Microbial biomass carbon in grassland soil aggregates of Biratnagar, eastern Nepal

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Abstract

Soil aggregation analysis was done in the grassland community of Degree Campus of Biratnagar, eastern Nepal. Physico-chemical and microbial biomass carbon were assessed in microaggregate and macroaggregate soil components. Estimation of soil organic carbon (SOC) was done by dichromate digestion method, total nitrogen (TN) by micro-Kjeldahl method and soil microbial biomass carbon (MB-C) by chloroform fumigation-extraction method. Macroaggregate component was dominant over microaggregate exhibiting 65:35 ratio in the soil. Soil organic carbon was higher in microaggregate than macroaggregate but C:N ratio was narrow (8.2-9.2) in macroaggregate indicating the concentration of nitrogen was relatively higher in macroaggregate. Conversely, the microbial biomass carbon was higher in macroaggregate than microaggregate which is also reflected in higher percentage of MB-C in soil organic carbon. Dominance of macroaggregate in soil with high value of MB-C as percent of SOC (2.50-3.82%) represent a more suitable component of soil in comparison to microaggregate. Because of high value of active and functional fraction of SOC, the macroaggregate component of soil may contribute a greater role in the development of grassland community.

Keywords: Macroaggregate, Microaggregate, Soil organic carbon, Total nitrogen

1. Introduction

Grasslands have multiple functions and values in terms of ecosystem services. They reduce soil erosion through a permanent soil cover and dense rooting system, favor soil fertility, water quality and water retention [1]. Grassland soil rich in organic matter enhances soil quality by its positive effect on aggregation, microbial activity and diversity as well as nutrient and water availability [2]. Soil organic matter (SOM) acts as source of energy for soil organisms and as a nutrient reservoir for intra-system cycling. Soil organic matter is divided into three fractions: active, slow and passive on the basis of turn over time [3]. Active fraction with most rapid turnover time (0.14 y) is represented by soil microbial biomass which contributes in the formation of soil aggregates [4].

Soil aggregates are the basic unit of soil structure that comprising a group of primary particles that cohere each other more strongly [5]. Aggregate hierarchy model developed by Tisdall and Oades [6] categorized soil aggregates into macroaggregates (>250 mm) and microaggregates (<250 mm) without any mesoaggregates size class. Aggregation of soil particles is mainly governed by five major factors: soil fauna, soil microorganisms, roots, inorganic binding agents and environmental variables [7]. However, organic binding agents can significantly improve the water-stability of soil aggregates compared to inorganic binding agents [6, 8]. The mean weight diameter of soil aggregates is often used to quantify the soil aggregate stability as it is mainly determined by macroaggregate proportion [9].

Soil microbial biomass is a live, labile and active fraction of soil organic matter with most rapid turnover time [3]. The rapid turnover rate of soil microbial biomass enables to release the available nutrients at fast rate [10]. Labile organic matter binds microaggregate into macroaggregate which could be the soil microbial biomass [4, 11]. Soil aggregation is associated with soil aggregate stability which provides a physical protection to soil organic matter. It may influence water holding capacity, soil aeration, rhizosphere development, infiltration of soil water and thus aggregate stability is generally regarded as an indicator of soil quality [12].

Documentation on microbial biomass associated with macro and microaggregates along a forest-savanna-cropland gradient was done by Singh and Singh [13] in a seasonally dry tropical region of India in which both macro and microaggregates, mean microbial biomass C, N and P were maximum in forest and minimum in cropland soils. The information on such an active and functional fraction of soil organic matter is limited especially in the soil aggregates of grassland ecosystem. Grassland is essential source for the feeding and management of livestock. Soil aggregate with high value of soil microbial biomass is supposed to play a significant role in the development of grassland community. Unfortunately, there is encroachment in the areas of grassland. Works related to the development of grassland community becomes vital, especially when its area is limited. In this context an attempt has been made to assess the concentration of microbial biomass carbon in concerned with soil organic carbon within the microaggregate and macroaggregate components in the grassland soil.

2. Materials and Methods

2.1 Study area

Study was carried out in and around the grassland community of Degree Campus of Biratnagar (26°23'-26°30' N latitudes and 87°14'-87°18' E longitudes, elevation 72 m asl), a plain area of south-west corner of Morang district, in Koshi Province, eastern Nepal. The climate is tropical with great variation in the annual temperature and precipitation. Biratnagar is characterized by very hot summer, during which the temperature rises up to 43°C and cold winter, during which temperature falls below 11°C with an annual average temperature of 24.96°C. Monthly average maximum and minimum precipitation exhibited 365.6 mm in July and 0.7 m in December respectively with 80% rainfall occurred in rainy season. Relative humidity ranged between 59.5 - 83.72% with higher percentage exhibited in rainy season [14]. The grassland areas are limited in Biratnagar. The present study area contains a large tract of grassland used for grazing purpose.

2.2 Soil sampling

Soil was collected from three randomly selected plots. At each sampling plot, soil was collected from three depths i.e., 0-5 cm, 5-10 cm and 10-15 cm using 10 cm × 10 cm pit. Soil samples were brought to Ecology Research Laboratory of Degree Campus, Biratnagar where further analysis was carried out. Soil samples were spread on blotting paper for air drying maintaining a depth of approximately 1 cm, a drying method recommended to estimate the distribution of aggregate size fraction. Dried soil samples were sieved through 2 mm mesh screen. Fine root components were removed. Finally, soil samples were separated into macroaggregate (>250µm) and microaggregate (<250µm) soil component using sieve method [6]. Both soil samples were separately used for further analysis.

