

## Study on Microbial and Ecophysiological Indices of Tea Soils in Chattogram and Khagrachari Districts of Bangladesh

Sanjida Akter<sup>1</sup>, Shuva Das<sup>2</sup>, Sajal Roy<sup>1\*</sup> and Nasrin Chowdhury<sup>1</sup>

<sup>1</sup> Department of Soil Science, Faculty of Biological Sciences, University of Chittagong, Chattogram-4331, Bangladesh

<sup>2</sup> Bangladesh Tea Research Institute, Fatickchhari, Chattogram-4353, Bangladesh

\* Correspondence to: Sajal Roy; Email: sajal.roy@cu.ac.bd

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### Abstract

The evaluation of soil microbial and ecophysiological properties is highly significant to maintain soil health and sustainable production of crops. Thus, this research was carried out to observe the status of different microbial and ecophysiological indices of tea soils belonging to Oodaleah tea garden (OTG) and Tin Tohori tea garden (TTTG) located in Chattogram and Khagrachari districts of Bangladesh. A total number of thirty seven and twenty four surface soil (0-9 cm) samples were collected from OTG and TTTG, respectively. The areas of both the tea gardens are mostly covered by low hill ranges to terraces. Irrespective of the sampling sites, the mean values of microbial biomass carbon (MBC), microbial activity (MA), substrate induced respiration (SIR), microbial quotient (qMic) and mineralization quotient (qM) were found to be 3.73%, 6.25%, 2.15%, 26.19% and 20.0% higher, while metabolic quotient (qCO<sub>2</sub>) was 7.69% lower in TTTG compared to OTG. The findings of this study clearly showed the highest microbial responses at toeslope except MA in TTTG. As for OTG, the MA and qM at toeslope significantly differed ( $p < 0.05$ ) from the values found at summit. However, all the microbial and ecophysiological indices did not differ significantly ( $p > 0.05$ ) among the three different slopes for TTTG. All the microbial indices were positively correlated, while a significant ( $p < 0.01$ ) negative correlation was found between MBC and qCO<sub>2</sub> both for OTG and TTTG. Besides, the correlation between qCO<sub>2</sub> and qMic was also found negative and significant ( $p < 0.05$ ) both for OTG and TTTG.

**Keywords:** Ecophysiological indices, microbial activity, microbial biomass carbon, tea soils

### Introduction

Tea (*Camellia sinensis* L.), one of the cheapest and popular temperance drinks throughout the world, is made from the young leaves of tea plants<sup>1</sup>. In Bangladesh, tea gardening is practiced in the hilly regions of Sylhet, Moulavibazar, Chattogram (previously named Chittagong), Rangamati, Khagrachari and Panchagarh districts as tea plant enjoys plentiful rainfall. The consumption of tea among Bangladeshi population is increasing at a significant level because of its health benefits<sup>2</sup>. A total amount of 102.92 million kilogram tea was produced from 168 tea gardens in 2023 which was ever the highest record of tea production in Bangladesh. Of the total, there are 22 tea gardens in Chattogram district of Bangladesh<sup>3</sup>.

Oodaleah tea garden (OTG) has been playing significant role in tea production in Bangladesh since 1962<sup>4</sup>. On the other hand, the cultivation of tea in the Tin Tohori tea garden (TTTG) was started in 2016.

Tea plants grow best in well-draining light textured soils with acidic (4.5-5.5) reaction<sup>4,5</sup>. Tea soils of Bangladesh are often lack of organic matter and readily available nutrients required for optimum growth of tea plant<sup>6,7</sup>. Maintaining the quality of soil is very critical for achieving soil-related ecosystems and sustainable tea crop production. The ability of soil to perform a particular function can be influenced by several soil quality attributes such as physical, chemical and biological<sup>8</sup>. Microbial activity (MA), microbial biomass

carbon (MBC) and substrate induced respiration (SIR) are considered the most important microbial attributes that determine soil health by influencing biological functions as well as the physical and chemical properties of soils and ultimately regulating the productivity of agricultural ecosystems<sup>9,10</sup>. The MBC represents the labile soil carbon fraction used to assess the biological activities in soil<sup>11</sup>. The MA and SIR also contribute to the growth of plants by recycling nutrients and energy under various ecological settings<sup>12</sup>. Besides, different ecophysiological indices such as metabolic quotient ( $qCO_2$ ), microbial quotient ( $qMic$ ) and mineralization quotient ( $qM$ ) are considered important determinants of soil health. The  $qCO_2$  indicates the metabolic efficiency of soil microbial communities, while the  $qM$  indicates the fraction of organic carbon mineralization over a period of time<sup>13</sup>. Soil microbial and ecophysiological properties can be used as the important determinants in maintaining soil health and ecosystem services providing important insights to

farmers, policymakers, and stakeholders in making sustainable production of tea<sup>14,15</sup>.

