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Spatio-Temporal Variation of Soil Respiration and Its Association with Environmental Factors in Bluepine Forest of Western Bhutan

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Abstract

We investigated Soil respiration in Bluepine forest of western Bhutan, in relation to soil temperature, moisture content and soil pH and it was aimed at establishing variability in space and time. The Bluepine forest thrives in the typical shallow dry valleys in the inter-montane Bhutan Himalaya, which is formed by ascending wind from the valley bottom, which carries moisture from the river away to the mountain ridges. Stratified random sampling was applied and the study site was classified into top, mid, low slope and further randomized sample of n=20 from 30 m×30 m from each altitude. The overall soil respiration mean for the forest was found 2248.17 CO_2 g yr^{-1} and it is ∼613.58 C g yr^{-1}. The RS from three sites showed a marginal variation amongst sites, lower slope (2,309 m) was 4.64 μ mol m^{-2} s^{-1}, mid slope (2,631 m) was 6.78 μ mol m^{-2} s^{-1} and top slope (3,027 m) was 6.33 μ mol m^{-2} s^{-1} and mean of 5.92 μ mol m^{-2} s^{-1}, SE=0.25 for the forest. Temporal distribution and variations were observed more pronounced than in the space variation. Soil respiration was found highest during March and lowest in September. Soil temperature had almost inverse trend against soil respiration and dropped a low in February and peak in July. The moisture in the soil changed across months with precipitation and pH remained almost consistent across the period. The soil respiration and soil temperature had significant relationship R^2=-0.61, p=0.027 and other variables were found insignificant. Similar relationship are reported for dry season in a tropical forest soil respiration. Soil temperature was found to have most pronounced effect on the soil respiration of the forest under study.

Key Words: carbon dioxide, spatio-temporal, moisture, temperature, soil respiration

Introduction

Respiration is biochemical process within cells of an organism and this process are influenced by different biotic and abiotic factors (Luo and Zhou 2006). The forest soil respiration (R_S) is characterized by three major fluxes: photosynthesis, autotrophic respiration and heterotrophic respiration. R_S counter carbon sink processes and determines whether the ecosystem is a sink or source of carbon (Bolstad et al. 2004; Nirola 2016; Wangdi et al. 2017).

R_S is accounted as the second largest contributor to the global carbon flux and subtle change in processes of R_S will impact the global carbon dynamics and climate. The precise and reliable estimates of carbon dynamics is significant to understand impact on climate (Bond-Lamberty and Thomson 2010; Kim et al. 2020). Knowledge on R_S trends and fac-

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tors in different environment will underpin to comprehend carbon cycle of the ecosystem (Wang et al. 2022). The R_S dynamics in different ecosystems are complex; highly varied in time and space (Rustad et al. 2001; Davidson and Janssens 2006). The variability in R_S reflect differences among the ecosystems and considerable proportion account to variety of measurement techniques. The lowest rates of R_S recorded in the coldest and driest biomes and the highest at the tropical moist forests (Raich and Schlesinger 1992; Rochette et al. 1999; Lee et al. 2003).

The anthropogenic activities in Bhutan is observed as a main factors that change land use and land cover (LULC), affecting soil organic carbon storage in montane ecosystems (Dorji et al. 2014b). With a few studies on R_S and carbon carried out in Bhutanese forest ecosystems (Dorji et al. 2014a, 2014b; Nirola 2016; Wangdi et al. 2017; DoFPS 2018; Yangka et al. 2019) there is still dearth of knowledge on R_S for different forest ecosystems in Bhutan that need to uncover. Therefore, with the objective to better understand spatial and temporal variation of R_S in the Bluepine forest ecosystem, we studied R_S and factors affecting R_S in this type of forest of Thimphu District Bhutan. Rai and Zangpo (2022), Bhutan has 71 percent forest cover of the total geographical area of the country and Bluepine forest is 4% of the total.

