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Seasonal fluctuation of autotrophic and heterotrophic soil respiration in the subtropical *Schima-Castanopsis* forest, central Nepal

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Abstract

Soil respiration, the carbon dioxide emitted by the biological activity of soil, is one of the primary ways of carbon release from forest ecosystems. Soil respiration can be partitioned into its two components*:* autotrophic respiration by roots (R_A) and heterotrophic *respiration* by soil microorganisms (R_H). This study aims to assess the seasonal fluctuations of R_A and R_H in the subtropical forest of Nepal dominated by *Schima wallichii* and *Castanopsis indica.* The trenching method (root exclusion method) was applied to partition R_A and R_H. The closed chamber method was applied to measure soil carbon emission. A Portable Infra-Red Gas Analyzer (IRGA) was used to measure CO_2 concentration in control and trenched chambers every month for one-year period. Soil temperature and soil water content were measured to analyze the effect of these environmental variables on soil respiration rate. The results revealed that the mean R_A and R_H were 116.1±10.61 and 130±6.9 mg $CO_2 m^{-2} h^{-1}$, respectively, with higher contribution (52%) of R_H to total soil respiration. Significant seasonal variation with summer peak was remarkable in both components of soil respiration. R_A and R_H showed significant exponential relationships with soil temperature and soil water content. Soil temperature had a greater influence on R_H than R_A. Correspondingly, the temperature sensitivity of soil respiration (Q10) values of R_A and R_H were 3.218 and 2.083, respectively. This suggests that the soil CO_2 emissions in these subtropical forests may increase with future climate warming with more temperature sensitivity of autotrophic respiration.

1. Introduction

Carbon dioxide (CO_2) emission from soil respiration (R_S) represents one of the major mechanisms of carbon loss from forest ecosystems (Jian et al., 2018). Soil respiration represents the second largest carbon (C) efflux process in terrestrial ecosystems, which even exceeds the carbon released by fossil fuel combustion (Bond-Lamberty and Thomson, 2010). The amount of CO_2 in the atmosphere can vary greatly even by small changes in R_S (Davidson and Janssens, 2006). So, studies on soil respiration and its governing factors are necessary to understand the carbon balance of forest (Bond-Lamberty and Thomson, 2010).

Soil respiration is derived from metabolic activity by root (autotrophic respiration) and biological decomposition by microbial organisms (heterotrophic respiration) (Rankin et al., 2023). Hence, carbon emission from soil can be partitioned into autotrophic respiration (R_A) and heterotrophic respiration (R_H), which contribute differently to total soil respiration (R_S) (Chen et al., 2017; Ryan and Law, 2005). There have been numerous studies on R_S in different forest types of the world but little is known about the relative contributions of R_A and R_H components to R_S in the forest ecosystems (Yan et al., 2021). Existing studies present wide variations of soil respiration and contribution of autotrophic and heterotrophic respiration depending on forest type and geographical variation (Wei et al., 2010). The estimates of R_A in different forest ecosystems show variations with most values ranging between 45 to 60% (Jiang et al., 2017; Hanson et al., 2003). Some studies have reported higher R_H up to 60 % (Yang et al., 2018). On the global scale R_H has been re-

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ported to be 54% of the R_S (Hanson et al., 2000). Understanding the rate of R_A and R_H is necessary for accurate prediction of CO_2 emissions from forest ecosystems of particular geographic regions.

In addition, it is necessary to understand how soil carbon emissions from forests can be affected by the changes in environmental factors (Dhital et al., 2022a). Among many factors, soil respiration rate is well correlated with soil temperature and soil water content (SWC). These factors influence plant productivity, substrate supply, and microbial decomposition (Ise and Moorcroft, 2006; Kravchenko Irina et al., 2019). Soil respiration is temperature sensitive and increases with temperature rise (Hanson et al., 2000; Luo and Zhou, 2006). As global temperature is increasing, it is expected that soil respiration will also increase in warmer climates. Although various studies reported influence of soil respiration to vegetation type and environmental factors, the response of R_A and R_H to soil temperature and moisture content could be different (Kaur et al., 2010). R_A depends upon vegetation type and photosynthetic metabolism of the plant, while R_H is influenced by the decomposition of soil organic matter (Högberg and Read, 2006). The estimation of soil respiration components R_A and R_H and their response to environmental variables help to predict changes in the carbon cycling and storage in response to climate change. Soil respiration represents the primary pathway to releasing soil carbon back into the atmosphere. Hence understanding of R_S, and its components, as well as their response to a changing climate is important to assess the future ecosystem carbon balance (Rodtassana et al., 2021).