Although research on tea soils regarding physico-chemical properties is available, very limited research was found in the existing literature on tea soils *vis-à-vis* the microbiological aspect. Furthermore, no study has yet been done on such microbiological indices as MBC, MA and SIR of tea soils in Chattogram Hill Tract. Therefore, the present study was carried out to evaluate the status of MBC, MA and SIR of tea soils from two different tea gardens i.e., OTG and TTTG located in Chattogram and Khagrachari districts. Besides, such ecophysiological indices as  $qCO_2$ ,  $qMic$  and  $qM$  were assessed in the tea soils of the aforementioned tea gardens.

## Materials and Methods

### Study area

The soil samples were collected from two different tea gardens hereinafter OTG and TTTG. The OTG is situated in the hilly region of Suabil union of

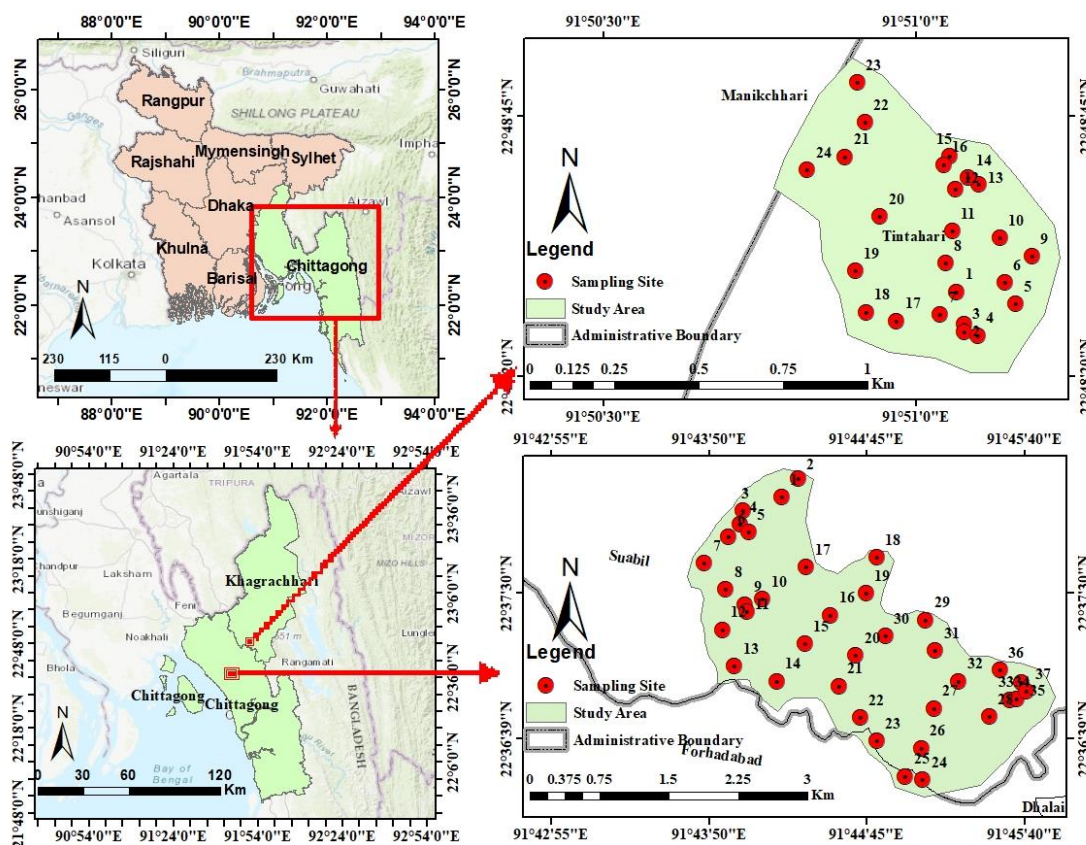


Figure 1. Sampling sites of OTG (right bottom) and TTTG (right top).

Fatikchhari upazila in the Chattogram district, while TTTG is situated at the village of Kumari Boro Tilla in Tin Tohori union of Manikchhari upazila in the Khagrachari district of Bangladesh. The OTG has an area of 2995.72 acres, while TTTG has an area of 43 acres<sup>3</sup>. The soil sampling sites belonging to OTG and TTTG are shown in Figure 1.

