



Research article

Seasonal variations in soil characteristics control microbial respiration and carbon use under tree plantations in the middle gangetic region

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ABSTRACT

Seasonal variations directly impact the biochemical and microbial properties of the soil, influence carbon and nutrient cycling within the soil system. Soils under tree plantation (TP) are rich in organic matter and microbial population, making them more susceptible to seasonal variation. We studied the effect of seasonal variations in soil chemical properties (pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN), C/N ratio etc) and microclimate (moisture and temperature) on microbial respiration (SR), biomass, and carbon (C) utilization efficiency under 13 years old Kadamb (*Anthocephalus cadamba* Miq.), Simaraubha (*Simarouba glauca* DC), and Litchi (*Litchi chinensis* Sonn.) based TPs in middle Gangetic region. In contrast to higher SR and metabolic quotient (qCO₂) in winter, the microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in fall > summer > spring > winter, irrespective of TPs. The positive relationship between qCO₂ and C/N ratios strongly supports the dependence of microbes on soil carbon for respiration. qCO₂ had a significantly positive relationship with soil moisture (MC) and Electrical conductivity (EC), but a significantly negative relationship with temperature and pH. Higher MBN/TN and MBC/TOC ratios fall under simaraubha, and litchi-based TPs indicated more nitrogen (N) and carbon accumulation into microbial biomass. The seasonal variation of MBC/MBN ratios signifies the changes in microbial communities and fungi dominate over bacteria during winter, as bacteria have a lower C/N ratio than fungi. Stepwise regression analysis suggested that soil properties and micro-climate regulated microbial biomass and SR differ with TPs. Thus, the study indicates that microbial activities and biomass production can significantly influence by soil properties and seasonal variations under TPs.

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1. Introduction

Microbes in soil are one of the most important building blocks of terrestrial ecosystem. The soul of any ecosystem and control nutrient cycling by regulating various biochemical processes like the decomposition of organic remains, immobilization and mineralization of nutrients [1–4]. These processes are related to microbial population contained in the soil, that is, microbial biomass. Microbial biomass provides an easily available supply of soil organic carbon (SOC), nitrogen and phosphorus [5,6]. Although, soil microbial biomass containing only around 5 % of total soil organic matter (SOM), but it is considered as an important factor in determining changes in biochemical properties of soil [7].

The climatic factors, microbial biomass constituent and their actions affect the degradation process of plant litter to release available nutrients [8,9]. Microbes play a crucial role in the transformation and mineralization of soil carbon. Thus, soil microbial biomass carbon (MBC) production and SR are influenced by climatic factors [9,10]. The efficiency of MBC depends on the ratio of carbon used for microbial growth and carbon consumed. Hence, microbial carbon use efficiency signifies the progress of microbial biomass production and respiration to generate energy for the growth and development of microorganisms [11,12]. Microbial metabolic quotient (qCO_2) is the ratio of microbial respiration to MBC. Microbial metabolic quotient denotes an indicator of carbon produced per unit of microbial biomass to reveal the ecological condition of microorganisms and the status of microbial processes [13]. Though qCO_2 cannot measure biomass accumulation and changes of carbon addition into biomass to respired, it can be used to determine microbial carbon use efficiency [14]. On the other hand, some recent studies have identified qCO_2 as an indicator of microbial carbon use efficiency and carbon cycle [14–17]. A low value of qCO_2 usually signifies high microbial carbon use efficiency, and the ecosystem is stable [18–20].

Trees can influence the variations and intensity of microorganisms and soil biochemical characteristics in multiple ways, mainly because of the differences in the amount and composition of litter and root exudates [21,22]. On the other hand, seasonal variations in microbial processes significantly control soil nutrient release and plant accessibility. Seasonal microbial actions and biomass variations are directly linked with seasonal soil water presence and temperature [23,24]. Furthermore, seasonal changes in environment and soil characteristics directly regulate qCO_2 and microbial biomass production, emphasizing deep seasonal relationships in microbial carbon use efficiency as qCO_2 usually increases with litter addition [25].

The Middle Indo-Gangatic region is one of the most highly populated regions in the world and is of great importance in South Asia's agricultural production. The area has huge agricultural potential, as most crops' average productivity is lower than national average. Extensive agriculture is not prominent in this region as small landholding resource poor farmers cannot sustain enough production with the required input supply. Annual monsoon floods and off-season water scarcity very often affect crop yields leading to economic loss to the small and marginal farmers. Thus, tree plantation is considered a better option in this environment to reduce input loss and make farming more resilient by providing additional byproducts, such as fodder, fuel and fruits [26]. However, understanding the factors that influence soil microbial activities is important for developing sustainable tree plantation (TP) systems with different trees and crops associations.

Microbial biomass can be considered a consistent and responsive indicator for soil management under TP [27–29]. Hence, qCO_2 indicates the influence of climatic factors (moisture, temperature) and other factors (organic carbon, total nitrogen, C/N ratio, pH, EC) controlling microbial actions and residue decomposition [30,31]. The ratios of MBC to TOC, MBC to MBN, and MBN to TN are valuable indices for studying SOM dynamics and are considered better indicators of soil microbial status over only monitoring SOC [14,32]. The changes in these ratios reveal a change in comparative biomass between fungal and bacterial populations, and a higher MBC/MBN ratio suggests the presence of more fungal population [33,34]. In addition, these ratios also signify the effect of seasonal changes on microbial actions in soil.

We demonstrated that MBC and qCO_2 in soil are effective indices of microbial carbon utilization and microbial activity, which vary significantly with seasonal changes in soil attributes such as water content, temperature, pH, and electrical conductivity (EC) which would influence soil biological indices in TP systems. The selected trees for TPs are prevalent and grows well under regional agro-climatic conditions. This experiment aimed to i) study how the three TPs influence seasonal trends in SR and microbial biomass in the Indo-Gangetic region, ii) determine whether qCO_2 can be a reliable parameter of microbial carbon use under different TPs and seasons, iii) investigate the relationships between microbial indices (MBC, respiration, nitrogen, etc.) and soil physiochemical characteristics. This study helps to understand the importance of various parameters and their interrelations concerning seasons and TP systems.

2. Materials and methods

2.1. Tree plantation sites and characteristics

The experiment site was situated bank of river Burhi Gandak, one of the northern tributaries of the Ganga under the Indian state of Bihar (26° 61' 34" N; 84° 60' 29" E). All three TPs have similar climatic features. The three different TPs under this study include 13 years tree species of Kadamb (*Anthocephalus cadamba* Miq.), Simarauba (*Simarouba glauca* DC), and Litchi (*Litchi chinensis* Sonn.). Kadamb and Simaraubha are commonly found in this region, mostly in forests and roadsides. Turmeric (*Curcuma longa*) was grown an intercrop during the initial ten years, which later stopped as shady ground making it inferior for turmeric cultivation. This region's climatic conditions and soil naturally favours the cultivation of Litchi tree plantation, making the region the top producer of litchi in India [35]. The features of TPs and soils under this experiment are listed in Table 1. The soil texture reported in all three TPs was sandy

loam. The variations of sand, silt, and clay proportions under TPs are negligible. Based on the Köppen-Geiger climate classification, the location falls under "subtropical monsoon and hot summer" (<http://koeppen-geiger.vu-wien.ac.at/applications.htm>). Weather data were collected from the India Meteorological Department, Patna (<https://mausam.imd.gov.in/patna/>) (Fig. 1). Recorded annual precipitation was 937 mm, with the highest recorded value in August (388 mm) and no rainfall received during November and December. The mean annual temperature was 32 °C. The study site is situated on plain land at an elevation of 70 m from sea level. Mean soil pH was high (8.4) and contained a high amount of CaCO₃ in the topsoil.