Different methods have been devised to separate root and microbial respiration. Root exclusion techniques are widely used such as trenching method, girdling method, clipping methods etc. (Epron, 2010). In root exclusion methods, roots are isolated physically by digging trench and estimation of root respiration can be done by subtracting microbial respiration (trenched plots) from total soil respiration in control plots (Lee et al., 2003). Trenching method is cost effective, simple to use and reliable for estimates of root respiration in forest ecosystems (Epron, 2010; Lee et al., 2003). However, some limitations, such as soil disturbances and changes in soil moisture conditions, should be considered.

Nepal, a Himalayan country, is rich in forest resources with diverse forest types contributing to carbon sequestration. Subtropical forests (*Schima-Castanopsis* forest) are found within the elevation range of 1000–2000 m above sea level and represent the dominant forest type in Nepal. The subtropical forests are important for their significant storage of atmospheric carbon due to their high productivity and carbon sequestration potential (Yu et al., 2014). Previously, studies have investigated carbon sequestration by plant biomass and soil in different forest types of Nepal. However, there are limited studies conducted on soil respiration in forests of Nepal Himalaya (Dhital et al., 2019, 2022a, b). There is no information on the contributions of root respiration and microbial respiration in the forests of the Nepal Himalaya. Separating autotrophic and heterotrophic contributions helps in the prediction of carbon fluxes as plants and microorganisms respond differently to environmental changes such as climate warming (Beverly and Franklin, 2015). The studies conducted across different seasons would reflect the effects of changes in temperature and rainfall patterns.

In this study, we attempted to quantify the autotrophic (R_A) and heterotrophic soil respiration (R_H) and their response to environmental factors in a subtropical mixed forest of Nepal with following specific research questions (i) How do R_A and R_H rates vary with seasonal change in subtropical forests?, and (ii) What is the relationship between these components and soil temperature and soil water content? This investigation will provide valuable insights into soil respiration components and address the research gap in the dominant forest type of the central Himalaya.

2. Materials and methods

2.1. Experimental site

The study was carried out in subtropical *Schima-Castanopsis* forest of Phulchoki hill, south-east of Kathmandu valley, Bagmati Province, Nepal. The Phulchoki hill is the part of lesser Himalaya and represents typical midland hill of Nepal. The experimental sites were situated within the forest at the base of Phulchoki Hill (27°34'49"N, 85°22'46"E), at an elevation of 1,600 meters above sea level (Fig. 1). The forest is dominated by the tree species such as *Schima wallichii* and *Castanopsis indica* and therefore, the forest is known as *Schima-Castanopsis* forest (TISC, 2002). Other associated trees are *Castanopsis tribuloides*, *Morella esculenta, Eurya acuminata, Carpinus viminea, Engelhardia spicata*.

The climate of the experimental site is subtropical with a mean annual temperature of 13.3°C and mean annual precipitation of 1527 mm from 2012 to 2022 AD (Fig. 2). Approximately 70% of the precipitation occurs in the monsoon season, from June to September. The forest consists of clayey loam soil type with pH ranging from 5.1 to 6. The average soil organic carbon content of the studied forest was 4.3%.

2.2. Measurement of soil respiration

An area of 1 hectare was selected for the experiment within the forest. A trench was made in five randomly selected subplots within the experimental site to measure root respiration (R_H). Trenching involving the root removal method was adopted for separation of the R_H and R_A (Epron, 2010; Hanson et al., 2000) To prepare trenched plots, a narrow ditch of 50 cm length, 50 cm breadth, and 40 cm depth was dug. Four metal plates, 2 mm thick were inserted into the vertical cuts of ditch to prevent root regrowth and a plastic lining was placed at the base of ditch. The set up overcomes the limitation of waterlogging as water can seep through the joints of the vertical plates and horizontal sheet in slopped terrain. The roots were removed manually and the root-free soil was carefully refilled into the ditch avoiding soil compaction and mixing of horizons. The general assumption of this method is that heterotrophic respiration is the only source of CO_2 in the trenched plots (Epron, 2010). Chambers made of polyvinyl chloride (18 cm in diameter and 16 cm in height were perma-

