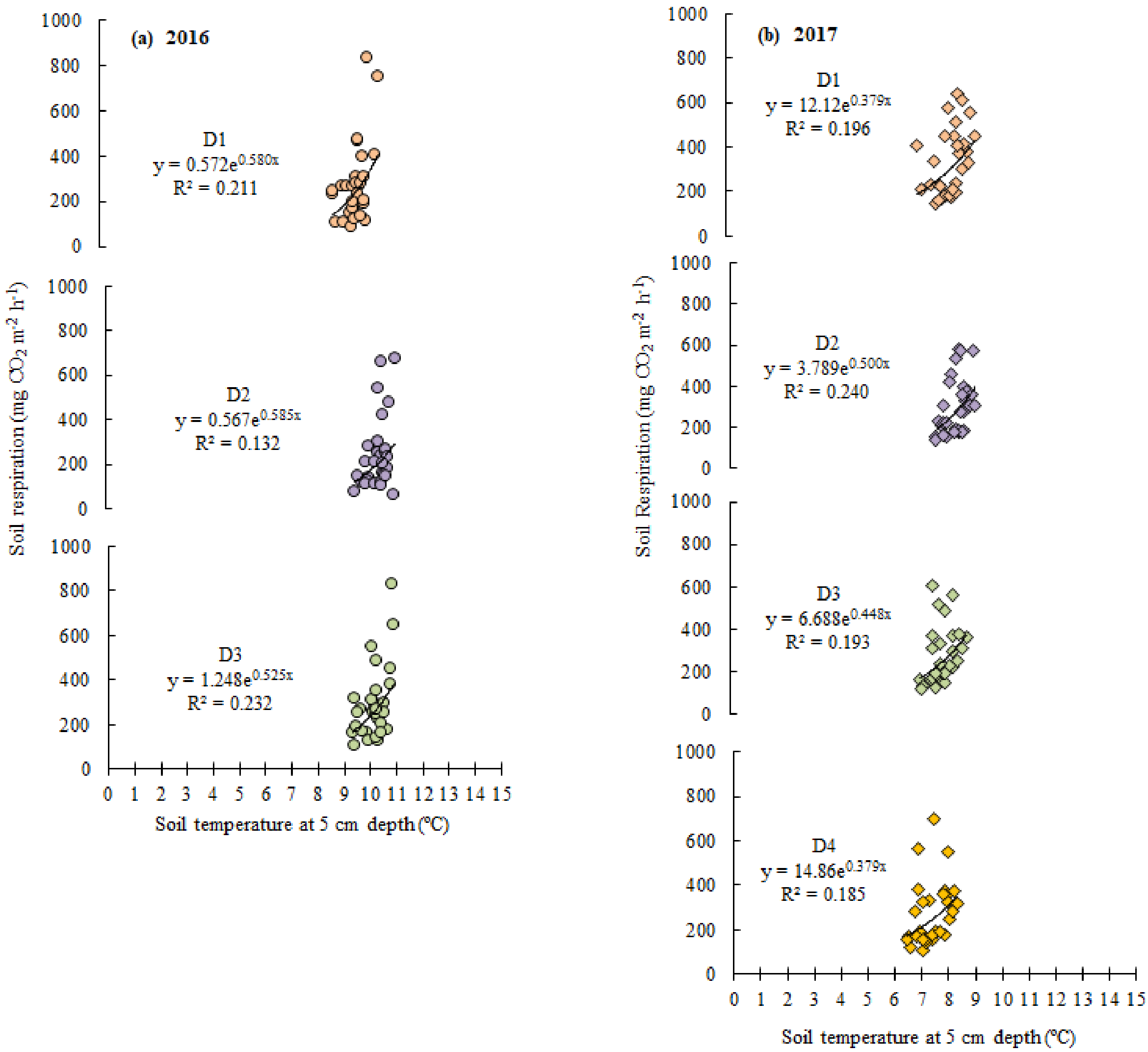

Figure 3

Variations of soil respiration with soil temperature in different dates (D1, D2, D3, D4) of measurements in 16th , 17th, 18th and 27th, 28th, 29th, 30th in October (a) 2016 (b) 2017

