

Soil CO₂ Efflux Dynamics and Its Relationship with the Environmental Variables in a Sub-Tropical Mixed Forest

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Abstract

Soil CO₂ efflux is an ongoing process of respiration from soil; plant parts/microbes below the ground to the atmosphere which is known for faster cycling of carbon sources. A large portion of carbon sequestered and fixed by forests is returned to the atmosphere through soil CO₂ efflux and multiple controlling parameters mainly temperature, precipitation, and growth factors interact with the soil CO₂ efflux variation. This study assessed the soil CO₂ efflux every month for consecutive 2-years (August 2015 to July 2017) by using the closed chamber method to determine the role of ecological parameters that govern the soil CO₂ efflux and its temporal modification in a sub-tropical mixed forest of central region in Nepal. The results of this study manifested that soil CO₂ efflux accounted 63.2% ($y = 31.96e^{0.128x}$), 71.3% ($y = 44.77e^{0.123x}$) and 64.5% ($y = 44.11e^{0.117x}$) variations in soil temperature with significantly ($p < 0.05$) exponential positive relation in the year 2015/2016, 2016/2017 and the two years when merged. And the temperature sensitivity value (Q_{10}) of the soil CO₂ efflux was 3.6, 3.4, and 3.2, respectively. Soil water content also expressed significantly ($p < 0.05$) positive exponential effect on soil CO₂ efflux and accounted 62.0% ($y = 138.3e^{0.057x}$), 46.1% ($y = 88.42e^{0.052x}$) and 40.5% ($y = 133.1e^{0.0447x}$) in its variability in different years and the merged years. Evident variations of soil CO₂ efflux, soil temperature, soil water content, and litter were observed in the forest seasonally and inter-annually. Two years mean total annual soil CO₂ efflux of the forest was estimated at 904.76 g C·m⁻²·y⁻¹. The study revealed that sub-tropical forests could be more influenced by precipitation regimes in progressing warm climates i.e. vulnerable to climate change, illustrating the comprehensive dynamics of the representative forest carbon cycle in the tropical region.

Keywords

Soil CO₂ Efflux, Environmental Parameters, Temperature Sensitivity (Q₁₀), Sub-Tropical Forest, Climate Change

1. Introduction

The soil which is rich in soil organic carbon (SOC) is climate-sensitive and supports to mitigate the increasing level of atmospheric carbon dioxide (CO₂) (Baveye et al., 2020), and the global SOC stocks storage (Minasny et al., 2017). Increasing atmospheric CO₂ concentration and advancement of industrialization cause warming (Pachauri et al., 2014) and further increases with the high temperatures which in compilation stimulate decomposer activity (Kravchenko et al., 2021). Complications arise owing to multiple controlling mechanisms that interact over numerous temporal scales and depend on the complex plant, microbial and environmental parameters such as temperature, precipitation, sunlight, and growth factors (Rodbassana et al., 2021). The soil temperature and water content are the key environmental factors responsible for soil CO₂ efflux variation and the process plays an important role in the regulation of the carbon cycle (Hursh et al., 2017; Chen et al., 2022). The soil CO₂ efflux is a continuum mechanism from soil to plant and to the atmosphere that arises from faster cycling carbon sources (Högberg & Read, 2006), considered one of the most important components of the carbon cycle in forest ecosystems (Raich, 2017). The soil CO₂ efflux can have a huge impact on the strength and future direction of the forest soil carbon storage and its sink capacity, with changes in CO₂ efflux in response to predicted climate change scenarios (IPCC, 2013). The magnitude of soil carbon feedback to climate change mostly depends on the temperature sensitivity of soil CO₂ efflux (Q₁₀) in regulating the carbon emission (Dhital et al., 2010b; Klimek et al., 2021) and the soil organic matter in decomposition (Kravchenko et al., 2021). The Q₁₀ becomes most determinate in ecosystem soil CO₂ efflux in combating the soil temperature, soil water content, solar radiation, and plant litter (Dhital et al., 2019).

Understanding the forest's carbon cycle is important for the scientific elucidation of the global carbon cycle and climatic warming. Carbon absorbed from the atmosphere is processed in several nutritional steps in the forests, and a large portion of the carbon is returned to the atmosphere. The quantitative analysis of CO₂ is therefore fundamental in the understanding of the material cycle of ecosystems (Lee et al., 2017). The soil CO₂ efflux comes with the processes of root respiration, heterotrophic respiration through the decomposition of soil organic matters by microorganisms, and efflux of CO₂ from the animals (Crowther et al., 2016). For the purpose to access the overall impacts of warming on ecosystem carbon fluxes (Dhital et al., 2010a), it is crucial to evaluate and understand the effects of temperature, moisture, and photosynthesis on soil CO₂ efflux (Marcolla

et al., 2011; Rubio & Detto, 2017). Factors such as litter quantity, soil microbial biomass, plant physiological activity (Hopkins, 2013), forest types (Klimek et al., 2021), composition and quantity of litter (Chen et al., 2014), soil organic carbon (Huang et al., 2014), soil nitrogen (Zhang et al., 2021) and fine root biomass (Luan et al., 2012) have also very common and interactive impact on the CO₂ efflux. Therefore, environmental and biological elements potentially provide effective parameter inputs for the determination and assessment of soil atmosphere carbon exchange (Zhang et al., 2018; Warner et al., 2019) and the inter-annual variations of the carbon either with the emission or the absorptions (Dhital et al., 2014).

Forests ecosystem are considered a major source of atmospheric carbon and even the tropical (including sub-tropical) forest area cover about 12% of Earth's total land surface (Lieth, 1973; Whittaker, 1975), and 45% of the total forest is rich in biodiversity and much productive due to its warm and wet climate (Brown, 2014; FAO, 2020). They contribute to the global carbon by storing one-third (40%) of the world's terrestrial carbon stocks (Beer et al., 2010; Pan et al., 2011), 56% of which is found in green biomass, 32% in soils (Ngo et al., 2013). For the major role and contribution in the global carbon cycle, tropical forests could strongly influence future concentrations of the atmospheric level of carbon dioxide (Sayer et al., 2011; Cox et al., 2013) and affect sparsely in the global carbon budget (Erwin, 1982; Brown, 2014); play a significant role in soil CO₂ efflux (Warner et al., 2019; Cui et al., 2020). Tropical forest ecosystems are underrepresented in reviews of soil CO₂ efflux research and insufficient to publish, considering the cause of ecosystems intricacy in various factors of climate to the seasonal/temporal entities and essentially limited due to lacking facilities (Takahashi et al., 2011; Wood et al., 2013; Rubio & Detto, 2017). However, few of the researches are accepted and recognized that have examined soil CO₂ efflux, presenting ecological parameters and their interdependence on the tropical ecosystem (Dhital et al., 2020).

The significance of exploring the tropical forest is considered relevant for the benefit of its ecological impacts such as deforestation, land-use change, and over-exploitation with understanding the climate variations and its seasonal changes. The climatic factors such as temperature and soil water content and the plants are considered major ecological parameters that represent the key component of forest carbon balance. They are still not well understood and their relationships remain empirical to explain how they interact with climate, soil, and especially in the vegetation and forest type (Rubio & Detto, 2017; Cui et al., 2020). In tropics, the moisture control becomes a prominent and much stronger parameter than the temperature that induce more effect of less dry periods in soil CO₂ efflux (Zhang et al., 2018), however, it was different in the temperate regions, where the temperature control overcomes the soil moisture control on soil CO₂ flux (Dhital et al., 2019; Klimek et al., 2021). It has been hypothesized that the soil temperature could influence the variation of soil CO₂ efflux for short period

but soil water content could have a limiting long period effect on the variation of soil CO₂ efflux in tropical forests. The ecosystem balance of carbon and its response to the changes in temperature and the water balance is quite uncertain, and much important to know the function of the biosphere in the future global carbon budget (Moore et al., 2008). The coverage and biodiversity richness form complexity in ecosystem carbon fluxes and its interaction with climatic and plant factors of tropical forest is still very less and almost not implied especially in Nepal, and needs to be explored and expanded. Therefore, we aimed to determine the soil CO₂ efflux in a sub-tropical mixed forest of central Nepal by using the closed chamber method of measurement for consecutive 2 years. We further aimed to address two integral issues 1) the role of ecological parameters that govern the soil CO₂ efflux and 2) seasonal and inter-annual modification in soil CO₂ efflux variations of the forest. To achieve this goal, the sub-tropical mixed forest was selected for soil CO₂ efflux measurement from August 2015 to July 2017. The continuous measurements conducted over the 2 years helped to determine the carbon emission from the forest soil and observe the influence of environmental parameters on the temporal variations of soil CO₂ efflux.

