



## OPEN Integrating seasonal dynamics and human impact on microbial biomass carbon across deep soil profiles in tropical Sal forest of Achanakmar-Amarkantak Biosphere Reserve, India

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Forest soil is crucial in climate change mitigation, food security, and biogeochemical nutrient cycling. Mixed Sal forests enhance soil organic matter, improve nutrient availability, and regulate pH dynamics. However, anthropogenic disturbances, including deforestation and land-use changes, significantly alter forest cover, leading to shifts in soil physicochemical and microbial properties. These impacts necessitate rigorous monitoring and comprehensive assessment. Therefore, we investigated the effects of contrasting conditions- closed (no human activities) and open (human interferences) mixed Sal Forest on the vertical and seasonal dynamics of microbial biomass carbon (SMBC). Results revealed that the closed mixed Sal Forest had significantly higher SMBC than the open mixed Sal Forest across the soil profile (D1–D5) with a strong seasonal effect. Closed mixed Sal Forest had 60% higher SMBC in D1 than open mixed Sal Forest while it reduced with depth and 17.1 to 56.7% higher SMBC in the subsurface to bottom-most soil profile (D2–D5). Moreover, SMBC was higher in the monsoon period in both forests. The SMBC reduced by 24.2 to 45.1% in the post-monsoon period while reduction was more intense in the pre-monsoon period (48.1 to 68.2%) compared to the monsoon period under closed mixed Sal Forest. Similarly, the decline was more intense in the open mixed Sal Forest, where SMBC declined 12.1 to 54% in the post-monsoon period and 56.1 to 76.2% in the pre-monsoon period compared to the monsoon period. The study indicates that human interference in mixed Sal forests leads to loss of forest cover, negatively affecting microbiological properties and reducing soil fertility, which weakens the forest's resilience to climate change. Additionally, SMBC exhibits seasonal variations, reflecting responses to environmental conditions. These results underline the need to reduce human disturbances and enhance forest conservation strategies to ensure soil sustainability and ecosystem stability.

**Keywords** Mixed forest, SOC, SMBC, Seasonal variation, Vertical distribution

Tropical forests are renowned for their exceptional biodiversity, biomass, productivity, and ecological benefits<sup>1</sup>. These ecosystems are incredibly diverse and enhance soil quality<sup>2</sup>. However, human activities pose a significant threat, leading to ongoing degradation that alters microclimatic conditions such as soil properties, temperature, and moisture levels<sup>3,4</sup>. This degradation also disrupts microbial diversity, ultimately affecting soil microbial characteristics within these ecosystems<sup>5,6</sup>. Soil quality is shaped by its physical and chemical properties, along with the presence of soil microbes and microbial biomass, which are essential for litter decomposition, nutrient cycling, and organic carbon dynamics<sup>7</sup>. Achankmaar Amarkantak Biosphere Reserve (AABR) is a unique

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reserve located at the intersection of Madhya Pradesh and Chhattisgarh. It represents tropical deciduous forest ecosystems and has been recognized as a UNESCO heritage site in Central India. Consequently, it was designated as the 14th Biosphere Reserve in India on March 30, 2005, by the Ministry of Forests & Environment, Government of India<sup>8</sup>. Regrettably, various areas within AABR, especially those near forest settlements, have witnessed ecological degradation due to continuous human interference. This interference includes issues like excessive grazing, deliberate forest fires, overexploitation of forest resources, and illegal logging over the past few decades<sup>9</sup>. As a result, the forest cover has dwindled to 19.72 km<sup>2</sup>, with an additional conversion of 14.77 km<sup>2</sup> of Dense Sal mixed forest into open mixed forest and cultivable land between 2008 and 2018<sup>9</sup>. Such transformations in the forest and the decline in forest cover have significant impacts on the physicochemical and microbial properties of the soil<sup>10,11</sup>. While some studies on soil microbial biomass carbon have been conducted in this region, they have been limited to the agroecosystem<sup>12,13</sup>, fire severity in the forest ecosystem<sup>14</sup>, and confined only to the surface soil layer (0–20 cm depth). Consequently, there is a lack of understanding regarding the dynamics of soil microbial biomass carbon concerning forest conversion or degradation, seasonal variations, and deep vertical soil layers in this region.

Soil microbial biomass represents a vital labile pool of soil-available nutrients; it comprises approximately 1–4% of organic matter<sup>15</sup>. This biomass is highly responsive to changes in its environment and actively prevents nutrient loss in forest soils<sup>16</sup>. Climatic factors, microbial biomass composition, and their activities significantly influence the degradation of plant litter, releasing nutrients into the soil. Microorganisms play a vital role in transforming and mineralizing soil carbon. Consequently, the production of soil microbial biomass carbon (MBC) and soil respiration (SR) are affected by anthropogenic activities and climatic conditions<sup>7</sup>. The efficiency of MBC is determined by the balance between carbon utilized for microbial growth and carbon consumed. Thus, microbial carbon use efficiency reflects the extent to which microbial biomass production and respiration generate energy to support the growth and development of microorganisms<sup>17</sup>.

