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## Soil microbial characteristics in sub-tropical agro-ecosystems of North Western Himalaya

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**Eight predominant land use systems, viz. agriculture ( $T_1$ ), horticulture ( $T_2$ ), agrisilviculture ( $T_3$ ), silvopastoral ( $T_4$ ), agrihorticulture ( $T_5$ ), agrihortisilviculture ( $T_6$ ), forest ( $T_7$ ) and grassland ( $T_8$ ) of subtropical parts of Himachal Pradesh were selected along two altitudinal ranges  $A_1$  (365–635 m amsl) and  $A_2$  (636–914 m amsl) to observe the variation in soil microbial activity and microbial characteristics. Agroforestry land uses and forest ecosystems displayed significantly higher microbial counts and microbial biomass carbon than agriculture and grasslands. The  $\text{CO}_2$  evolution (soil microbial activity) was found higher in**

**agrisilviculture, agrihortisilviculture, forest and grassland use systems at both altitudinal ranges. Soil biological properties (microbial count, microbial biomass and microbial activity) were maximum in forest land-use system. Among the agroforestry land-use systems, agrisilviculture had significantly higher microbial counts. The maximum microbial count ( $164.50 \times 10^5 \text{ cfu g}^{-1}$  soil) was recorded in forest and remained statistically at par with agrisilviculture ( $162.34 \times 10^5 \text{ cfu g}^{-1}$  soil). Minimum microbial count ( $80.66 \times 10^5 \text{ cfu g}^{-1}$  soil) was observed in agriculture land use. At both the altitudinal ranges, the  $\text{CO}_2$  evolution was highest at 48 h time interval and decreased thereafter. The metabolic quotient ( $q\text{CO}_2$ ) indicated that C-use efficiency is higher in grassland use and agriculture land use systems than other studied systems.**

**Keywords:** Microbial biomass carbon,  $\text{CO}_2$  evolution, metabolic quotient, land uses.

THE importance of microorganisms in ecosystem functioning has led to an increased interest in determining soil microbial biomass<sup>1</sup>. The soil microbial biomass is the active component of soil organic pool, responsible for organic matter decomposition, soil nutrient content and consequently primary productivity in most biogeochemical processes in terrestrial ecosystems<sup>2–4</sup>. Therefore, measuring microbial biomass is a valuable tool for understanding and predicting long-term effects on changes in land use and associated soil conditions<sup>5</sup>. Land use, climate change, habitat destruction as well as other human perturbations strongly alter natural ecosystems and understanding these responses is crucial to forecast sustainability of environmental services. Climatic, edaphic, topographic and biotic factors influence litter fall production in tree-based land-use systems and have direct or indirect effect on mitigation rate and soil carbon production.

Many studies have been carried out globally and in India on vegetation and soil carbon biomass of varying land use systems. However, studies on microbial biomass and  $\text{CO}_2$  evolution have been rarely reported in different agroecosystems in the Indian Himalayan region. Due to its unique topographical conditions, Himalayan ecosystem offers outstanding potential to investigate microbial biomass variations in prevailing land uses along the altitudinal level. Microbial biomass being an important component of ecosystem carbon needs to be assessed and examined across different land-use systems and crop combinations and best land use from a mitigation perspective needs to be recommended to forest and farming dependent communities. In the present study, we report the effect of major land-use systems of subtropical ecosystems of North-Western Himalayas on bacterial counts, microbial biomass carbon, their activities and metabolic quotient.

The study area is located between  $32^\circ 50'$  and  $30^\circ 22' \text{N}$  lat. and  $76^\circ 18'$  and  $77^\circ 47' \text{E}$  long. at 365 to 914 m amsl

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**Table 1.** Effect of land-use systems and altitudinal range on microbial count and bacterial biomass carbon

Land-use systems (T)	Microbial count ( $\times 10^5$ cfu $g^{-1}$ soil)	Microbial biomass carbon ( $\mu g/100 g^{-1}$ soil)
$T_1$ (Agriculture)	80.66	281.50
$T_2$ (Horticulture)	117.66	358.15
$T_3$ (Agrisilviculture)	162.34	380.19
$T_4$ (Silvopastoral)	125.33	414.75
$T_5$ (Agrihorticulture)	142.17	437.04
$T_6$ (Agrihortisilviculture)	140.17	464.96
$T_7$ (Forest)	164.50	506.63
$T_8$ (Grass land)	81.00	323.73
SEm $\pm$	5.14	27.66
CD <sub>0.05</sub>	14.93	80.27
$A_1$ (365–635 m)	136.79	400.41
$A_2$ (636–914 m)	116.67	391.33
SEm $\pm$	2.57	13.83
CD <sub>0.05</sub>	7.46	NS

