



# Soil CO<sub>2</sub> efflux variability influenced by different factors in the subtropical sacred groves of Manipur, North-East India

Chongtham Sanjita<sup>1</sup> · Rojen Singh Thounaojam<sup>1</sup> · Th. Binoy Singh<sup>1</sup> · N. Dhirendra Singh<sup>2</sup>

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## Abstract

The influencing factors on the carbon dynamics of low latitude regions remain unclear in the global carbon budgets. Studies on the carbon budget of sacred groves of north east India as well as influencing factors on rate of carbon dioxide efflux are scanty. This study explores the spatial and temporal seasonality trend in the rate of soil CO<sub>2</sub> efflux in relation to biotic and abiotic factors in subtropical sacred groves of Manipur. An automatic chamber system was used in measurement process in six selected sacred groves over two consecutive years. In the present study, soil CO<sub>2</sub> efflux rates showed a positive exponential correlation with soil temperature, soil moisture and root biomass, having greater sensitivity to soil temperature. The rate of soil CO<sub>2</sub> efflux showed strong positive correlation to seasonal soil organic carbon content indicating greater role in mineralization with higher carbon dioxide emission. Litter biomass showed negative correlation with soil CO<sub>2</sub> efflux depicts trade-off carbon budget. This indicates the sensibility of soil CO<sub>2</sub> efflux with soil temperature surpassing the influence of soil moisture and other biotic factors. It suggests that the persisting carbon sink will be weakening with the increase in temperature, giving the feedback mechanism regarding carbon cycle under global warming scenario in the subtropical region.

**Keywords** Global warming · Respiration · Sacred groves · Seasonality · Temperature sensitivity

## Introduction

The efflux of soil carbon dioxide (CO<sub>2</sub>) is the respiratory by-product derived from the soil by the activities of roots, soil microorganisms and oxidation of carbon from organic compounds (Lundegardh 1927). Around 60–80% of photosynthetic product is respired into the atmosphere through soil respiration (Bond-Lamberty and Thomson 2010; Hashimoto et al. 2015). Soil, being largest carbon stock of 2700 Gt more than both atmosphere and biomass combined, which

contribute almost 10% CO<sub>2</sub> to the atmosphere through respiratory process (Raich and Potter 1995; Lal 2008). Small scale changes in soil efflux has the potentiality of altering ecosystem C sequestration rate expecting a positive feedback to global warming (Davidson and Janssens 2006) which plays an important role in global carbon cycle (Raich et al. 2002). Among other factors combustion of fossil fuel and changes in the land use pattern also affect in altering global carbon cycle and thereby influences in the relationship between environmental factors and ecosystem carbon dynamics (Vitousek et al. 1997; Magnani et al. 2007). Rising temperature will increase CO<sub>2</sub> emission from the soil which will lead this system to be a contributor to global warming, instead a carbon sink, rivaling the CO<sub>2</sub> emission through fossil fuel combustion and land use changes (Schlesinger and Andrews 2000; Forster et al. 2007). It released 5.4 Pg C year<sup>-1</sup> and 1.6 Pg C year<sup>-1</sup> during 1980–1989 respectively (Folland et al. 1992).

Global fluctuation in the soil respiration rate relates to complex temporal variations of temperature anomalies and soil C stocks (Lei et al. 2021). The study about the magnitude of soil CO<sub>2</sub> efflux and its controlling factor may predict potential feedback of climate change in the present scenario

✉ Chongtham Sanjita  
chsanjita@gmail.com

Rojen Singh Thounaojam  
rjemeitei@gmail.com

Th. Binoy Singh  
thingbajamb@yahoo.com

N. Dhirendra Singh  
singnd@gmail.com

<sup>1</sup> Ecology Laboratory, Centre of Advanced Study in Life Sciences, Manipur University, Canchipur, Manipur 795003, India

<sup>2</sup> Waikhom Mani Girls' College, Thoubal, Manipur, India

of emission of CO<sub>2</sub> in the global carbon cycle (Rustad et al. 2001; Raich et al. 2002). 86% of the global vegetation carbon pool is maintained by forest soil whereas 73% is from the soil carbon pool (Tans et al. 1990; Dixon et al. 1994; Deluca and Boisvenue 2012). Soil respiration is the primary path of returning carbon fixed by land plants to the atmosphere (Barba et al. 2018). Effect of abiotic factors besides substrate availability and litter fall turnover in CO<sub>2</sub> efflux rate had been analyzed in temperate and tropical forest ecosystem (Davidson et al. 2000; Reichstein et al. 2003; Hibbard et al. 2005). Several studies in subtropical forest ecosystem conclude that soil CO<sub>2</sub> efflux is vulnerable to climate change due to increase of soil temperature (Chang et al. 2008; Yan et al. 2009; Tan et al. 2013). There was temperature sensitivity in rate of soil respiration along elevation gradients indicating temperature as one leading factor in the rate limiting (Ma et al. 2017). There was variation in the relationship between soil respiration and soil temperature and moisture in different ecosystems (Mosier et al. 1998; Rustad et al. 2000) which was controlled by precipitation and mean annual temperature (Chang et al. 2008). Thus, precipitation, relative humidity and mean annual temperature directly or indirectly influence the variation of soil respiration.

Biotic factors such as fine root and litter biomass also affect the soil respiration rate as well as in the regulation of ecosystem function involving manipulation in soil CO<sub>2</sub> efflux (Ohashi et al. 2000; Hogberg et al. 2001; Lee and Jose 2003; Metcalfe et al. 2011; Lei et al. 2021). This variability proposes the need for more measurements of soil respiration to explore its environmental dependence on a regional scale (Tang et al. 2006; Bond-Lamberty and Thomson 2010). Even there is large uncertainty while estimating soil respiration rate since soil respiration is regulated by several biotic and abiotic factors (Chen et al. 2017). Therefore, accurate quantification of carbon emissions through soil respiration is of great significance for understanding climate change and the carbon cycle in the earth system (Zeng et al. 2018).