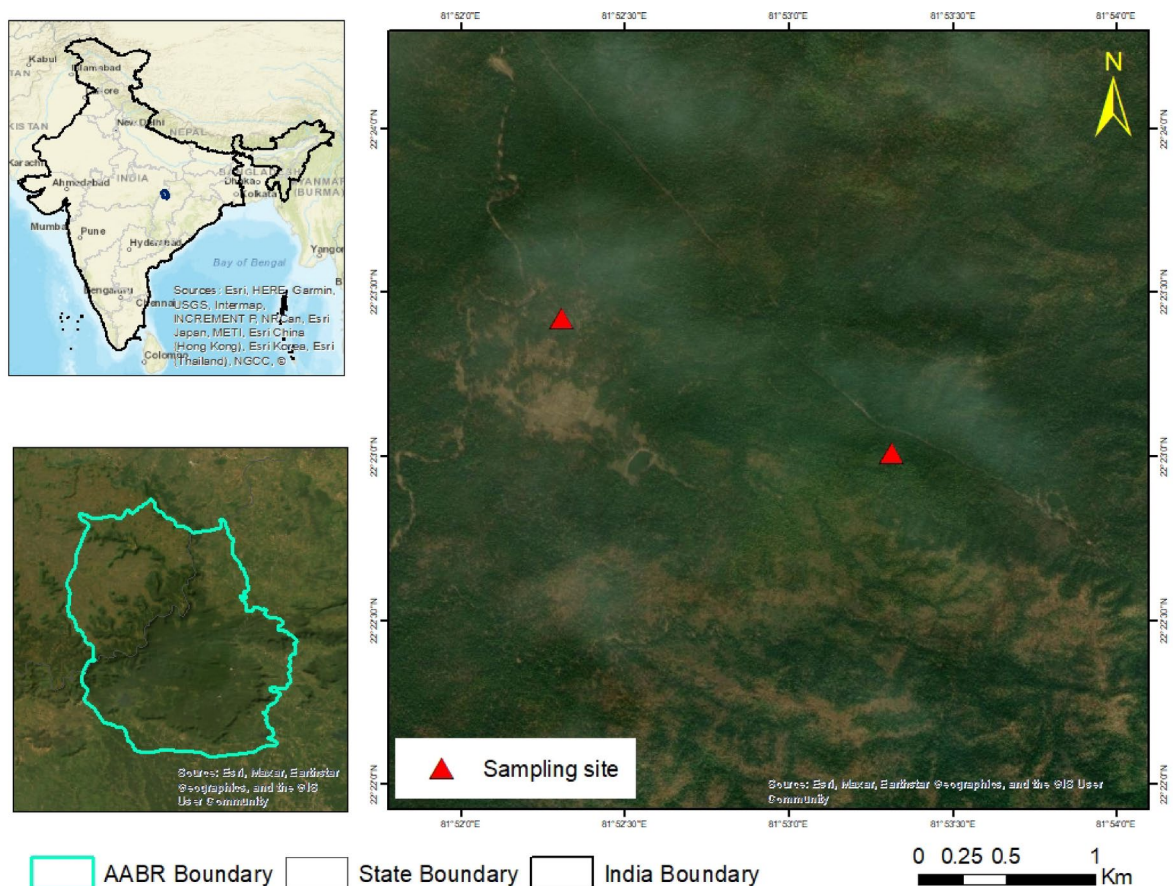
Understanding the shifts in microbial biomass and carbon due to forest degradation is of paramount importance<sup>18</sup>. It serves as a keystone biological indicator to detect alterations in soil organic matter and ecosystem functioning<sup>19</sup>. Trees' components significantly impact the diversity and activity of microorganisms, as well as soil biochemical properties, primarily due to variations in the quantity and composition of litter and root exudates<sup>20</sup>. Additionally, seasonal fluctuations in microbial processes play a crucial role in regulating soil nutrient release and plant nutrient availability<sup>21</sup>. These seasonal microbial dynamics are closely associated with changes in soil moisture and temperature. Moreover, seasonal shifts in environmental and soil conditions directly influence qCO<sub>2</sub> and microbial biomass production, highlighting strong seasonal correlations with microbial carbon use efficiency, as qCO<sub>2</sub> often increases with the addition of litter<sup>22</sup>. Vegetation cover is crucial to ecosystem health, playing a vital role in enhancing soil fertility and promoting microbial biomass. In addition to vegetation loss caused by human activities, seasonal changes significantly influence microbial indices through variations in soil moisture, temperature<sup>23</sup>, and soil depth<sup>24</sup>. Seasonal shifts in natural conditions are essential for sustaining ecosystem diversity and ensuring its proper functioning<sup>25</sup>. Mixed Sal forests play a vital role in shaping soil properties, positively influencing organic matter content, nutrient availability, and pH levels. Additionally, seasonal variations impact the vertical distribution of soil microbial biomass carbon, reflecting the adaptability of microbial communities to changing conditions. Understanding the ecological significance of Sal mixed forests and their impact on soil properties contributes to better ecosystem management and conservation practices. This research is the pilot study in the Achanakmar Amarkantak Biosphere Reserve and highlights the dynamic nature of forested ecosystems and the importance of considering seasonal variations in soil microbial processes. Therefore, the present study aims to assess the impact of disturbance and season on soil microbial biomass of Sal mixed forest. Here, we hypothesized that soil physicochemical properties and microbial biomass carbon would be higher in closed forest compared to open forest because of least or no human activities. We also hypothesized that the disturbance and season effects on the soil microbial biomass would vary with soil depths. To check the above hypothesis, the objectives of the study were: (1) To determine the soil physicochemical properties and microbial biomass carbon in closed and open forest of Sal mixed forest; (2) Effect of different seasons on the vertical distribution of soil microbial biomass carbon in Sal mixed forest of Achanakmar Amarkantak Biosphere Reserve in Chhattisgarh, India.

## Material and methods

### Study area

The present study was conducted in Achanakmar Amarkantak Biosphere Reserve (AABR), a UNESCO natural heritage site in Madhya Pradesh and Chhattisgarh states of India. The study area lies between 22° 23' 00.7'' to 22° 23' 25.4'' N latitude and 81° 52' 18.4'' to 81° 53' 18.9'' E longitude, falling under the Kota range of the Biosphere Reserve (Fig. 1). The forests in this reserve are tropical deciduous forests that can be further classified as Northern Tropical Deciduous and Southern Dry Mixed Deciduous forests. *Shorea robusta* (Sal) is the dominant species associated with *Terminalia tomentosa*, *Syzygium cumini*, *Lagerstroemia parviflora*, *Diospyros melanoxylon*, *Pterocarpus marsupium*, *Anogeissus latifolia*, *Schleichera oleosa*, *Mallotus philippensis*, *Madhuca longifolia*, *Buchnanania lanzan*, *Cleistanthus collinus*, *Miliusa tomentosa*, *Bauhinia Malabarica*, *Ougenia oojenensis*, *Terminalia chebula*, *Adina cordifolia*, *Lannea coromandelica*, etc.<sup>26</sup>. Sal mixed forest was selected for the study which was further divided into two sites i.e., open forest (OS) and closed forest (CS) based on the biotic interferences. Cattle grazing, illegal logging, burning, and the collection of different Minor Forest Produce (MFPs) by forest inhabitants are all common occurrences at the open forest. In the close forest, there is no trace of anthropogenic activities.

The study area has a typical monsoon climate and experiences three distinct seasons comprising summer/pre-monsoon (March–June) which are quite dry, rainy/monsoon (July–October) damp and humid season with heavy clouds, and winter (November–February). However, October and November are the transition months



**Fig. 1.** Location of the open and closed mixed sal forests at Achanakmar Amarkantak Biosphere Reserve (AABR), a UNESCO natural heritage site, located in Madhya Pradesh and Chhattisgarh states of India (Generated by ArcMap 10.4 (ESRI) and incorporates high-resolution imagery from ESRI, Maxar Technologies and Field data. <https://www.esri.com/arcgis/arcmap>).

between monsoon and winter and can be recognized as post-monsoon season. Generally, the warmest month is May, while the coldest months are December and January. During July and September, the humidity stays quite high, with an average relative humidity ranging from 50 to 85%. The mean annual precipitation of the area is more than 1600 mm, whereas, the mean temperatures in winter and summer remain 16.1 °C and 31 °C, respectively. The geology of the region is distinct since it is part of the Deccan Peninsula. The rock types are schists and gneisses with granite intrusion rocks, sandstone, shales, limestone, basaltic lava, and bauxite. The soils are generally lateritic, characterized by excessive amounts of iron oxide, alluvial and black cotton soils are also found along the numerous streams and Nalas.

#### Soil sampling protocol

We selected 10 quadrates measuring 5 m X 5 m each (three quadrates for each land use) and exposed soil profiles in each quadrate corner up to a depth of 1.0 m using a random stratified selection approach<sup>27</sup>. We collected soil samples at 5 vertical levels (D1–D5) to capture the lateral variation of soil carbon distribution in the profiles because the lateral variation of soil properties, including organic C, fluctuates within a single pedon<sup>28</sup>. Each layer depth was 0–20 cm (D1), 20–40 cm (D2), 40–60 cm (D3), 60–80 cm (D4), and 80–100 cm (D5), respectively. For each profile, layer-wise mixing of samples from each quadrate corner ( $L1 + L1 + L1 + L1 = L1$ ) resulted the one composite sample<sup>7</sup>. During the pre-monsoon, monsoon, and post-monsoon months, we collected a total of 50 soil samples (5 depths, 2 forest types, 5 quadrates = 50 samples) from the surface 0–20 cm (D1) to a depth of 100 cm (D5) (May–November). The collection of composite soil samples up to a depth of 1.0 m allowed for a comprehensive assessment of lateral variations in soil properties, including soil carbon, SMBC, and other important soil attributes. This provides valuable insights into the spatial distribution of different forest types' soil properties and can help formulate appropriate land management strategies for sustainable soil use and conservation.