2. Material and Method

2.1. Study Area

The study was conducted over a 2-years (i.e., 2015/2016, 2016/2017) period in a sub-tropical mixed forest named Manthali community forest (27°39'00.8"N and 085°25'18"E) in Suryabinayak of Bhaktapur district located in central Nepal. It is the primary natural dense *Schima-Castanopsis* mixed forest. The study site comprises mixed sub-tropical trees and is commonly dominated by *Schima wallichii*, *Castanopsis indica* and *Castanopsis tribuloides* followed by *Rhododendron arboreum*, *Myrsine capitellata*, *Myrica esculenta*, etc. The forest floor is almost clear with sparse small plants and it is the source of firewood, medicinal plants, flowers, wild fruits, etc. (DFRS, 2015) and was declared as a Community Forest in 1998 for its protection by the local community and user groups. The altitude of the 68.75 ha forest ranges from 1426 to 1722 m a.s.l. (Figure 1). The forest area was declared for the construction of the National Zoological Garden in 2016 for *ex-situ* conservation and protection of wildlife, and breeding of endangered and exotic animals.

The area falls under the sub-tropical monsoon climatic zone, feature high temperature with the wet rainy summer (i.e., June to August) and lower temperature with the dry winter (i.e., December to February) (Figure 2). According to the meteorological station (Department of Hydrology and Meteorology, DHM), the 12 years (2006-2017) average monthly air temperature of the study area ranged from 11.4°C in January to 24.8°C in June, July, and August. They vary from 28°C to 30°C during the summer season and in winter season ranged from 8°C to 12°C (CBS, 2012) with an annual average air temperature of 19.6°C. In addition, the 12-years average monthly precipitation ranged from 1.6 mm in

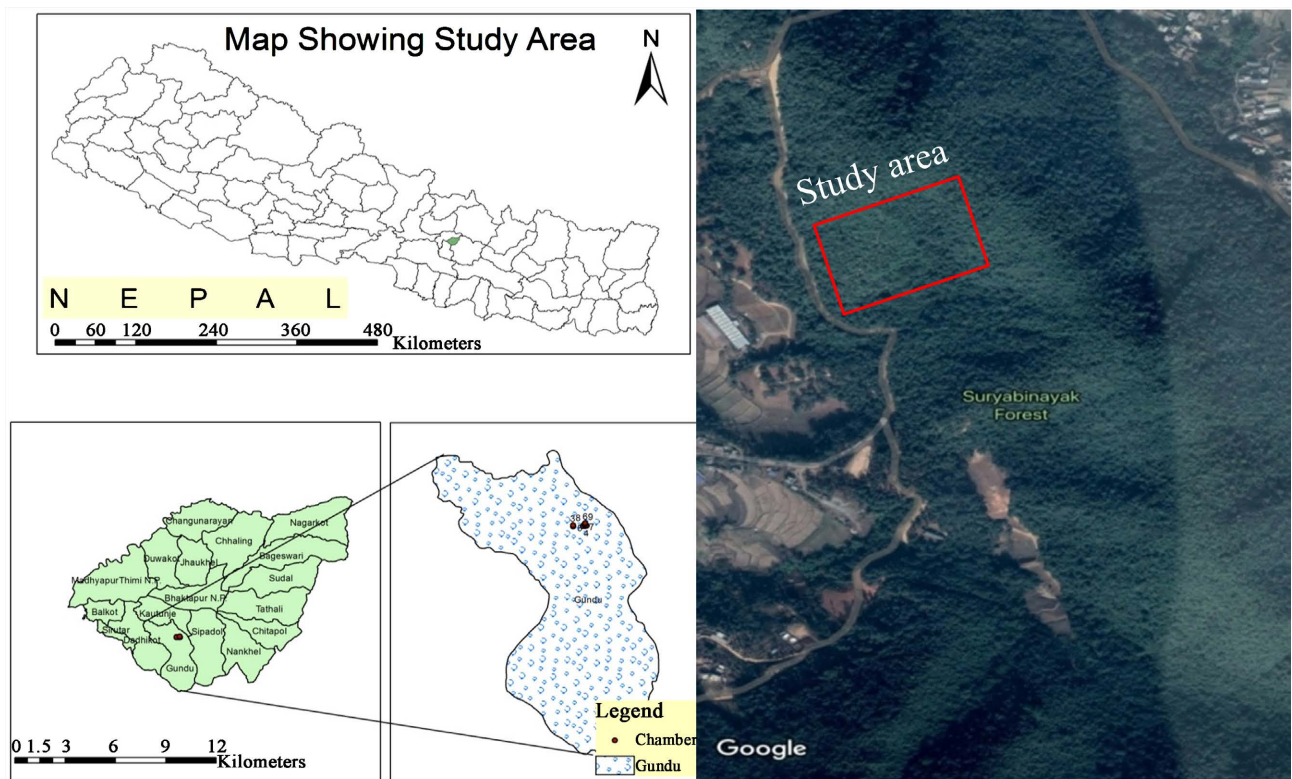


Figure 1. The map showing the study region and location of the study area in the Suryabinayak forest of Bhaktapur.

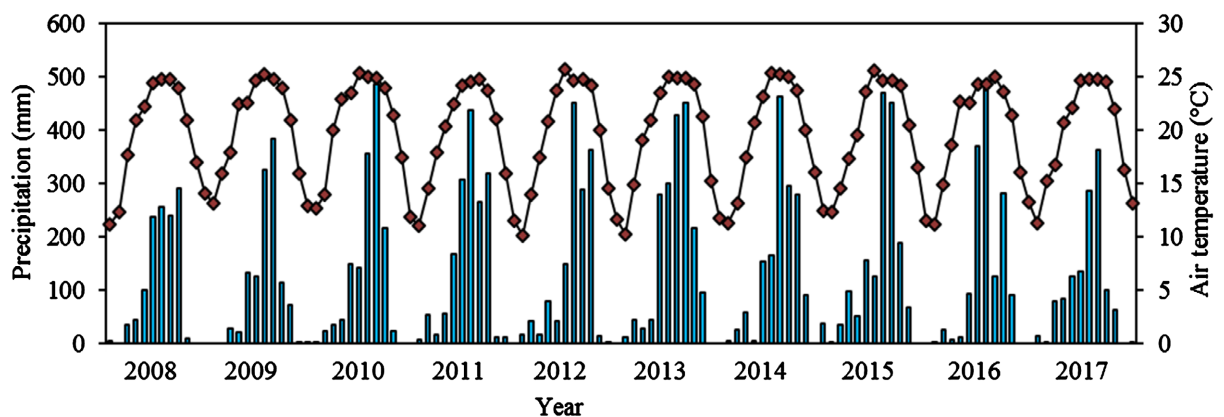


Figure 2. Seasonal and inter-annual patterns of monthly mean precipitation and monthly mean air temperature of the study area from 2008 to 2017 (source: department of hydrology and meteorology, DHM, Kathmandu). Bar, precipitation; filled squares, air temperature.