#### *Collection and processing of soil samples*

A total number of 37 soil samples were collected from a depth of 0-9 cm from OTG. From the same depth, a total number of 24 soil samples were collected from TTTG. Soil samples were brought to the laboratory just after the collection from the field. Bulk soil samples were made homogenous and kept in the refrigerator immediately until the experiment was set up.

#### *Analyses of soil samples*

The MBC of soil was measured by fumigation-extraction method<sup>16</sup>. According to this method, two sets of all soil samples with a 5 g amount were taken in plastic bottles immediately after pre-incubation. One set of samples was fumigated with alcohol-free chloroform for 24 hours at  $\approx 25^{\circ}\text{C}$  in a sealed desiccator to kill the microbial cells in the soil. Other sets or the non-fumigated sets of samples were stored in the fridge. After fumigant removal, both fumigated and non-fumigated soils were extracted with freshly prepared 0.5M  $\text{K}_2\text{SO}_4$  at 1:4 ratios and filtered. Samples were digested in the presence of 0.0667M  $\text{K}_2\text{Cr}_2\text{O}_7$  and concentrated  $\text{H}_2\text{SO}_4$ . Dissolved organic carbon in the extracts was determined by titrating with 0.033M acidified  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2(\text{H}_2\text{O})_6$ . The amount of MBC was calculated according to the following equation<sup>16</sup>:

$$\text{MBC} = [(\text{organic carbon extracted from fumigated soils}) - (\text{organic carbon extracted from non-fumigated soils})] \times 2.46$$

The MA was determined by soil microbial respiration, by trapping the  $\text{CO}_2$  in NaOH which evolved from the soil during incubation in a closed system<sup>17</sup>. The trapped  $\text{CO}_2$  was determined by measuring electrical conductivity. For this purpose, 50g pre-incubated soil was placed in plastic jars. A plastic bottle containing 20

ml of 0.5M NaOH solution was placed in each jar as the  $\text{CO}_2$  trap. An amount of 10ml water in 50ml plastic bottles was also placed inside the jar to maintain the soil moisture. Jars were made airtight immediately. Two jars with 20ml 0.5M NaOH but without soil were used as control. All jars were incubated at  $\approx 25^{\circ}\text{C}$ . The  $\text{CO}_2$  absorbed in the traps was analyzed at 11, 28, and 60 days of NaOH placement. Each time fresh NaOH solution (20 ml) was replaced to trap  $\text{CO}_2$  for the next incubation period. In this method,  $\text{CO}_2$  evolved from each sample was calculated as the difference between the initial and the  $\text{CO}_2$  concentration after each measurement period. The amount of soil organic carbon was determined by the wet oxidation method as described in Huq and Alam<sup>18</sup>.

The SIR of the soils was assessed according to the rate of the maximal initial respiration of the microorganisms after the enrichment of the soils with 2% glucose<sup>19</sup>. Over the first 2 h, the increase in  $\text{CO}_2\text{-C}$  was proportional to the size of the initial MBC concentration. Respiration was determined by trapping the  $\text{CO}_2$  in NaOH as done for MA.

The ecophysiological analyses were performed following the equations given by Anderson and Domsch<sup>20</sup>.

The  $q_{\text{Mic}}$ , the ratio of MBC to SOC, was determined by the following equation:

$$q_{\text{Mic}} (\text{C}_{\text{mic}} : \text{C}_{\text{org}}) = \text{mg of MBC} \times \text{Kg C}^{-1}.$$

The  $q_{\text{M}}$  expresses the fraction of total organic carbon mineralized and was calculated from basal respiration by soil organic carbon ratio.

$$q_{\text{M}} = \text{mg CO}_2\text{-C cumulative} \times \text{Kg total organic C}^{-1}$$

The  $q_{\text{CO}_2}$ , the ratio of basal soil respiration by microbial biomass ( $\text{C}_{\text{mic}}$ ) was calculated according to the procedure as follows:

$$q_{\text{CO}_2} = \text{mg CO}_2\text{-C basal} \times \text{mg C}_{\text{mic}} \text{ h}^{-1}.$$

#### *Statistical analysis of data*

All analytical data of soil samples were expressed on an oven-dried basis. Descriptive statistics and correlations of the soil parameters were done through Statistical

Packages for Social Sciences (SPSS) software. The differences in the means were also tested through one-way analysis of variance (ANOVA) SPSS software 16 version.