#### Laboratory analysis of soil carbon, carbon SOC stock, SMBC and other soil properties

The soil bulk density (BD) of oven-dried ( $104 \pm 1$  °C) soil samples was estimated by using the core sampler (5.15 cm height and 4.7 cm diameter)<sup>29</sup>. The following formula was used:

$$\text{Bulk density (Mg m}^{-3}\text{)} = \text{Mass of dry soil (Mg)} / \text{Volume of dry soil (m}^{-3}\text{)} \quad (1)$$

After removing debris, stones, roots, and other foreign materials, half of the pre-processed samples were immediately stored in sealed plastic bags and kept in the deep freezer ( $-80^{\circ}\text{C}$ ), the remaining half of the samples were air dried, crushed, and sieved. Sieved samples of 2.0 mm were used for pH (at a ratio of 1:2.5 soil: water) and the particle size distribution was determined by international pipette<sup>30</sup>. While 0.5 mm sieved samples were analysed for SOC estimation by the wet digestion method<sup>31</sup>, water holding capacity (WHC) was estimated by the Raczkowski box method<sup>32</sup>. Following standard procedures, the samples were analyzed for available N-nitrogen, Brays 1 phosphorus  $\text{P}_2\text{O}_5$  and available potash ( $\text{K}_2\text{O}$ )<sup>33</sup>. The soil microbial biomass C (SMBC) from refrigerated samples was determined by the chloroform fumigation extraction method<sup>34</sup>. The soil carbon stocks for each soil profile layer, soil profile average (PWA), and soil profile sum (up to 1.0 m) were estimated through the measured corresponding concentrations (0.15 mm sieved), BD values, coarse fragments (CF: coarse roots/ gravels/ rocks) larger than the 2.0 mm diameter (fraction by mass, %/100) and from the respective thickness of layers following Ellert et al.<sup>35</sup>.

$$\text{C Stock (mg ha}^{-1}\text{)} = [\text{BD (Mg m}^{-3}\text{)} \times \text{C (\%)} \times (1 - \text{CF}) \times \text{soil depth (m)} \times 10^4 \text{ m}^2 \text{ ha}^{-1}/100] \quad (2)$$

$$\text{Soil profile (0.0–1.0 m depth) C stock} = \sum_{D=1}^{D=5} \text{C stock for each soil depth} \quad (3)$$

### Stratification ratio (SR)

The Stratification Ratio (SR) measures the value of a soil parameter in the surface soils divided by the value in the subsurface soils, as described by Qua et al.<sup>36</sup>. For soil quality assessment, the SR of soil carbon is often used as an indicator, as an increase in SR is associated with greater rates and amounts of organic carbon sequestration<sup>37</sup>. In this study, the SRs of various soil properties were calculated between the surface layer (D1: 0–20 cm) and four subsurface layers: SR1 (D1: 0–20 cm/D2: 20–40 cm), SR2 (D1: 0–20 cm/D3: 40–60 cm), SR3 (D1: 0–20 cm/D4: 60–80 cm), and SR4 (D1: 0–20 cm/D5: 80–100 cm, the bottom-most layer).

### Statistical analysis

All the collected data of both forest types were checked for normality of distribution, and the design of sampling across land use systems led to a simple balanced two-way analysis of variance (ANOVA) with 2 forest types and 5 vertical soil depths (D1–D5) as factors/ treatments. All the soil properties including soil microbial properties across the forest types and soil depths were compared by two-way ANOVAs followed by Duncan's Multiple Range Test (DMRT) at the Post Hoc stage with a 5% probability level.

## Results

### Effect of closed and open mixed Sal forest on different soil properties

Data obtained from open and closed mixed Sal forest revealed that the soil bulk density ranged from 1.31 to 1.40  $\text{Mg m}^{-3}$  (Table 1). Open mixed Sal forest recorded 6% higher soil bulk density (averaged up to 1.0 m soil depth) compared to the closed mixed Sal forest, while the closed mixed Sal forest soils were significantly ( $p < 0.05$ ) 41.5% higher in the soil moisture (Table 1). In contrast, open mixed Sal forest soils were higher ( $p < 0.05$ ) in soil pH, water holding capacity (WHC), sand and silt particle size with 0.37 unit in pH, 6.8% in WHC, 7.8% in the sand and 11.6% in silt content respectively (Table 1). Similarly, closed mixed Sal forest were significantly

Soil Properties	Closed mixed Sal forest	Open mixed Sal forest
BD( $\text{Mg m}^{-3}$ )	1.31 <sup>b</sup> ± 0.13	1.40 <sup>a</sup> ± 0.13
Moisture (%)	15.88 <sup>a</sup> ± 1.08	9.30 <sup>b</sup> ± 1.17
pH	4.93 <sup>b</sup> ± 0.18	5.30 <sup>a</sup> ± 0.17
WHC (%)	35.83 <sup>b</sup> ± 3.33	38.46 <sup>a</sup> ± 4.47
Sand (%)	63.20 <sup>b</sup> ± 3.17	68.51 <sup>a</sup> ± 3.94
Silt (%)	16.35 <sup>b</sup> ± 1.65	18.50 <sup>a</sup> ± 3.07
Clay (%)	20.45 <sup>a</sup> ± 2.46	12.99 <sup>b</sup> ± 2.61
SOC (%)	0.49 <sup>a</sup> ± 0.31	0.35 <sup>b</sup> ± 0.17
Av. N (kg/ha)	186.73 <sup>a</sup> ± 53.24	177.77 <sup>b</sup> ± 47.84
Av. P (kg/ha)	15.80 <sup>a</sup> ± 6.24	11.79 <sup>b</sup> ± 3.11
Av. K (kg/ha)	253.59 <sup>a</sup> ± 112.84	223.18 <sup>b</sup> ± 94.01
SOC Stock (Mg/ha)	12.43 <sup>a</sup> ± 7.04	9.51 <sup>b</sup> ± 4.20

**Table 1.** Profile average of different soil properties under closed and open mixed Sal forest (1.0 m soil profile depth). \*BD, Bulk density; WHC, Water holding capacity; SOC, Soil organic carbon; Av. N, Available nitrogen; Av. P, Available phosphorus; and Av. K, Available potassium; Means in the column followed by different small letters in superscript (a–e) based on values represent statistical significant differences among the land uses at  $p < 0.05$ .



Soil Depth	BD (Mg m <sup>-3</sup> )	Moisture (%)	pH	WHC (%)	Sand (%)	Silt (%)	Clay (%)	SOC (%)	Av. N (kg/ha)	Av. P (kg/ha)	Av. K (kg/ha)
D1:0-20 cm	1.18 <sup>c</sup> ± 0.10	16.80 <sup>a</sup> ± 1.08	4.72 <sup>c</sup> ± 0.06	38.00 <sup>a</sup> ± 3.53	59.82 <sup>c</sup> ± 1.34	16.71 <sup>ab</sup> ± 1.44	23.47 <sup>a</sup> ± 1.89	1.03 <sup>a</sup> ± 0.07	259.56 <sup>a</sup> ± 20.80	23.90 <sup>a</sup> ± 1.72	405.44 <sup>a</sup> ± 21.54
D2:20-40 cm	1.25 <sup>d</sup> ± 0.09	16.12 <sup>b</sup> ± 0.67	4.76 <sup>d</sup> ± 0.04	37.68 <sup>a</sup> ± 1.78	62.31 <sup>b</sup> ± 3.60	17.19 <sup>a</sup> ± 2.14	20.50 <sup>b</sup> ± 1.99	0.57 <sup>b</sup> ± 0.04	219.65 <sup>b</sup> ± 7.08	22.08 <sup>b</sup> ± 1.20	344.96 <sup>b</sup> ± 14.60
D3:40-60 cm	1.31 <sup>c</sup> ± 0.11	15.29 <sup>c</sup> ± 0.76	4.97 <sup>c</sup> ± 0.09	35.19 <sup>b</sup> ± 2.71	64.06 <sup>a</sup> ± 3.01	16.23 <sup>b</sup> ± 1.98	19.71 <sup>c</sup> ± 1.77	0.40 <sup>c</sup> ± 0.03	190.09 <sup>c</sup> ± 5.35	12.97 <sup>c</sup> ± 1.05	238.88 <sup>c</sup> ± 21.07
D4:60-80 cm	1.37 <sup>b</sup> ± 0.10	15.10 <sup>d</sup> ± 0.91	5.03 <sup>b</sup> ± 0.08	33.48 <sup>c</sup> ± 3.02	64.82 <sup>a</sup> ± 1.64	15.59 <sup>c</sup> ± 1.40	19.58 <sup>c</sup> ± 2.23	0.26 <sup>d</sup> ± 0.02	151.69 <sup>d</sup> ± 8.18	10.96 <sup>d</sup> ± 0.90	173.80 <sup>d</sup> ± 9.85
D5:80-100 cm	1.42 <sup>a</sup> ± 0.10	16.12 <sup>b</sup> ± 1.27	5.17 <sup>a</sup> ± 0.05	34.83 <sup>c</sup> ± 3.94	64.98 <sup>a</sup> ± 3.20	16.03 <sup>b</sup> ± 1.36	18.98 <sup>d</sup> ± 2.23	0.20 <sup>e</sup> ± 0.02	112.66 <sup>e</sup> ± 5.97	9.07 <sup>e</sup> ± 0.72	104.88 <sup>e</sup> ± 6.80
Mean	1.31 ± 0.10	15.88 ± 0.69	4.93 ± 0.19	35.83 ± 1.94	63.20 ± 2.17	16.35 ± 0.62	20.45 ± 1.77	0.49 ± 0.33	186.73 ± 57.25	15.80 ± 6.74	253.59 ± 122.58