2.2. Experimental Design and sampling

Soil sampling was done in four seasons, i.e., in summer (June), fall (September), winter (December) 2018, and spring (March) 2019 at each TP. Each sample was a composite of eight separate soil samples collected (four each at 50 cm and 2 m away from tree bases in four directions) from a depth of 15 cm and 30 cm. A total of 720 soil samples were taken, 30 samples from two soil depths of three TP systems during four seasons. The study site consists of a total area of 1.5 ha, which is equally divided into three tree plantation plots specifically developed to conduct experiments. The spacing maintained in all three plantations was 7 × 7 m.

2.3. Laboratory analysis

Soil pH and EC were determined using 1:2 (soil: water) suspension with a pH and EC meter [36]. Soil moisture was estimated by oven drying at 100 °C until a constant weight was achieved [37]. Soil temperature (°C) was collected using Temp 4/5/6 Thermistor Thermometer at a depth of 10 cm during noon. The sample soils were further grounded and sieved (2 mm) for nutrient analysis. TOC and TN were determined through the potassium dichromate oxidation method and the Kjeldahl digestion method [36]. Fresh soil samples were shed dried to adjust moisture content to 35%–40 % of total water holding capacity and kept in a cool place for microbial analysis. The alkali trap method was used to estimate SR [38]. A 10 ml glass beaker was placed on top of the aluminium plate containing 20 g of soil on double filter paper (with the help of a stand) inside a wide-mouth mason jar. The beaker contained 9 ml of 0.5 M KOH to trap released CO₂ from the soil. Distilled water was pipetted into the jar onto the side so that the water run down and was wicked up into the soil through the filter paper. The jar was sealed and remained incubated for 4 days. After the incubation electrical conductivity of the KOH solution was measured using an EC meter. The CO₂ released was calculated by comparing the EC of the blank trap solution with solution representing soil samples.

The chloroform fumigation extraction method was used to estimate MBC and MBN [39]. Each soil sample was divided into two portions (10 g each). The fumigation of the first portion of the soil sample with chloroform was done at 25 °C for 24 h in a desiccator. The second portion of the soil sample was mixed with 0.5 M K₂SO₄ (40 ml), followed by shaking the suspension (200 rpm for half hr) and filtering through the Whatman-42 filter paper. Similarly, the first portion of the soil sample goes through this extraction process. Total organic carbon present in both fumigated and non-fumigated extracts was estimated as mentioned in the Walkley Black method [39,40]. Microbial biomass carbon was estimated by Ref. [41]:

$$MBC = \frac{C_e}{K_c} \quad (1)$$

Where, C_e is the difference between the total organic carbon (TC) of two soil portions and K_c is the factor related to biomass carbon produced by the fumigation method, i.e. 0.45. The total nitrogen in the fumigated and non-fumigated extracts were determined through the Kjeldahl digestion method, and MBN was estimated as [39,42]:

$$MBN = \frac{N_e}{K_n} \quad (2)$$

Where, N_e is the difference between total nitrogen (TN) of two soil portions and K_n is the factor related to biomass nitrogen produced by the fumigation method, i.e. 0.54. The microbial metabolic quotient (qCO₂) was calculated by following [43,44].

Table 1

Characteristics of tree plantation systems and soil at the experiment site.

Characteristics/Properties	Tree species		
	Kadamb	Simaraubha	Litchi
Longitude	25° 60'27"	25° 60'29"	25° 59'32"
Latitude	85° 49'43"	85° 49'44"	85° 50'08"
Altitude (m)	12.04	12.04	11.92
Stand age (year)	13	13	13
Stand canopy (%)	55–84	60–91	63–94
Sand (g kg ⁻¹)	665 ± 39	672 ± 21	689 ± 96
Silt (g kg ⁻¹)	180 ± 20	177 ± 09	196 ± 39
Clay (g kg ⁻¹)	155 ± 15	151 ± 72	115 ± 67
Soil texture	Sandy loam	Sandy loam	Sandy loam
pH classes	Moderately alkaline	Moderately alkaline	Moderately alkaline

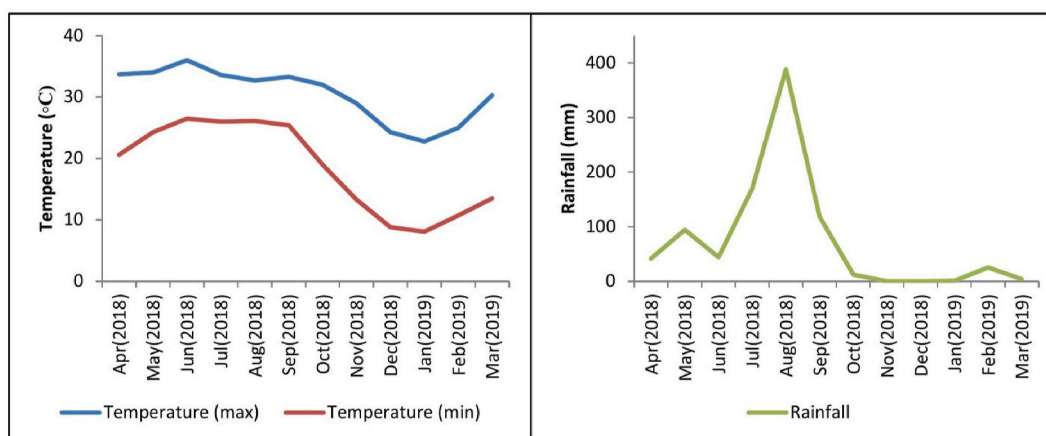


Fig. 1. Temperature and Rainfall during the study period.

$$qCO_2 = \frac{\text{Microbial respiration}}{\text{Microbial biomass carbon}} \quad (3)$$

2.4. Data analysis

To check normal distribution before conducting the analysis of the variance test, Skewness-Kurtosis and Shapiro-Wilk tests were performed [45]. A two-way analysis of variance (ANOVA) was used to evaluate the influence of TP and seasons on the recorded parameters, and Tukey's post hoc test was employed for multiple mean comparisons. Bivariate plots were used to perform regression analysis to show relations between microbial characteristics and soil properties. Stepwise multiple regression was utilized to identify the variables with the highest variations in SR, MBC and MBN under TP. Principal component analysis (PCA) was conducted to visualize the relationships between soil properties and microbial parameters across different TPs and seasons. All the tables and figures presented mean values ($n = 30$) of the data.

3. Results

Tree plantations, seasons, and their interactions significantly influenced soil properties and microbial activity indicators under investigation (Table 2). The influence of TPs on soil temperature, moisture, qCO_2 , MBC/MBN ratio was insignificant.

The highest soil temperature was observed under The Kadamb based TP in all seasons except spring. On the other hand, soil temperature in the winter season was significantly lowest, irrespective of TPs (Table 3). Soil temperature under the Simaraubha TP remained intermediate in all seasons. The lowest soil moisture was found under the litchi TP in all seasons. Simaraubha TP had the highest soil moisture content in all seasons except winter. As predictable, all TPs had the highest soil moisture during the fall season due to monsoon rainfall. The fall season had 94 % higher soil moisture than the summer (Table 3). Soil pH and EC were the highest

Table 2

Effects of TP, seasons, and their interactions on soil and microbial characteristics (Two-way ANOVA, F statistics).