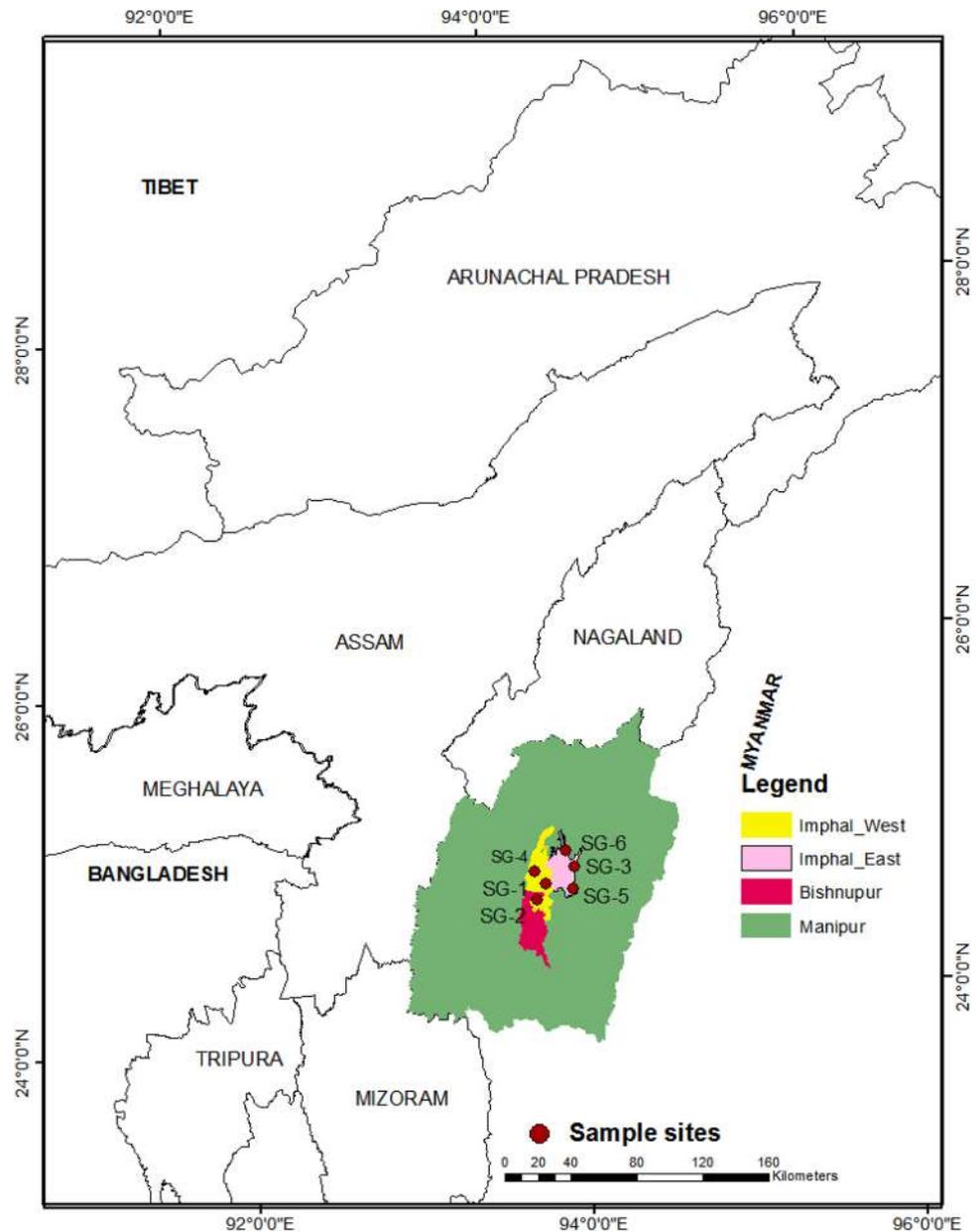
Ecosystems of low latitude and mid latitude regions were estimated to be potential repositories of the missing carbon sink (Tans et al. 1990). The sacred groves of Manipur lie in low latitude region which is maintained by the ecological dynamics of the subtropical ecosystem. It was reported that sacred groves have high diversity of trees (Bhandary and Chandrashekar 2003; Ramanujam and Cyril 2003) with great supportive role in species maintenance while performing different ecological functions (Chima and Nuga 2011). The community composition and species diversity had confounded relationship with soil respiration (Craine et al. 2001; Johnson et al. 2008). Therefore, the understandings of soil respiration rates and influencing factors particularly in northeastern Indian region have a solid empirical base and its accurate quantification becomes very important. Though few researches on factors affecting soil respiration have been

initiated in subtropical forests of north east India however, carbon budget in the sacred grove ecosystem is still lacking. It is highlighted that data pertaining to soil respiration rate was important for synthetic studies (Bond-Lamberty and Thomson 2010; Mahecha et al. 2010). It is very uncertain whether these carbon sinks still persist or weaken under a warming climate in the subtropical region (Tan et al. 2012). The accurate quantification of soil CO<sub>2</sub> efflux in the subtropical forest ecosystem particularly in sacred groves is necessary in the context of warming trend in global atmosphere besides uncertainty on the relative contribution to soil CO<sub>2</sub> efflux by biotic and abiotic factors. It was hypothesized that: (1) soil temperature factor plays crucial role in the determination of rate of soil CO<sub>2</sub> efflux in the subtropical sacred grove forest ecosystems of Manipur, north eastern India, and (2) soil moisture factor plays synergistic role in the CO<sub>2</sub> efflux. It is expected that rate of CO<sub>2</sub> efflux may be high at the sites where high tree density and diversity occurred during warm and moist period because of higher litter decomposition and microbial growth. Thus, the present investigation aims (1) to find out the biophysical parameters including climatic factors controlling the temporal variation of soil CO<sub>2</sub> efflux, and (2) role of litter and fine root biomass in the seasonal variability of soil CO<sub>2</sub> efflux. This study will reflect the role of soil CO<sub>2</sub> efflux in the soil carbon budget in the subtropical sacred grove ecosystem.

## Materials and method

### Study area and general climate

The present study was undertaken at Chajing Lakpa (SG 1), Chaning Lairembi (SG 2), Kalika Lairembi (SG 3), Ibudhou Loiyalakpa (SG 4), Panam Ningthou (SG 5) and Nongpok Ningthou (SG 6) sacred groves conserved and protected by the local people of Manipur, north east India (Fig. 1). These forests patches belong to subtropical broad leaved hill forest type (Champion and Seth 1968) occurring at elevations (797.9–901.29 m above mean sea level) (Table 1). Total of 88 woody species have been found in all the studied sacred groves out of which maximum number of occurrence were observed in SG 3 and least in SG 6 with an average of 28 species per hectare, considering all plots (Table 1). However, maximum stem density was observed in SG 6 and least in SG 1. Mean basal area were observed similarly with other tropical non sacred groves forest ecosystem ranging from  $16.56 \pm 3.28$  as in SG 4 to  $33.83 \pm 14.27$  in SG 5. Shannon Weiner index (H'), concentration of dominance (Cd), species richness index (SR), species evenness index (SE), distribution pattern (DP) for each of the grove were represented along with litter biomass (Table 2). Each studied grove was characterized

**Fig. 1** Location map of the study area**Table 1** Location and physical characters showing mean annual soil temperature (ST) °C, Mean annual soil moisture (SM) %, Soil organic carbon (SOC) % and Mean annual Fine root biomass (Fr) g m<sup>-2</sup> of the studied site

Sacred groves	Latitude/longitude	Altitude (m)	Mean SM (%)	Mean ST (°C)	Mean SOC %	Mean Fr (g m <sup>-2</sup> )	Soil type
SG 1	24° 43' 13.64" N 93° 55' 50.13" E	797.9	24.96 ± 2.46	22.31 ± 2.41	1.67 ± 0.26	116.02 ± 8.53	Loamy sand
SG 2	24° 43' 49.46" N 93° 50' 31.69" E	802.8	23.85 ± 3.66	22.68 ± 2.04	1.54 ± 0.10	83.21 ± 7.11	Sandy loam
SG 3	24° 51' 8.79" N 94° 04' 32.60" E	807.8	23.71 ± 3.55	22.39 ± 2.03	1.50 ± 0.16	128.75 ± 6.03	Sandy loam
SG 4	24° 50' 48.73" N 93° 48' 37.83" E	854.05	25.00 ± 3.77	23.97 ± 1.69	1.51 ± 0.22	154.44 ± 10.30	Sandy loam
SG 5	24° 45' 24.61" N 94° 2' 41.04" E	901.29	24.20 ± 3.28	21.70 ± 2.09	1.54 ± 0.18	152.91 ± 14.15	Sandy loam
SG 6	24° 58' 51.71" N 94° 1' 50.51" E	825.39	24.77 ± 3.02	22.79 ± 2.00	1.53 ± 0.28	147.74 ± 9.75	Sandy loam

by abundant occurrence of tree species such as *Schima wallichii* (DC.) Korth, *Lithocarpus dealbatus* (Hook. F.