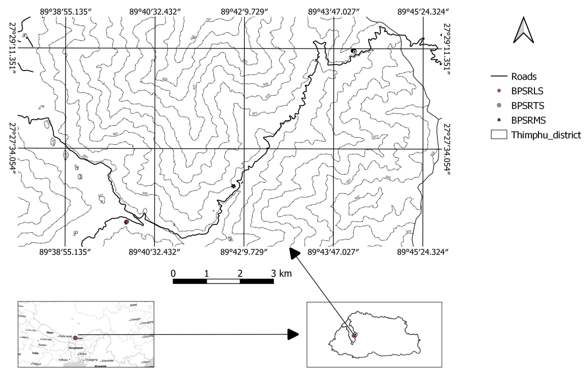

Fig. 1. Showing sampling sites on an altitudinal gradient for the measurement of soil respiration (SR) and affecting factors in Bluepine (BP) forest in Thimphu district Bhutan; Tope slope (TS), Mid slope (MS) and Low slope (LS).

Materials and Methods

Site description

The Bluepine forest representative ecosystem for the Bhutan Himalaya was studied, under Thimphu district western Bhutan. Grierson and Long (1983) the forest occupies the inner shallow dry valleys at an altitudinal range 2,100-3,100 m, with rainfall of 700-1,200 mm, in west and central Bhutan. The tree species is one of the significant timber preferred and utilized by the local inhabitants.

The study area covers lower slope at Semtokha, mid slope at Memelakha and top slope above Hongtsho (Fig. 1) under Thimphu district and it is one of the typical shallow dry valleys that occur in Bhutan. The site characteristics of three study locations are described in Table 1 and *Pinus wallichina* is dominant species (78.76%) from the eight tree species enumerated in the research plots, all above 1.3 m diameter at breast height (dbh).

The forest is classified as cool dry conifer, with *Pinus wallichina* species dominance and associate understory species of: *Quercus lanata, Rhododendron arboretum, Populus rotundifolia, Berberis aristata, Jasminum humile, Lyonia ovalifolia* and the stand basal area struutre is given in Table 2. The slope is climatically much drier due to its short range

Table 1. Site characteristics of the study area

Parameter	Top slope (TS)	Mid slope (MS)	Lower slope (LS)
Elevation	3,027	2,631	2,309
Latitude (N)	27° 29' 14"	27° 27' 05"	27° 26' 37"
Longitude (E)	89° 44' 15"	89° 42' 06"	89° 40' 04"
Aspect	180° (S)	140° (SE)	40° (NE)
Slope degrees	40	25	40
Litter accumulation (t ha^{-1})	58.08	27.63	25.38
BA of tree species above 1.3 m ht.	54.23%	29.73%	16.04%

Table 2. Tree species distribution and percent cover of the research sites of height > 1.3 m BA in %

Tree species	Top slope (TS)	Mid slope (MS)	Lower slope (LS)
Lyonia ovalifolia		0.04%	0.07%
Picea spinulosa	10.28%		
Pinus wallichi	38.16%	27.68%	12.92%
Populus rotundifolia		0.08%	
Quercus griffithii			2.99%
Quercus semecarpifolia	5.79%		
Rhododendron arboreum		1.85%	0.06%
Rhus chinensis		0.09%	

from valley bottom to the mountain top, where ascending winds carries moisture to the ridge top making the lower altitudes dry (Wangda and Ohsawa 2006; Eguchi and Wangda 2012), it is also known as sub mesic type of forest based on the humidity condition and such dry valleys are considered to be formed due to low precipitation in the area (Ohsawa 1987).

The research site falls under state own forest land and it is subjected to different anthropogenic activities due to its accessibility. The forest is used as a cattle grazing by the local communities and the MS are usually frequented by the mushroom hunters during the season.

Sampling and measurements

The study area was stratified into three altitudinal range: TS, MS and LS, from each range a plot size of 30×30 m was randomly established, at least 50 meters away into the forest from the main road. The plot was further divided into three meters interval grid forming 10 rows and 10 columns and sample of one row and a column were chosen using MS excel function: RANDBETWEEN (1,10) for the measurement SR and other environmental factors of interest. The 20 nos of Polyvinyl chloride (PVC) circular collar were placed in each plot on a randomly selected row and column grids, which are called horizontal and vertical sample points, which served as subplots and all the measurements were done in the day.