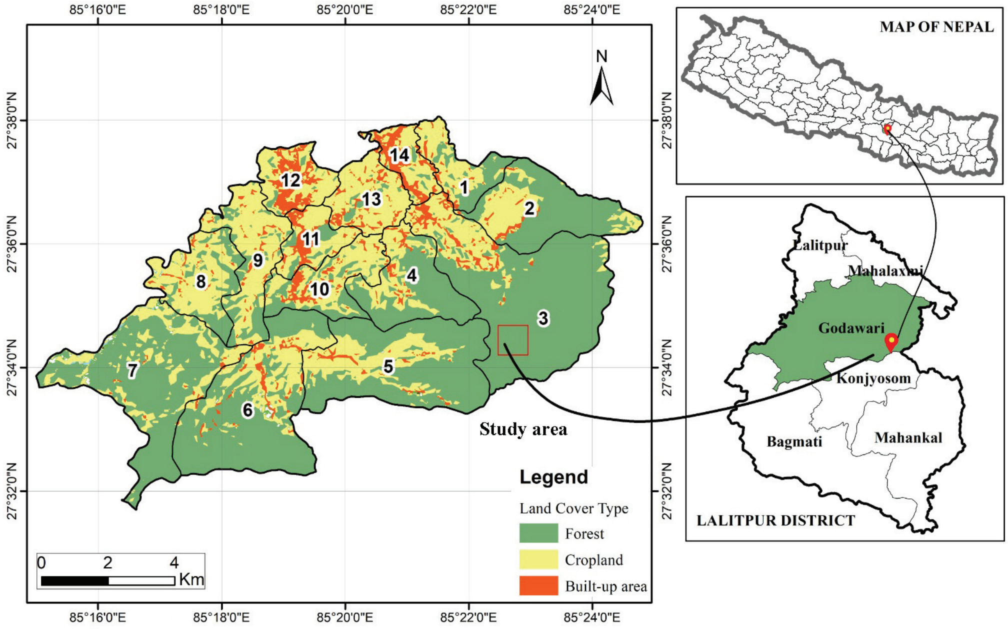

Fig 1. Map showing study area (Numbers in figure represent ward no. in Municipality)

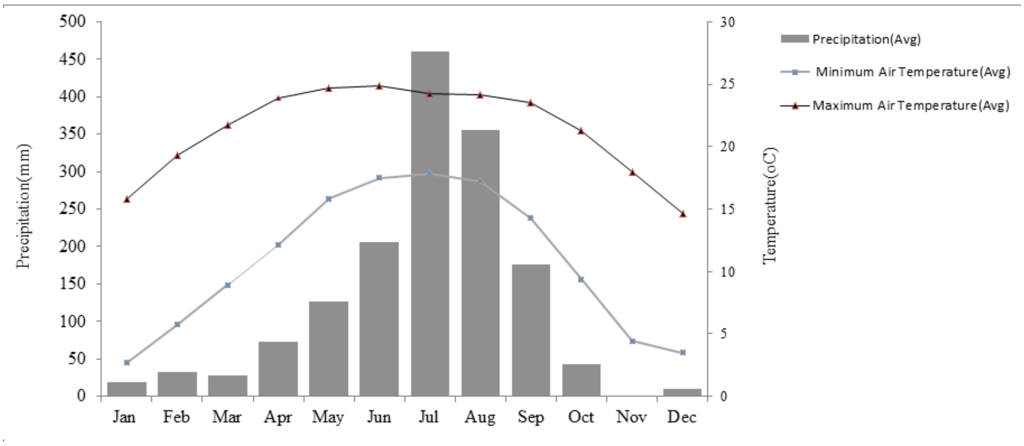

Fig 2. Climatic data (10 years average, 2013–2024) of study area (Data source: Department of hydrology and meteorology, GON)

nently installed 2 cm deep into the soil within the trenched plots. Similarly chambers were installed within 3 m of each trenched plot to measure total soil respiration, designated as control (untrenched) plots. Autotrophic respiration was estimated as the difference between the soil respiration rate recorded in the untrenched (control) plots and the trenched plots (Lee et al., 2003).