## Results and Discussion

Table 1 and Table 2 show the mean values of different microbial parameters of OTG and TTTG. Regardless of the sampling sites, the MBC in soils of OTG ranged from 52.48 mg C/Kg to 384.86 mg C/Kg with the mean value of 288.38 mg C/Kg. As for TTTG, the highest MBC content was 377.28 mg C/Kg and the lowest value was 73.25 mg C/Kg. The mean value of MBC was found 296.46 mg C/Kg of all the sampling sites. Irrespective of the sampling sites in OTG, the MA and SIR were found in the range of 0.04-0.64 mgCO<sub>2</sub>/g and 0.96-5.65 mgCO<sub>2</sub>/g respectively, with the mean values of 0.32 mgCO<sub>2</sub>/g and 3.75 mgCO<sub>2</sub>/g, respectively. On the other hand, MA and SIR in TTTG ranged from 0.04 mgCO<sub>2</sub>/g to 0.74 mgCO<sub>2</sub>/g and 1.08 mgCO<sub>2</sub>/g to 5.43

mgCO<sub>2</sub>/g, respectively with mean values of 0.34 mgCO<sub>2</sub>/g and 3.80 mgCO<sub>2</sub>/g irrespective of the land types. The mean values of qCO<sub>2</sub>, qMic and qM were 0.13 mg CO<sub>2</sub>-C/ mg Cmic h<sup>-1</sup> × 10<sup>-4</sup>, 0.42% and 0.05 mg CO<sub>2</sub>-C/mg Corg × 10<sup>-2</sup>, respectively as for OTG, whereas 0.12 mg CO<sub>2</sub>-C/ mg Cmic h<sup>-1</sup> × 10<sup>-4</sup>, 0.53% and 0.06 mg CO<sub>2</sub>-C/mg Corg × 10<sup>-2</sup>, respectively as for TTTG. The mean values of MBC, MA, SIR, qMic and qM were found to be 3.73%, 6.25%, 2.15%, 26.19% and 20.0% higher, while qCO<sub>2</sub> was 7.69% lower in TTTG compared to OTG. In our study, clear differences in microbial properties were observed between two tea gardens. Soil is a dynamic natural body which highly varies both spatially and temporally<sup>21</sup>. The differences in microbial indices between the two tea gardens could be due to microclimate, human management, edaphic factors, and soil biodiversity<sup>22</sup>. Khadka *et al.*<sup>23</sup> stated that the properties of soil even in a small land area may vary due to such factors as management practices, vegetation, topography etc. The variation in microbial

**Table 1.** Descriptive statistics of microbial parameters and ecophysiological indices of tea soils in OTG

Parameters	Minimum	Maximum	Mean	Range	Skewness	Kurtosis
MBC	52.48	384.86	288.38	332.38	-1.109	0.629
MA	0.04	0.64	0.32	0.60	-0.06	-0.72
SIR	0.96	5.65	3.72	4.69	-0.37	-1.11
qCO <sub>2</sub>	0.01	0.58	0.13	0.57	2.84	11.03
qMic	0.06	0.89	0.42	0.83	0.575	-0.27
qM	0.00	0.14	0.05	0.14	1.07	1.41

Note: MBC- mg C/Kg, MA- mg CO<sub>2</sub>/g, SIR- mg CO<sub>2</sub>/g, qCO<sub>2</sub>- mg CO<sub>2</sub>-C/ mg Cmic h<sup>-1</sup> × 10<sup>-4</sup>, qMic- %, qM- mg CO<sub>2</sub>-C/mg Corg × 10<sup>-2</sup>

**Table 2.** Descriptive statistics of microbial parameters and ecophysiological indices of tea soils in TTTG

Parameters	Minimum	Maximum	Mean	Range	Skewness	Kurtosis
MBC	73.25	377.28	299.13	304.03	-1.51	3.02
MA	0.04	0.74	0.34	0.70	0.48	0.10
SIR	1.08	5.43	3.80	4.35	-0.50	-0.45
qCO <sub>2</sub>	0.01	0.39	0.12	0.38	1.69	4.52
qMic	0.08	1.28	0.53	1.20	1.06	2.11
qM	0.01	0.14	0.06	0.13	0.53	-0.06