Characteristics	F-values		
	TP ($df = 2$)	Season ($df = 3$)	TP \times season ($df = 6$)
Soil moisture	2.17 ^{ns}	325 ^{***}	19.6 ^{***}
Temperature	1.94 ^{ns}	3169 ^{***}	29.4 ^{***}
pH	106 ^{***}	27.2 ^{***}	52.3 ^{***}
EC	57.9 ^{***}	8.61 [*]	5.11 ^{**}
TOC	26.1 ^{***}	20.8 ^{***}	4.96 ^{***}
TN	82.7 ^{***}	98 ^{***}	23.4 ^{***}
C:N ratio	39.2 ^{***}	371 ^{***}	42.3 ^{**}
Microbial respiration	12.6 ^{***}	59.7 ^{***}	9.14 ^{***}
MBC	17.5 ^{***}	72.6 ^{***}	7.35 ^{***}
MBN	62.4 ^{***}	22.3 ^{***}	5.82 ^{**}
MBC/MBN ratio	2.98 ^{ns}	7.42 ^{***}	3.45 [*]
MBC/TOC ratio	13.1 ^{***}	65.7 ^{***}	10.9 ^{***}
MBN/TN ratio	10.3 ^{***}	58.6 ^{***}	8.05 ^{***}
qCO_2	2.45 ^{ns}	37.8 ^{***}	4.98 ^{***}

df: degree of freedom; *P < 0.05, **P < 0.01, ***P < 0.001, ns: nonsignificant. EC: electrical conductivity, TOC: total organic carbon, TN: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass nitrogen.

Table 3

Seasonal temperature, moisture, pH, and EC of soil under TPs over four seasons.

Season	Temperature (°C)			Soil moisture (g kg ⁻¹)			pH			EC (μS cm ⁻¹)		
	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi
Summer	37.23 ± 0.21 ^d	37.14 ± 0.37 ^d	36.94 ± 0.15 ^d	409 ± 19 ^c	428 ± 46 ^c	424 ± 32 ^c	8.73 ± 0.09 ^g	8.47 ± 0.08 ^f	8.69 ± 0.09 ^g	295 ± 15 ^{ab}	321 ± 18 ^b	349 ± 10 ^{bc}
Fall	34.22 ± 0.26 ^c	34.12 ± 0.17 ^c	33.26 ± 0.14 ^c	846 ± 67 ^d	847 ± 59 ^d	756 ± 45 ^d	7.97 ± 0.05 ^d	8.13 ± 0.05 ^{de}	8.36 ± 0.07 ^{ef}	289 ± 13 ^{ab}	268 ± 17 ^a	452 ± 19 ^d
Winter	12.56 ± 0.07 ^a	12.35 ± 0.15 ^a	12.09 ± 0.21 ^a	268 ± 56 ^a	257 ± 35 ^a	246 ± 97 ^a	7.78 ± 0.04 ^c	7.61 ± 0.05 ^b	9.05 ± 0.09 ^h	308 ± 12 ^{ab}	345 ± 19 ^b	425 ± 23 ^{cd}
Spring	25.06 ± 0.11 ^b	24.70 ± 0.12 ^b	25.21 ± 0.16 ^b	378 ± 11 ^b	389 ± 28 ^b	338 ± 45 ^b	7.15 ± 0.06 ^a	8.46 ± 0.05 ^f	8.59 ± 0.06 ^f	316 ± 22 ^{ab}	234 ± 16 ^a	442 ± 22 ^d

EC: electrical conductivity.

under litchi TP in all seasons (Table 3). In the litchi TP highest mean soil pH (9.05) was recorded during the winter, which is also the overall highest soil pH. Whereas, the summer season recorded the highest soil pH under the Simaraubha and Kadamb-based TP. Simaraubha had the lowest soil EC in all seasons except the winter. There were no significant variations of soil EC values under Simaraubha and Kadamb TPs, but all values were significantly lower over the soil EC under Litchi TP, irrespective of seasons. The mean annual soil pH (8.67) and EC ($417 \mu\text{S cm}^{-1}$) under the Litchi TP were 6 % and 9 %, and 46 % and 42 % higher over the soil pH and EC in the Simaraubha and Litchi TPs, respectively. The soil pH ranged from neutral to moderately alkaline (7.15–8.73) under Kadamb and Simaraubha TPs, and moderate to strongly alkaline (8.36–9.05) under the Litchi TP. The TOC under the litchi TP (328 g kg^{-1}) was 3 % and 8 % higher over the Kadamb (316 g kg^{-1}) and Simaraubha TPs (302 g kg^{-1}), respectively (Table 4). The TOC under the Kadamb TP was greater than those under the Simaraubha and Litchi TPs in all seasons except in the fall season. In the case of TN, Litchi TP had higher contents over other TP during summer, fall and spring. The mean TN under the Litchi TP (15.2 g kg^{-1}) were 13 % and 15 % more than the Kadamb (13.5 g kg^{-1}) and Simaraubha (13.2 g kg^{-1}) based TPs, respectively. Furthermore, TN under Litchi was higher over other TP, except in winter (Table 4). The highest mean C/N ratio was found in soil under litchi TP (34.1), followed by Kadamb (31.2) and Simaraubha TPs (30.9) during winter. Overall, the lowest C/N ratio was also observed under Litchi TP (19.4) in summer. However, the mean annual C/N ratio under the Kadamb was 25.9, which was 4 and 8 % higher than the Simaraubha and Litchi TPs, respectively (Table 4).

Among the seasons, mean SR was higher in winter ($10.5 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) followed by spring, fall and summer (8.8, 8.4 and $7.8 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) (Fig. 2a). Simaraubha TP had significantly greater respiration rate in all seasons and Kadamb TP had the overall lowest respiration rate during summer. The mean MBC and MBN were recorded highest during fall, followed by summer and spring (Fig. 2b and c). During the winter, significantly the lowest MBC and MBN were recorded under all TP. The maximum MBC and MBN were observed during fall season in all TPs. It was also observed that Simaraubha TP had the overall maximum MBC and MBN during the fall season, which were 10.4 g C kg^{-1} and 1.37 g N kg^{-1} . Litchi TP generally had intermediate MBC and MBN values, while the Kadamb TP had the lowest values. The average MBC in the Simaraubha TP (8.2 g C kg^{-1}) was 19 % and 30 % more than the Litchi and Kadamb TPs, respectively. Similarly, the MBN content under Simaraubha TP (0.9 g N kg^{-1}) was 12 % and 28 % higher than those under the Litchi and Kadamb-based TPs, respectively. Based on microbial respiration rate and MBC, the average qCO_2 was found to be the highest during winter (6.7) and lowest during fall (2.8), respectively (Fig. 2d). During the winter, Simaraubha TP had the highest qCO_2 (4.9), which was significantly higher over those under Litchi (3.7) and Kadamb TP (4.0). The lowest mean qCO_2 was observed during summer under Litchi and Kadamb TPs, but during fall under Simaraubha TP. The violinplot indicated the depth wise distribution of SR, MBC, MBN and qCO_2 (Fig. 3a–d). Surface soil (15–30 cm) had a wider range and higher values of all four parameters over lower soil (15–30 cm).