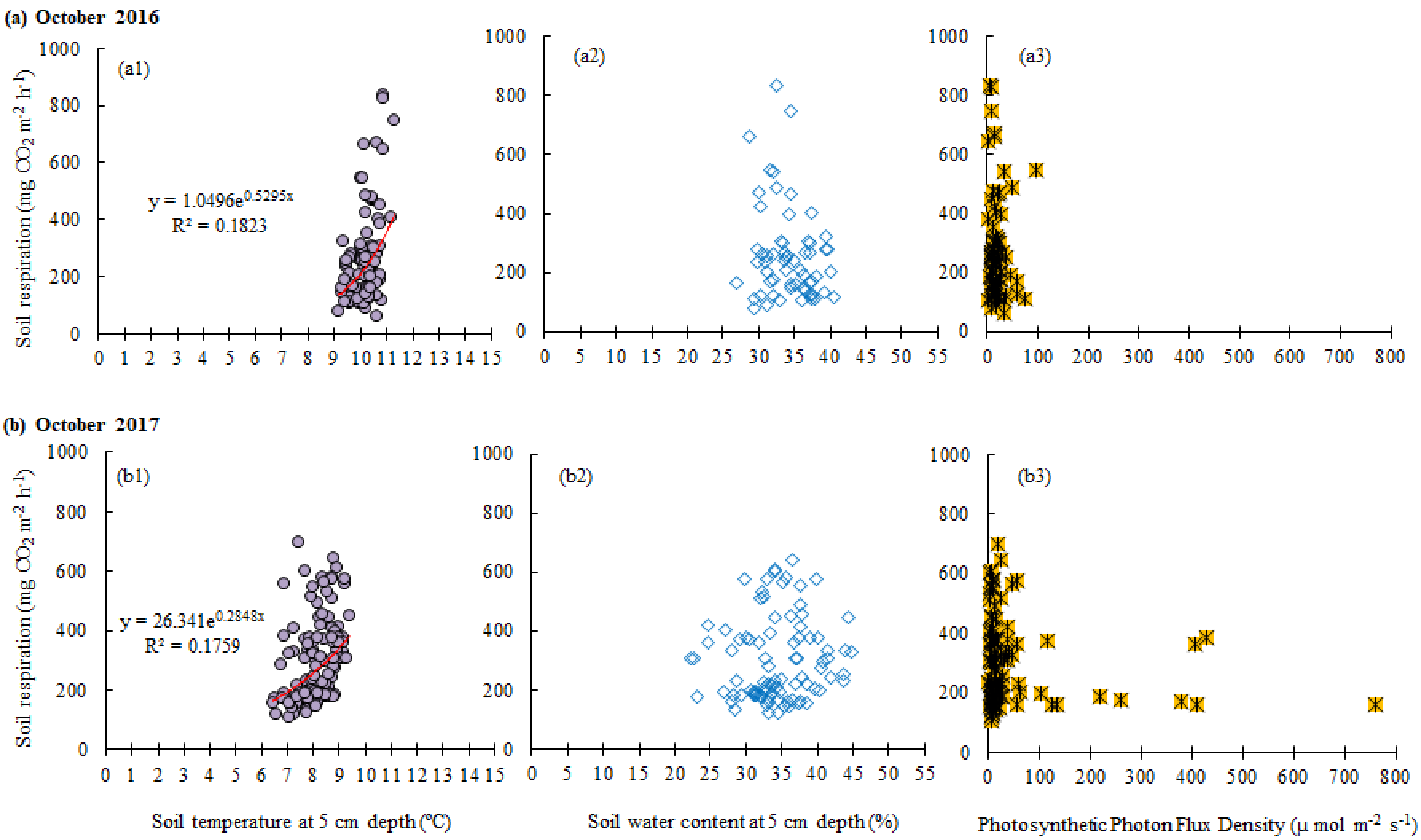

Figure 4

(a) Variations of soil respiration with soil temperature at 5 cm depth (a1,b1), soil water content at 5 cm depth (a2,b2) and Photosynthetic Photon Flux Density (PPFD) (a3,b3) in October (a) 2016 and (b) 2017. Measurements of different dates (16th, 17th, 18th and 27th, 28th, 29th, 30th) were combined in each year.

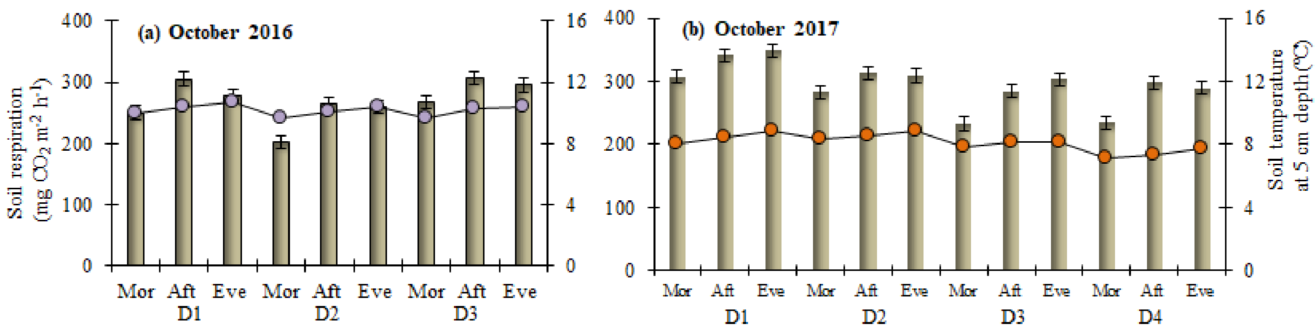

Figure 5