and Thomson ex Miq.) Rehder and *Quercus serrata* Murray along with several understorey woody shrubs and

**Table 2** Summary of the structure, diversity and litter biomass of the studied site

Sacred grove	No. of tree species	Average density (Ind Ha <sup>-1</sup> )	Average basal area (m <sup>2</sup> ha <sup>-1</sup> )	H'	Cd	SE	SR	DP	LB (g m <sup>-2</sup> )
SG-1	29	35.77 ± 7.76	25.36 ± 7.61	0.10 ± 0.01	0.003 ± 0.001	0.03 ± 0.003	16.32 ± 1.43	0.05 ± 0.003	90.46 ± 18.55
SG-2	26	35.58 ± 12.85	20.42 ± 11.02	0.01 ± 0.02	0.006 ± 0.003	0.03 ± 0.005	15.07 ± 1.44	0.08 ± 0.004	91.41 ± 21.81
SG-3	31	40.05 ± 8.92	19.81 ± 7.53	0.09 ± 0.01	0.003 ± 0.001	0.03 ± 0.003	15.75 ± 1.50	0.08 ± 0.008	101.01 ± 21.26
SG-4	27	54.79 ± 4.82	16.56 ± 3.28	0.12 ± 0.008	0.002 ± 0.0003	0.04 ± 0.002	8.24 ± 0.52	0.12 ± 0.008	92.68 ± 18.45
SG-5	30	44.58 ± 8.64	33.83 ± 14.27	0.10 ± 0.01	0.003 ± 0.001	0.03 ± 0.003	12.55 ± 1.26	0.05 ± 0.003	103.50 ± 20.17
SG-6	22	62.88 ± 12.03	25.64 ± 6.50	0.13 ± 0.01	0.003 ± 0.0009	0.04 ± 0.004	7.54 ± 0.70	0.06 ± 0.007	97.61 ± 21.03

H' Shannon-Wiener diversity Index, Cd Simpson dominance index, SE species evenness, SR species richness, DP distribution pattern, LB litter biomass

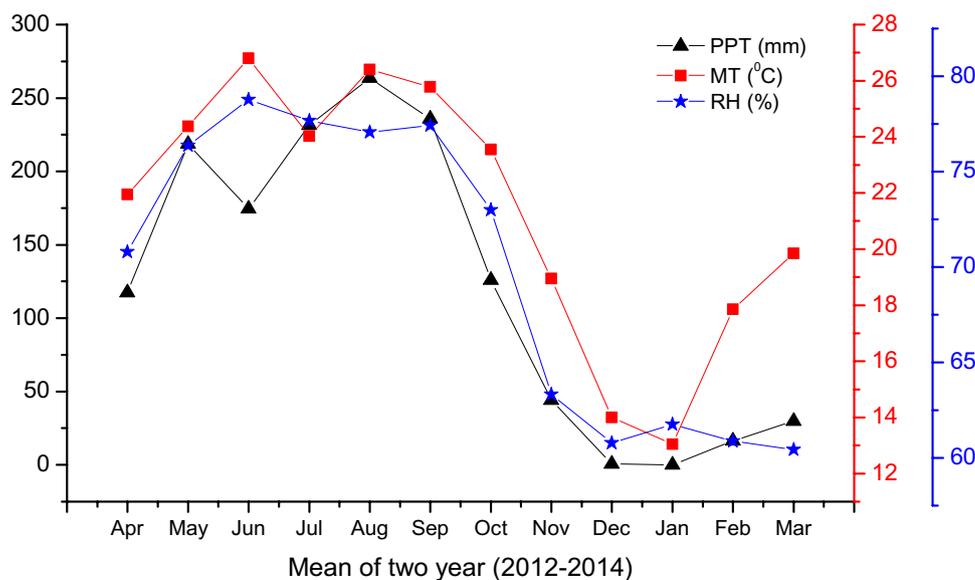
herbaceous species such as *Justicia adhatoda* L., *Capparis tenera* Dalz., *Crateva religiosa* G Forst, *Clerodendrum glandulosum* Lindl., *Lindera pulcherrima* (Nees) Hook. f, *Mussaenda erythrophylla* Schumacher and Thonn., *Holmskioldia sanguine* Retz, *Ocotea lancifolia* (Schott) Mez, *Wendlandia tinctoria* (Roxb.) DC, *Bonnaya brachiata* Link and Otto, *Viola serpens* Wall ex. Roxb, and *Artemisia nilagirica* (C. B. Clarke) Pamp. The lianas found in these forests are *Gymnema acuminatum* Wall, *Tinospora cordifolia* (Willd.) Miers., *Smilax zeylanica* Linn, *Ligustrum indicum* (Lour.) Merr., *Wendlandia wallichii* Wight and Arn, *Paederia foetida* Linn etc. Soil type is loamy sand and sandy loam and pH ranges from 4.43 to 5.43 in all the study sites. The mean temperature during the study period ranged from 13.05 to 26.80 °C and monthly mean rainfall and monthly mean relative humidity ranges from 0.00 to 263.65 mm and 60.43–78.78% showing variation in different season (Fig. 2). Here, the summer season (March–May) characterized as warm, dry period followed by warm moist rainy season (June–October) and winter

(November–February) is generally cold and dry where rainfall is scanty.

### Respiration measurement and data collection

Soil CO<sub>2</sub> efflux was measured using the soil respiration package Q-Box SR1LP (Canada). This system measure rate of CO<sub>2</sub> accumulation in situ using a soil chamber placed over the undisturbed soil surface (closed-flow recirculation system). The measurement was carried out at twelve replicates consecutively in 0.24 ha in every fortnight for two consecutive years (April 2012 to March 2014) in each studied site. The measurement was designed to quantify CO<sub>2</sub> efflux for each studied grove. Soil temperature (ST) °C and soil moisture (SM) % was measured at 0–10 cm depth concomitant with each CO<sub>2</sub> measurement. Six soil cores were collected from each study site using a soil corer and kept in plastic bags in the field itself for determining the total soil organic carbon (SOC %). To determine fine root biomass, twelve (12) soil cores (8.5 × 8.5 × 10 cm<sup>3</sup>) were taken again at six plots in each

**Fig. 2** Mean annual precipitation (PPT) mm, mean annual temperature (MT) °C and mean relative humidity (RH) % during the study period. Data obtained from the Weather station Imphal Airport



grove and kept in plastic bag. Litter were collected from 12 randomly located  $1 \times 1 \text{ m}^2$  litter trap next to the soil  $\text{CO}_2$  efflux measurement point in each of the studied sites. Data on soil respiration, moisture, temperature, litter and fine root biomass were collected on monthly basis for 2 years.

### Laboratory analysis

The soil cores were air dried, grinded and sieved using sieve of 2 mm pore size. Total soil organic carbon (SOC %) was determined using calorimetric analysis (Anderson and Ingram 1993). Soil cores for finding fine root biomass determination were subjected to running water to loosen the soil particles and washed out the particles completely. The sorted roots and collected litter were washed and dried at constant temperature until a constant weight is achieved. The collected litter were washed and dried at  $80^\circ\text{C}$  until to constant weight and weighted for final biomass determination.