**Table 2.** Vertical distribution of different soil properties under closed mixed Sal forest (Up to 1.0 m soil profile depth). \*BD, Bulk density; WHC, Water holding capacity; SOC, Soil organic carbon; Av. N, Available nitrogen; Av. P, Available phosphorus; and Av. K, Available potassium; Means in the column followed by different small letters in superscript (a–e) based on values represent statistical significant differences among the land uses at  $p < 0.05$ .

Soil Depth	BD	Moisture (%)	pH	WHC (%)	Sand (%)	Silt (%)	Clay (%)	SOC (%)	Av. N (kg/ha)	Av. P (kg/ha)	Av. K (kg/ha)
D1:0-20 cm	1.30 <sup>c</sup> ± 0.10	9.21 <sup>b</sup> ± 0.50	5.04 <sup>c</sup> ± 0.05	43.02 <sup>a</sup> ± 3.52	69.62 <sup>a</sup> ± 4.46	16.32 <sup>c</sup> ± 1.67	14.06 <sup>a</sup> ± 3.15	0.65 <sup>a</sup> ± 0.03	247.03 <sup>a</sup> ± 23.46	15.65 <sup>a</sup> ± 1.35	338.76 <sup>a</sup> ± 20.24
D2:20-40 cm	1.33 <sup>d</sup> ± 0.13	9.19 <sup>b</sup> ± 0.46	5.25 <sup>b</sup> ± 0.03	40.82 <sup>b</sup> ± 4.79	65.04 <sup>b</sup> ± 5.09	21.06 <sup>c</sup> ± 2.75	13.90 <sup>a</sup> ± 3.68	0.34 <sup>b</sup> ± 0.02	202.28 <sup>b</sup> ± 11.80	13.94 <sup>b</sup> ± 0.75	304.64 <sup>b</sup> ± 24.28
D3:40-60 cm	1.41 <sup>c</sup> ± 0.10	11.14 <sup>a</sup> ± 0.70	5.28 <sup>b</sup> ± 0.03	37.86 <sup>c</sup> ± 3.24	69.47 <sup>a</sup> ± 3.61	17.56 <sup>bc</sup> ± 2.99	12.96 <sup>b</sup> ± 2.18	0.31 <sup>c</sup> ± 0.02	176.36 <sup>c</sup> ± 9.98	11.41 <sup>c</sup> ± 0.68	224.0 <sup>c</sup> ± 17.71
D4:60-80 cm	1.45 <sup>b</sup> ± 0.11	8.91 <sup>c</sup> ± 0.73	5.41 <sup>b</sup> ± 0.05	35.16 <sup>d</sup> ± 2.59	69.22 <sup>a</sup> ± 3.15	18.92 <sup>b</sup> ± 4.03	11.86 <sup>c</sup> ± 2.33	0.24 <sup>d</sup> ± 0.02	149.04 <sup>d</sup> ± 7.10	10.96 <sup>d</sup> ± 0.99	153.16 <sup>d</sup> ± 9.77
D5:80-100 cm	1.53 <sup>a</sup> ± 0.12	8.04 <sup>d</sup> ± 0.56	5.53 <sup>a</sup> ± 0.03	35.43 <sup>d</sup> ± 2.98	69.19 <sup>a</sup> ± 2.39	18.66 <sup>b</sup> ± 2.34	12.15 <sup>bc</sup> ± 1.50	0.19 <sup>e</sup> ± 0.01	114.14 <sup>e</sup> ± 6.84	7.01 <sup>e</sup> ± 0.46	95.32 <sup>e</sup> ± 7.54
Mean	1.40 ± 0.09	9.30 ± 1.13	5.30 ± 0.18	38.46 ± 3.42	68.51 ± 1.95	18.50 ± 1.76	12.99 ± 0.99	0.35 ± 0.18	177.77 ± 50.68	11.79 ± 3.28	223.18 ± 101.55

**Table 3.** Vertical distribution of different soil properties under open mixed Sal forest (Up to 1.0 m soil profile depth). \*BD, Bulk density; WHC, Water holding capacity; SOC, Soil organic carbon; Av. N, Available nitrogen; Av. P, Available phosphorus; and Av. K, Available potassium; Means in the column followed by different small letters in superscript (a–e) based on values represent statistical significant differences among the land uses at  $p < 0.05$ .

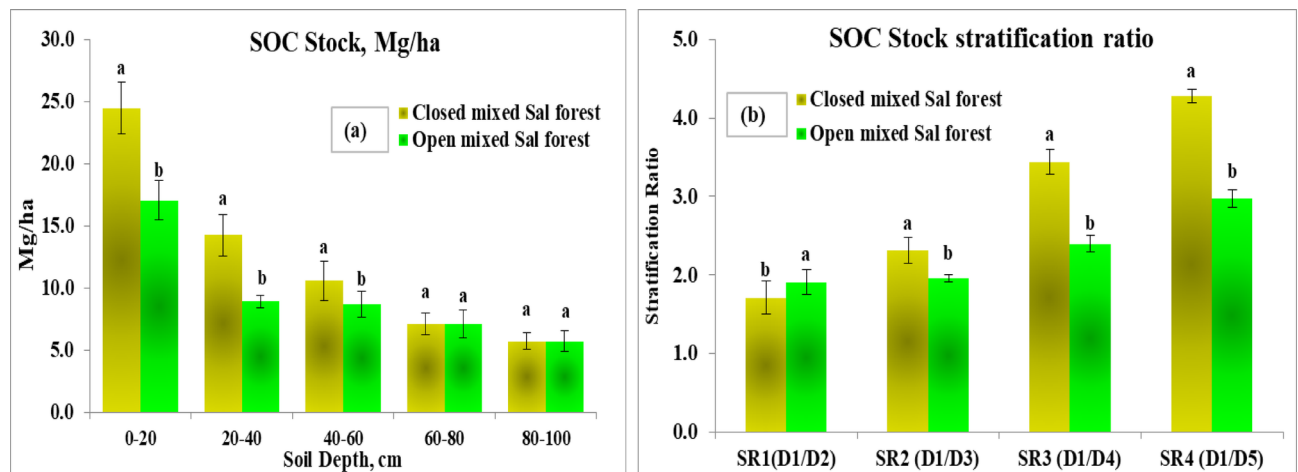
( $p < 0.05$ ) higher in the 36.5% in clay particles, 28.6% in soil organic carbon (SOC), 5% in available nitrogen (Av. N), 25% in available phosphorus (Av. P), 12% in available potassium (Av. K) and 23.5% in SOC stocks respectively (Table 1).