November to 376.3mm in July and the annual average precipitation was recorded 122.4 mm (Figure 2; DHM). The bulk density of the forest soil in the study site calculated in the summer season was 1.052 g·cm⁻³.

2.2. Experimental Design and Measurement

An experimental area of size 100 m × 50 m was established on the southern slope (about 30°) of the forest area. The chambers (n = 10) were randomly arranged between the altitude of 1430 and 1443 m a.s.l. in the experimental area

and inserted 2 cm below the ground surface into the soil to assure that the chambers were airtight without any leakage between the soil and the edge of the chambers. The frequency of soil CO₂ efflux measurement was maintained once every month and continued for 2-years from August 2015 to July 2017. To avoid strong diurnal fluctuations, measurements were always carried out between 11:00 and 14:00. The midday period was considered to provide rates of respiration that were representative of the average daily value (Mielnick & Dugas, 2000). Considering the sensitivity of the equipment, all measurements were conducted on rain-free days and taken mid-date of each month to maintain the days' difference between the two measurements. The soil CO₂ efflux was measured by using Vaisala CARBOCAP CO₂ probe GMP343 (Vaisala Oyj, FI-00421 Helsinki, Finland) and the chambers made of polyvinyl chloride with a diameter of 18 cm and height of 16 cm. The method involves placing a closed chamber over the soil surface covered with the cap having a tightly fitted CO₂ probe and the increase in the concentration of CO₂ within the chamber is measured as a function of time. An Infrared Gas Analyzer (IRGA) was fitted in the chamber to measure CO₂ and gas temperature recorded in the data logger. Air temperature recorded within the chamber was used to calculate the density of CO₂ within the chamber. Measurement of soil CO₂ efflux was conducted one day after the chamber was fixed on the forest floor for stabilization and to avoid the variability in measurements data due to the installation effects. Hence, it was assumed that there is no disturbance caused to the flux chamber during the measurement. The soil CO₂ efflux was calculated by using the following equation (Koizumi et al., 1999).

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

where,

F = Soil CO₂ efflux (mg CO₂·m⁻²·h⁻¹);

V = Volume of air within the chamber (m³);

A = Area of the soil surface within the chamber (m²);

$\Delta c/\Delta t$ is the time rate of change of the CO₂ concentration in the air within the chamber (mg CO₂·m⁻²·h⁻¹).

Three cycles of consecutive measurements were carried out on each soil chamber to prevent any systematic errors in measurements, and the average of three soil CO₂ efflux values was recorded for each chamber. The soil CO₂ efflux recorded from all chamber measurements recorded was averaged in a month as the representative monthly value and integrated for the seasonal and annual values. The missing data of soil CO₂ efflux in April 2016 due to technical glitches was estimated with the equation developed from the soil temperature effect on soil CO₂ efflux and the continuous records of the soil temperature data during study period. The soil water content for that month was calculated by averaging the previous and later months assuming that there is almost no change in soil water during the spring season without rain.

2.3. Estimation of Temperature Sensitivity (Q_{10}) of Soil CO₂ Efflux

Studies have indicated that soil temperature is the major regulating factor of soil CO₂ efflux variations (Dhital et al., 2010b; Wang et al., 2020; Klimek et al., 2021). Hence, prediction of soil CO₂ efflux would be possible with the values of soil temperature and calculated by using an equation of exponential regression (Dhital et al., 2020) as follows:

$$F = a * \exp(b * Ts) \quad (2)$$

where, F is the predicted soil CO₂ efflux (mg CO₂·m⁻²·h⁻¹) at soil temperature (T_s , °C) at a depth of 5 cm. The value a represents the intercept of soil respiration rate when ST is zero. The value b represents the temperature sensitivity of soil CO₂ efflux. The b value is used to calculate the index of temperature sensitivity for soil CO₂ efflux (Q_{10} value), which describes the changes in soil respiration over a 10°C increase in soil temperature and is calculated by using the following Equation (3).

$$Q_{10} = \exp(b * 10) \quad (3)$$

2.4. Measurement of Soil Temperature

The soil temperature at the depth of 5 cm was recorded using the digital lab stem thermometer (AD-5622, A & D, Japan) at three different points around the soil CO₂ efflux measurements chamber. The continuous soil temperature data was also recorded with the TidbiT v2 Temperature logger (Onset HOBO data logger, Australia) that was installed at the center point of the study site at 5 cm soil depth. The logger started to record the soil temperature data from August 2015 to July 2017 throughout the period when the soil CO₂ efflux was measured. The recorded data of the air temperature and precipitation of the study area were generated from the Department of Hydrology and Meteorology (DHM), Government of Nepal from 2006 to 2017.

2.5. Measurement of Soil Water Content (SWC)

The soil water content (SWC, %) at the depth of 5 cm was recorded using soil moisture sensor TRIME-FM (Imko, Germany). The measurements of soil water content were conducted monthly at three different points near and around the chambers of soil CO₂ efflux measurement for 2 years.

2.6. Measurement of Litter Biomass

Litter at the ground level of the forest floor was sampled every month from five random plots at the same date of soil CO₂ efflux measurements in the study area. The samples were collected inside the soil CO₂ efflux measurements chamber and the area was calculated for the square meter. The samples were oven-dried at 70°C for 48 hours and weighed with an electronic balance. The dry weight of litter biomass was calculated using the following formula:

$$\text{Biomass} = \text{Dry weight (gm)} / \text{Area (m}^2\text{)}$$

2.7. Measurement of Bulk Density

Soil samples from five different plots ($n = 5$) in the study area were randomly collected for calculating bulk density using a core sampler with standard volume. The following formula was used to calculate the bulk density of the soil (Cresswell & Hamilton, 2002).

$$\text{Bulk Density (g} \cdot \text{cm}^{-3}\text{)} = (\text{oven dry weight of soil}) / (\text{volume of soil in the core})$$

2.8. Data Analysis

The statistical analysis was made by using R software and GIS software of version 10.4.1 for mapping of sampling site and the study area arc. Statistical analysis such as correlation, the significance of the correlation, and linear regression was used to examine the relationship between the variables. The annual soil CO₂ efflux was estimated from the equation obtained from the temperature effect of soil CO₂ efflux in each year and the continuous (1 h interval) recorded data (daily average) from the temperature logger. The estimated daily soil CO₂ efflux values were integrated for annual and 2-years estimation, and then separated into seasonal values of soil CO₂ efflux.

3. Results and Discussion

3.1. Soil Temperature Effect on Soil CO₂ Efflux

The temperature sensitivity of soil CO₂ efflux was expressed by a positive exponential curve derived from the relationship between soil temperature at 5 cm soil depth and soil CO₂ efflux (Figure 3). The effect was statistically significant ($p < 0.05$, $y = 31.96e^{0.128x}$) where the soil temperature accounted for 63.2% monthly variation of soil CO₂ efflux in the year 2015/2016 (Figure 3(a)) and 71.3% of the variability with the significant ($p < 0.05$, $y = 44.77e^{0.123x}$) relation in 2016/2017 (Figure 3(b)). A similar trend of soil temperature effect on soil CO₂ efflux with a significant positive exponential curve ($p < 0.05$, $y = 44.11e^{0.117x}$) that accounted for 64.5% of the monthly variability was derived when both years' of the measurements were merged (Figure 3(c)). The significant exponential positive response of soil temperature on soil CO₂ efflux (Figure 3) well illustrates that the soil temperature better explain soil CO₂ efflux variations in this sub-tropical mixed forest and that was exceptionally supported by the outcomes of the previous research (Takahashi et al., 2011; Dhital et al., 2019; Wang et al., 2020). However, the linear regression between soil CO₂ efflux and soil temperature can also be used to interpret the sensitivity of soil temperature to soil CO₂ efflux (Shi et al., 2011). The claim that the soil temperature is proven to be the major component affecting soil CO₂ efflux is improved with this study and better accepted for the sub-tropical mixed forest (Tan et al., 2013; Urbanek & Doer, 2017). It was highly comparable to the different types of Korean deciduous, pine, and mixed forests (Kim et al., 2010).