**Table 2.** Effect of interaction between land-use systems and altitudinal range on soil microbial biomass carbon (MBC  $\mu g 100 g^{-1}$  soil)

Land-use systems (T)	Altitudinal range (A)	
	$A_1$ (365–635 m amsl)	$A_2$ (636–914 m amsl)
$T_1$ (Agriculture)	303.31	259.68
$T_2$ (Horticulture)	412.81	303.49
$T_3$ (Agrisilviculture)	426.87	333.50
$T_4$ (Silvopastoral)	390.12	439.38
$T_5$ (Agrihorticulture)	360.06	514.01
$T_6$ (Agrihortisilviculture)	441.57	488.36
$T_7$ (Forest)	473.60	539.66
$T_8$ (Grassland)	394.91	252.55
	SEm $\pm$	CD <sub>0.05</sub>
$T \times A$	39.12	113.51

altitudinal range at different locations of varying altitudes in the subtropical parts of districts Sirmaur, Solan and Kangra of Himachal Pradesh, India. This zone is affected by all three extreme climatic conditions, i.e. high temperature in summer (18–35°C), very low temperature in winter (5–21°C) and heavy rainfall in rainy season. The average annual rainfall varies from 1400 to 1800 mm and almost 80% of which is concentrated during July–August. The dominant soil texture is sandy loam and clay loam and soil pH is 6.3–6.6. Podzolic is parental material and soil types are brown, alluvial and grey brown.

Eight different land-use systems, viz. agriculture ( $T_1$ ), horticulture ( $T_2$ ), agri-silviculture ( $T_3$ ), silvopastoral ( $T_4$ ), agrihorticulture ( $T_5$ ), agrihortisilviculture ( $T_6$ ), forest ( $T_7$ ) and grassland ( $T_8$ ) were selected along two altitudinal ranges  $A_1$  (365–635 m amsl) and  $A_2$  (636–914 m amsl) to observe changes in soil microbial characteristics during 2011–2013. The soil samples were collected during July–October after rainfall, from three districts (Sirmaur, Solan and Kangra) at two altitudinal ranges. At each altitudinal range three sites were marked randomly on the basis of altitude and from each site three villages were selected

for soil sampling. Three replicates of soil samples were collected randomly from each land use system at 0–20 cm depth. Then these were stored in polythene bags, wrapped with cloth bags and transported in thermocol containers to the laboratory where they were stored in a refrigerator for further analysis.  $K_{EC}$  value of 0.38 as proposed<sup>6</sup> was used to calculate microbial biomass carbon (MBC). MBC was determined by soil fumigation–extraction method in fresh soil samples<sup>7</sup>. The soil samples were not pre-incubated. The difference in soil organic carbon with and without fumigation was considered as soil microbial biomass carbon. The population was expressed as colony forming units (cfu  $g^{-1}$  soil). Soil microbial activity was measured by  $CO_2$  evolution method<sup>8</sup>. In this method, 100 g of soil was taken in a one litre flask; then water was added to maintain 30–35% moisture. The flask was incubated at  $30 \pm 2^\circ C$  with appropriate control and then the test tube was removed at different time intervals (12, 24, 36, 48, 72 and 96 h). To this 1 ml of 50%  $BaCl_2$  was added and this was titrated against 1 N HCl with phenolphthalein as indicator. The results were expressed as mg  $CO_2 g^{-1}$  soil.

$$CO_2 \text{ evolution (mg } CO_2/g \text{ soil)} = (B - V) NE,$$

where  $B$  is the volume of HCl used for blank,  $V$  the volume of HCl used for sample flask,  $N$  the normality of acid and  $E = 22$  (equivalent weight of  $CO_2$ ).

For microbial count, the soil was analysed for total bacterial counts at the initiation and end of the experiment, i.e. after three days. The viable bacterial counts were carried out on nutrient agar medium by following the serial dilution and pour plate method. One gram soil mixture was taken in 9 ml of sterilized water blank and the soil suspension was diluted in 10 fold series; then the microbial count was determined by standard pour plate technique on different media<sup>9</sup>. The data obtained were subjected to statistical analysis using randomized block design (RBD)<sup>10</sup>. Wherever the effects exhibited

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**Table 3.** Effect of land-use systems and altitudinal range on soil microbial activity (CO<sub>2</sub> evolution) (µg CO<sub>2</sub> g<sup>-1</sup> soil) by CO<sub>2</sub> evolution method