The results revealed that Litchi based TP had a significantly higher MBC/MBN ratio during summer (13.85), fall (12.31), and winter (17.43) over other TP (Table 5). Although, the overall lowest MBC/MBN ratio was also observed under Litchi TP during spring (8.42). There were no significant differences between the MBC/MBN ratio of Kadamb and Simaraubha TPs in all seasons, except in winter. The highest MBC/TOC ratio was recorded during fall and the lowest during the winter under all TPs (Table 5). The mean annual MBC/TOC ratios were 2.67, 3.29, and 2.56 under Kadamb, Simaraubha and Litchi based TPs, respectively. Simaraubha TP had MBC/TOC ratio value of more than 4 during fall and spring seasons; those are also higher over other TPs. The winter season had significantly lower MBC/TN ratios over other seasons in all TPs. The range of MBC/TN ratios across seasons were 3.06–8.61, 3.41–9.49, and 3.38–9.67 under the Kadamb, Simaraubha and Litchi TPs, respectively (Table 5). Litchi TP (9.67) recorded the highest MBC/TN ratio followed by Simaraubha TP (9.34) in the fall season.

The bivariate scatterplots indicated that the MBC/MBN ratio had a significant positive correlation with the C/N ratio in all TP (Fig. 4a–c). A significant negative correlation was observed between qCO_2 and soil temperature, irrespective of TPs (Fig. 5a–c). On the other hand, soil moisture positively correlated with qCO_2 under all TPs (Fig. 5d–f). All TPs had shown significant negative correlations between pH and qCO_2 (Fig. 5g–i), but qCO_2 showed significant positive correlations between EC and qCO_2 (Fig. 5j–l). PCA biplots showed that the relationship between soil characteristics and microbial activity indicators varies among the TPs (Fig. 6a–c) and seasons (Fig. 7a–d). There was a positive correlation between SR and MC, while both had a negative correlation with soil temperature in all TPs (Fig. 6a–c). Soil EC negatively correlated with MBN and MBC, irrespective of TPs. The TOC and TN had a negative correlation in Kadamb and Litchi TPs, but a positive correlation in Simaraubha TP. In all seasons, MC was found to have a positive correlation with SR and MBC (Fig. 7a–d). The correlations between qCO_2 and soil C/N ratio were significantly positive in all TPs (Fig. 8a–c). The

Table 4
Seasonal TOC, TN, and C/N ratio of soil under TPs over four seasons.

Season	TOC (g kg^{-1})			TN (g kg^{-1})			C/N ratio		
	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi
Summer	$316 \pm 4.67^{\text{cd}}$	$315 \pm 5.21^{\text{cd}}$	$327 \pm 6.12^{\text{de}}$	$12.6 \pm 0.37^{\text{a}}$	$13.9 \pm 0.46^{\text{b}}$	$16.7 \pm 0.42^{\text{d}}$	$27.5 \pm 0.59^{\text{e}}$	$24.1 \pm 0.53^{\text{d}}$	$19.4 \pm 0.29^{\text{a}}$
Fall	$240 \pm 5.36^{\text{a}}$	$289 \pm 7.81^{\text{b}}$	$309 \pm 2.90^{\text{cd}}$	$15.1 \pm 0.37^{\text{c}}$	$14.2 \pm 0.32^{\text{b}}$	$16.5 \pm 0.40^{\text{d}}$	$20.5 \pm 0.21^{\text{ab}}$	$22.4 \pm 0.25^{\text{c}}$	$20.4 \pm 0.41^{\text{a}}$
Winter	$361 \pm 7.17^{\text{fg}}$	$326 \pm 6.09^{\text{de}}$	$353 \pm 7.53^{\text{fg}}$	$12.3 \pm 0.29^{\text{a}}$	$11.2 \pm 0.25^{\text{a}}$	$11.4 \pm 0.37^{\text{a}}$	$31.2 \pm 0.35^{\text{f}}$	$30.9 \pm 0.28^{\text{f}}$	$34.1 \pm 0.88^{\text{h}}$
Spring	$348 \pm 7.32^{\text{f}}$	$279 \pm 7.06^{\text{b}}$	$323 \pm 7.25^{\text{de}}$	$13.7 \pm 0.26^{\text{b}}$	$13.5 \pm 0.24^{\text{b}}$	$16.3 \pm 0.36^{\text{d}}$	$24.4 \pm 0.28^{\text{d}}$	$21.9 \pm 0.38^{\text{c}}$	$21.2 \pm 0.42^{\text{bc}}$

TOC: total organic carbon, TN: total nitrogen.

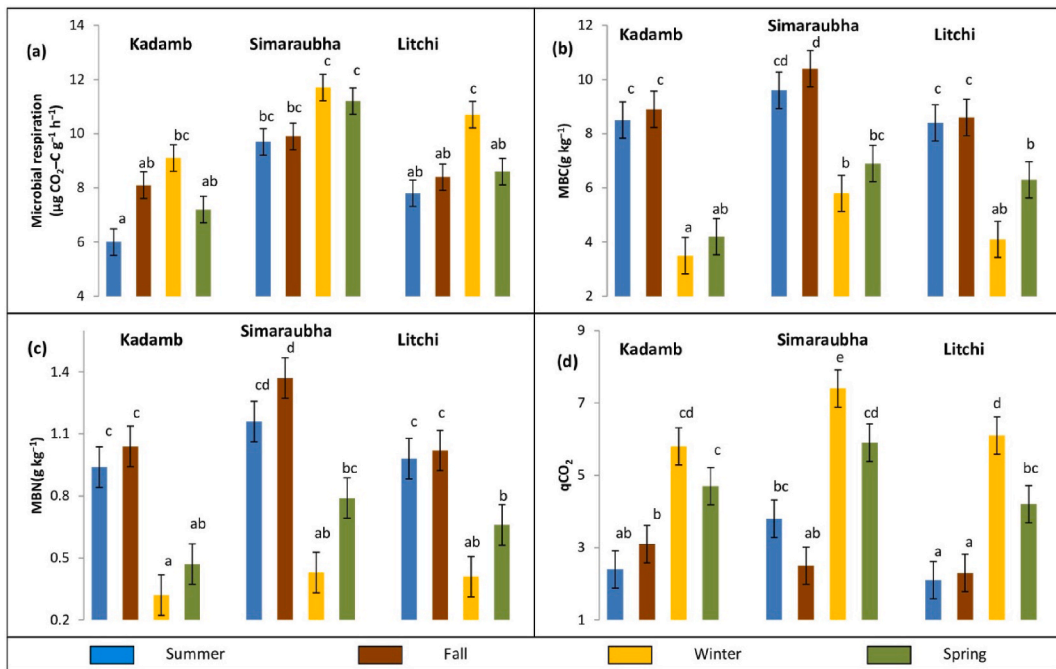


Fig. 2. Seasonal changes in (a) microbial respiration, (b) MBC (microbial biomass carbon), (c) MBN (microbial biomass nitrogen) and (d) $q\text{CO}_2$ of soils under TP over four seasons. Bars show mean values of 30 samples from each TPs and contain standard errors of means ($n = 30$).