The CO_2 effluxes were periodically measured every month in all 10 chambers for a year by the closed chamber method (Bekku et al., 1995), using a portable infra-red gas analyzer (IRGA) (Vaisala CARBOCAP Carbon dioxide Probe GMP343). The closed chamber method was adopted because this method is comparatively economical and easier to install as the chamber systems are portable (Liang et al., 2004). Also repeated measurements can be taken from same plots in different months as this method doesn't allow soil disturbances. CO_2 flux was measured at same time between 11 am and 2 pm of each day. The initial reading for soil CO_2 concentration was taken immediately (one minute) after the chamber cover (lid) was applied. The final reading was taken after other minute of first reading. Three replicates of soil respiration measurements were made in each chamber and averaged to obtain the accuracy. The soil carbon emission rate was calculated from the following equation (Bekku et al., 1995; Koizumi et al., 1999):

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

where,
F = Soil respiration (mg CO_2 m^{-2} h^{-1});
V = Air volume enclosed by chamber (m^3);
A = surface area of chamber (m^2);
$\Delta c/\Delta t$ is the rate of change of the CO_2 concentration.

Soil temperature (at 5 cm depth) was also measured during the time of soil CO_2 emission measurement using the digital lab stem thermometer (AD-5622, Japan). Similarly, measurement of soil water content (SWC) was done by using soil moisture sensor TRIME-FM (Imko, Germany) at 5 cm soil depth near the chamber. Repeatedly three measurements of soil temperature and soil water content was taken and averaged for each chamber. For the assurance of valid data continuous measurement of soil temperature was performed for a year by installing a Stowaway Tidbit Temperature Data Logger (HOBO) inside the soil. An exponential model was developed based on measured data to obtain the relationship between soil respiration fractions and soil temperature. Based on the continuous soil temperature values throughout the year daily soil CO_2 emission of each soil respiration component (R_S, R_A and R_H) was computed and summed to obtain the annual values (Yang et al., 2018) according to the following formula:

R_ST= a x $\exp^{bxT}$ …….. (i)

where, R_ST is soil respiration at a given temperature, a and b are constants, and T is temperature. The b value in equation (i) was used to calculate the index of temperature sensitivity for soil respiration (Q10 value), which describes the changes in soil respiration over a 10°C increase in soil temperature. The Q10 value was calculated by inserting the parameter b into the equation- Q10=$\exp^{10\times b}$

2.3. Data analysis

Two-way ANOVA was performed to observe seasonal variation and trenching effects on soil physical parameters. The seasonal variation of R_A and R_H was analyzed using One-way ANOVA. Post-Hoc comparison using the Tukey test was generated to observe the variations in soil temperature and moisture among the seasons and between treatments. Regression was performed to analyze the relationship of soil respiration with environmental variables. The *P*-value <0.05 was considered as the significant in the test variables. The statistical analyses were performed using R (version 4.3.2) (R Core Team, 2023).

3. Results

3.1. Soil temperature and moisture

Soil temperature and soil water content (SWC) of the forest varied significantly with seasons ($p < 0.05$) (Table 1). Soil temperature was higher during the summer season and the lowest in winter in both trenched and control plots. Soil water content (SWC) also demonstrated seasonal variation with the highest SWC during summer (26.3%) and lowest (9.1%) in winter. Both soil temperature and SWC were moderate in the spring and autumn seasons (Fig. 3a, b). No effect of trenching treatment was detected for soil temperature and SWC variation. Although soil temperature exhibited high seasonal variation during the studied period, there were no significant differences in soil temperature between trenched and untrenched plots ($p > 0.05$) (Table S1). The interaction of both factors was also not significant (Table 1).

Table 1
Two-way ANOVA results

Soil Water Content (SWC)					
	Sum of squares	df	Mean Square	F value	P value.
Treatment	46.557	1	46.557	1.721	0.192
Season	4197.243	3	1399.081	51.726	<0.001
Treatment * Season	188.656	3	62.885	2.325	0.079
Soil temperature					
Treatment	0.331	1	0.331	0.126	0.723
Season	2126.749	3	708.916	269.945	<0.001
Treatment * Season	1.315	3	0.438	0.167	0.918