**Vertical distribution of different soil properties under closed and open mixed Sal forest**

Forest type significantly ( $p < 0.05$ ) influenced the distribution of different soil properties across the soil profile (up to 1.0 m soil depth). Open mixed Sal forest soils had significantly ( $p < 0.05$ ) higher (5.8 to 10.3%) bulk density across the soil profile depths (D1:0–20 cm to D5:80–100 cm), the maximum bulk density (1.53 Mg m<sup>-3</sup>) was found in the bottom-most soil profile layer (D5) of the open mixed Sal forest while the lowest (1.18 Mg m<sup>-3</sup>) was found in the surface soil of the closed mixed Sal forest (Tables 2, 3). Soil bulk density decreased constantly with depth irrespective of forest types. In contrast, closed mixed Sal forest had ( $p < 0.05$ ) higher (33.7 to 46.7%) soil moisture content compared to the open mixed Sal forest. The maximum soil moisture content (16.12%) was in the bottom-most soil profile depth (D5) of the closed mixed Sal forest while the lowest was in the soil depth (D4:60–80 cm) of the open mixed Sal forest (Tables 2, 3). The open mixed Sal forest was higher ( $p < 0.05$ ) 0.31 to 0.49 units in pH, 1.7 to 13.8% in water holding capacity, 4.4 to 16.4% in sand particles, and 8.2 to 22.5% in silt particles respectively under different soil profile depths (D1–D5) compared to the closed mixed Sal forest soils (Tables 2, 3). The closed mixed Sal forest was significantly higher ( $p < 0.05$ ), 32.2 to 40% in clay content, 5.3 to 41.3% in soil organic carbon (SOC), 2 to 8% in available nitrogen (Av. N), 1 to 36.9% in available phosphorus (Av. P) and 6.2 to 16.4% in the available potassium (Av. K) under different soil depths compared to the open mixed Sal forest soils (Tables 2, 3).

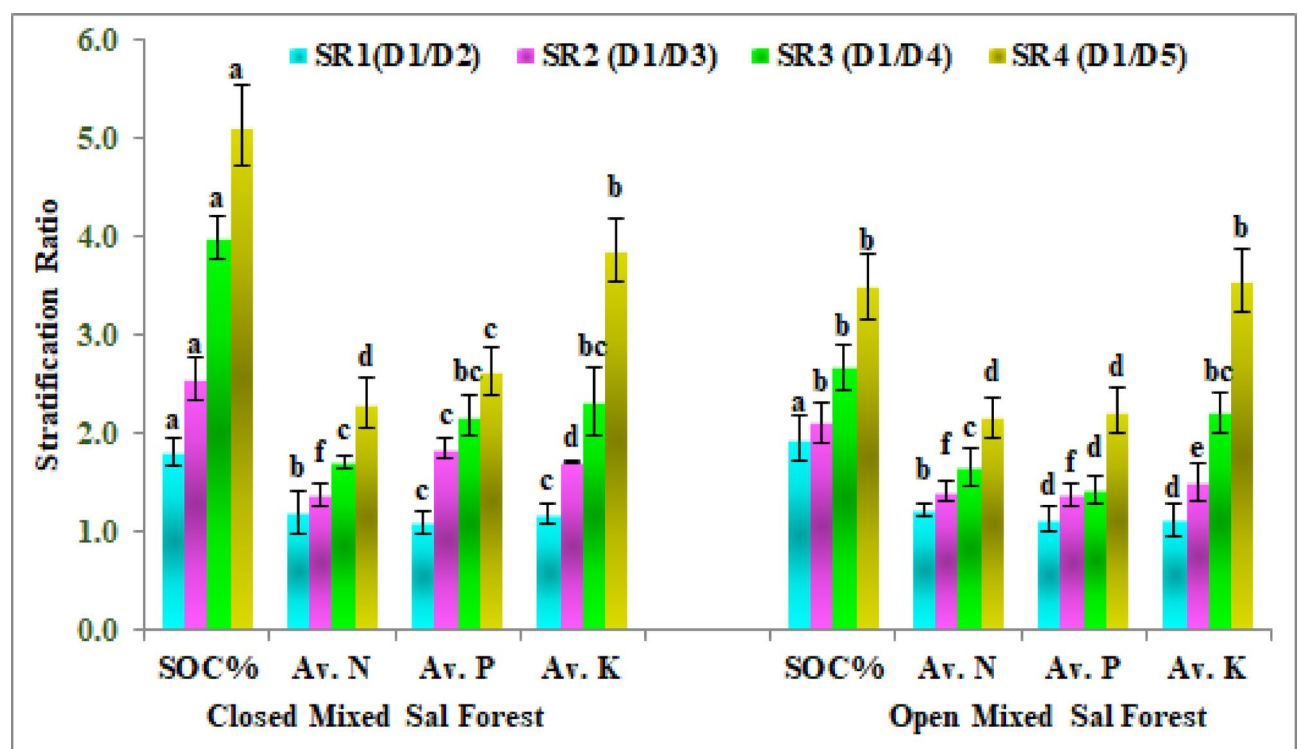
**Soil organic carbon stocks, stratification ratio and correlation of different soil properties**

Soil organic carbon stock (SOC stock) varied between 5.7 and 24.5 Mg ha<sup>-1</sup> irrespective of forest types and soil profile depths (Fig. 2a). SOC Stock was significantly ( $p < 0.05$ ) higher in the closed mixed Sal forest compared to the open mixed Sal forest across the soil profile depths (D1 to D5). Closed mixed Sal forest had 43% higher



**Figure 2.** a. SOC stock distribution of closed and open mixed sal forest and b. Stratification ratio of SOC stocks under closed and open mixed sal forest

**Fig. 2.** (a) SOC stock distribution of closed and open mixed sal forest and (b) Stratification ratio of SOC stocks under closed and open mixed sal forest.

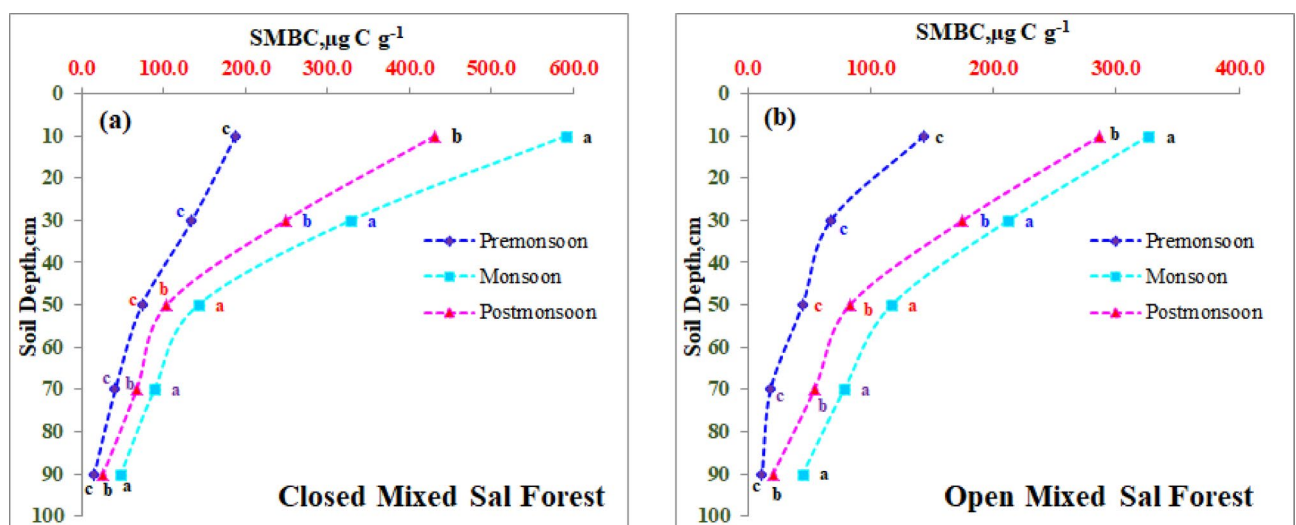


**Fig. 3.** Stratification ratio of different soil properties under closed and open mixed sal forest at different seasonal variations.