Land use systems	Altitudinal range								Mean
	A <sub>1</sub> (365–635 m amsl)				A <sub>2</sub> (636–914 m amsl)				
	24 h	48 h	72 h	Average	24 h	48 h	72 h	Average	
T <sub>1</sub> (Agriculture)	8.37	11.65	10.90	10.30	7.31	10.55	7.71	8.52	9.41
T <sub>2</sub> (Horticulture)	8.47	13.80	15.07	12.44	6.15	8.41	7.31	7.29	9.86
T <sub>3</sub> (Agrisilviculture)	18.33	21.63	19.54	19.83	5.58	7.31	6.10	6.33	13.08
T <sub>4</sub> (Silvopastoral)	8.25	11.77	9.21	9.74	8.44	10.19	9.31	9.31	9.52
T <sub>5</sub> (Agrihorticulture)	7.82	10.68	8.44	8.98	6.74	8.47	7.14	7.45	8.21
T <sub>6</sub> (Agrihortisilviculture)	10.02	17.10	14.62	13.91	10.61	12.83	11.08	11.50	12.70
T <sub>7</sub> (Forest)	13.80	18.31	15.07	15.72	14.59	17.37	16.13	16.02	15.87
T <sub>8</sub> (Grassland)	11.70	19.41	16.11	15.74	11.60	13.97	12.83	12.80	14.27
Mean	10.84	14.28	11.60	12.24	8.87	11.13	9.70	9.90	

**Table 4.** Microbial quotient (at the peak rate of microbial activity) in relation to land use and altitudinal gradient

Land-use systems	Altitudinal range		Mean
	A <sub>1</sub> (365–635 m amsl)	A <sub>2</sub> (636–914 m amsl)	
	T <sub>1</sub> (Agriculture)	3.88	
T <sub>2</sub> (Horticulture)	3.34	2.79	3.06
T <sub>3</sub> (Agrisilviculture)	5.07	2.19	3.63
T <sub>4</sub> (Silvopastoral)	3.01	2.32	2.66
T <sub>5</sub> (Agrihorticulture)	2.96	1.64	2.30
T <sub>6</sub> (Agrihortisilviculture)	3.87	2.62	3.24
T <sub>7</sub> (Forest)	3.87	3.21	3.54
T <sub>8</sub> (Grassland)	4.92	5.54	5.23
Mean	3.86	3.04	

significance at 5% level of probability, the critical difference (CD) was calculated.

It is evident from the data presented in Table 1 that various tree-based land-use systems under study, viz. agrisilviculture, agrihorticulture, agrihortisilviculture and forest ecosystems displayed significantly higher microbial biomass as well as bacterial counts than monoculture-based land-use systems of agriculture, horticulture and grassland, but significant differences were recorded with respect to bacterial counts only. The maximum bacterial counts ( $164.50 \times 10^5$  cfu g<sup>-1</sup> soil) were recorded in the forest which, however, remained statistically at par with agrisilviculture ( $162.34 \times 10^5$  cfu g<sup>-1</sup> soil) only. Whereas minimum bacterial counts ( $80.66 \times 10^5$  cfu g<sup>-1</sup> soil) were observed in agriculture land use followed by grassland ( $81.00 \times 10^5$  cfu g<sup>-1</sup> soil) (Table 1). Higher microbial biomass carbon in forest ecosystem may be due to production of more leaf litter and deep root system of trees allowing more microbial activities than in monoculture-based agricultural system. Maximum microbial biomass carbon (506.63 MB-C µg 100 g<sup>-1</sup> soil) was found in forest land-use system followed by agrihortisilviculture (464.96 MB-C µg 100 g<sup>-1</sup> soil), agrihorticulture (436.04