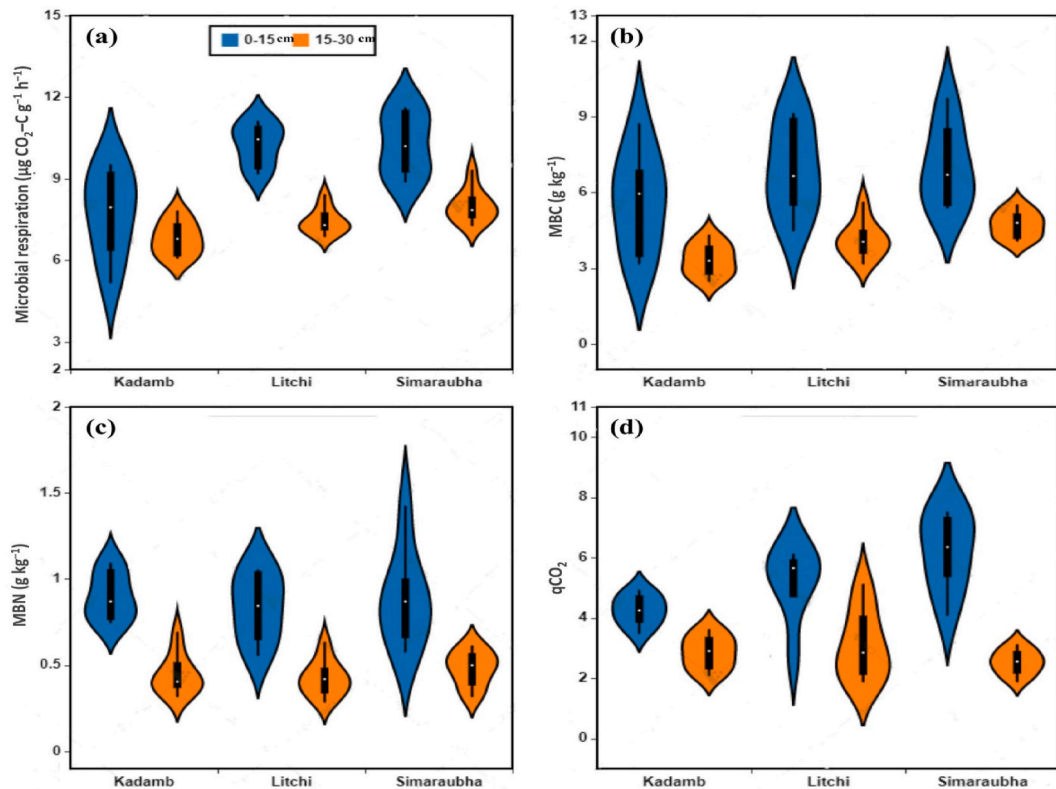


Fig. 3. Depth-wise distribution of (a) microbial respiration, (b) MBC (microbial biomass carbon), (c) MBN (microbial biomass nitrogen) and (d) $q\text{CO}_2$ of soils under TPs.

Table 5
Seasonal variations of soil MBC/MBN, MBC/TOC, and MBN/TN ratios under TP.

Season	MBC/MBN ratio			MBC/TOC ratio			MBN/TN ratio		
	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi	Kadamb	Simaraubha	Litchi
Summer	10.32 ± 1.32 ^{ab}	9.87 ± 1.34 ^{ab}	13.85 ± 1.92 ^{cd}	3.45 ± 0.31 ^{ef}	2.81 ± 0.35 ^{cd}	3.40 ± 0.19 ^{ef}	8.61 ± 0.67 ^{de}	7.72 ± 0.64 ^{cd}	7.92 ± 0.78 ^{cd}
Fall	10.51 ± 1.23 ^{ab}	9.73 ± 0.93 ^{ab}	12.31 ± 1.74 ^{bc}	3.47 ± 0.29 ^{ef}	4.27 ± 0.36 ^g	3.92 ± 0.26 ^{fg}	6.72 ± 0.36 ^c	9.34 ± 0.69 ^e	9.67 ± 0.81 ^e
Winter	11.21 ± 0.89 ^{ab}	15.20 ± 1.21 ^c	17.43 ± 1.87 ^d	1.82 ± 0.09 ^a	2.04 ± 0.14 ^{ab}	1.07 ± 0.08 ^a	3.06 ± 0.15 ^a	3.41 ± 0.12 ^a	3.38 ± 0.25 ^a
Spring	11.45 ± 1.91 ^{ab}	9.75 ± 0.93 ^{ab}	8.42 ± 0.48 ^a	1.92 ± 0.08 ^a	4.02 ± 0.25 ^g	1.67 ± 0.14 ^{bc}	3.70 ± 0.46 ^a	9.49 ± 0.64 ^e	5.21 ± 0.38 ^b

MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, TOC: total organic carbon, TN: total nitrogen.

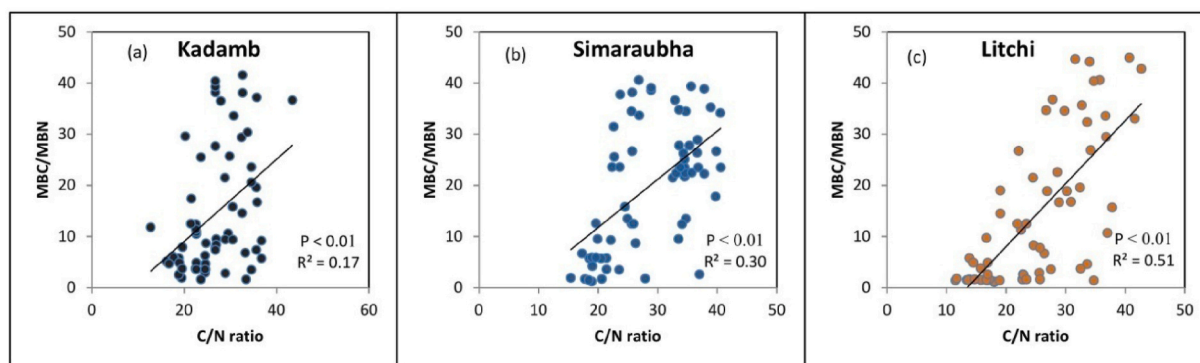


Fig. 4. Correlations of soil MBC/MBN ratios and C/N ratios in (a) Kadamb, (b) Simaraubha, and (c) Litchi based TPs.

influence of soil characteristics on SR, MBC and MBN varies differently in each TP, as shown through stepwise regression analysis (Table 6). The variations in MBC due to temperature, pH, C/N ratio and MC in Kadamb TP was 27 %, due to temperature, TOC, C/N ratio and MC in Simaraubha TP was 31 %, and due to the MC and C/N ratio in Litchi TP was 35 %. In the case of MBN, the influence of soil characteristics differs in Kadamb (MC, TOC and pH), Simaraubha (TOC and C/N ratio), and Litchi (TOC and C/N ratio) TPs.

4. Discussion

Soil microbial carbon and microbial respirations are important biological indicators impacted by a range of factors comprising seasonal variations, trees and soil properties [14,46]. Microbial carbon directly indicates the pool of microbial biomass in soil. Soil microbial respiration denotes the oxidation status of the soil by microbes and is regarded as one of the most valuable indicators of carbon cycling [47,48]. Moreover, it also monitors microbial actions, organic matter status and nutrient cycling [49]. Babur et al. (2022) [14] observed that the SR was $8.96 \mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$ in soil under TP during winter was more than in fall ($6.85 \mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$) and summer ($6.19 \mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$). This trend is attributed to our study. Other experiments on tree plantations also suggest increased soil SR during spring and winter due to optimum temperature and moisture content in the soil, which accelerate microbial activities [50]. In contrast to our findings, Arora et al. (2021) reported rainy season had more SR than winter under Litchi TP [51]. On the other hand, the findings of Yan et al. (2018) recorded higher SR under mango TP in summer, contradicting our findings which could result from microbial cell death due to water stress during summer [52]. This reason seems reasonable as soil microbes are very sensitive to moisture and temperature variations causing low respiration in summer [53].