### Statistical analysis

All the measured data were normally distributed and used for further analysis with no transformations. One-way ANOVA were calculated for soil respiration, fine root biomass, litter biomass, soil temperature and soil moisture. Exponential function was fitted to find the degree of association between rate of  $\text{CO}_2$  efflux with soil temperature and soil moisture content as well as litter biomass using Statistica 10. The principal component analysis was applied to find relationship between average monthly soil  $\text{CO}_2$  efflux rate and soil physico-chemical parameters as well as average of monthly air temperature, monthly rainfall and relative air humidity recorded at weather stations using software SPSS 23 (2015). The analysis also enables us to find the respective contributions of each measured parameters of the study sites as well as observation for each month to the variability explained by PCs.

## Result

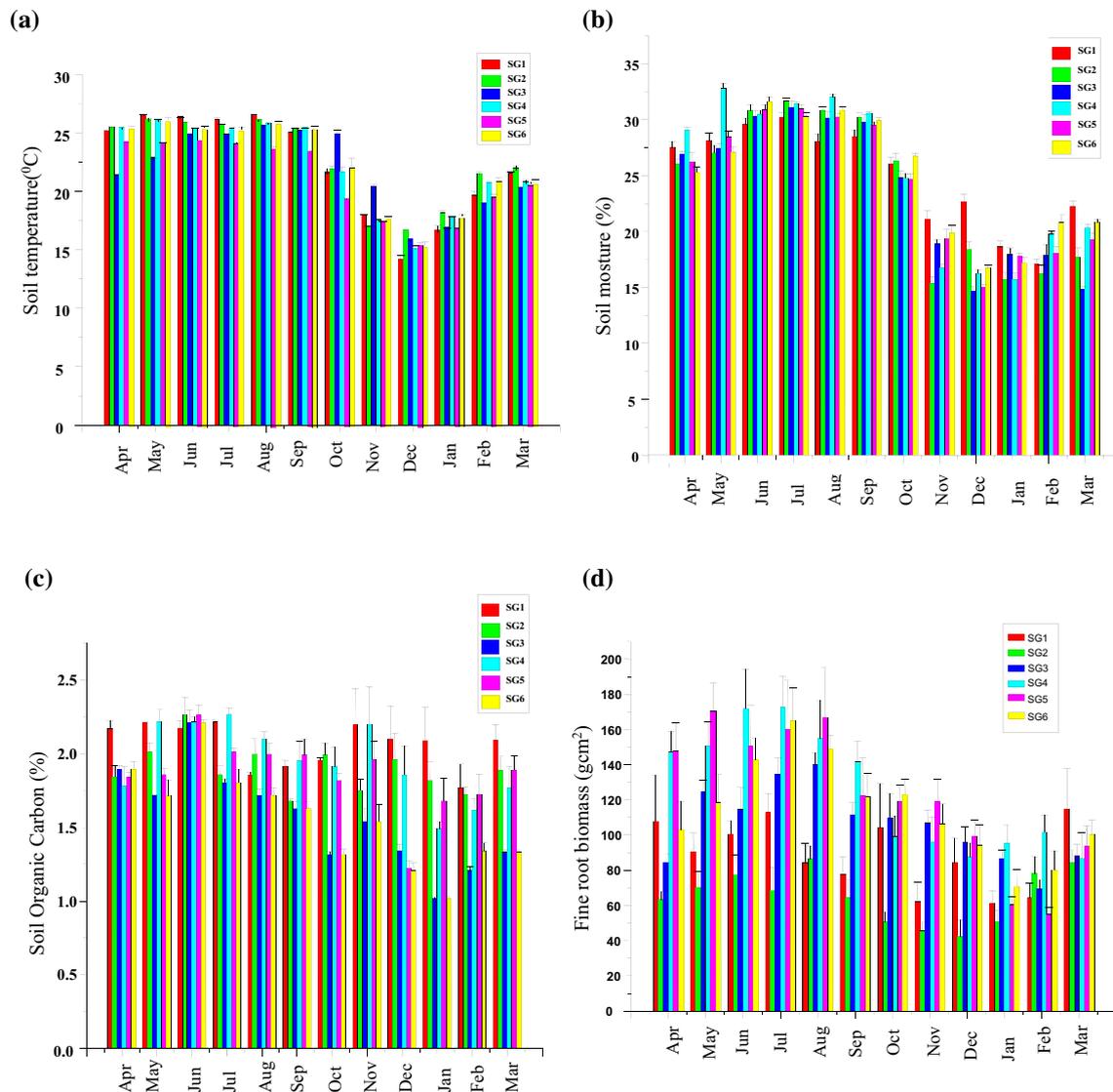
### Soil temperature and soil moisture content

The mean soil temperature ( $^\circ\text{C}$ ) for both years showed strong seasonality in all the study sites, highest during August in all the study sites (SG 1 =  $26.58 \pm 0.48$ ; SG 2 =  $26.18 \pm 0.00$ ; SG 4 =  $26.78 \pm 0.94$ ; SG 5 =  $25.78 \pm 2.41$ ; SG 6 =  $26.89 \pm 0.75$ ) except SG 3 as  $25.93 \pm 0.93$  (June). However, the lowest soil temperature was recorded during December in all the study sites (Fig. 3a). Similar trend of seasonality in soil moisture content was observed during the study period for both years where highest mean soil moisture content was observed during July in SG 1, SG 2, SG 3 and SG 5

( $30.19 \pm 1.27$ ;  $31.65 \pm 0.03$ ;  $31.12 \pm 4.45$  and  $30.93 \pm 3.35$ ). Slight deviation in soil moisture % were observed in SG 4 and SG 6 where highest soil moisture was measured during August ( $32.00 \pm 0.04$ ) and June ( $31.63 \pm 1.24$ ) (Fig. 3b). The minimum soil moisture content was observed during winter, however variation exists among the studied groves in December and January. One-way ANOVA analysis reveals that there was no significant variation in the 2-year data of ST and SM ( $F_{(1, 142)}$  at  $P > 0.05$ ) while ST which showed significant variation ( $F_{(5, 858)} = 2.85$  at  $P > 0.05$ ) but not for SM ( $F_{(5, 858)} = 166.129$ ,  $P > 0.05$ ) among all the studied groves.