MB-C µg 100 g<sup>-1</sup> soil) and silvopastoral system (414.75 MB-C µg 100 g<sup>-1</sup> soil), agrisilviculture (380.19 MB-C µg 100 g<sup>-1</sup> soil) respectively (Table 1). Minimum MBC was noted in agriculture system (281.50 MB-C µg 100 g<sup>-1</sup> soils). Compared to conventional practices, organic farming practices have shown to promote higher microbial biomass<sup>11,12</sup>. MBC was also significantly higher under agroforestry-based land use systems, viz. agrisilviculture, silvopastoral, agrihorticulture and agrihortisilviculture systems than agriculture. Higher biomass in forest ecosystem and other tree-based land use systems may be due to production of more leaf litter and deep root system of trees allowing more microbial activities than in agricultural system<sup>11</sup>. MBC values obtained in the present study coincide well with those reported for temperate (61–2000 µg g<sup>-1</sup>)<sup>13</sup> and tropical (102–2073 µg g<sup>-1</sup>)<sup>14</sup> regions. MBC values found by other studies range from 279 to 910 µg g<sup>-1</sup> for stands of different age, soil type and species composition<sup>15</sup>, and from 219 to 864 µg g<sup>-1</sup> for different land uses (forest, agroforestry, agriculture and wasteland)<sup>5</sup>. MBC values range from 166 to 1539 µg g<sup>-1</sup> for pasture land<sup>16</sup>, 726.70 to 1529.14 µg g<sup>-1</sup> for wasteland<sup>17</sup>, and are at mean values of 1684 µg g<sup>-1</sup> for sacred grove forest, 806.1 µg g<sup>-1</sup> for grassland<sup>16</sup>, 248 µg g<sup>-1</sup> for agricultural soil, and 1326 µg g<sup>-1</sup> for forest soil<sup>18</sup>. The bacterial counts showed decline with increase in the altitudinal range. The higher microbial counts at lower altitude (A<sub>1</sub>) than at upper ones (A<sub>2</sub>) can be ascribed to decrease in viable counts with increasing altitude. Interaction effect between land-use system and altitudinal range depicts that different land-use systems did not show any consistent trend with increase in the elevation range (Table 2). In land-use systems, agriculture, horticulture, agrisilviculture and grassland the MBC declined with the increasing altitude and increased under silvopastoral, agrihorticulture, agrihortisilviculture and forest land use with the increase in altitude (Table 2).

The CO<sub>2</sub> evolution through microbial activity showed marked variation in different land-use systems after 24, 48 and 72 h. It is clear from Table 3 that at both the

altitudinal ranges, CO<sub>2</sub> evolution showed maximum value at 48 h period and then decreased thereafter. At the A<sub>1</sub> altitudinal range, maximum (21.63 µg CO<sub>2</sub> g<sup>-1</sup> soil) CO<sub>2</sub> evolution at 48 h was recorded under agrisilviculture, followed by grassland (19.41 µg CO<sub>2</sub> g<sup>-1</sup> soil), forest (18.31 µg CO<sub>2</sub> g<sup>-1</sup> soil), agrihortisilviculture (17.10 µg CO<sub>2</sub> g<sup>-1</sup> soil), horticulture (13.80 µg CO<sub>2</sub> g<sup>-1</sup> soil), silvopastoral (11.77 µg CO<sub>2</sub> g<sup>-1</sup> soil), agriculture (11.65 µg CO<sub>2</sub> g<sup>-1</sup> soil) and agrihorticulture (10.68 µg CO<sub>2</sub> g<sup>-1</sup> soil), respectively. However, at altitudinal range of A<sub>2</sub> (636–914 m amsl), maximum (17.34 µg CO<sub>2</sub> g<sup>-1</sup> soil) CO<sub>2</sub> evolution was recorded in forest land use system followed by grassland, agrihortisilviculture, agriculture, silvopastoral, agrihorticulture, horticulture and agrisilviculture. This variation in microbial activity under different land-use systems with altitudinal range may be due to the combined effect of quantity of leaf litter, amount of organic matter and prevailing temperature conditions. Accelerated microbial activity may be due to addition of litter in large quantity, huge amount of organic matter and elevated temperature<sup>18,19</sup>.

Table 4 reflects that the metabolic quotient for the land-use systems, viz. agriculture and grassland is markedly higher than silvipastoral and agri-horticulture. The metabolic quotient of the land uses, viz. horticulture, agri-horticulture and forest is however in the middle category. The metabolic quotient for CO<sub>2</sub> (qCO<sub>2</sub>) has found world-wide application in soil microbial ecology. In ecological terms, however, a high qCO<sub>2</sub> reflects a high maintenance carbon demand, and if the soil system cannot replenish the carbon which is lost through respiration, microbial biomass must decline<sup>20</sup>. This means that agriculture, agrisilviculture and grasslands which have higher metabolic quotient should be supplied/replenished with adequate amount of carbon source in order to maintain microbial carbon demand, whereas in systems like silvipastoral, agrihorticulture and horticulture where there is annual accretion of C in the form of leaf litter, the level of stress is lower. The value of metabolic quotient at lower altitudinal range (A<sub>1</sub>) is higher than at the upper altitudinal range (A<sub>2</sub>). This implies that there is high C maintenance demand at low altitude than at high altitude, probably due to higher temperature.

From the present study, it can be concluded that there is need to adopt tree-based systems on mass scale in place of monocultures, like agriculture and grassland on account of better status of microbial count, microbial biomass carbon, soil microbial activity and lower microbial quotient for maintaining better soil health and sustainability.

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