R_S was measured from February 2022 to September 2022 from three plot locations once every month from randomly chosen sample points (n=20, horizontal ten and vertical ten-subplots). Permanent PVC collars were installed (height 5cm, 2-3 cm inserted in the soil, diameter 10 cm) in December 2021 to minimize disturbances and it served as the spot for the R_S measurements and reference point for other variables. We used a portable infrared gas analyser (EMG-5, PP systems, Amesbury, USA) for measurement of R_S, which took 2-3 hours complete at each site. R_S was estimated by applying a linear fit model that estimates increasing CO_2 concentration in the SRC head space, over time with chamber closure time (DT) of 90 seconds and delta carbon (DC) of 50 ppm (Wangdi et al. 2017).

Volumetric soil water content (VWC) 0-20 cm soil depth in (vol. %) was measured at the adjacent of each plot, using a portable Field Scout TDR 100 system (Spectrum Technologies, Inc. 6640FS, USA). Soil temperature (T_S) at 5 cm soil depth was measured with a handheld thermometer probe (Hanna Instruments, USA) at each measurement spot and at Mid slope plot HOBO Tidbit v2 temperature logger was used to log T_S at every 15 minutes interval. Soil pH was also measured using portable Kelway Soil Tester (Kel Instruments Co. Inc. Japan) against each R_S measurements; composite soil sample was collected from each site (n=20) and the samples were test for pH and EC at soil and plant analytical laboratory (SPAL), Thimphu Bhutan, with pH-H_2O in 1: 2.5 soil－water suspension method.

Basal area for each site was measured, using the conventional measurement of diameter at breast height over bark diameter at breast height (DBHOB) of the trees with height more than 1.3 m within the plot. The accumulation of litter was assessed in 40×40 cm plot, collecting and weighing the weight in grams using Pesola spring scale (lightline, 500 g, Switzerland), one time of (n=20) at each site.

Results and Discussion

Variation among site

The forest soil CO_2 efflux respiration rate, measured in mu mole meter square per second (μ mol m^{-2} s^{-1}) and rate of CO_2 efflux and carbon was determined for the forest as; 187.35 g CO_2 and 51.13 g C per month and 2248.17g CO_2 yr^{-1} , which is equivalent to 613.58 C g yr^{-1}. This falls within the typical range of respiration rates observed in temperate coniferous forests, as reported in (Raich and Schlesinger 1992). An examination of respiration rates across three different sites within the forest revealed minimal variations. The lowest rate was found at an altitude of 2,309 m at lower slope, measuring 4.64 μ mol m^{-2} s^{-1}. The rate at the mid-slope location of 2,631 m was 6.78 μ mol m^{-2} s^{-1}, and at the top slope location of 3,027 m it was 6.33 μ mol m^{-2} s^{-1}. The average rate for the entire forest ecosystem was 5.92 μ mol m^{-2} s^{-1}, SE=0.25. It is apparent that the respiration rate of the different sites is influenced by factors such as moisture and temperature. The lower slope appears to have a lower rate of respiration due to low levels of moisture and low temperatures that might have impeded the microbial activities. The mid plot, however appears to have optimal conditions in terms both temperature and moisture leading to higher rate of respiration compared to the other sites, as seen in Fig. 2A-C.

The temperature at the mid slope was slightly higher than the lower slope by about one degree Celsius. The average temperature across all the sites was mean of 14.97°C, SD=5.1. The temperature at top slope plot was distinct from the other two plots as shown in Fig. 2B. The mean moisture in the site was 12.16 % measured in volumetric water content, SE=0.27, from the total n=540. It was observed that the moisture levels increased as the altitude increased, with the highest levels recorded at thee tope slope of the study area at 16.45% Fig. 2C. The paradigm of temperature and moisture content observed in the slope, may be attributed to the typical local climatic conditions prevail in the inner Himalayan dry valleys as described in (Eguchi and Wangda 2012) ascending wind carrying moisture from the valley floor to the mountain top.