The MBC/MBN, MBN/TN and MBC/TOC ratios and qCO_2 values/trends recorded in our experiment are similar to those reported by Liu et al. (2018) [54] and de Moraes et al. (2021) [55]. In our experiment, MBC contents ranged from 3.54 g kg^{-1} to 10.41 g kg^{-1} , considering seasonal and TP variations. Hossain et al. (2019) reported that soil MBC was 37.52 mg kg^{-1} under a litchi-based plantation with turmeric as an intercrop in a hot and humid region of Bangladesh, whereas sole litchi orchard's soil MBC value was $290.11 \text{ mg kg}^{-1}$ [56]. However, such high variation may be due to the influence of several abiotic factors [57]. The variations of MBC under TPs and seasons may be because of interactions of multiple factors like tree species, climate, root system development, root secretion, agricultural practices, and time of sampling [58,59]. The positive correlation observed between the MC and MBC under all TPs in our experiment during all seasons affirmed the works of Tomar & Baishya (2020) [48]. These variations of MBC could also be linked with soil microbial respiration, as both (MBC and soil microbial respiration) parameters had a positive correlation in all seasons. TOC was more during winter under all TPs, which is attributed to the findings of Watanabe et al. (2019) [60] and, Bargali & Bargali, (2020) [61]. This could be low temperature and slow microbial activities. Jeihanipour et al. (2018) reported more TOC value in the fall season, which could be due to litterfall received during winter and spring increasing TOC in fall under high soil moisture presence [62]. The

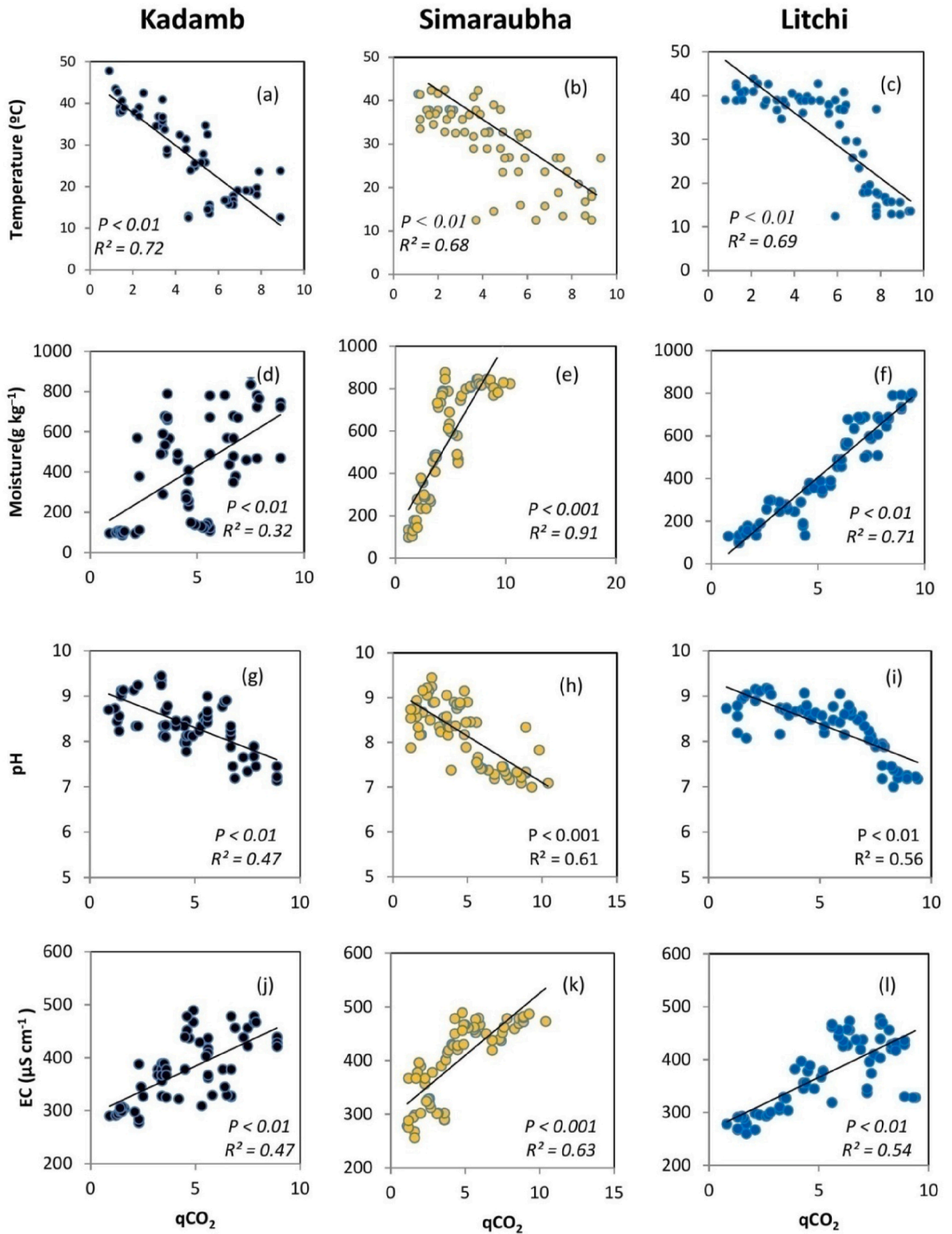


Fig. 5. Correlations of temperature, moisture, pH, and EC (electrical conductivity) with qCO₂ in Kadamb (a, d, g, j), Simaraubha (b, e, h, k) and Litchi (c, f, i, l) based TPS.