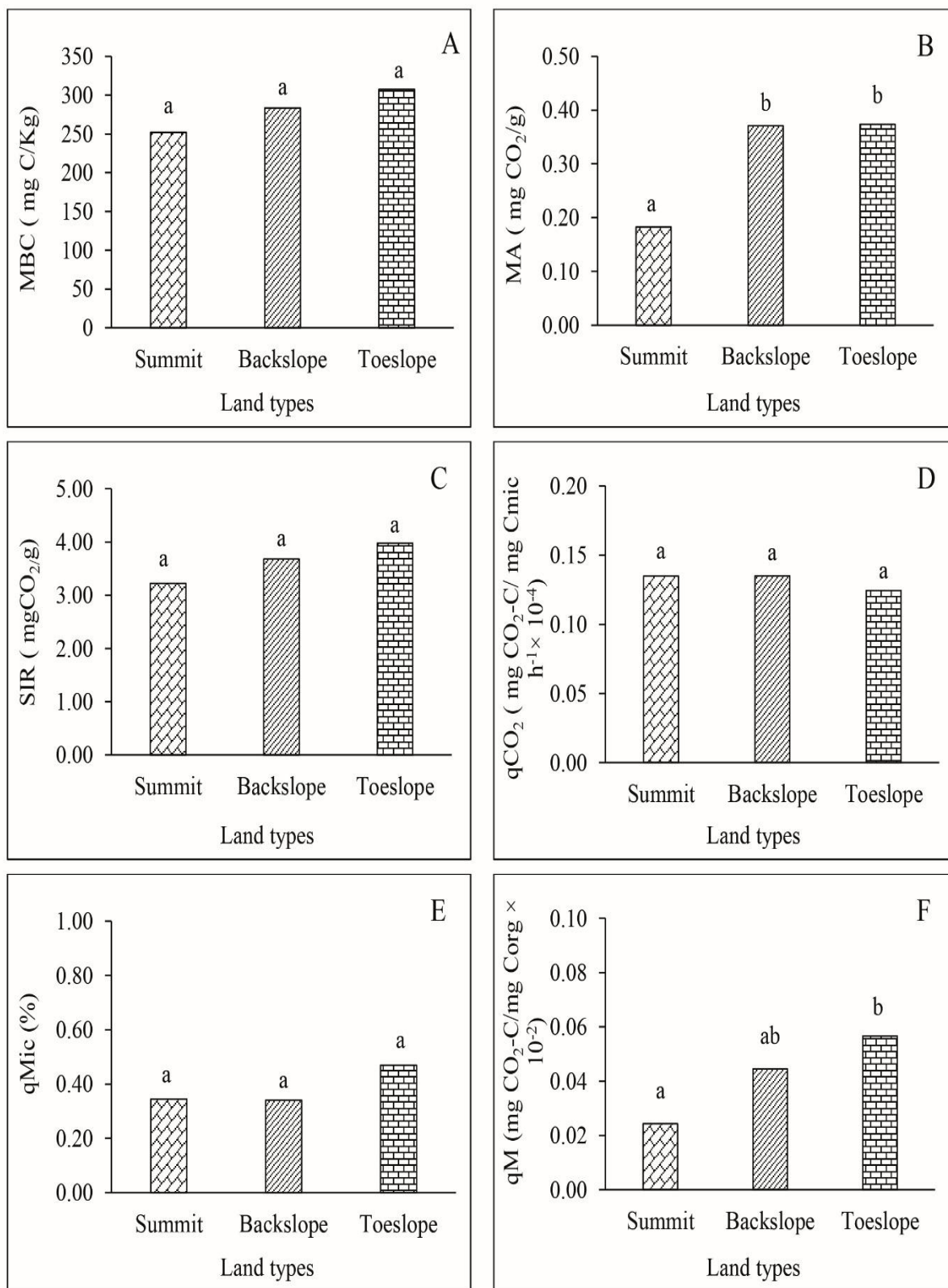
Note: MBC- mg C/Kg, MA- mg CO<sub>2</sub>/g, SIR- mg CO<sub>2</sub>/g, qCO<sub>2</sub>- mg CO<sub>2</sub>-C/ mg Cmic h<sup>-1</sup> × 10<sup>-4</sup>, qMic- %, qM- mg CO<sub>2</sub>-C/mg Corg × 10<sup>-2</sup>

properties of different tea gardens was also found by Rahman and Solaiman<sup>24</sup>. Moreover, climate variables, temperature and moisture in particular, have long been thought to be the main regulators of soil organic carbon breakdown regulating the activity of microorganisms and microbial biomass content among different types of soils<sup>25</sup>. The lower microbial biomass along with qMic in OTG could be due to higher rates of carbon mineralization from the microbes to support vegetation in OTG. Such a finding was also supported by Lepcha and Devi<sup>26</sup>. The qCO<sub>2</sub>, as an important index of soil stress, was found higher in soils of OTG. Sharma *et al.*<sup>13</sup> also reported that stressed soils produce greater qCO<sub>2</sub> values than less-stressed soils since qCO<sub>2</sub> measures how effectively soil microorganisms exploit carbon resources in the soil. However, depending on soil management, organic matter levels are capable of either decreasing or increasing the qCO<sub>2</sub> of soils<sup>27</sup> reducing the use of chemical fertilizers and pesticides to minimize their negative impact on soil microbial communities.