No significant difference in pH were observed, the overall pH level of the forest floor was acidic with a median value of 6.4, max=7, min=5.7 were found from n=380, as seen pH distribution variation among the different sites Fig. 2D.

Variation along horizontal and vertical grids

The data was analysed by dividing it into horizontal and vertical grid points for each site to determine variations in the mean values between and within the grids of each plot. The overall R_S for the horizontal grid had a mean of 6.16 μ

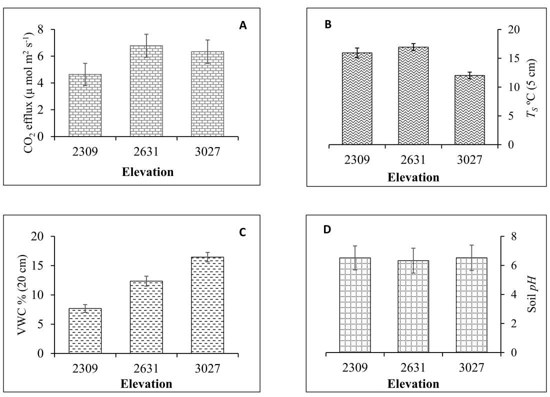

Fig. 2. Altitudinal variation and existence of soil respiration and associate environmental factors and error bars of CI 95%, a comparison among the sites, x-axis is the different site's altitude masl. (A) RS mean in different sites of carbon dioxide in mole meter square per second. (B) Mean of T_S in degree Celsius measured at 5cm soil depth. (C) Mean of volumetric water content measured at 20 cm soil depth. (D) Soil pH of three sites.

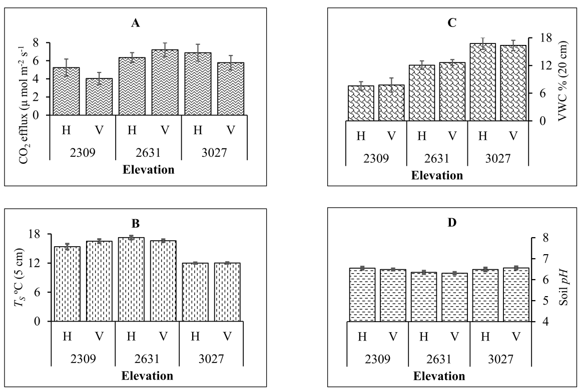

Fig. 3. Across grid spatial variation and distribution of soil respiration with other environmental factors in horizontal and vertically sampled in the Bluepine forest ecosystem and error bars are CI 95%. The x-axis is horizontal and vertical sample grid with elevation of the site. (A) Cumulative R_S at horizontal and vertical sample points. (B) T_S space distribution measured at 5 cm depth of the soil. (C) VWC % (volumetric water content) measured at the soil depth of 20 cm distribution along the grids. (D) Soil pH spatial variation among the grids points.

mol m^{-2} s^{-1}, SD=0.96 and the vertical grid had a slightly lower mean of 5.68 μ mol m^{-2} s^{-1}, SD=0.96 and n=30. In the separate site wise grids, lowest mean 4.04 μ mol m^{-2} s^{-1}, SD=0.96 was observed in the lower slope and n=10 as in the Fig. 3A.

The soil temperature in the horizontal and vertical grid points were found to be quite similar, with horizontal grid of M=14.88, SD=0.38 and vertical grid slightly higher M=15.05, SD=0.16 and n=30. However, when comparing the data from the different sites, it was found that the middle slope had the highest record M=17.26, SD=0.61 and n=10 in the Fig. 3B and The unusual temperature observed in the study can be attributed to the inversion layer in the atmosphere, which is climatic phenomenon that occurs in dry valleys according to research (Eguchi and Wangda 2012). The moisture content between the grids were almost similar, without much difference and followed the plot wise distribution of altitudinal gradient increment in Fig. 3C. The distribution of pH in both the vertical and horizontal grids were similar among the different sites, observed in the Fig. 3D.