### Soil $\text{CO}_2$ efflux, total soil organic carbon and fine root biomass

There was an increase in the  $\text{CO}_2$  efflux rate of the soil from moist summer to rainy season (April to August), but attain its highest values in different months of rainy season in most of the study areas (Fig. 4). A slight decrease in  $\text{CO}_2$  efflux took place during the mid-rainy and decreased in winter in all the studied groves. Maximum annual rate of soil  $\text{CO}_2$  efflux in all the six studied sites are SG 1 =  $758 \pm 354.98$ ; SG 2 =  $798.96 \pm 100.50$ ; SG 3 =  $728.70 \pm 19.06$ ; SG 4 =  $789.35 \pm 172.88$ ; SG 5 =  $950.97 \pm 41.15$  and SG 6 =  $761.70 \pm 139.37 \mu\text{mol m}^{-2} \text{min}^{-1}$ . One way analysis of ANOVA results shows significant variation in  $\text{CO}_2$  efflux rate among months ( $F_{(11, 132)}$  at  $P > 0.05$ ) and there observed significant variation of soil  $\text{CO}_2$  efflux rate ( $F_{(1, 286)}$  at  $P > 0.05$  in SG 1) while rest of the studied groves did not show significant variation in the soil  $\text{CO}_2$  efflux rate between years. However, the monthly soil  $\text{CO}_2$  efflux rate varied significantly among all the studied groves ( $F_{(5, 1722)} = 2.92$  at  $P > 0.05$ ). The percentage of total organic carbon (SOC) content ranged from  $0.97 \pm 0.44$  to  $2.22 \pm 0.00\%$  in (0–10) cm soil depths throughout the study years (Fig. 3c). The mean annual SOC among the studied grove ranges from  $1.50 \pm 0.16$  to  $1.67 \pm 0.26$  (Table 1). There was significant variation in SOC content between years in SG 1 to SG 5 ( $F_{(1, 73)}$  at  $P > 0.05$ ) while SG 6 did not show significant variation at  $P > 0.05$ . However, we found significant difference in SOC content among the studied groves ( $F_{(5, 444)} = 10.66$  at  $P > 0.05$ ). The highest fine root (Fr) biomass at (0–10) cm depth was found in April ( $162 \pm 5.15 \text{ g m}^{-2}$ ) in SG 1 and in May ( $163 \pm 23.96 \text{ g m}^{-2}$ ) in SG 3. Maximum fine roots biomass ( $122.46 \pm 18.73$ ;  $203.14 \pm 28.57$ ;  $210.05 \pm 23.43$  and  $203.32 \pm 35.45$ )  $\text{g m}^{-2}$  in SG 2, SG 4, SG 5 and SG 6, respectively, were observed during rainy season (Fig. 3d). The mean annual fine root biomass ranged from  $83.21 \pm 7.11$  to  $154.44 \pm 10.30 \text{ g m}^{-2}$  among the studied groves (Table 1). One way-ANOVA reveals significant difference between sampling year ( $F_{(1, 118)}$  at  $P > 0.05$ ) in all the studied sacred groves except SG 2 and there exist significant variation among the studied groves ( $F_{(5, 714)} = 27.75$  at  $P > 0.05$ ). Litter



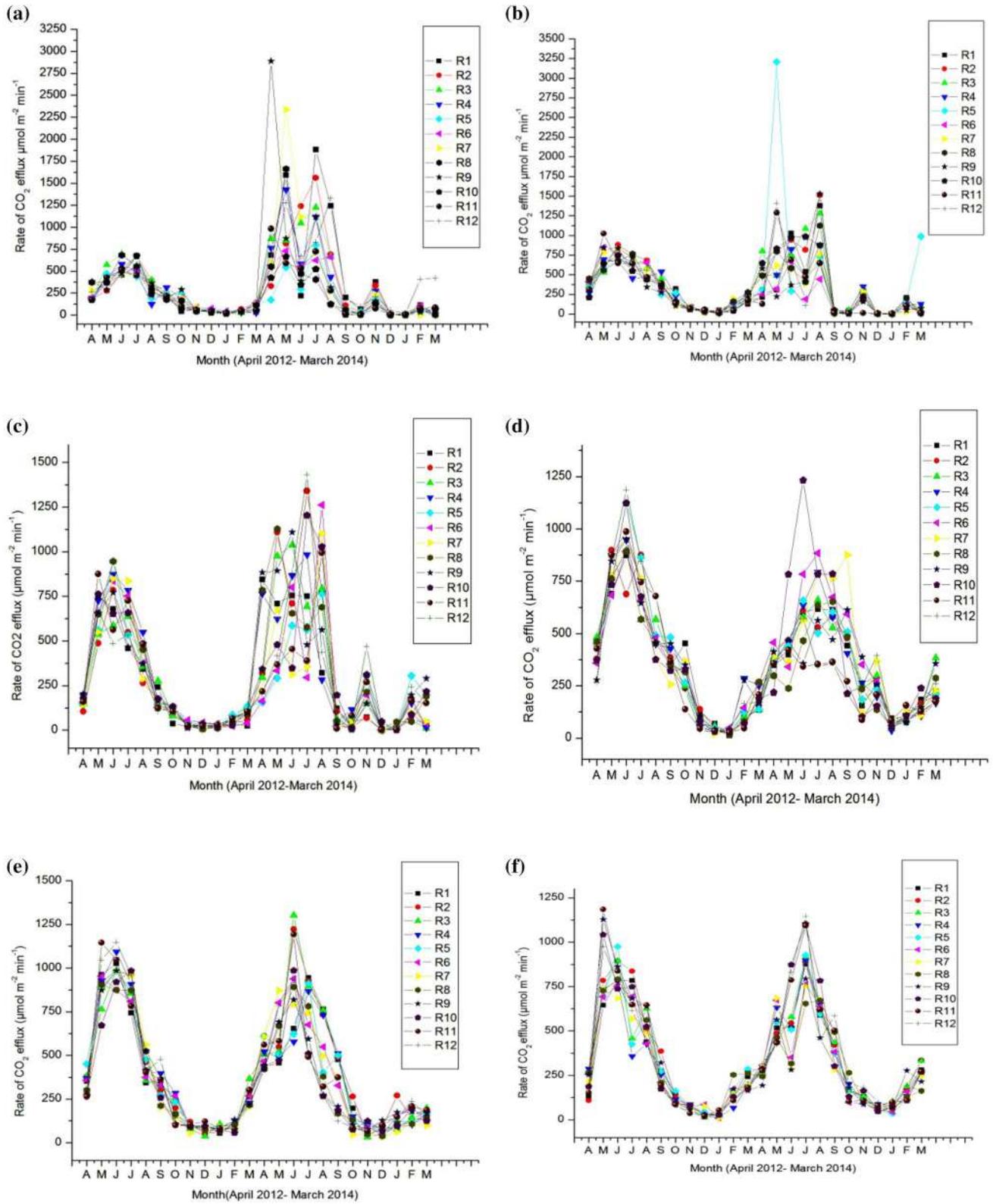
**Fig. 3** Soil temperature (ST) °C, Soil moisture (SM) %, Soil organic carbon (SOC) %, Fine root biomass (Fr) g m<sup>-2</sup> of studied site during the study period. **a–d** ST, SM, SOC, Fr biomass respectively and mean of 2 years data was represented

biomass varied from  $90.46 \pm 18.55$  in SG 1 to  $103.50 \pm 20.17$  in SG 5 (Table 2). Monthly variation in litter biomass was observed in all the studied grove ( $F_{(11, 132)}$  at  $P > 0.05$ ) and significant 2-year variation was observed in SG 3, SG 4 and SG 5 ( $F_{(1, 286)}$  at  $P > 0.05$ ), however no significant variation was found among the studied groves ( $F_{(5, 1722)} = 1.63$  at  $P > 0.05$ ). Soil organic carbon content and fine roots biomass were also high during summer and rainy seasons.