Effects of topography on microbial and ecophysiological indices of OTG are shown in Figure 2. The MA and qM in the summit were found to be significantly ( $p < 0.05$ ) different from the toeslope. Otherwise, no significant ( $p > 0.05$ ) variation was found for MBC, SIR, qCO<sub>2</sub> and qMic among different topographical position. The MBC and SIR were found to be highest in toeslope which were 22.06% and 23.44% higher compared to the corresponding lowest values (251.92 mg C/Kg and 3.22 mg CO<sub>2</sub>/g, respectively for MBC and SIR) found in the summit. The highest MA was found in both the backslope and toeslope, while the lowest was found in the summit. The MA in the summit was about 51.15% lower compared to other two landforms. Both the qMic and qM were also found higher in the toeslope which were 36.20% and 132.83% relative to their corresponding lowest values in the summit. On the other hand, the highest qCO<sub>2</sub> was found in the summit as well as in the backslope and lowest in the toeslope.

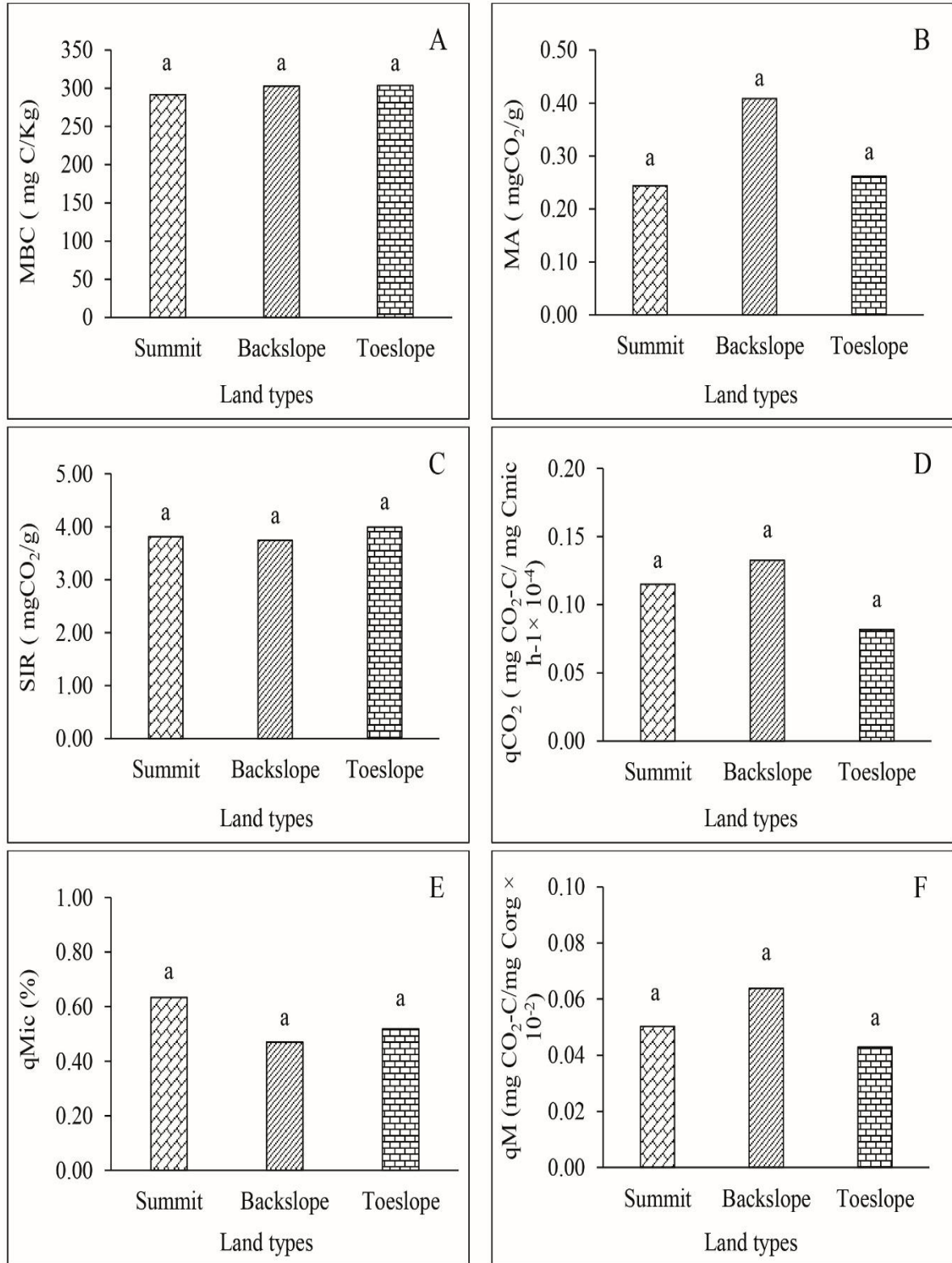
Figure 3 shows different microbial and ecophysiological indices for TTTG under different topography. All the microbial and ecophysiological indices did not differ significantly ( $p > 0.05$ ) among the three different land types. The highest values of MBC and SIR were also found in toeslope as found in OTG. The MBC and SIR were found to be 4.01% and 6.67% higher in toeslope compared to their corresponding lowest values found in either summit (MBC, 291.70 mg C/Kg) or backslope (SIR, 3.75 mgCO<sub>2</sub>/g). Like OTG, the highest MA of 0.41 mgCO<sub>2</sub>/g was found in backslope which was 67.49% higher compared to lowest MA (0.24 mgCO<sub>2</sub>/g) found in summit. The qCO<sub>2</sub> and qM were found highest in backslope which were 65.32% and 48.68% higher compared to their corresponding lowest values, while qMic was 34.81% higher in summit compared to the corresponding lowest value in backslope.