The temperature sensitivity of soil CO₂ efflux, Q_{10} value was obtained (Equation

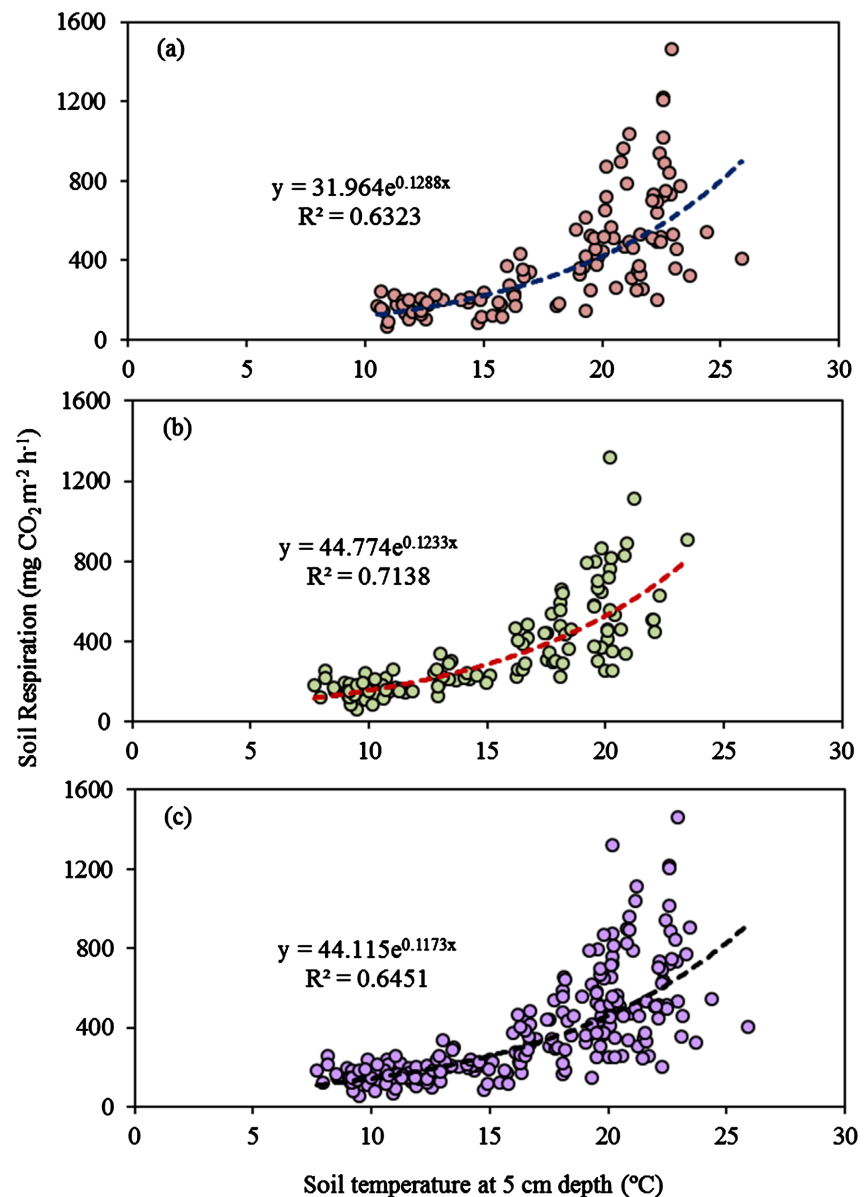


Figure 3. Soil temperature effect on soil CO₂ efflux, (a) 2015/2016, (b) 2016/2017 and (c) combined (a) & (b).

(3)) at 3.6 (**Figure 3(a)**), 3.4 (**Figure 3(b)**), and 3.2 (**Figure 3(c)**) in 2015/2016, 2016/2017 and when both years values merged. The annual Q_{10} values determined in this study were well ranged (1.84 to 3.78) as observed in Alpine ecosystem (Liang et al., 2019), warm temperate Oak forest (1.7 to 5.12), pine plantation (2.3 to 6.21) (Luan et al., 2013) and close to the higher global range of 1.3 to 3.3 (Raich & Schlesinger, 1992). This could most likely be compared to different ecotypes in Korean forests (Kim et al., 2010) and solely higher than the nearby sub-tropical grassland (Dhital et al., 2020). According to Hashimoto et al. (2004), the mean calculated Q_{10} value of 1.91 for Japanese cedar plantations versus 2.40 for broad-leaved forests was less sensitive to temperature than was the case for deciduous forests in the same region ($Q_{10} = 3.10$ (Lee et al., 2002); $Q_{10} = 2.58$ –

3.26 (Mo et al., 2005)). The observations overall revealed that Q_{10} is more sites specific and determined to indicate the seasonal and inter-annual variation. The higher soil CO_2 efflux of our study was concentrated between the soil temperatures 20°C and 25°C (Figure 3) that defined the optimum level of soil temperature for the major release of soil CO_2 efflux in this forest. It is known that moderate soil water content is the most functional condition for soil CO_2 efflux (Luo & Zhou, 2006). This impression was more consistent with the observation of the sub-tropical monsoon climatic ecosystem in China (Wang et al., 2020). The cumulative observations of this study revealed well-defined temperature sensitivity of the soil CO_2 efflux of sub-tropical mixed forest.

The value of Q_{10} in the year 2016/2017 (Figure 3(b)) was slightly lower than the year 2015/2016 (Figure 3(b)). This variation commonly depends on location and the monthly differences in temperature sensitivity of soil CO_2 efflux (Q_{10}) controlled by the combination of soil temperature and soil moisture (Cui et al., 2020). The inter-annual variation of the Q_{10} in this study is owing to the effect of multiple associated ecological parameters, but the priority for dependency was given to the soil temperature. However, studies also established that the soil CO_2 efflux was mediated by various soil parameters such as soil temperature (Klimek et al., 2021), soil moisture (Jiang et al., 2015; Dhital et al., 2020), decomposition of organic matter and litter (Kravchenko et al., 2021), properties of soil and plants by vegetation cover and fine root mass (Li et al., 2008), and soil physical parameters such as soil organic carbon, bulk density, litter fraction, nitrogen, etc. (Luan et al., 2012). Compared to the forgoing research, the value of Q_{10} determined to intricate the temperature sensitivity of the soil CO_2 efflux was not much far from the previous observations made in the forests (Takahashi et al., 2011; Luan et al., 2012; Rubio & Detto, 2017).