### Relationship between soil respiration and soil temperature, soil moisture, total organic carbon and fine roots biomass

Pearson's correlation analysis reveals that soil temperature (ST) factor showed most significant correlation to the rate of

soil CO<sub>2</sub> efflux in all the studied sites ( $r = 0.80, 0.74, 0.82, 0.92, 0.90, 0.87$ ). There was less relationship between soil CO<sub>2</sub> efflux and soil moisture to monthly rate of CO<sub>2</sub> efflux ( $r = 0.65, 0.54, 0.62, 0.87, 0.82, 0.79$ ) (Table 3). Principal component analysis revealed 80–90% of variability on data sets explained by both PC 1 and PC 2 in the entire study sites. Principal component (PC 1) alone explained the maximum variability (Table 4). Among the predictor variables soil temperature (ST), soil moisture (SM) and soil organic carbon (SOC) contributed maximum percentage of variance explained by PC 1 and PC 2 and least contribution was shown by fine roots (Table 5). Whereas the observations obtained during summer, rainy and winter period showed maximum percentage of contribution to PC 1. The observations during spring season and post rainy season did not



**Fig. 4** Rate of CO<sub>2</sub> efflux (R) μmol m<sup>-2</sup> min<sup>-1</sup> for the six studied sacred grove during the sampling period. Here, R1 to R12 indicates the respiration rate of each sampling in a month for two years. **a-f** represents SG-1 to SG-6 respectively

**Table 3** Correlation (Pearson's) between soil CO<sub>2</sub> efflux (R)  $\mu\text{mol m}^{-2} \text{min}^{-1}$  and soil organic carbon (SOC) %, Fine root biomass (Fr)  $\text{g m}^{-2}$ , Soil moisture (SM) %, Soil temperature (ST) °C, Mean annual precipitation (PPT) mm, Mean annual temperature (MT) °C, Mean annual relative humidity (RH) %

Variables	SG-1	SG-2	SG-3	SG-4	SG-5	SG-6
	R1	R2	R3	R4	R5	R6
SOC	0.83**	0.63*	0.85**	0.92**	0.83**	0.96**
Fr	0.61*	0.69*	0.77**	0.96**	0.74**	0.78**
SM	0.65**	0.54**	0.62**	0.87**	0.82**	0.79**
ST	0.80**	0.74**	0.82**	0.92**	0.90**	0.87**
PPT	0.73**	0.80**	0.75**	0.86**	0.74**	0.83**
MT	0.65*	0.75**	0.71**	0.87**	0.72**	0.77**
RH	0.75**	0.78**	0.77**	0.90**	0.80**	0.82**

\*\* and \*correlation is significant at the 0.01 and 0.05 level (2-tailed)

**Table 4** % of variability explained by Eigenvectors in Principal component 1 (PC1) and Principal component 2 (PC2)

Component	SG 1	SG 2	SG 3	SG 4	SG 5	SG 6
PC1	84.18	81.73	86.97	90.34	86.87	88.64
PC2	7.79	7.50	5.27	5.41	7.05	5.06

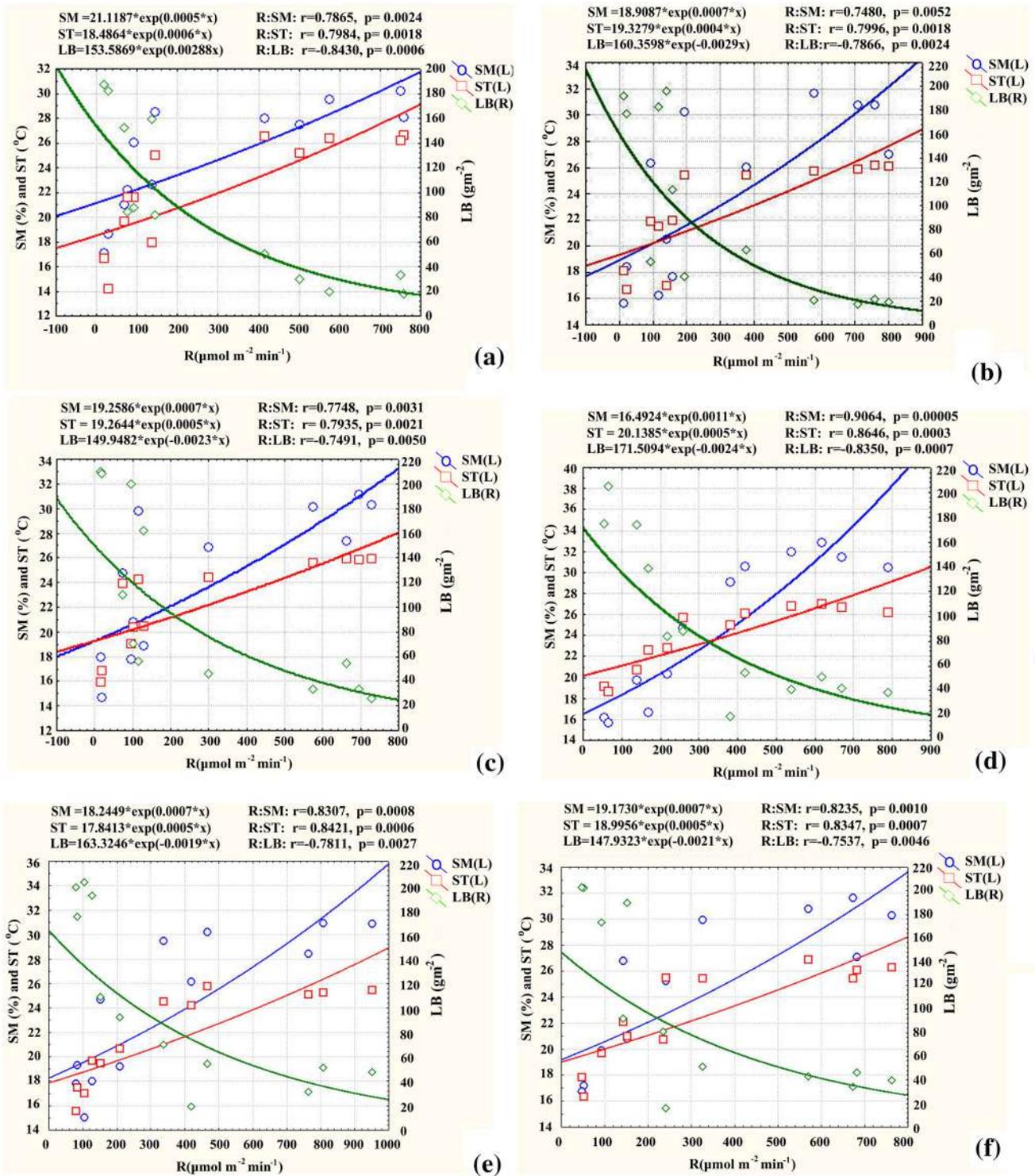
**Table 5** Multicollinearity between variables of soil CO<sub>2</sub> efflux (R)  $\mu\text{mol m}^{-2} \text{min}^{-1}$ , soil organic carbon (SOC) %, fine root biomass (Fr)  $\text{g m}^{-2}$ , soil moisture (SM) %, soil temperature (ST) °C, mean annual precipitation (PPT) mm, mean annual temperature (MT) °C, mean annual relative humidity (RH) % with principal component 1 (PC1) and principal component 2 (PC2) derived from factor analysis