SOC stock in the surface soil (D1:0–20 cm) while in immediate subsurface soil (D2–20–40 cm) and middle of the soil profile (D3–40–60 cm), closed mixed Sal forest had 60% and 22% higher ( $p < 0.05$ ) SOC stocks than the open mixed Sal forest (Fig. 2a). Stratification ratio (SR) was higher in the open mixed Sal forest (1.9) compared to the closed mixed Sal forest (1.7) in the SR1 while in the subsurface to the bottom-most soil profile depths (SR2 to SR4), closed mixed Sal forest had higher SR than the open mixed Sal forest (Fig. 2b). Similarly, SR for other soil properties also behaved similarly to the SOC stocks, where open mixed Sal forest soils had higher ( $p < 0.05$ ) SR1 for SOC, available nitrogen, phosphorus, and potassium compared to the closed mixed Sal forest while, closed mixed Sal forest soils had higher SR2–SR4 for the same soil properties than the open mixed Sal forest (Fig. 3). Correlation analysis revealed that the sand and silt particles had a strong and significant positive

Soil Properties	BD	Moisture	pH	WHC	Sand	Silt	Clay	SOC	Av. N	Av. P	Av. K
BD	1										
Moisture	-0.42317	1									
pH	0.657975	-0.80204	1								
WHC	-0.03939	-0.21188	-0.08599	1							
Sand	0.313471	-0.61911	0.58985	0.088661	1						
Silt	0.149109	-0.42159	0.318004	0.057457	-0.26801	1					
Clay	-0.39543	0.855889	-0.76613	-0.12085	-0.82318	-0.32639	1				
SOC	-0.58562	0.435982	-0.7541	0.35715	-0.41369	-0.15854	0.499319	1			
Av. N	-0.64016	0.230175	-0.69743	0.503972	-0.28327	-0.02062	0.290072	0.857655	1		
Av. P	-0.66454	0.476434	-0.82792	0.301006	-0.46982	-0.11788	0.530425	0.884468	0.836715	1	
Av. K	-0.66201	0.275367	-0.73276	0.451882	-0.32542	-0.03803	0.341689	0.864275	0.948141	0.896357	1

**Table 4.** Association of different soil parameters across all the Land use practices estimated through Pearson's correlation coefficient. \*BD, Bulk density; WHC, Water holding capacity; SOC, Soil organic carbon; Av. N, Available nitrogen; Av. P, Available phosphorus; and Av. K, Available potassium.

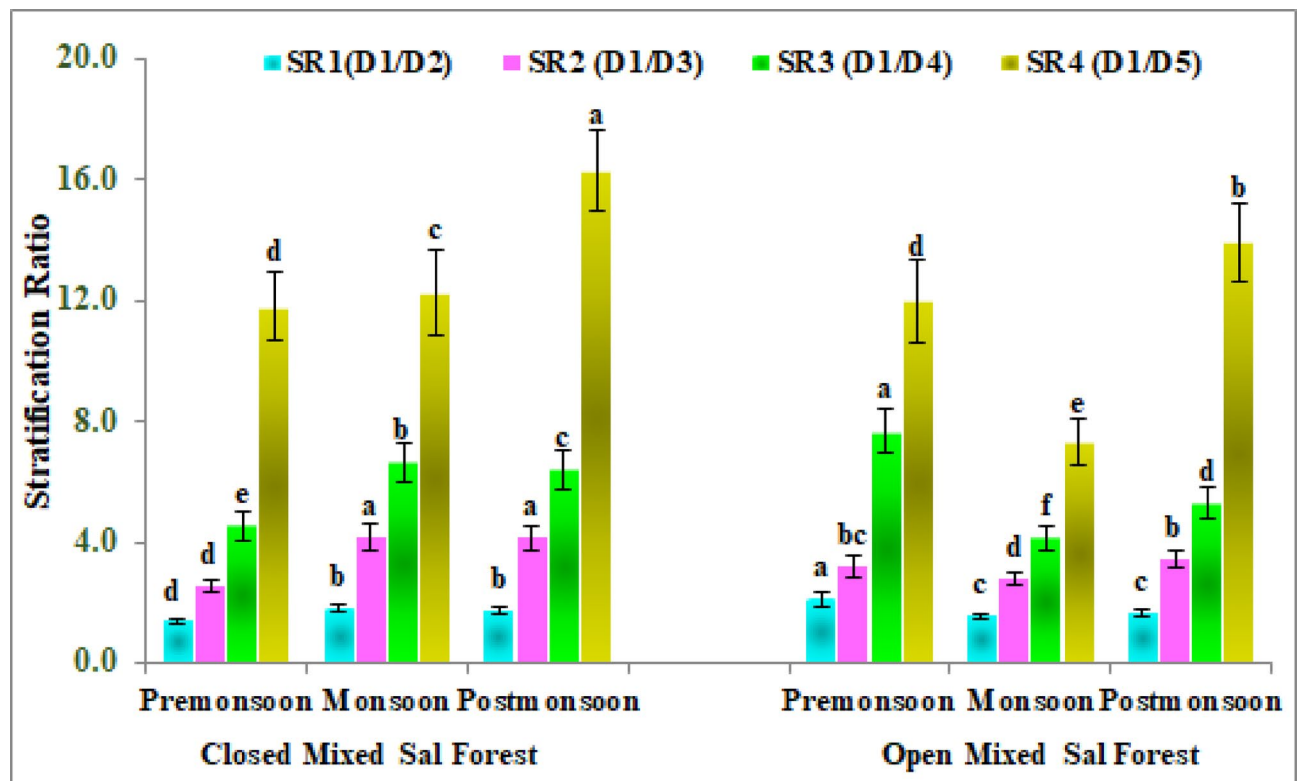


**Fig. 4.** Vertical distribution of soil microbial biomass carbon (SMBC) under open and mixed sal forest at different seasonal variations.

influence on bulk density ( $r = +0.31$ – $0.15$ ). In contrast, SOC had a strong and positive relation with the clay content ( $r = +0.50$ ) (Table 4). Similarly, SOC also showed a highly positive relation with the major soil nutrients (nitrogen, phosphorus, and potassium) ( $r = +0.86, +0.88, +0.86$ ) respectively, which defines that if the SOC concentration in soil increases, the concentration of available nitrogen, phosphorus, and potassium will also increase significantly (Table 4).