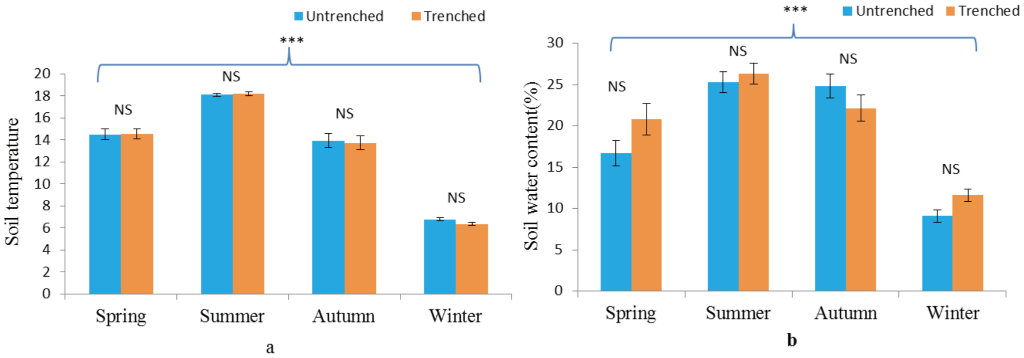

Fig 3. Seasonal variation in soil parameters: a) soil temperature; b) soil water content (***represents p<0.001 and 'NS' represents non significant)

3.2. Autotrophic and heterotrophic respiration

Based on the monthly measurements, the mean R_A and R_H were 116.1±10.61 and 130±6.9 mg CO_2 m^{-2} h^{-1} (n = 60), respectively. The contributions of R_A and R_H to total soil respiration were 47.2% and 52.8%, respectively (Fig. 4). The R_H rate was higher than that of R_A, but the difference was not significant ($p > 0.05$).

3.3. Monthly variation in R_A and R_S

Both R_A and R_H exhibited monthly variation with minimum value in winter and maximum in summer. The R_A showed the lowest rate in February (42.6 mg CO_2 m^{-2} h^{-1}) and peaked in August (226.9 mg CO_2 m^{-2} h^{-1}) (Fig. 5). The R_H rate also showed a similar curve with its summer peak in July. Moreover, R_H rate was higher than R_A in winter and dry months whereas R_A exceeded R_H in wet months during August and September (Fig. 5).

3.4. Annual soil respiration

Total annual soil respiration is often derived from R_S measured for a short period of time which is mostly done in day time; hence soil respiration is more accurately predicted using mean annual temperatures (Jian et al., 2020). Annual CO_2 emission derived from R_A and R_H were 429.9 and 695.7 g CO_2 m^{-2} yr^{-1} respectively. This emission, when converted to carbon, results in 116.07 and 187.8 g C m^{-2} yr^{-1}, emitted by R_A and R_H, respectively (Table 2).

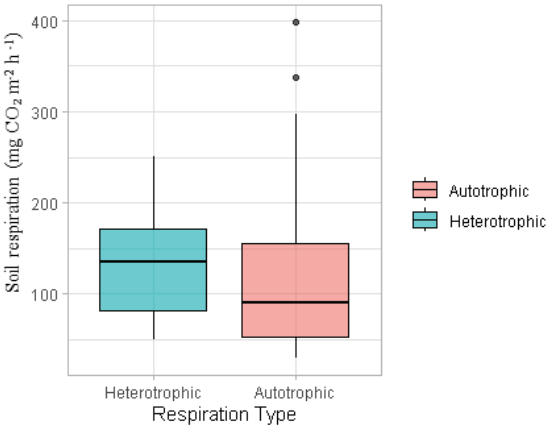

Fig. 4. Rate of autotrophic respiration (R_A) and Heterotrophic respiration (R_H)

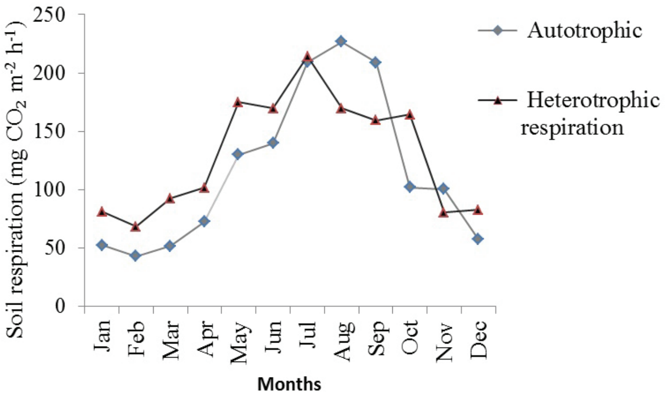

Fig. 5. Monthly variation of soil respiration (n=5 each autotrophic and heterotrophic respiration)