3.2. Soil Water Content Effect on Soil CO_2 Efflux

The soil water content of this forest expressed a positive exponential curve with soil CO_2 efflux (Figure 4). In the year 2015/2016, the soil water content accounted for 62.0% of the monthly variability with soil CO_2 efflux that was statistically significant ($p < 0.05$, $y = 138.3e^{0.057x}$) (Figure 4(a)). Similarly, a statistically significant ($p < 0.05$, $y = 88.42e^{0.052x}$) exponential curve was detected in the year 2016/2017 while accessing soil water effect on soil CO_2 efflux which accounted for 46.1% of the monthly variability (Figure 4(b)). The soil CO_2 efflux variability with the changes in soil water content was comparatively higher in 2015/2016 than in the year 2016/2017. The effect of both years' when merged was significantly exponential ($p < 0.05$, $y = 133.1e^{0.0447x}$) as well and accounted for 40.5% variability of the soil CO_2 efflux (Figure 4(c)). Our results comfortably support that soil moisture is the most probable environmental parameter influencing soil CO_2 efflux and contributes a major role in the variation of the forest soil CO_2 efflux, apart from the associated temperature effect. This is supported in continuation within the tropical forests (Li et al., 2006; Rubio & Detto, 2017; Yu et al., 2019). The well explained and visible effect of soil water content with the soil

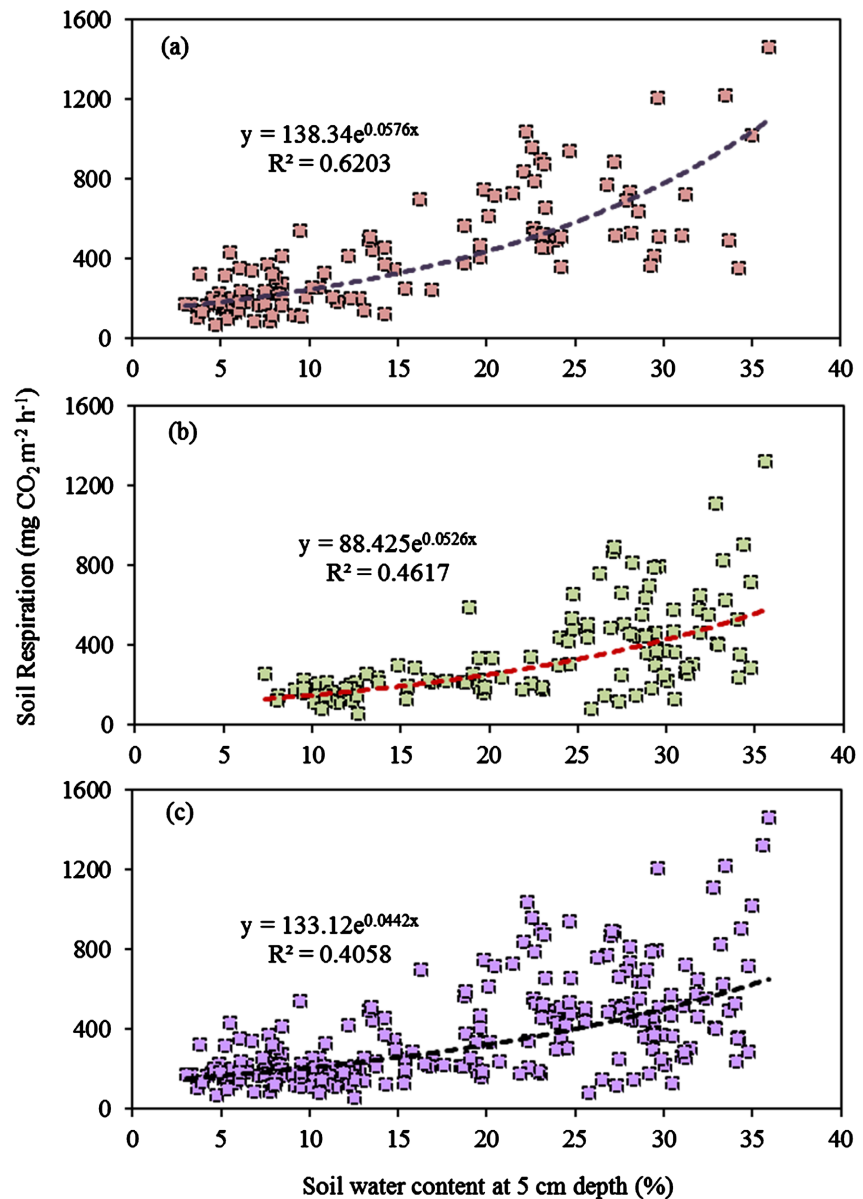


Figure 4. Soil water content effect on soil CO₂ efflux, (a) 2015/2016; (b) 2016/2017; (c) combined (a) & (b).

CO₂ efflux of this sub-tropical mixed forest (**Figure 4**) better explained the sensitivity of soil water and its availability in similar tropical regions disclosed by Rubio & Detto (2017) and Dhital et al. (2020). However, it was also varying with negative exponential relation (Dhital et al., 2019) and the invisible scattered relationship (Dhital et al., 2010b) in different temperate regions. The soil CO₂ efflux does not always respond positively to precipitation change because water deficiency due to drought has increased the soil CO₂ efflux by alleviating the oxygen deficit (Liu et al., 2016; Zhang et al., 2018). The sensible soil CO₂ efflux with soil water in the tropical region was due to the occurrence of differentiated dry and wet seasons (Yu et al., 2019; FAO, 2020), and the soil water sometimes overcomes and mask the effect of temperature on soil CO₂ efflux (Hashimoto et al.,

2004). The effect of soil water availability beyond and below the limit could suppress the soil CO₂ efflux due to reduce production and diffusion of CO₂ (Davidson et al., 2012; Cui et al., 2020). Therefore, the soil water effect on soil CO₂ efflux becomes a major determinant of ecosystem function that varies depending on the ecotype and vegetation (Meir et al., 2015; Raich et al., 2017). Similar to this study, as compared to temperate forests (Kim et al., 2020), seasonal variations in the soil moisture better represent the seasonal soil CO₂ efflux in the tropics (Vargas & Allen, 2008). The soil water availability is hence importantly common in the ecological functioning of the carbon cycle, and it is adequately more prominent in the tropical ecosystem.

3.3. Variations of Soil CO₂ Efflux, Soil Temperature, Soil Water Content, and Litter Biomass

The soil CO₂ efflux showed well explained w-shaped monthly and seasonal variations along with a similar trend observed for soil temperature and the soil water content (Figure 5). This appearance illustrating the dynamics of soil CO₂ efflux was comprehensible and resembled the temperature and precipitation seasonal variation. The results could be better explained that besides the soil temperature, precipitation also behaved as an effective determinant of the soil CO₂ efflux in sub-tropical mixed forests. In this study, it was attributed due to pronounced seasonal variation of soil CO₂ efflux towards the soil temperature and soil moisture as in the prior research that was considered the differences in monthly/seasonal soil temperature and soil water content (Urbanek & Doer, 2017; Dhital et al., 2020). Our result is very much consistent with the different sub-tropical ecosystems (Yan et al., 2006; Hanpattanakit, 2015; Raich et al., 2017). However, the fluctuated soil CO₂ efflux induced due to increased precipitation or prolonged wet season and decreasing precipitation (Meir et al., 2015; Liu et al., 2016).

On a monthly scale, mean (n = 10) soil CO₂ efflux ranged from 448.66 to 892.16 mg CO₂·m⁻²·h⁻¹ during the growing season in summer (Jun-August) was

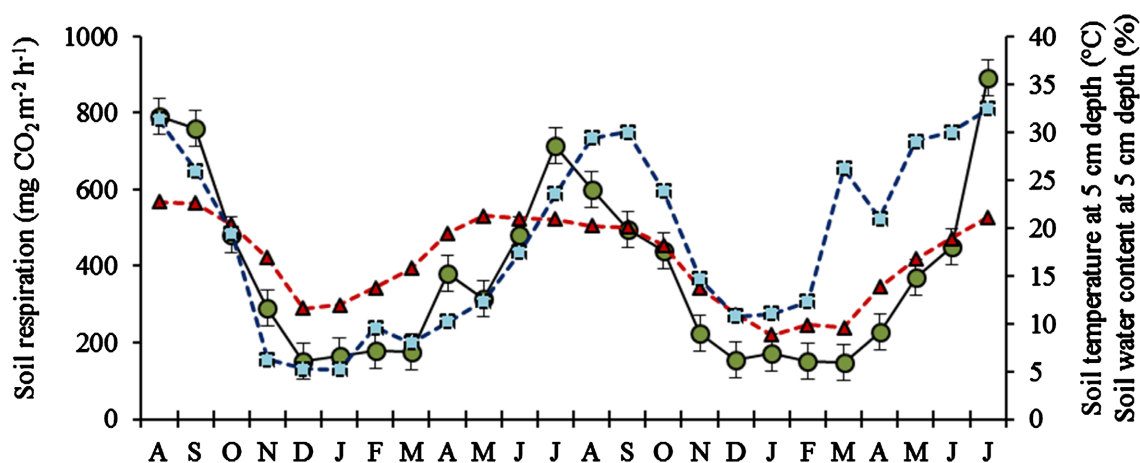


Figure 5. Seasonal and inter-annual variations of (a) soil CO₂ efflux and soil temperature and (b) soil CO₂ efflux and soil water content in the year from August 2015 to July 2017. Filled circle, soil CO₂ efflux; filled triangle, soil temperature; filled square, soil water content.