#### Seasonal effect on vertical distribution of soil microbial biomass carbon (SMBC) under closed and open mixed Sal forest

Results revealed that the SMBC had a strong seasonal effect under both forest types. The SMBC content ranged from  $12.0$  to  $591 \mu\text{g C g}^{-1}$  irrespective of forest type and season. The closed mixed Sal forest had significantly ( $p < 0.05$ ) higher SMBC content than the open mixed Sal forest across the soil profile (D1–D5) (Fig. 4). Closed mixed Sal forest had 60% higher ( $p < 0.05$ ) SMBC in surface soil (D1) than the open mixed Sal forest while it reduced with depth and 17.1 to 56.7% higher SMBC in the subsurface to bottom-most soil profile (D2–D5) compared to the open mixed Sal forest (Fig. 4). The SMBC content was higher in the Monsoon period ranged  $48.2$  to  $591 \mu\text{g C g}^{-1}$  in closed mixed Sal forest and  $44.8$  to  $326.4 \mu\text{g C g}^{-1}$  in open mixed Sal forest (Fig. 4). The SMBC content declined significantly ( $p < 0.05$ ) with seasonal change. The SMBC content reduced by 24.2 to 45.1% in the post-monsoon period while the reduction was more intense in the pre-monsoon period (48.1 to 68.2% compared to the monsoon period under the closed mixed Sal forest (Fig. 4). Similarly, the decline was more intense in the open mixed Sal forest, where SMBC declined 12.1 to 54% in the post monsoon and 56.1 to 76.2% in pre-monsoon period compared to the monsoon period (Fig. 4). The Stratification ratio for soil microbial biomass carbon (SMBC) was higher in the monsoon period across the soil profile (SR1 to SR4) compared to the SRs of pre and post-monsoon period under closed mixed Sal forest (Fig. 5). However, it was



**Fig. 5.** Stratification ratio of soil microbial biomass carbon (SMBC) under closed and open mixed sal forest at different seasonal variations.

reversed in the open mixed Sal forest, where the SRs of SMBC during monsoon period were lesser than in the pre and post-monsoon periods (Fig. 5).

## Discussion

### Effect of closed and open mixed Sal forest on different soil properties

The results of the present study revealed that the soil physicochemical (bulk density, moisture, pH, WHC, particle size, SOC, Av. N, Av. P, and Av. K) properties and SOC stocks differ significantly in open and closed mixed Sal forest. Soil physicochemical properties vary with land use and changes in soil management practices<sup>38–40</sup>; space and time because of varied topography, climate, vegetation cover, weathering process, and microbial activities<sup>41</sup>, and by a group of interactive controls such as disturbance regime and human activities<sup>42</sup>. Thus, Parent rocks, vegetation cover, and land use all influence soil properties within short distances. In our study, the average bulk density ( $1.31$  to  $1.40 \text{ Mg m}^{-3}$ ) was found in  $1.0 \text{ m}$  soil profile depth, similar to the value ( $1.12$  to  $1.38 \text{ Mg m}^{-3}$ ) up to  $1.5 \text{ m}$  soil depth<sup>43</sup>, close to the value  $1.28 \text{ Mg m}^{-3}$  reported by<sup>44</sup> up to  $1.0 \text{ m}$  soil depth. Higher bulk density was found in open mixed Sal forest compared to closed mixed Sal forest (Table 1) this is due to the greater organic matter deposition on the floor of the closed forest while in open forest less accumulation of organic matter was observed. The organic matter acts as a sponge, increases pore spaces, and reduces the bulk density<sup>7,45,46</sup>. Furthermore, anthropogenic activities and intensive grazing significantly affect the bulk density<sup>14,47,48</sup>. Soil moisture % was significantly higher in closed mixed Sal forest than open mixed Sal forest, which may be due to closed mixed Sal forest providing shade with high canopy cover as well as higher accumulation of plant litter that reduces direct sunlight<sup>49</sup>, temperature, and evaporation loss<sup>50</sup> on the soil surface. Moreover, open canopy resulted in a significant loss of soil moisture<sup>7,51</sup>. Similar results were reported by Thakrey et al.<sup>52</sup> and Shankar and Garkoti<sup>11</sup>, they found opening in the forest canopy leads to reduction in soil moisture. The regenerating capacity of Sal makes its pH acidic in nature<sup>53</sup>. The pH was moderately acidic ( $4.93$ – $5.30$ ) within the range reported by Jha et al.<sup>54</sup> ( $4.65$ – $5.97$ ), and significantly ( $p < 0.05$ ) higher in open mixed Sal forest. This could be associated with several factors including rapid leaching of the basic cations due to the faster rate of litter decomposition in undisturbed forests and higher accumulation of organic matter<sup>41</sup>, release acids, which decreases the soil pH<sup>55</sup>. Soil particle size distribution affects podzolization<sup>56</sup>, hydraulics, productivity, and soil erosion<sup>57</sup>. In our study sand and silt particles were higher in open mixed Sal forest, while clay particles were higher ( $20.45\%$ ) in closed mixed Sal forest than in open mixed Sal forest ( $12.99\%$ ) (Table 1). Soil texture is a static property that remains stable even with management measures, but it can be affected by soil erosion or human activities<sup>58</sup>. Soil organic carbon, available (N, P, K), and SOC stocks were found to be higher in closed mixed Sal forest as compared to open mixed Sal forest which agrees with Burke<sup>59</sup>, he proposed that the presence of N, P, K, soil physical characteristics, and organic matter was higher near the forest canopy and lower in areas with low tree density. Similar finding



was also reported by Singh et al.<sup>7</sup>, mentioned that nutrient availability in soil was significantly higher under the tree canopy than in open forest areas or non-planted sites. The nutrient availability in forest soil basically depends on the balance between nutrient incorporation<sup>60</sup> and decomposition rates in the forest ecosystem<sup>61</sup>. The amount of litterfall can positively impact the addition of primary limiting nutrients (N, P, and K)<sup>62</sup> and organic carbon<sup>5</sup> into the soil. However, the removal of vegetation cover and biomass due to anthropogenic activities harms litter production and accumulation of organic matter on the forest floor further affecting decomposition and nutrient cycling which accelerates nutrient loss<sup>48,63</sup>, as well as vegetation degradation significantly reduces the organic carbon concentration into the soil<sup>64</sup>.

### Vertical distribution of different soil properties under closed and open mixed Sal forest

Anthropogenic activities in mixed Sal forest have resulted in various observable and significant ( $p < 0.05$ ) changes in soil physiochemical properties across the soil profile (up to 1.0 m soil depth) (Tables 2, 3). Open mixed Sal forest soils had significantly ( $p < 0.05$ ) higher (5.8 to 10.3%) bulk density across the soil profile depths (D1:0–20 cm to D5:80–100 cm), the maximum bulk density ( $1.53 \text{ Mg m}^{-3}$ ) was found in the bottom-most soil profile layer (D5) of the open mixed Sal forest while the lowest ( $1.18 \text{ Mg m}^{-3}$ ) was found in the surface soil of the closed mixed Sal forest (Tables 2, 3), and it increased with soil depth in both forests. An increase in bulk density down the soil depth has been reported by several authors<sup>65–67</sup>. Increase in bulk density with soil depth due to less organic matter and overburden of overlying layers<sup>68</sup>. The maximum soil pH ( $5.53 \pm 0.03$ ) was recorded in the bottommost profile layer (0.8–1.0 m) of open mixed Sal forest and the minimum ( $4.72 \pm 0.06$ ) in the surface layer (0–0.2 m) of closed mixed Sal forest with a gradual increment in pH with soil depth up to the 1.0 m depth was found in the present study (Tables 2, 3). A similar trend in pH was also reported by Han, et al.<sup>69</sup>, He suggested that the increase in soil pH with depth may be due to neutralization by humic acids in the upper soil and a decrease in the loss of carbonate minerals in the deeper soil. The studied chemical properties SOC and Available (N, P, and K) of the soil were found to be significantly ( $p < 0.05$ ) different between different soil layers (Tables 2, 3). The closed mixed Sal forest was significantly higher ( $p < 0.05$ ), 5.3 to 41.3% in soil organic carbon (SOC), 2 to 8% in available nitrogen (Av. N), 1 to 36.9% in available phosphorus (Av. P) and 6.2 to 16.4% in the available potassium (Av. K) under different soil depths compared to the open mixed Sal forest soils (Tables 2, 3). The gradual reduction in SOC% and plant-available nutrients has been seen throughout the soil profile depth. This might be linked to increased intake and decreased contribution of nitrogen, phosphorus, and potassium nutrients by the litters<sup>70–72</sup>. In forest soil of the USA Jobbágy and Jackson<sup>73</sup> reported an 86% decrease in SOC in deeper depth compared to the surface layer. A larger amount of soil nutrients such as nitrogen, phosphorus, and potassium in the topsoil profile can be attributed to significant litter decomposition and increased organic matter inputs from litterfall<sup>72</sup>.