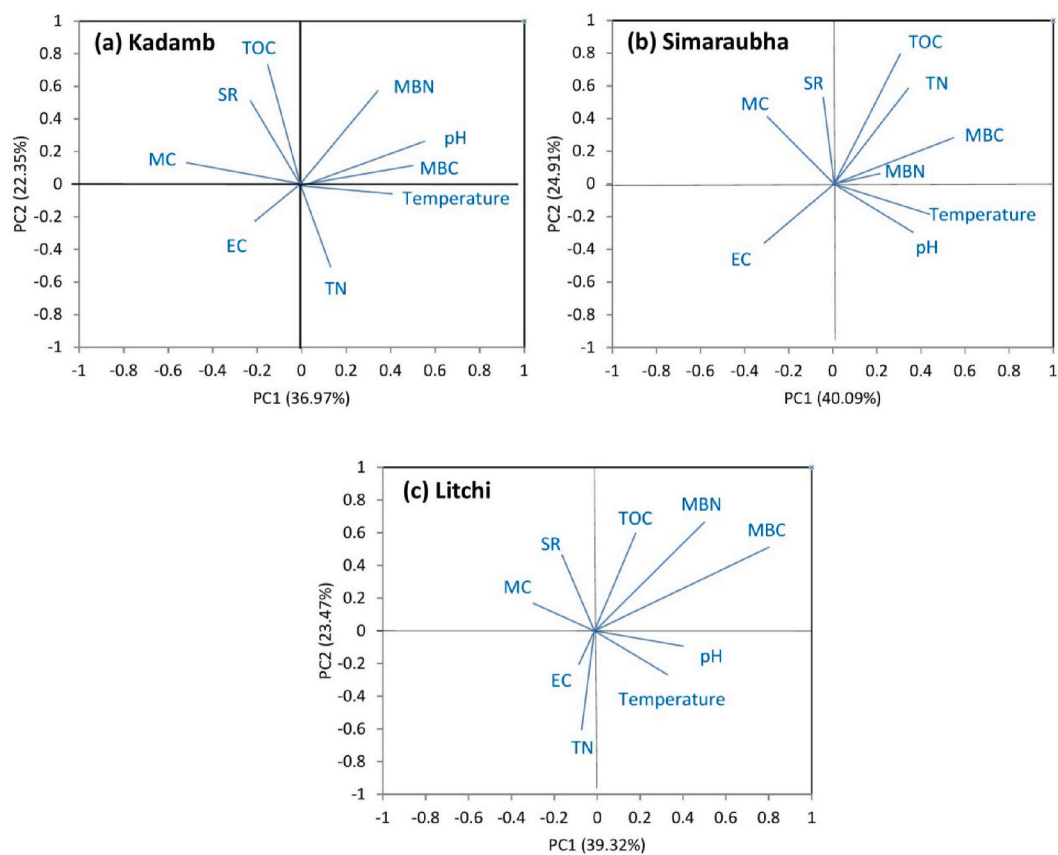


Fig. 6. PCA biplots of soil characteristics and microbial properties in (a) Kadamb, (b) Simaraubha and (c) Litchi based TPs. MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, TOC: total organic carbon, TN: total nitrogen, SR: microbial respiration, EC: electrical conductivity, MC: soil moisture.

soil MBN observed in our experiment is similar to the findings of Cai et al. (2018), i.e. 0.41–0.73 g kg⁻¹ [63]. Though, Mgelwa et al. (2019) recorded higher MBN (1.78–2.09 g kg⁻¹) with significant seasonal variations [64]. This study reported the highest MBN during summer, whereas our study showed the highest MBN in winter may be due to higher organic matter deposition during winter. In another study, soil moisture was reported to have more influence on microbial population over soil temperature [65]. On the other hand, our study shows both soil temperature and moisture positively correlate with MBN during spring, but temperature negatively correlated with MBN in fall and winter. Although the MBN values differ widely among TPs and seasons, it is impacted by soil moisture, temperature, litterfall and sampling time [66].

The MBC/MBN ratio may signify the diversity and status of soil microbial communities, such as the domination of bacterial or fungal populations [55,67,68]. The MBC/MBN ratio of 10–12 denotes fungal domination, whereas 3–5 signifies bacterial dominance [14]. In the present study, the MBC/MBN ratio was generally greater than 10, which suggests fungal dominance among the microbial community. The high MBC/MBN ratio during the winter is related to low temperature and high soil carbon supply, benefiting the fungal population. Furthermore, fungi had more tolerance over bacteria to adverse climatic parameters [69]. The MBC/MBN ratio obtained in the current study is similar to those reported by Srivastava et al. (2023) [70]. In addition, higher MBC/MBN ratios during winter followed by spring and summer indicate a decrease in fungal dominance over bacteria across the seasons. This statement is further affirmed by the positive correlation between C/N and MBC/MBN ratio under all TPs, as higher fungal populations contribute to higher biomass carbon. The MBC/TOC ratio also signifies variations in climatic conditions, SOM, and soil carbon dynamics [61,71]. The MBC/TOC ratio ranged from 1.07 to 4.27 among the TPs in the present study, similar to the findings of Gualberto et al. (2023) [72]. The high MBN/TN ratio is related to more nitrogen availability for microbes, and a low MBN/TN ratio denotes low accessibility of organic matter for microbes [70,73,74]. The recorded MBN/TN ratios were 3.06–9.67 in the current investigation showing wide variability in nitrogen availability, similar to the work of Farooq et al. (2022) [75]. During the fall season, there were higher MBN/TN ratios under all TPs, signifying more immobilization of carbon and nitrogen into microbial biomass [5].

The increase in qCO₂ values can be taken as a sign of microbial reaction with the change in the soil environment [76,77]. The qCO₂ can help to evaluate soil living microbes' ecological status and estimate their quantity [78,79]. Generally, the values of qCO₂ differ from 0.5 to 2.0 [15], though the present study had the values exceeded 2.0 in spring and winter. This may be because the low temperature responsible for decreasing the decomposition rate, which is also supported by the negative correlation between qCO₂ and

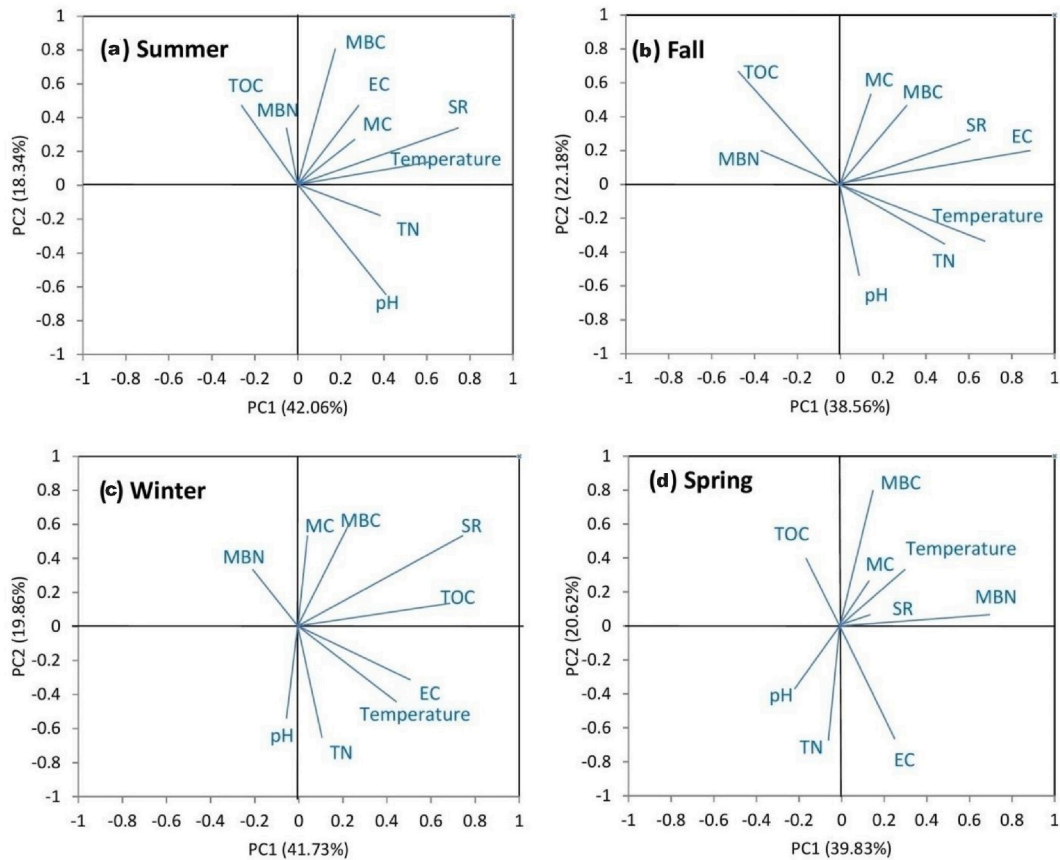


Fig. 7. PCA biplots of soil characteristics and microbial properties in (a) summer, (b) fall, (c) winter and (d) spring seasons. MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, TOC: total organic carbon, TN: total nitrogen, SR: microbial respiration, EC: electrical conductivity, MC: soil moisture.