higher during the period of increased precipitation and soil temperature, and it gradually lowered from 151.97 to 179.02 mg CO₂·m⁻²·h⁻¹ with reduced soil temperature and soil water content in the winter season (December–February). However, the lowest record of monthly mean (n = 10) soil CO₂ efflux was 148.45 mg CO₂·m⁻²·h⁻¹ in March (Spring season) after February (151.97 mg CO₂·m⁻²·h⁻¹) and the highest in July 2017 at 892.16 mg CO₂·m⁻²·h⁻¹. The variations in soil temperature and soil water content also exhibited a similar trend to the monthly and seasonal variations of soil CO₂ efflux. The lower mean soil temperatures (8.8°C - 13.7°C) were recorded during the months of the winter season and higher (18.9°C - 22.7°C) in the summer season, with the maximum value reached in August 2015 and the minimum in January 2017 (**Figure 5**). Similar, higher (17.4% - 32.4%) mean soil water content values were recorded during summer and the lower (5.2% - 12.3%) in the winter season, with maximum recorded in July 2017 and the minimum in January 2016 because high precipitation recorded was from June to August and low from December to February (**Figure 1**, DHM data). The observations revealed soil CO₂ efflux presented well-defined monthly variations and seasonal trends of variations among the years with the environmental factors; soil temperature and soil water content. Our study suggested that there was a slight variation in monthly average soil temperature and soil water content in 2-years. The higher mean soil CO₂ efflux in 2015/2016 corresponds to the high soil temperature in 2015/2016 than the year 2016/2017. Inversely, the average soil water content recorded was lower in 2015/2016. The comparatively lower soil water content of the year 2015/2016 could be the optimum level of soil CO₂ efflux in this forest. Increasing soil water content caused an increase in soil CO₂ efflux which decreased further with the increasing soil water content was also recognized in the Chinese Plateau (**Chen et al., 2016**). The suppressive effect of soil temperature on soil respiration under soil water deficit conditions was as well observed in the studies (**Wan & Luo, 2003; Inoue & Koizumi, 2012**), however, the reverse result was obtained in this study, where the higher soil CO₂ efflux was determined at the time of lower soil water level and high soil temperature.

The litter biomass in each month presented a fluctuating trend and less smoothly followed the seasonal trend of the soil CO₂ efflux, ranging from a minimum of 490.8 g d w·m⁻² in April 2017 to a maximum of 872.3 g d w·m⁻² in July 2017 (**Figure 6**). The increasing rate of soil CO₂ efflux observed in 2015/2016 of this study might be supported by the high litter biomass that in decomposition caused an additional increment in soil CO₂ efflux. This result conveyed that prevailing environmental characteristics of the forest such as the soil temperatures and water availability were predominant and remarkably attributed to the variations of soil CO₂ efflux. This could be assumed that it was further enhanced due to the above-ground plant productivity (**Tiwari et al., 2021**). The higher amount of litter biomass during summer and autumn season were observed (**Figure 6**) that caused higher soil CO₂ efflux with the root and microbial activity

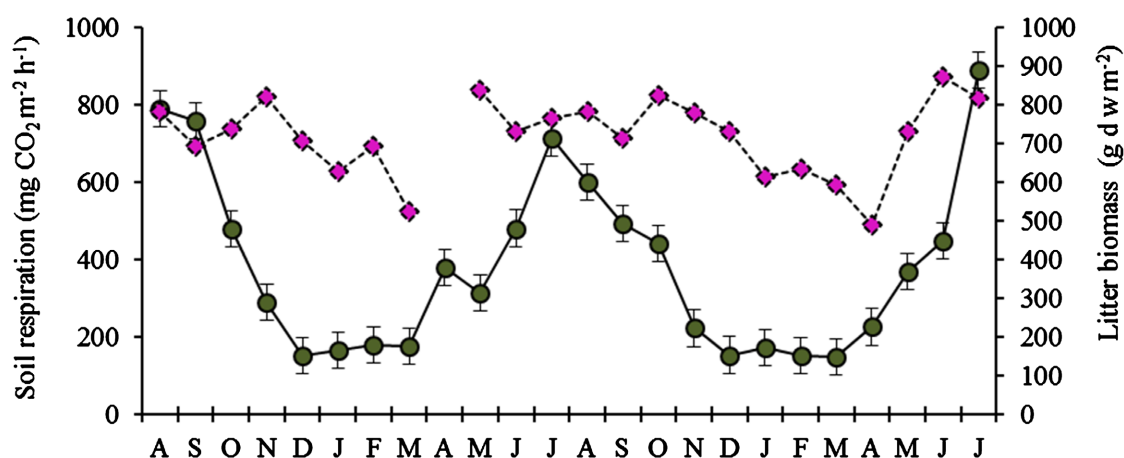


Figure 6. Seasonal and inter-annual variations of soil CO₂ efflux and litter biomass in the year from August 2015 to 2017. Filled circle, soil CO₂ efflux; filled diagonal, litter biomass.

and the plant growth because soil CO₂ efflux is generally well correlated with soil microbial biomass (Zhao et al., 2018), and litter decomposition rates (Wang et al., 2017). Because the litterfall contributes a key role to transfer elements from vegetation to soils during decomposition, acting as a major source of soil carbon (Kravchenko et al., 2021) and a direct substrate of microbial respiration accumulated under the canopy (Luan et al., 2012). In this study, the nature of the moving curve of the soil CO₂ efflux variations with the changes in above-ground litter biomass was comparable but specifically neither clear in growing nor in the non-growing season. The timely collection of litter from above soil surfaces before getting decomposed might be the cause of the invisible and not understandable relationship between the litter and the soil CO₂ efflux in this study. Similar to this study, a negative effect on soil CO₂ efflux by the removal of litter and organic layers was observed in *Picea abies* forest stand (Buchmann, 2000). The overall study suggested that seasonal variation of soil CO₂ efflux is directed by the plant litterfall, apart from climatic variables in the tropical forest.