### Soil organic carbon stocks, stratification ratio and correlation of different soil properties

In the perennial woody trees, the carbon forms are controlled by a complex interaction between the carbon inputs (leaf litter, twig branches, etc.), carbon stabilization processes, and carbon loss (decomposition) in the soil<sup>74,75</sup>. The higher SOC stocks in closed mixed Sal forest and variation between the forest types could be attributed to the different quantities and qualities of organic substances through fresh leaf litter fall, living organisms, and root activity (e.g. turnover and exudates)<sup>76</sup>. However, SOC stocks were higher in surface soils under both land uses. This is in line with the earlier studies of the northeastern and northwestern Himalayan regions under similar climatic conditions<sup>7,14,70</sup>. In Germany, a study involving more than 2500 sites by Vos et al.<sup>77</sup> revealed that land use, land-use history, and clay content influenced the carbon content in surface soil and subsurface soils (L1:0–0.15 m to L2:0.15–0.30 m), whereas stratigraphy, parent material, and relief influenced the carbon content in subsoil to deeper soil profiles (L3:0.30–0.45 to L6:0.80–1.0 m). Leaf litter, ripening fruits, twigs, and branches had a major impact on the carbon content (TC, TOC, and SMBC) in the surface soil profile layer, but contributions from plant roots are more significant in the subsurface to deeper soil profile layers<sup>74,75</sup>. The higher SRs values in closed mixed Sal forests may have resulted from higher SOC stocks in the surface soils, whereas lower SRs in open mixed Sal forests may have resulted from open Sal forests themselves having much lower SOC stocks in the surface soil, with the decline being much more minor than in closed Sal forests<sup>58</sup>.

### Seasonal effect on vertical distribution of soil microbial biomass carbon (SMBC) under closed and open mixed Sal forest

Besides the importance of physicochemical and biological processes to maintain soil health, SMBC may be the 'keystone' biological driver of ecosystem functioning and an important index for soil health and environmental sustainability<sup>19</sup>. The closed mixed Sal forest had significantly ( $p < 0.05$ ) higher SMBC content than the open mixed Sal forest, range of SMBC reported in the present study ( $12.0$  to  $591 \mu\text{g C g}^{-1}$ ) irrespective of forest type and seasons, close to the value ( $17.08$  to  $484.52 \mu\text{g C g}^{-1}$ ) reported by<sup>78</sup>. Higher amount of SMBC in closed mixed Sal forest could be due to the presence of more organic matter and SOC content while in open mixed Sal forest various anthropogenic activities like collection of minor forest produce, surface burning, and grazing reduce the depositions of organic matter on the forest floor. Soil microbes require sufficient carbon, nitrogen, and energy sources for their synthesis and metabolism, which can be provided by high levels of soil organic matter and total nitrogen<sup>79</sup>. Soil microbial properties are influenced by soil moisture, temperature, nutrient supply, and human disturbances<sup>80</sup>. The distinct seasonal pattern of SMBC was similar in open and closed Sal mixed forest, the value being highest during monsoon and lowest during pre-monsoon (Fig. 4). Several authors found higher SMBC content during monsoon<sup>51,81–85</sup>. This might be due to the high temperature and moisture during the monsoon significantly promote the growth of soil microbes and contribute to the soil microbial biomass<sup>51,81,84–87</sup>. Also, during the wet period, high relative humidity accelerates fungi growth, which increases microbial biomass carbon<sup>88</sup>. Diaz-Ravina et al.<sup>89</sup> found that microbial biomass was lower in dry periods due

to water limitation rather than temperature. The availability of water has a significant impact on the survival and activity of soil microorganisms<sup>90</sup>. When water is scarce, it reduces intracellular water potential, leading to dehydration and inhibition of microbial activity<sup>91</sup>. During periods of moisture limitation, microbial communities experience starvation. Therefore, drought stress is considered the most common environmental stress for soil microorganisms<sup>92</sup>.

Besides land use type and season, soil depth is another crucial factor that regulates the SMBC. In the present study, SMBC was more in the topmost soil layer and less in the bottommost (Fig. 4). This pattern arises due to lower carbon and nitrogen concentrations in the subsoil and higher organic matter content in the topsoil, which provide the energy source to the microbial community, and hence promote microbial biomass. Similar findings on SMBC across soil depth in various land use types were reported by several authors<sup>58,83,93,94</sup>. The study offers critical insights into the effects of forest type and seasonal variability on soil properties, organic carbon stocks, and microbial biomass, showcasing its global relevance to forest conservation and sustainable management practices. The comparative analysis between open and closed mixed Sal forests demonstrates the significant impact of forest density on soil health, organic carbon sequestration, and microbial activity, with closed forests consistently showing higher levels of soil organic carbon (SOC), nutrient availability, and soil microbial biomass carbon (SMBC)<sup>95</sup>. The observed correlations between SOC and essential nutrients (nitrogen, phosphorus, and potassium) further emphasize the role of forest management in enhancing soil fertility and ecosystem productivity<sup>96</sup>. Moreover, the seasonal dynamics of SMBC underscore the sensitivity of soil microbial communities to climatic variations, offering valuable data to model the impacts of climate change on forest soils<sup>97</sup>. By addressing critical aspects of soil-forest interactions and highlighting the importance of forest density, the findings contribute to global efforts in carbon sequestration, soil health restoration, and combating land degradation, making them highly relevant for forest conservation strategies worldwide.

## Conclusion

Our comprehensive study highlights the significant impact of human activities on soil health in mixed Sal forests. It emphasizes the critical importance of preserving intact forest ecosystems for maintaining global ecological balance. The loss of forest cover due to anthropogenic activities decreases SMBC, which is essential for sustaining soil fertility, nutrient cycling, and overall ecosystem stability. Seasonal dynamics indicate that SMBC reaches its peak during the monsoon, further revealing the intricate relationship between climate, soil health, and microbial activity. These findings not only underscore the ecological importance of conserving closed mixed Sal forests but also provide valuable insights for global forest management and restoration efforts. There is an urgent need to minimize human-induced disturbances, such as illegal logging, grazing, and land conversion, to sustain soil microbial health and enhance carbon storage. Implementing protective measures, such as controlled land use, afforestation programs, and sustainable forest management practices, can help mitigate soil degradation and preserve biodiversity. Additionally, integrating SMBC monitoring into forest conservation strategies can serve as an early indicator of ecosystem health, guiding policymakers toward evidence-based conservation efforts. Protecting and restoring these ecosystems is crucial for mitigating climate change, enhancing biodiversity, and ensuring the long-term sustainability of soil and forest resources. By highlighting the ecological role of SMBC in forest ecosystems, this study contributes to a broader understanding of tropical forest sustainability. It provides actionable insights for improving soil quality, enhancing carbon sequestration, and strengthening forest resilience against climate change. Future research should focus on long-term monitoring of microbial carbon dynamics, including fire disturbance impacts, SOC fractions, and enzyme activities, to enhance understanding of carbon cycling and support sustainable forest management strategies.

## Declaration

### Data availability

Data will be made available on request, please get in touch with the corresponding author.

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## Author contributions

S.S.: conducted the study, collected the periodic soil sample, analysed it in the laboratory, prepared tables and figures, reviewed literature, and assisted in manuscript preparation. A.S. and B.D.: Conceptualized the study, data analysis (statistical), and figures, and wrote the main manuscript. S.C., L.P. and AT: Helped in the experiment, facilitated field and laboratory studies, and overall supervision.

## Competing interests

The authors declare no competing interests.

## Additional information

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