Further analysis of data from the horizontal and vertical grids revealed that there was little variation between the two settings, as shown by the results of the paired sample t-test. For R_S horizontal M=6.16, SD=0.96 and vertical M=5.68, SD=0.96, t (9)=0.95, p=0.37; T_S horizontal M=14.8, SD=0.38 and vertical M=15.05, SD=0.16, t (9)=-1.62, p=0.14; VWC% horizontal M=12.13, SD=1.05 and vertical M=12.18, SD=0.88, t(9)=-0.13, p=0.90. This suggests that other factors may be influencing the measurements and more detailed micro-level site stratification would be necessary to account and deepen understanding, which is in agreement of the findings greater 'small scale variability than at larger landscape scales' (Nirola 2016).

Temporal distribution and variations

The data of all sites were pooled and averaged into monthly means for all the variables to analyse the variation that occurred during the study period from February to September 2022. The overall monthly means of variables were; R_S of M=5.92, SD=3.83; T_S of M=14.70, SD=4.37; VWC% of M=12.28, SD=4.36; pH of M=6.52, SD=0.20 and n=60. The mean R_S was observed highest in the month of March and lowest during the September and T_S mean a low in February and highest in the month of August refer Fig. 4A. The volumetric water content (VWC%) reached peak in August, lowest in April

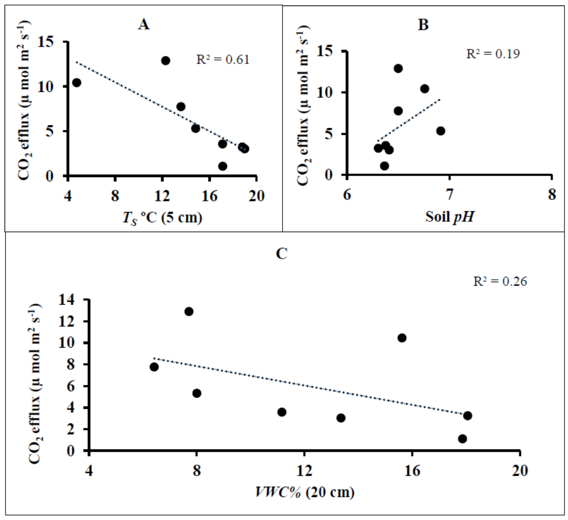

Fig. 5. Bivariate scatter plot assessing the relationship of the dependent and independent variables. (A) Rs CO_2 (μ mol m^{-2} s^{-1}) as dependent variable (DV) and Ts °C (5 cm) as independent variable (IV); (B) Rs CO_2 (μ mol m^{-2} s^{-1}) as DV and Soil pH as IV; (C) Rs CO_2 (μ mol m^{-2} s^{-1}) as DV and VWC% (20 cm) as IV.

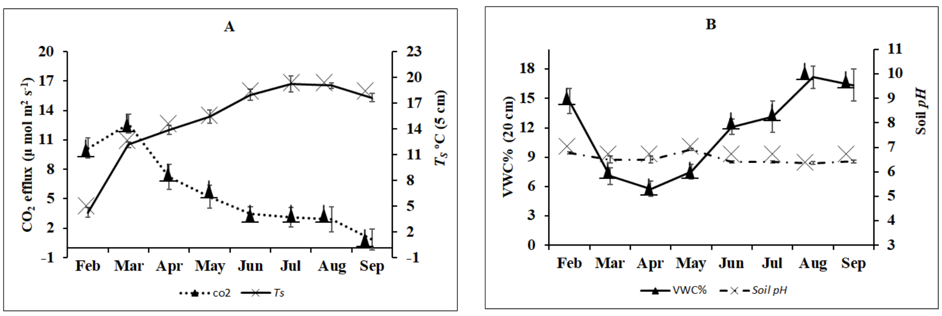

Fig. 4. Temporal variation of different variables. (A) CO_2 μ mol m^2 s^{-1} and Soil temperature measured in 5 cm depth over month of the study period; (B) Volumetric water content (VWC) in percentage measured in 20 cm depth and soil pH over the study period; Error bars are the ± CI 95 % plot.

and Soil pH were almost similarly distributed across the month Fig. 4B.