The differences in microbial and ecophysiological indices at different topographic position were in consistent with the findings of other authors<sup>26,28</sup>. Tian *et al.*<sup>29</sup> also found highest MBC content at the slope bottom, whereas the lowest at the slope top. The amount of microbial biomass is influenced by a number of variables that affect the input and breakdown of carbon, including the kind of vegetation, land-use type, season, soil depth, texture, mineralogy, aeration, bulk density, porosity, and soil organism<sup>26,30,31,32</sup>. Weralupitiya *et al.*<sup>11</sup> also stated that the MBC can primarily be affected by such soil factors as organic carbon, water retention capacity, and pH. Relatively higher MA, MBC and SIR at toeslope may be due to relatively greater moisture content. Tian *et al.*<sup>29</sup> stated that the soils on the bottom slope often have better moisture. Devi and Yadava<sup>33</sup> also observed a strong impact of soil moisture on the content of soil microbial biomass. Besides, toeslopes of the tea gardens are subjected to nutrient accumulation because of intense rainfall-driven runoff from the backslope and summit. Nutrient limitations at summit could be another reason for low MBC in both the tea gardens.



**Figure 2.** (A) Microbial biomass carbon (MBC), (B) microbial activity (MA), (C) substrate reduced respiration (SIR), (D) metabolic quotient (qCO<sub>2</sub>), (E) microbial quotient (qMic) and (F) mineralization quotient (qM) in different land types of tea soils in OTG. Bars with the same letters within the land types are not significantly different from each other at  $p < 0.05$ .





**Figure 3.** (A) Microbial biomass carbon (MBC), (B) microbial activity (MA), (C) substrate reduced respiration (SIR), (D) metabolic quotient ( $qCO_2$ ), (E) microbial quotient ( $qMic$ ) and (F) mineralization quotient ( $qM$ ) in different land types of tea soils in TTTG. Bars with the same letters within the land types are not significantly different from each other at  $p < 0.05$ .

A similar result was found by Ren *et al.*<sup>34</sup>. In an experiment, Roy *et al.*<sup>35</sup> observed that better nutritional quality improves the microbial dynamics of the soil.

Table 3 and Table 4 show the correlation co-efficient values among different parameters of OTG and TTTG. The relationships of microbial parameters were positive with each other. Besides, MBC and SIR had positive relations with all ecophysiological indices except for qCO<sub>2</sub>. This is true for both OTG and TTTG. Among the ecophysiological indices, all the correlations were positive with the exception between qCO<sub>2</sub> and qMic. The positive relation of MBC with MA and SIR indicated that the increase in MBC was responsible for the increase in the activity of organisms. A significant positive correlation of MBC with that of MA and SIR was in consistent of the findings of Lepcha and Devi<sup>26</sup> indicating that soil MA and SIR were highly influenced by soil biomass. Moreover, the positive and significant ( $p < 0.05$ ) relation of qMic with that of the MBC and qM

was agreed with the findings of Sharma *et al.*<sup>13</sup>. On the other hand, a significant negative correlation between MBC and qCO<sub>2</sub> was supported by the findings of Roy *et al.*<sup>35</sup>. This indicated that soil samples of both the tea gardens with low microbial biomass were in more stressful conditions.