Table 2
Total annual soil respiration

	R_S	R_A	R_H
Annual respiration (g CO_2 m^{-2})	1125.60	429.90	695.70
Mean daily respiration (g CO_2 m^{-2} day^{-1})	3±0.04	1.13±0.02	1.87±0.02
Annual carbon release from soil respiration (g C m^{-2})	303.91	116.07	187.80

R_A: Autotrophic soil respiration; R_S: Total soil respiration; R_H: Heterotrophic soil respiration

3.5. Seasonal variation of autotrophic and heterotrophic soil respiration

The rates of both soil respiration components had obvious seasonality, with the maximum record in summer and the minimum in winter (Fig. 6). Both R_A and R_H varied significantly with seasonal changes ($p < 0.001$) (Table 3). R_A ranged from minimum (50.8 mg CO_2 m^{-2} h^{-1}) in winter to maximum (192 mg CO_2 m^{-2} h^{-1}) in summer. Similarly, R_H ranged from a minimum of 77.5 to a maximum of 184.5 mg CO_2 m^{-2} h^{-1} in winter and summer seasons respectively. R_H exceeded R_A in the three dry seasons: spring, autumn and winter, while a contrasting pattern was observed in summer with higher R_A than R_H.

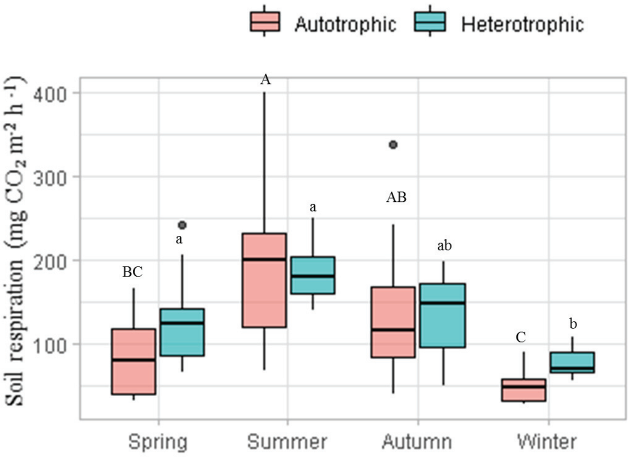

Fig. 6. Box plots showing seasonal variation of R_A and R_H. (The bar inside the box represents median value. The box indicates range of the data. The maximum and minimum values are shown by vertical lines; box shows interquartile range)

Table 3
One-way ANOVA results for seasonal variation of R_A and R_H

	Sum of Squares	df	Mean Square	F-value	P-value
RA	148090.123	3	49363.374	8.257	<0.001
RH	63872.574		21290.858	8.327	<0.001

R_A: Autotrophic soil respiration; R_S: Total soil respiration; R_H: Heterotrophic soil respiration

3.6. Relationship between environmental variables and soil respiration components

There were similar trends in the variation of soil respiration components with soil temperature. Both the R_A and R_H increased with soil temperature exhibiting significant exponential relationships ($R^2 = 0.53$ and $R^2 = 0.69$ respectively, and $p < 0.001$) (Fig. 7).

Correspondingly, the temperature sensitivity of soil respiration (Q10) values of R_A and R_H were 3.218 and 2.083 respectively. Moreover, the Q10 value of R_A was higher than that of R_H.

The R_A and R_H showed significant exponential relationships with soil water content (SWC) but the relationship was weaker than with soil temperature (Fig. 8). The model explained 36% variability of R_A to soil water content ($R^2 = 0.36$; $p < 0.001$). On the other hand, the R_H showed a higher correlation with SWC than R_A ($R^2 = 0.49$).

4. Discussion

4.1. Soil carbon efflux and contribution of R_A and R_H

Studies show that there are variations in the contribution of R_A and R_H to total soil respiration depending on forest types, climate and organic substrates, and methods of measurement. The global relationships show that R_A and R_H are strongly correlated to annual R_S across a wide range of forest ecosystems (Bond-Lamberty et al., 2004). The present findings showed 52.8% contribution of R_H to total soil respiration, slightly higher than R_A (Fig. 4). The contribution of R_H to R_S averaged 58.1%, ranging from 45 to 81% in a subtropical camphor tree forest in China (He et al., 2023). Also, R_A and R_H accounted for 63–65% and 40–49%, in subtropical mixed broadleaved forest respectively (Yu et al., 2017). Similarly, R_A and R_H contributed about 50% of the total soil respiration in the Spruce forest (Comstedt et al.,