Temporal variations of soil respiration and soil temperature in October (a) 2016 and (b) 2017. Vertical bar – soil respiration (mg CO2 m-2 h-1), filled circle – soil temperature (°C)

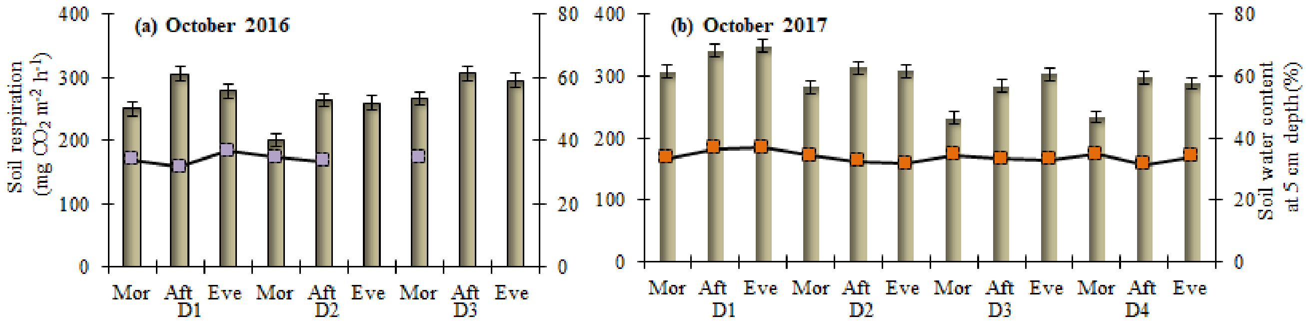

Figure 6

(a) Temporal variations soil respiration and soil water content in October (a) 2016 and (b) 2017 . Vertical bar – soil respiration (mg CO2 m-2 h-1), filled square – soil water content (%)