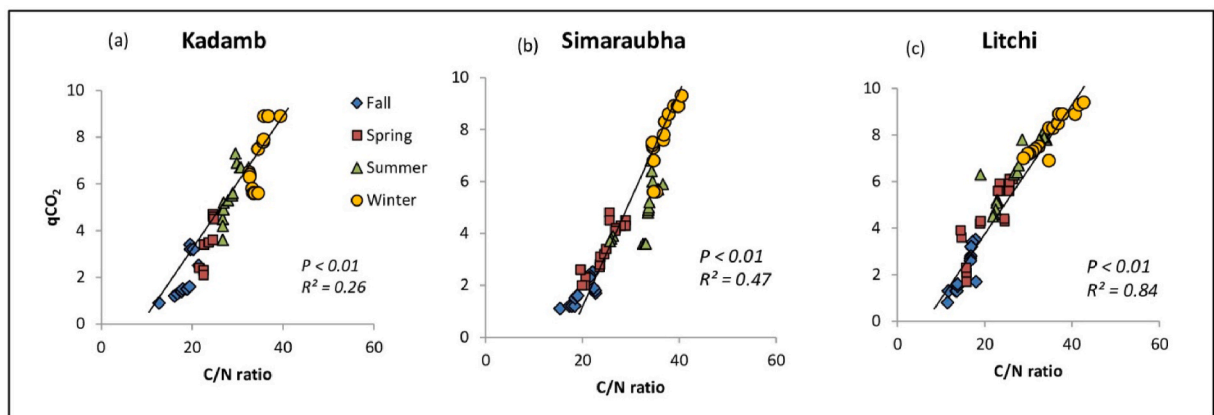


Fig. 8. Relationships of soil C/N ratio with qCO_2 across seasons under (a) Kadamb, (b) Simaraubha and (c) Litchi TPs.

temperature. The qCO_2 was found to have a positive relationship with soil moisture. The TP has unique seasonal micro-climates in soil moisture and temperature, impacting the litterfall decomposition [80]. The type and volume of organic matter available for SR also influence qCO_2 in soil. The trend of qCO_2 values change indicates the shift in microbial activities [81,82]. A high qCO_2 can also reflect a huge rivalry for carbon uptake by microbes and states better utilization of carbon energy for growth [15]. In general, qCO_2 positively correlates with TOC and microbial biomass, glucose, carbohydrate, protein and other substrates [83–85]. This current study also shows a positive relationship between qCO_2 and C/N in all TPs, referring to the positive relationship between qCO_2 and biomass carbon. The

Table 6

Effects of soil characteristics on microbial respiration and biomass in each TP, independent variables are temperature, pH, EC (electrical conductivity), TOC (total organic carbon), TN (total nitrogen), MC (soil moisture) and C/N ratio, (stepwise regression analysis).

TP	Dependent variables	Independent variables included	Standard error of estimates	R ²	F
Kadamb	MBC	Temperature, pH, C/N ratio, MC	1.94	0.27	22.9
	MBN	MC, TOC, pH	0.29	0.41	43.7
	Microbial respiration	Temperature, MC	1.87	0.15	15.2
Simaraubha	MBC	Temperature, TOC, C/N ratio, MC	2.73	0.31	39.5
	MBN	TOC, C/N ratio	0.45	0.42	134
	Microbial respiration	Temperature, MC	1.78	0.38	25.8
Litchi	MBC	MC, C/N ratio	3.81	0.35	30.5
	MBN	TOC, C/N ratio	0.65	0.32	22.4
	Microbial respiration	Temperature, MC, TOC, pH	3.08	0.37	24.2

Probability of *F* to include 0.05–0.10, *P* < 0.001. MBC: microbial biomass carbon, MBN: microbial biomass nitrogen.

differences in soil temperature and moisture due to seasonal variations control microbial biomass decomposition and nutrient availability.

This experiment reported that qCO_2 had a negative correlation with soil pH and a positive correlation with soil EC. Higher SR can be due to higher pH stress resulting in lower qCO_2 [86]. The current investigation reported a positive correlation between qCO_2 and EC, attributed to the works of Ebrahimi et al. (2022) [87]. On the other hand, Akburak et al. (2018) observed a negative relationship between qCO_2 and EC, and argued that lower EC improved microbial activities resulting in higher qCO_2 [88]. Our findings suggested a positive correlation of qCO_2 with C/N ratios in all TPs, which is similar to the findings of Brzezińska et al. (2018) [89] and Cai et al. (2022) [90]. However, Liu et al. (2020) reported no change in the C/N ratio with increase in qCO_2 [91]. In addition, Zhou et al. (2018) summarized from several field and lab studies that a positive relationship existed between qCO_2 and C/N ratios under different environments [92]. Generally, SR per unit MBC is higher in soil with a higher C/N ratio than in soil with a low C/N ratio [93]. The low availability of microbial nitrogen may be a reason for the positive correlation of qCO_2 with the C/N ratio and seasonal changes of SR in soil [87]. Therefore, low C/N ratios, high nitrogen, and high fungal to bacterial ratio could yield a low value of qCO_2 [94,95], since the residue degradation rate by fungi is relatively slower, resulting in lower qCO_2 [96]. However, Spohn (2015) was unable to determine the exact reason behind the positive correlation of qCO_2 with C/N ratio in different TP soils [97].

We confess that there are limitations to considering qCO_2 as a microbial carbon use efficiency parameter as it cannot reasonably explain MBC buildup and is unclear about the possible differences of carbon respired and carbon added in soil [14]. Additionally, qCO_2 estimates carbon use efficiency without determining microbial growth rate, which is inaccurate [15,98]. However, qCO_2 is easy to estimate and can be considered as an index of microbial carbon use efficiency to evaluate the status of microbial population in experiments where data on MBC growth rate is unavailable or chemical-based analysis is not feasible [93,98]. Hence, looking at the possible scopes, we emphasize that qCO_2 must be interpreted with the particular microbial ecosystem to eliminate various errors, as Sarkar et al. (2022) reported [99].

5. Conclusions

This experiment demonstrated that the soil properties and microbial indicators differ primarily among the TPs and seasons. The highest SR rates were found in winter under all three TP systems, which positively correlated with MBC. The significant positive relationship between MBC/MBN ratio and C/N ratio in each TP denotes the profound influence of microbes on regulating available nitrogen and carbon in the soil. The seasonal changes in MBC/TOC and MBN/TN ratios signify the relative domination between microbes (fungi to bacteria) and their adaptability to climatic variations. Along with some adjustable limitations, the qCO_2 was a useful parameter to measure the impact of soil properties on microbial carbon use efficiency and the positive relationship between the C/N ratio and qCO_2 supporting the microbial dependency of biomass carbon for respiration throughout the year. This study indicated the importance of microbial characteristics for evaluating the seasonal influences and soil properties on nitrogen and carbon dynamics and practically needed for sustainable TP development. Moreover, the generated data can be useful for modeling and monitoring the efficiency of TP development in the Gangatic region with climatic variations. Hence, future studies are suggested for deeper evaluation of the influence of a range of soil properties on microbial carbon utilization in different seasons.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Sudip Sarkar: Methodology, Investigation, Formal analysis, Conceptualization. **Dipty Kumar Das:** Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Abhinandan Singh:** Writing – review & editing, Visualization. **Ranjan Laik:** Supervision, Resources, Investigation. **Santosh Kumar Singh:** Validation, Supervision, Resources, Investigation. **Harold M. van Es:** Writing – review & editing, Software, Data curation. **Kavya Krishnan:** Writing – review & editing. **Amit Kumar Singh:** Writing – review & editing, Formal analysis. **Anup Das:** Writing – review & editing. **Utkarsh Singh:** Methodology, Data curation. **Hosam O. Elansary:** Software, Resources, Funding acquisition. **Eman A. Mahmoud:** Software, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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