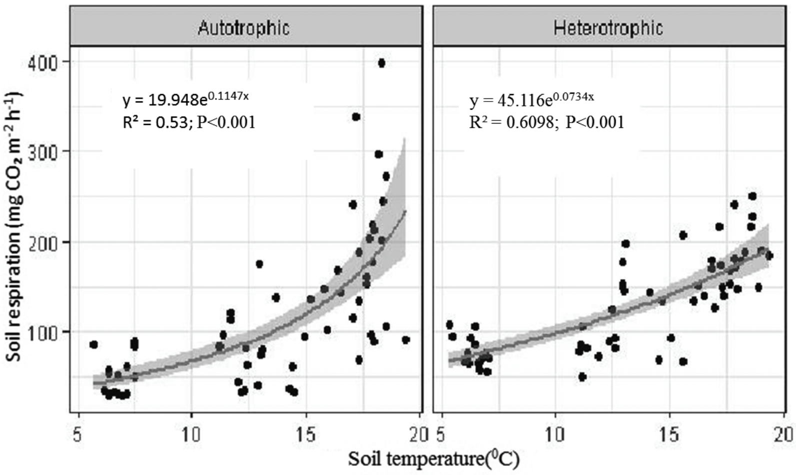

Fig. 7. Relationship of Autotrophic respiration (R_A) and Heterotrophic respiration (R_H) with temperature

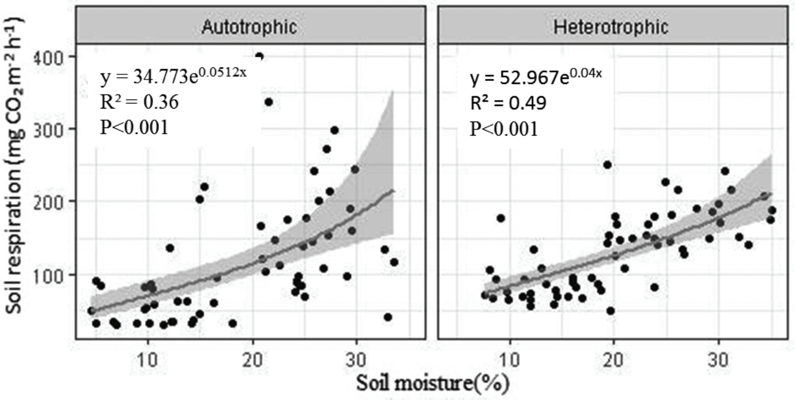

Fig. 8. Relationship of Autotrophic respiration (R_A) and Heterotrophic respiration (R_H) with soil moisture

2011) but in a semi-natural forest, R_H component accounted for 66% of annual CO_2 efflux (Ferréa et al., 2012). These estimates demonstrate variation in the contribution of R_A and R_H depending upon ecosystem type and the different techniques and time scales used (Zhou et al. 2009). Hence the evidences support that the present findings on R_H and R_A rates are similar to those reported in many forest ecosystems (Hanson et al., 2000; Lee et al., 2010).

4.2. Seasonal variation of R_A and R_H

The pattern of temperature and moisture variation during seasonal change influences soil respiration rate. R_S and its components (R_H and R_A) increased during the warm and moist summer season. The seasonal patterns in forests are regulated by several abiotic and biotic factors (Hanson et al., 1993). Soil temperature and moisture are dominant environmental controls of soil respiration and their extent of influence varies with substrate conditions and seasons (Shi et al., 2012). Soil moisture can limit R_S in very dry or wet soil conditions (Chang et al., 2014). However, tropical forests which are mostly wet generally have high soil respiration rates (Bond-Lamberty and Thomson, 2010). Distinct variations in soil respiration rates have been observed across different vegetation types (Raich and Tufekciogul, 2000).

In our study, both R_A and R_H exhibited similar seasonality patterns with their rates in decreasing order summer > autumn > spring > winter. Autotrophic respiration is closely linked with plant photosynthetic rate and phenological patterns of plants (Han et al., 2014). The enhanced belowground supply of carbon with abundant growth of plants in the summer season increases autotrophic (root) respiration (Song et al., 2012).

Further, the higher R_A during the rainy season may be due to an increase in root biomass and vegetation cover density (Kumar et al., 2023). The microbial activity resulting in R_H depends on carbon input, temperature conditions and moisture limitations in the progressive seasons. The seasonal differences between R_A and R_H were found because microorganisms and roots respond to environmental factors differently (Liu et al., 2023). High moisture content and optimum temperatures in the rainy season favor microbial activity and litter decomposition, thus increasing soil respiration (Yan et al., 2022).