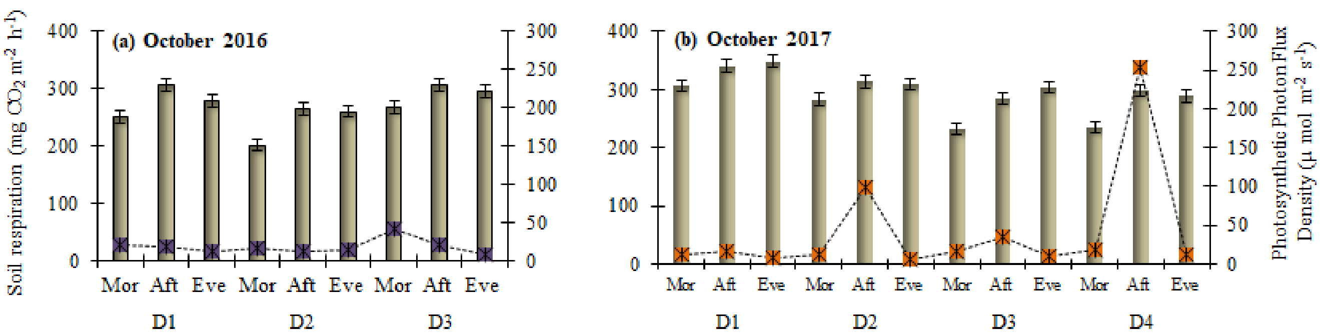

Figure 7

Temporal variations soil respiration and Photosynthetic Photon Flux Density (PPFD) in October (a) 2016 and (b) 2017. Vertical bar – soil respiration (mg CO2 m-2 h-1), filled star – Photosynthetic Photon Flux Density (μ mol m-2 s-1)

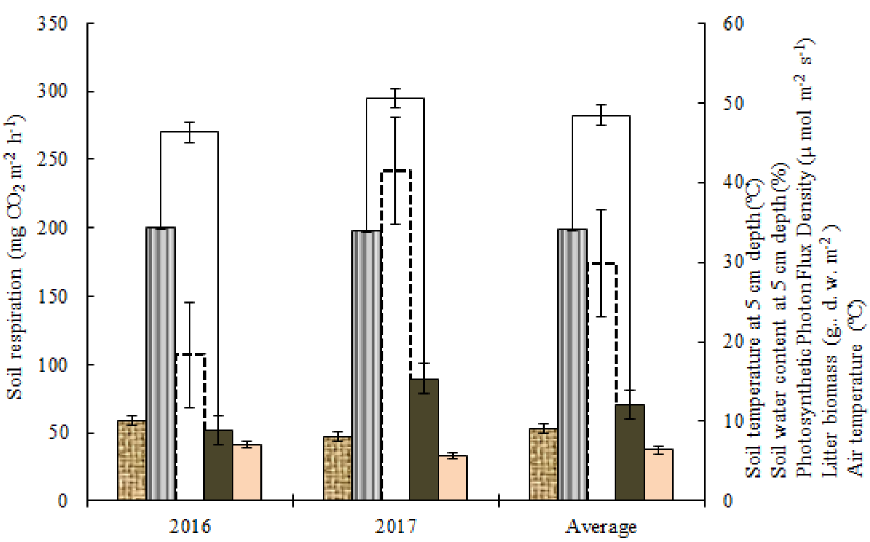

Figure 8

Inter-annual variations of the soil respiration, soil temperature, soil water content, Photosynthetic Photon Flux Density (PPFD), litter biomass and air temperature in October 2016 and 2017, and the average of both years. Non filled bar – soil respiration (mg CO2 m-2 h-1), gradient filled bar – soil temperature (°C), vertically stripped bar – soil water content (%), dash typed border non filled bar – Photosynthetic Photon Flux Density (μ mol m-2 s-1), dark coloured filled bar – litter biomass (g. d. w. m-2), light coloured filled bar – air temperature (°C)