Variables	SG 1		SG 2		SG 3		SG 4		SG 5		SG 6	
	PC1	PC2										
R	0.836	0.098	0.854	0.201	0.857	0.351	0.957	0.197	0.869	0.333	0.904	0.386
SOC	0.955	0.223	0.831	-0.314	0.925	-0.107	0.890	0.434	0.843	0.494	0.925	0.356
Fr	0.777	0.596	0.776	0.577	0.856	0.422	0.966	0.193	0.931	-0.265	0.905	0.018
SM	0.983	-0.038	0.942	-0.169	0.973	-0.171	0.979	-0.027	0.989	-0.117	0.973	-0.177
ST	0.964	-0.023	0.935	0.205	0.975	-0.087	0.950	-0.244	0.959	0.158	0.96	-0.038
PPT	0.923	-0.347	0.952	-0.132	0.958	-0.027	0.954	-0.175	0.950	-0.242	0.964	-0.126
MT	0.941	-0.147	0.966	-0.110	0.943	-0.264	0.942	-0.263	0.945	-0.103	0.940	-0.246
RH	0.941	-0.256	0.956	-0.167	0.967	-0.039	0.965	-0.091	0.960	-0.175	0.960	-0.140

contribute much in the variability explained by PC 1. There was a strong positive correlation between PC 1 and predictors in the entire study sites. Monthly average CO<sub>2</sub> efflux rate showed significant positive correlation with PC 1 (Table 5). In the present investigation, ST and SM for all the studied sites were significantly correlated ( $r=0.87$  at  $P>0.005$ ) as indicated in the correlation analysis. Exponential modelling shows that litter biomass has significant negative relation with soil CO<sub>2</sub> efflux in all the studied groves as SG 1 (-0.84), SG 2 (-0.78), SG 3 (-0.74), SG 4 (-0.83), SG 5 (-0.78) and SG 6 (-0.75) at  $P>0.005$  (Fig. 5).

## Discussion

The mean annual rate of soil respiration in the present study ( $326.96 \pm 163.22$  to  $372.78 \pm 237 \mu\text{mol m}^{-2} \text{min}^{-1}$ ) was comparatively higher than the tropical dry deciduous forest ecosystem of semi-arid area in India ( $205.2 \mu\text{mol m}^{-2} \text{min}^{-1}$ ) (Ahirwal et al. 2021) while the present finding was comparable with the tropical forest in the Amazon that lies in the range of  $261\text{--}585.6 \mu\text{mol m}^{-2} \text{min}^{-1}$  (Sotta et al. 2004),  $324 \mu\text{mol m}^{-2} \text{min}^{-1}$  in 1995/1996 (Malhi et al. 2014),  $318 \mu\text{mol m}^{-2} \text{min}^{-1}$  (Davidson et al. 2000) where temperature lies between 22 and 25 °C. The differences observed in the soil CO<sub>2</sub> efflux rate in the studied sites was due to large and small scale variation in biological, physical and chemical properties of soil (Reichstein et al. 2003; Hibbard et al. 2005). There was no significant inter-annual variability in



**Fig. 5** Exponential relation between Soil CO<sub>2</sub> efflux (R)  $\mu\text{mol m}^{-2} \text{min}^{-1}$  with soil temperature (ST) °C, soil Moisture (SM) % and litter biomass (LB)  $\text{g m}^{-2}$  (a = SG 1, b = SG 2, c = SG 3, d = SG 4, e = SG 5, f = SG 6)

the rate of soil CO<sub>2</sub> efflux in the studied sites except SG 1 which agreed that soil moisture and litterfall was a major

controlling factor in explaining inter-annual variation of soil

respiration as there was no inter-annual variation of SM content and litterfall in the studied groves.

Soil water content may limit CO<sub>2</sub> production during the drying-down period that appeared to be an important factor controlling the efflux rate (Sotta et al. 2004). Inter-annual variation found in SG 1 (Fig. 4a) may be due to variation in slope as it accounted for minor variation besides soil texture class, fine root biomass (Davidson et al. 2000; Hogberg 2001). Monthly variation of soil respiration in the studied site may be due to differences in ST, Fr (Fine root) biomass, SOC content as it was exhibited by significant variation of ST, Fr, SOC within the studied sites. Soil temperature factor alone accounted for a major fraction of the variation in soil respiration when soil moisture was within a site-specific threshold value concomitant with the other studies in subtropical region (Davidson et al. 1998; Rey et al. 2002).

The rate of CO<sub>2</sub> efflux showed an exponential trend with the temperature factor although CO<sub>2</sub> emissions was closely associated with the relative humidity as the evidence of temperature effect was not strong because of the effect of moisture overlaps (Fig. 5). Soil temperature regulates soil respiration rate, mainly by affecting the role of the root system and the decomposition rate of organic matter in the studied sites (Levigne et al. 2003; Wang et al. 2014). In the present investigation, the increased temperature can increase CO<sub>2</sub> efflux, not only because of increased autotrophic respiration but also because of increased respiration of the organic matter in the soil (heterotrophic respiration), especially CO<sub>2</sub> emissions from the litter layer, since the largest carbon stocks in terrestrial ecosystem are in the soil. If the labile part of this carbon is worldwide mobilized, we can have large impacts on the atmospheric CO<sub>2</sub> concentrations. Similar observations were also found in boreal forest (Kelsey et al. 2012). Other studies from subtropical region also reported relationship with soil moisture (Pandey et al. 2010). This may be explained as different vegetation type that showed significant changes in the rate of CO<sub>2</sub> efflux in terms of spatial and temporal pattern (Han et al. 2014). The co-variation of soil moisture and temperature observed (Fig. 5) may be affected by the seasonal patterns of precipitation and air temperature. It was difficult to distinguish the relative importance of moisture and temperature in controlling soil respiration based on current field observations. Confounding factors may play role in the rate of respiration (Davidson et al. 1998; Illeris et al. 2004). Delayed wet season will have significant impacts on soil respiration associated ecosystems components (Yu et al. 2020). In the present investigation the positive significant relationship between precipitation, RH, MT and soil respiration probably might have influenced in the CO<sub>2</sub> efflux as similar findings was also reported (Rey et al. 2002) that the persistent warm moist summer of subtropical climatic condition favor the congenial conditions for microbial growth. Studies also reveal that the soil microbial respiration was responsible

for higher CO<sub>2</sub> efflux under higher rainfall conditions (Sotta et al. 2004). The present investigation exhibited increased soil CO<sub>2</sub> efflux during the wet season suggesting rapid fluctuation in microbial growth and activity that also increase decomposition of organic debris including litter (Valentini et al. 2008; Yu et al. 2020) although abrupt fluctuations were observed in July. It may probably be explained by the fact that intensive precipitation might have affected the microbial activities under unfavourable environmental cues.