4.3. Relation of R_A and R_H to soil temperature and moisture

This study demonstrated that soil temperature and soil water content (SWC) are significant drivers of changes in heterotrophic respiration (R_H) and autotrophic respiration (R_A). The two components of soil respiration- R_A and R_H were significantly correlated ($p < 0.05$), with soil temperature and SWC. Soil temperature had a greater influence causing changes in soil respiration rate. These findings are consistent with the findings of Yu et al. (2017) in subtropical forests ecosystem. The global pattern of response of soil respiration components also concludes temperature as being important factor causing an increase in soil respiration (Wang et al., 2014). The effect of temperature on R_S is determined by the responses of R_A and R_H to temperature increases, although the responses may differ (Noh et al., 2016). A strong correlation between R_H and R_A to temperature was supported by Yan et al. (2021). Temperature affects the supply of carbon available for photosynthesis, thereby affecting carbon used for root respiration (Tang et al., 2005). R_A show positive response to temperature as increasing temperature enhances root growth and root exudate production (Leuschner et al., 2022). Temperature affects R_H as the decomposition of organic matter is positively correlated with temperature (Conant et al., 2004).

Our results suggest that temperature sensitivity (Q10) of R_A is greater than that of R_H. Higher temperature sensitivity of root respiration (R_A) has been observed in some studies of forest soil (Bhupinderpal-Singh et al., 2003; Lavigne et al., 2003). Field warming experiments have reported R_H to be more sensitive to warming than R_A (Liu et al., 2018). The effects of roots on Q10 also largely depend on climatic condition, vegetation type (Li et al., 2020; Yu et al., 2017) and effect of seasonality on tree below ground carbon allocation (Högberg and Read, 2006).

Soil respiration is sensitive to temperature and indirectly to SWC although SWC is not directly correlated with soil respiration. Our study showed a significant increase in R_A and R_H with SWC. R_A is stimulated by increased precipitation due to increase in substrate supply and soil nutrient availability (Liu et al., 2016). In semiarid grassland types seasonal changes in soil respiration are largely controlled by the moisture content of the soil (Meena et al., 2020). Low SWC impedes microbial activity by reducing solute transport through soils (Manzoni, 2012). Soil respiration generally increases with soil moisture, higher moisture conditions may reduce soil respiration due to decline in oxygen availability when soil pores are filled with water (Cook and Orchard, 2008). In the future temperature warming, soil respiration may increase significantly but the effect may be weakened by warming-induced soil drying as well.

Moreover, soil respiration is controlled by other factors such as soil organic carbon (Li et al., 2019) and carbon inputs from litter (Li et al., 2022) which supplies substrate to microbial activities. Similarly, R_S is also dependent on photosynthetic rate which provides substrate for root respiration (Moyano et al., 2008). Changes in temperature, precipitation and length of the seasons results in changes in CO_2 flux in the future (Cox et al., 2000).

5. Conclusions

This study evaluates the seasonal fluctuation of soil respiration from the forest of the subtropical region of Nepal Himalayas. A higher contribution of heterotrophic respiration (R_H) to total soil respiration than autotrophic respiration (R_A) was found in the *Schima-Castanopsis* forest. Significant seasonal variation with summer peak was remarkable in both components of soil respiration. Soil temperature and moisture have stronger effects on R_H than R_A. Higher Q10 value suggests R_A is more sensitive to temperature than R_H resulting in greater CO_2 emission from roots in future climate warming. The results of this study would be helpful in understanding the mechanism of carbon balance within the soil systems.

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Conflict of interest

The authors declare no known competing financial interests or personal conflicts that could have appeared to influence the work reported in this paper. The research doesn't pose damage and risks to humans and nature.

Author Contributions

Sanu Raja Maharjan: Conceptualization, data collection, data analysis, writing original draft. **Chandra Prasad Pokhrel:** Conceptualization, funding acquisition, data validation, review and editing, supervision. **Lal Bahadur Thapa:** Data validation, visualization, review and editing, supervision. **Ram Kailash Prasad Yadav**: Data validation, review and editing, supervision. **Deepa Dhital:** Conceptualization, data collection, funding acquisition, data validation, review and editing, supervision. All authors read and approved the final manuscript.

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