The maximum and minimum monthly mean ($n = 10$) soil CO₂ efflux observed was 789.88 mg CO₂·m⁻²·h⁻¹ in August 2015 and 892.17 mg CO₂·m⁻²·h⁻¹ in July 2017, and 152.45 mg CO₂·m⁻²·h⁻¹ in December 2015, and 148.45 mg CO₂·m⁻²·h⁻¹ in March 2017 (Figure 7(a)), respectively. Similarly, the maximum monthly mean ($n = 10$) soil temperature and the soil water content were 22.7°C and 31.28% in August 2015 and 21.12°C and 32.42% in July 2017, and the minimum was 11.60°C in December 2015, 5.18% in January 2016, 8.80°C in January 2017 and 10.77% in December 2016 (Figure 7(b) and Figure 7(c)). As compared to our study, the trend of inclined soil CO₂ efflux in hot and wet summer during plant growing season was described in a sub-tropical mixed oak forest (Devi & Yadava, 2008), temperate forests (Klimek et al., 2021) and semi-arid (Saraswathi et al., 2008) and black soil (Shi et al., 2011). In summer, the soil CO₂ efflux might decrease immediately after a rainfall event due to the filled soil pores with water in the upper layers and creating a barrier of gas exchange

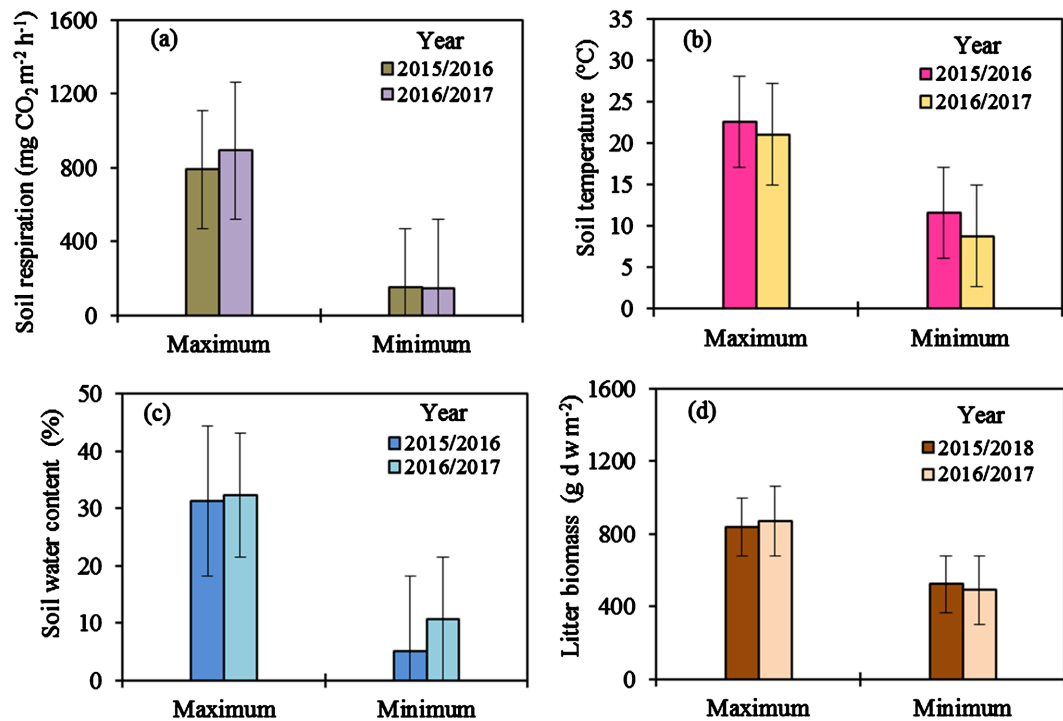


Figure 7. Variations of maximum and minimum values of soil CO₂ efflux, soil temperature, soil water content and litter biomass in the year 2015/2016 and 2016/2017.

between the soil and the atmosphere (Sotta et al., 2004). However, the rewetting process rather than soil water content becoming a major factor in controlling soil CO₂ efflux during dry summer in the sub-tropical forest could be conflicting, but multiple years of varying climatic regularities could be observed to understand the soil CO₂ efflux and the climatic parameters (Almagro et al., 2009).

The litter biomass was maximum in May 2016 averaged (monthly, n = 10) at 838.48 g d w·m⁻² and 872.30 g d w·m⁻² in June 2017, and the minimum values were 524.65 g d w·m⁻² in March 2016 and 490.84 g d w·m⁻² in April 2017 (Figure 7(d)). The soil CO₂ efflux, soil temperature, and soil water content was increased from a spring season and attained their peak in summer (June-August) and gradually declined to their lowest value in winter (December-February) in each year (Figure 5), whereas such trend of litter biomass behaved differently in the months and among the years (Figure 6). The maximum litter biomass recorded during the early growing season in May and the rainy season in June was late by a month to have the higher soil CO₂ efflux and precipitation due to rain events. This seems the litter biomass on the forest floor ultimately undergoes decomposition when the conditions become favorable with little rains, increasing soil temperature, and soil moisture accumulating the soil organic matter (Borken & Matzner, 2009; Kravchenko et al., 2021).

The wavy seasonal variations exhibited by the soil CO₂ efflux resembled the soil temperature measured continuously (1 h data, TidBiT data logger) at 5 cm soil depth throughout 2 years. Higher soil CO₂ efflux was recorded in summer when the soil temperature was higher and the lower soil CO₂ efflux was recorded

during the period of lower soil temperature (Figure 8). The soil CO₂ efflux started to decline from October till the winter period that again began to increase from March when the soil temperature get to rise and the lowest soil temperature was observed in January. The trend of receiving the highest soil CO₂ efflux was maintained in summer (August) when the soil temperature was recorded maximum in both of the years, which showed that the rising temperature contributes to bear increasing soil CO₂ efflux. Thus, our result expanded to the earlier version (Meyer et al., 2018; Cui et al., 2020; Klimek et al., 2021) of the seasonal trend of soil temperature and soil CO₂ efflux which was better visible to know the temperature sensitivity of the forest.

3.4. Soil CO₂ Efflux, Soil Temperature, Soil Water Content, and Litter Biomass of the Forest

The average annual soil CO₂ efflux measured in each month presented individually for 2-year (Table 1) was 407.21 mg CO₂·m⁻²·h⁻¹ in 2015/2016 and 360.41 mg CO₂·m⁻²·h⁻¹ in 2016/2017 which explained well define inter-annual variations (13%) of the forest soil CO₂ efflux between the years. Similarly, the soil temperature and soil water content as well followed the evident inter-annual

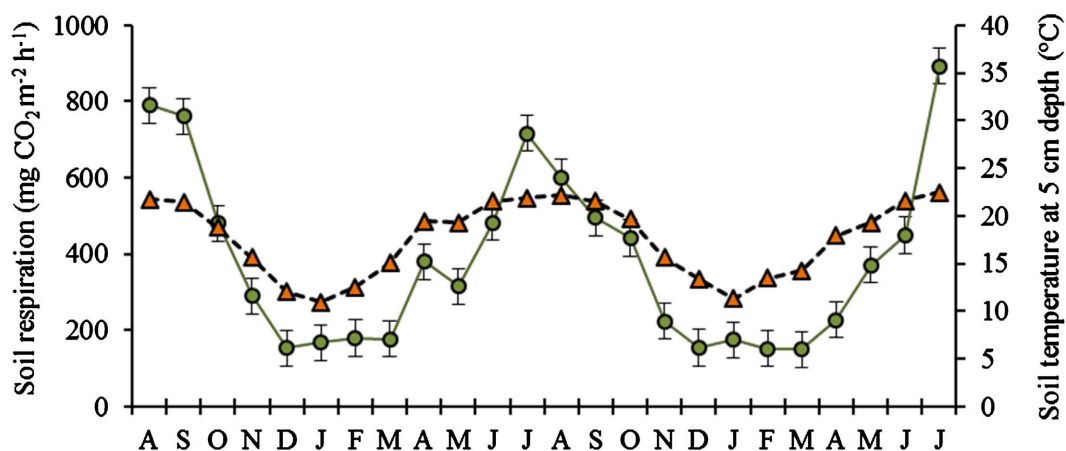


Figure 8. Seasonal and inter-annual variations of soil temperature (recorded data of TidBiT temperature logger at 5 cm soil depth) and soil CO₂ efflux (measured monthly) in the year from August 2015 to 2017. Filled circle, soil CO₂ efflux; filled triangle, soil temperature.

Table 1. Annual, two years average and seasonal soil CO₂ flux, soil temperature, soil water content and litter fall measured in the years of 2015/2016 and 2016/2017 of the study site.