The decreasing trend in the rate of soil CO<sub>2</sub> efflux towards winter season in the study sites probably due to decreased activity of both soil microorganisms and fine root biomass. It is found that maximum surface layer is covered by litter fall and minimizing insolation thereby reducing soil temperature and soil moisture during winter. In the present study, litter fall attains its maximum during winter when soil temperature and moisture decreases during dry period. Light intensity gave strong effects on the litter decomposition but effect varied with tree species composition (Ma et al. 2017). It is reported that pattern of litterfall were significantly affected by soil temperature and soil moisture in the subtropical forest ecosystem of north east India (Pandey et al. 2007). Besides litterfall, low precipitation during winter unable to saturate soil moisture and thereby RH alone could not reach optimum soil moisture condition which explains low soil CO<sub>2</sub> efflux rate in the present investigation during this period (Davidson et al. 1998). The combine factors of both edaphic as well as ambient climatic conditions as exhibited in the study sites showed positive effect in the rate of CO<sub>2</sub> efflux. All the environmental factors were closely related in controlling the soil CO<sub>2</sub> efflux as revealed in PCA analysis (Table 4). PC 1 showed significant relationship to the rate of soil CO<sub>2</sub> efflux in all the sites. A strong contribution of summer, rainy and post winter seasons measured variables were observed in the variance explained by PC 1 which indicated their greater role in the seasonal variability of soil CO<sub>2</sub> efflux. Significant positive relationship between soil CO<sub>2</sub> efflux and soil temperature and moisture content suggests that both the factors play synergistic effect in CO<sub>2</sub> efflux of the studied sites (Raich and Schlesinger 1992).

Strong relationships between soil respiration and soil temperature and moisture are considered to be the two most important biophysical parameters controlling the temporal variation of soil respiration in the present investigation and similar findings had also been reported by other workers including tropical forest (Davidson et al. 1998, 2000; Fang et al. 2001; Peixoto et al. 2017). Soil temperature of May to July and December to February showed higher association with soil CO<sub>2</sub> efflux (Fig. 5). Generally, the rate of CO<sub>2</sub> efflux may increase when soils become warmer during summer, post winter period and decrease when moisture content is below the saturation point (Davidson et al. 1998). The increasing trend in the rate of CO<sub>2</sub> efflux from the month

of February onwards and reaching peak during rainy season (June–August) may be explained that the approaching warmer months had congenial climatic condition that enhanced the activity of the soil microbes as well as the fine roots biomass (Janssens et al. 2001). This showed the dependence of soil respiration on the amount of living root biomass mainly fine root since most of the fine roots were distributed in the uppermost layer of the soil (Tripathi et al. 1999). The increase in fine-root biomass is likely due to proportional increases in all plant parts (i.e., bigger plants), because elevated CO<sub>2</sub> has been shown to have little effect on partitioning of biomass between root fractions (King et al. 1996) or other plant parts (Gebauer et al. 1996; Curtis and Wang 1998). Further it may be due to higher tree density and larger diameter trees contributing more autotrophic respiration (Kelsey et al. 2012). There was also strong positive correlation between soil respiration and soil organic carbon (SOC) in the present study. The relationship between soil respiration and soil organic carbon was derived from the component of heterotrophic respiration because heterotrophic respiration is a result of the mineralization of SOC that is stored in large stocks (Fang et al. 2005; Knorr et al. 2005; Reichstein et al. 2005). High organic carbon content under optimum soil moisture in warm temperature induce high respiration rate by increasing the rate of decomposition in subtropical forest ecosystem (Tan et al. 2013) and quantity of dead root as well as soil organic carbon content (Rustad et al. 2000). Further the soil carbon pool that has been accumulated in the studied sacred groves might be largely mineralized and released as CO<sub>2</sub> during the favourable season (Chang et al. 2008).

Litter biomass also influenced on soil CO<sub>2</sub> efflux in all the study sites indicating the positive feedback about functional role of litter biomass to soil atmosphere carbon budget (Fig. 5). Soil respiration and litter fall was found inversely proportional in the present investigation which is in contrast with the lowland tropical Dipterocarp Forest ecosystem (Katayama et al. 2009). In fact, it is observed that soil CO<sub>2</sub> efflux is inversely associated with litter layer biomass not only in cases where there is simply greater litterfall production but also because of decreased respiration rates, indicating the functional role of litterfall in the emission reduction of soil respiration during winter and spring season. The functional role of other biotic factor that contributed in the CO<sub>2</sub> efflux was also reduced considerably and emitted CO<sub>2</sub> help in the mineralization of litter while others remaining carbon get retained in the soil during the cool winter. However, during warm and moist season, the emitted CO<sub>2</sub> help in the rapid mineralization and much of the organic carbon get infiltrated to the soil though this process was influenced by soil type, textural class (Chang et al. 2008) and because of this soil respiration have positive relationship with SOC in the current study. While biotic factors such as

microbial population, fine root, large living roots and soil fauna also contributes in soil respiration. Autotrophic respiration and heterotrophic decomposition of organic matter are all affected by rise and fall in soil temperature (Bond-Lamberty et al. 2004; Bond-Lamberty and Thomson 2010; Melillo et al. 2011). Besides sharing in CO<sub>2</sub> efflux during warm and wet season, litter biomass played a key role in the emission reduction of CO<sub>2</sub> during dry season. It was one of the factors responsible for temporal variation of soil respiration in the studied sacred groves. This study showed that litter biomass gives negative feedback to the variability of soil CO<sub>2</sub> efflux as in fact, it is not the main driver of CO<sub>2</sub> emissions, but it is itself a consequence of low decomposition and/or high litter production. The accumulation of carbon in the ecosystem is a function of factors other than the litter layer itself, which is actually a consequence of the decomposition rates. The humidity and the temperature of the environment have mainly controlled decomposition rate. Thus, soil temperature factor played significant role in the rate of CO<sub>2</sub> efflux in the study sites as predicted earlier. Whereas, soil moisture factor played synergistic effect in determining the rate of CO<sub>2</sub> efflux as soil moisture is not a limiting factor in the rate of efflux of CO<sub>2</sub> since the region received intermittent showers as well as north-east monsoon during the dry seasons. The study sites SG 4, SG 5 and SG 6 experienced higher rate of soil CO<sub>2</sub> efflux than the other three sites as it was expected earlier which may be due to the presence of higher tree density. The environmental conditions enable the increase of CO<sub>2</sub> efflux not only from autotrophic respiration, but also from the decomposition of litter layer and organic compounds in the soil profile. Therefore, forest vegetation along with litter layer should be conserved in order to reduce the rate of CO<sub>2</sub> efflux and most of the soil carbon should get retained in the soil in the present warming trend of surface temperature.

## Conclusion

The seasonal variation in soil respiration rate is influenced by abiotic and biotic derived factors that strongly correlated with soil temperature rather than soil moisture. It suggested that temperature is the main factor driving the respiratory processes of these subtropical ecosystems and the climate change can strongly influence these CO<sub>2</sub> emissions rates. Considering that the increase in CO<sub>2</sub> efflux is directly proportional to the temperature increase, this process could result in increased CO<sub>2</sub> emissions from the ecosystem in the global warming scenario as both autotrophic and heterotrophic respiration is also influenced by temperature factor. Simultaneously seasonal rate of soil respiration is strongly related to the rate of soil organic carbon addition that depends on the quality of the organic material and

decomposition rates. Higher vegetation litter layer production is often associated with decreased respiration rate in the subtropical forest ecosystem. Further work on temperature sensitivity to soil respiration rate will enhance our understanding of the rate limiting factor in soil respiration in subtropical sacred grove forest. The counter act mechanism of litter dynamics and related soil nutrients with soil CO<sub>2</sub> efflux rate, it should also be investigated further on the CO<sub>2</sub> efflux from these regions that will enhance our understanding on the carbon source-sink mechanism of sacred grove ecosystem in the subtropical region. It further can recommend the need for conservation and protection of such sacred grove in these regions so as to check emission of carbon dioxide due to global warming.

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