The lower rate of CO<sub>2</sub> production than microbial biomass in OTG toeslope can be attributed to the efficient use of organic matter by microbial communities, as seen by the low qCO<sub>2</sub> value and greater soil microbial characteristics. This suggests a soil ecosystem that is more developed and stable than the TTTG toeslope, with effective carbon sequestration and nutrient cycling. However, according to Yao *et al.*<sup>36</sup>, soil microorganisms are known to be inhibited by tea soils. Due to plant polyphenol accumulation in the tea gardens, soil sickness is linked to a change in microbial communities in older tea plantations<sup>37</sup>. Additionally, as tea plantations age, the quality of the

**Table 3.** Correlation co-efficient (r) values among microbial and ecophysiological indices of tea soils in OTG

	MBC	MA	SIR	qCO <sub>2</sub>	qMic	qM
MBC	1					
MA	0.346*	1				
SIR	0.972**	0.403*	1			
qCO <sub>2</sub>	-0.603**	0.344*	-0.457*	1		
qMic	0.739**	0.280	0.762**	-0.377*	1	
qM	0.380*	0.781**	0.433*	0.184	0.677**	1

\*\* Correlation is significant at 1% level

\* Correlation is significant at 5% level

**Table 4.** Correlation co-efficient (r) values among microbial and ecophysiological indices of tea soils in TTTG

	MBC	MA	SIR	qCO <sub>2</sub>	qMic	qM
MBC	1					
MA	0.160	1				
SIR	0.971**	0.121	1			
qCO <sub>2</sub>	-0.548**	0.616**	-0.467*	1		
qMic	0.445*	-0.136	0.399*	-0.420*	1	
qM	0.110	0.683**	0.033	0.338	0.467*	1

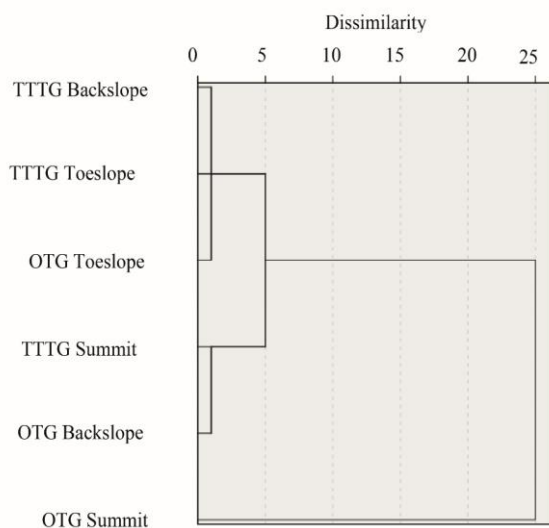
\*\* Correlation is significant at 1% level

\* Correlation is significant at 5% level



soil decreases overall<sup>38,39</sup>. Consequently, given that the tea plantation in TTTG was younger than in OTG-a finding corroborated by the dendrogram-the total soil health of TTTG was found to be superior than that of OTG soils.

Dendrogram grouping of tea soils characterized by similar responses of soil microbial and ecophysiological indices were performed (Figure 4). Among the different topographical positions, there were two clusters and a cluster that contained only one element OTG-Summit was significantly different from the other layers. The soil of this layer is at the highest stress as supported by high  $qCO_2$  along with the related responses of microbial activities by low MA, low MBC, low SIR and the lowest  $qM$ . The top two clusters of the TTTG soils demonstrated an overall better state of health than the OTG soils.



**Figure 4.** Similarity dendrogram for different topographical positions in tea soils of OTG and TTTG

## Conclusion

Scientists are paying great attention to microbial indices as active and sensitive indicators of soil health because of the quick response of microorganisms to changes in soil quality. The value of biomass and the activity of microorganisms in soils are vital microbial indicators determining soil health and ultimately

regulating the efficiency of agrarian frameworks. In our present study, differences in microbial properties were found between two tea gardens as well as among different slopes. The mean values of MBC, MA and SIR were found to be 288.38 mg C/Kg, 0.32 mgCO<sub>2</sub>/g and 3.75 mgCO<sub>2</sub>/g soil in OTG, while that of 296.46 mg C/Kg, 0.34 mgCO<sub>2</sub>/g and 3.80 mgCO<sub>2</sub>/g soil in TTTG. The MBC and SIR at toeslope in OTG were found to be 22.06% and 23.44% higher in comparison to summit, while the MBC and SIR at toeslope in TTTG were found to be 4.01% and 6.67% higher relative to summit. Moreover, microbial and ecophysiological indices indicated better soil health at the toeslope compared to the backslope and summit. This comprehensive study on the microbial and ecophysiological indices of tea soils in the Chattogram and Khagrachari districts will provide valuable insights into the soil health and microbial dynamics of these regions. However, extended research is recommended to understand the responses of microbial indices to tea soils at greater scale under different physiographic regions of Bangladesh.

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