Bivariate relationship analysis was conducted using excel scatter plots, with R_S as the dependent variable and the other variables as independent variables. The results showed that T_S explains and predicts 61 % of the R_S and has negative relationship in the Fig. 5A. Soil pH among the independent variables had minimal bivariate relation with the R_S Fig. 5B. The moisture content of the soil measured as VWC% in 20 centimetres depth predicts only 26% of the R_S Fig. 5C.

A multiple regression analysis was conducted to confirm the prediction of the R_S as the dependent variable from independent variables; T_S, VWC% and pH. Result showed 70% variances in the soil respiration can be accounted to the three predictor variables in the model collectively, $F(3,4)=6.35$, $p=0.05$. The R_S as DV was predicted to 64.90-0.80 (T_S 5 cm)-0.44 (VWC% 20 cm)-6.42 (Soil pH), where temperature (T_S) is measured in degree Celsius, Soil moisture content (VWC%) is measured in percentage and soil pH as degree of acidity or alkalinity. T_S amongst the IVs had significant negative relationship with R_S ($\beta=-0.912$, $t=-3.421$, $p=0.027$) moisture content and pH were insignificant predictor of R_S Table 3. The result showed that temperature had a significant relationship with R_S, while soil moisture content and pH were not found to be significant predictors in a temporal state.

Temporal distribution and variations were more pronounced than the space variations, showing significant relationship between the variables. It is expected to have different relationship among the variables with different situations; low and high soil water content affects diffusion on soluble substrates and oxygen, which limit the microbial respiration. Seasonal availability of soil moisture in the soil tend to affect the threshold temperature for the microbial action and substrate availability interact with these different variables affects the soil respiration (Davidson et al. 2006).

The relationship between the DV and IVs were assessed and T_S had significantly negative relationship $R^2=-0.61$, $p=0.027$ and other IVs had no significant relationship with DV Fig. 5, Table 3. The results could be inferred to the moisture and temperature distribution which could have impeded the soil respiration and lead to the negative relationship. February month had high, March the highest and gradually decline on the mean values of soil respiration, can be well related to the change in moisture availability and the temperature of the study site. The first month measurement record of moisture was high due to the snowfall in the January month and there after the gradual decrease in moisture and increase in temperature have likely reduced the microbial activity and thus limited soil respiration. The exitence of such phenomenon would be well speculated to the climatic conditions that prevail in this typical dry valleys. The ascending wind from thee valley floor toward the afternoon, carries moisture to the ridge top making the lower slopes drier (Wangda and Ohsawa 2006; Eguchi and Wangda 2012). Davidson et al. (2006) The relationship can vary with site conditions, the variables and thus affect can be both negative and positive on soil respiration, our study show the negative relation of temperature and soil efflux.

Simliar inverse relationship of soil respiration and temperature is been observed and reported from Caatinga Brazil and during dry season in Sakaerat tropical forest of Thailand (Ribeiro et al. 2016; Boonriam et al. 2021).

Table 3. Soil respiration (Rs) prediction as outcome from predictor variables; Ts, VWC% and pH.

Variables	B	SE B	β
Soil temperature (Ts)	-0.797	0.233	-0.912*
Soil moisture (VWC%)	-0.441	0.200	-0.502
Soil pH	-6.383	5.507	-0.332

$R^2=0.83$, $*p<0.05$.

Conclusion

The Bluepine forest under study showed presence of both space and temporal variations. Soil temperature had the most pronounced significant negative effect on the soil respiration in the temporal scale, than other variables measured in the Bluepine forest of our study area. The moisture gradient over season have had impacted the temperature that likely reduced the microbial activity with typical localised climatic condition prevailed in the area.

Although there exist good relationship between Soil respiration and temperature, it is not possible to determine the specific processes involved in the CO_2 efflux production. The limitation of this study was accouting the physiological processes affected by the moisture and temperature were not examined.

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