	2015/2016 Year	2016/2017 Year	Average of 2-years	Winter (Dec-Feb)	Spring (Mar-May)	Summer (Jun-Aug)	Autumn (Sep-Nov)
Total soil CO ₂ flux* (g C·m ⁻²)	786.71	1022.81	904.76	105.65	206.61	346.98	245.51
Average soil temperature (°C)	18.16	15.15	16.65	11.14	16.08	20.79	18.60
Average soil water content (%)	14.52	22.55	18.53	9.00	17.77	27.34	20.03
Average litter fall (g d w ⁻²)	721.06	715.97	718.51	668.97	635.42	792.82	762.57

*Estimated.

variations among the years. The average soil temperature recorded was 18.16°C and 15.14°C, and the soil water content was 14.51% and 22.54% in the year 2015/2016 and 2016/2017, respectively. The soil CO₂ efflux average was higher in 2015/2016 when the average soil temperature was higher compared to the year 2016/2017. But, the soil water content behaved differently with the record of averaged lower soil water content in the year 2015/2016 when the soil CO₂ efflux was higher and the averaged soil water content was higher when soil CO₂ efflux was lower in 2016/2017. Thus, with this altered behavior of the soil moisture, the temperature sensitivity of the soil CO₂ efflux could better explain the precipitation pattern of the forest to the large regional scale as indicated by Meyer et al. (2018). A similar trend of inter-annual variations of annual average litter biomass was obtained at 721.05 and 715.97 g d w·m⁻¹ in 2016 and 2017, respectively but the variation accounted for was minor between the years. The annual soil CO₂ efflux in 2015/2016 was 786.71 g C·m⁻²·year⁻¹ and it was comparatively lower than the year 2016/2017 at 1022.81 g C·m⁻²·year⁻¹ which explained the understandable inter-annual variations of soil CO₂ efflux (Table 1). However, the soil CO₂ efflux estimation might reach near to accurate after several more years of continuous observation and better represent the soil carbon efflux (Dhital et al., 2014). The variations of soil CO₂ efflux of this forest among the years are acceptable, which is ultimately dependent on the multiple environmental parameters and the geographical variability. The annual soil CO₂ effluxes of this sub-tropical mixed forest were correspondingly lower than (1300 - 1700 g C·m⁻²·year⁻¹ (Metcalf et al., 2007)) the tropical rain forest, (2560 g C·m⁻²·year⁻¹, (Hashimoto et al., 2004) and 1591 g C·m⁻²·year⁻¹ and 1602 g C·m⁻²·year⁻¹ (Rubio & Detto, 2017)) tropical monsoon forest, (794.8 - 1186.2 g C·m⁻²·year⁻¹ (Kiese & Butterbach-Bahl, 2002)) tropical forests, and higher than (601 g C·m⁻²·year⁻¹ (Mo et al., 2007)) tropical forest and (831 g C·m⁻²·year⁻¹, 600 - 1000 g C·m⁻²·year⁻¹ and 984 g C·m⁻²·year⁻¹ (Fernandes et al., 2002)) Brazilian Amazon pasture land. The variation in cumulative values of soil CO₂ efflux among the forest and ecological region with the years was predominantly owing to the soil temperature and soil water content variations and in addition seasonal variance of the amount of litterfall in different years. The analysis however suggests that the rainfall rather than the temperature drive the annual variability which is most visible in the tropics (Thomas et al., 2016; Cui et al., 2020). Because soil water directly affects the respiration of the living plant roots and the soil microbial community (Cook & Orchard, 2008) and it is more prominent in the tropical region.

The average of soil CO₂ efflux measured for 2-years was 483.81 mg CO₂·m⁻²·h⁻¹ and the highest was observed in the summer season (June-August) at 654.77 mg CO₂·m⁻²·h⁻¹ and lowest during the winter season (December-February) at 163.005 mg CO₂·m⁻²·h⁻¹. However, the spring (March-May) and autumn seasons (September-November) were intermediate at 269.481 mg CO₂·m⁻²·h⁻¹ and 448.13 mg CO₂·m⁻²·h⁻¹, respectively. The 2-years average soil temperature was 16.65°C and seasonal values were 11.14°C, 16.00°C, 20.79°C and 18.60°C in winter, spring, summer and autumn, respectively. The soil water content was averaged at 18.53%

and the seasonal soil water content was 9.00% in winter, 17.77% in spring, 27.34% in summer and 20.03% in autumn. It was highest during the summer season and lowest during the winter. The 2-years average litter biomass of the forest was 718.51 g d w·m⁻². It was lower in winter and spring at 668.97 g d w·m⁻² and 635.42d g w m⁻², and higher during the summer and autumn at 792.82 g d w·m⁻² and 762.57 g d w·m⁻², respectively (**Table 1**) indicating the seasonal trait of the plant growth. This record of the litter biomass explained the amount of litter added to the forest floor soil each year. The total annual soil CO₂ efflux of the forest in a year was estimated at 904.76 g C·m⁻²·y⁻¹ averaged for 2-years. The summer season summed soil CO₂ efflux was higher at 346.98 g C·m⁻² and was lower during the winter season at 105.65 g C·m⁻². However, the intermediate efflux estimated for the spring and the autumn season was at 206.61 g C·m⁻² and 245.51 g C·m⁻², respectively. The cause for the highest and lowest soil CO₂ efflux of this study incurred by the maximum and minimum level of the soil temperature during wet summer and dry winter season corresponds to the reviews of prior research in tropical forests (Rubio & Detto, 2017) and the temperate ecosystems (Dhital et al., 2014, 2019). The values of soil CO₂ efflux, soil temperature, soil water content, and the litter biomass detected in the spring and autumn season of this study were exactly in-between the winter and summer season that was attributed due to the pre-monsoon and the post-monsoon seasonal entities of the annual cycle. The wide range of variation in temperature and precipitation, at both short (hours to a day) to long (seasonal and inter-annual) temporal scales are most common in tropical forests and is responsible for the inter-annual variation of the soil CO₂ efflux that is driven by the changes in biophysical and biogeochemical conditions (Sotta et al., 2004).

4. Conclusion

The significant positive exponential curve between the soil CO₂ efflux and soil temperature, and the adequate temperature sensitivity of soil CO₂ efflux (Q_{10}) of this study explained the higher dependency of soil CO₂ efflux on soil temperature. Soil water content and the soil CO₂ efflux expressed significant positive exponential relations as well, which better expanded the knowledge of the sensitivity of soil water on soil CO₂ efflux and its availability in a subtropical forest ecosystem. The soil CO₂ efflux, soil temperature, and soil water content well explained the temporal variations of the forest, and the trend of correlations varied among the months and years. The variations of the soil CO₂ efflux with the changes in the above-ground litter biomass were well explained. The higher count of litter biomass in the summer and autumn seasons owing to the indirect but influential growth activity of the plant, root, and microbes, and the associated climate of the forest caused higher soil CO₂ efflux. The annual soil CO₂ efflux of the forest varied over the years with the variations of influencing factors/parameters that explained the soil carbon emission is directed by the ecological variables. Thus, years of measurements better proved that soil temperature and soil water content

solely with litterfall are the determinant factors for the soil CO₂ efflux variability in a mixed forest of the sub-tropical region. The result revealed sub-tropical forests could be the most influenced by changing precipitation regimes in the progression of a warming climate. This illustrates the forest is vulnerable to the climate change and the study demonstrates the comprehensive dynamics of the representative forest carbon cycle of the tropical region. The detailed studies are further required to examine the remaining environmental parameters like soil microbial activities, root growth, and its respiration, photosynthesis and carbon allocation, soil physicochemical properties, anthropogenic effects on forest and litter, tree phenology, and vegetation which affect the soil CO₂ efflux and understand the overall carbon exchange mechanism of the forest that directly effects on the carbon budget of the sub-tropical eco-region.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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