2.2.1 Physico-chemical analysis of soil

Soil texture was determined by sieve method. Soil moisture and water holding capacity (WHC) were estimated following Piper [15]. Water holding capacity was estimated by allowing perforated box filled with compacted soil to stand overnight over water until saturated. Weight of saturated soil and oven dried soil were taken to estimate % WHC. Soil pH was measured by pH meter using a glass electrode (1:5, soil: water).

Soil organic carbon was determined by digestion of soil samples with concentrated sulfuric acid along with potassium dichromate and titration with ferrous ammonium sulphate [16]. Total soil nitrogen was determined by micro-Kjeldahl method following three steps: digestion, distillation and titration [17].

2.2.2 Determination of soil microbial carbon (MB-C)

Soil samples were pre-conditioned for 7 days at room temperature. For pre-conditioning, 300 g of each soil sample was taken in open polythene bag and then kept in a large air-tight container. Soil was watered up to its water holding capacity. Then, two small beakers, one filled with 20 ml distilled water to maintain relative humidity and the other filled with 20 ml KOH solution to absorb CO_2,were kept in the container. The container was opened daily for 15 minutes for the aeration. After pre-conditioning the soil samples were used for the determination of microbial biomass carbon.

Soil microbial biomass carbon was estimated by chloroform fumigation-extraction method [18]. 25g of pre-conditioned soil sample was saturated with purified liquid $CHCl_3$ for 20 h [19]. After 20 h, the $CHCl_3$ was removed by evacuation and the soil was extracted with 100 ml of 0.5M K_2SO_4 (1:4, soil: extractant) for 30 minutes and filtered. This represented the fumigated sample. Another set of un-fumigated soil sample (without $CHCl_3$) was also extracted with 0.5M K_2SO_4 in the same way. Microbial biomass carbon was estimated from these fumigated and un-fumigated soil extracts.

Soil microbial biomass C was determined in the soil extracts of fumigated and un-fumigated samples by dichromate oxidation in a reflux system and titration with ferrous ammonium sulphate. For this purpose, 8ml filtered extract (aliquot) was taken in round bottom reflux flask then 2 ml $K_2Cr_2O_7$ (66.7 mM), 70 mg HgO and 15 ml of H_2SO_4 + H_3PO_4 (in 2:1 ratio) were added and heated for 10 minutes in low flame and then heated it in high flame for about half an hour. After cooling the flask, 25 ml distilled water was added with rinsing the condenser. It was proceeded for titration where 5 drops of phenanthroline, a redox indicator was kept and finally titrated with 33.3 mM ferrous ammonium sulphate [20].

Microbial biomass carbon (MB-C) was then estimated using the formula: MB-C = 2.64 EC, where EC is the difference between C estimated from fumigated and un-fumigated soils, both expressed as µgCg-1 dry soil [18].

3. Results and Discussion

3.1 Physio-chemical properties of soil aggregates of grassland

In the grassland soil, macroaggregate was dominant over microaggregate in their ratio in each soil depth from 0-5 cm, 5-10 cm and 10-15 cm (Table 1). The mean value exhibits 65:35 ratio between macroaggregate and microaggregate, representing that macroaggregate is 1.8 times greater than microaggregate in soil. Further, this ratio increased slightly along lower depth as microaggregate decreased depth-wise. It showed higher macroaggregate formation at 10-15 cm depth which may be due to higher accumulation of fine roots, the below-ground source of organic matter. Further, in the soil aggregation leading role of macroaggregate may be due to higher accumulation of soil microbial biomass carbon as obtained in the present study [13].

Organic residues have their own hierarchical system in enmeshing particles and forming aggregates. Microbial polysaccharides stabilize macroaggregates, whereas humic compounds stabilize microaggregates. The binding agents responsible for stabilizing and arranging the aggregates are classified as temporary, transient, and persistent agents [6]. Temporary agents comprise plant roots, fungal hyphae, mycorrhizal hyphae, bacterial cells, and algae. They develop simultaneously with the growth of plant roots and build up a visible organic skeleton to enmesh the mineral particles by adsorption to form young macroaggregates. Because temporary agents comprise large substances, they are mainly associated with macroaggregates.

Table 1: Ratio of Macro and Microaggregate in grassland soil of Biratnagar, eastern Nepal (% ± S.E)

Soil Depth (cm)	Soil Aggregates		Ratio of Macro: Micro
	Microaggregate (%)	Macroaggregate (%)	
0-5	39±3.6	61±3.6	1.5
5-10	37±1.2	63±1.2	1.7
10-15	30±2.1	70±2.1	2.3
Mean (x)	35±2.3	65 ±2.3	1.8

Table 2: Soil texture and soil moisture of grassland community of Biratnagar, eastern Nepal (x ± S.E)

Soil texture	Soil Depth (cm)			
	0-5cm	5-10cm	10-15cm	Mean (x)
Sand (%)	44.5±0.6	47.5±1.5	47.7±5.8	46.5±2.1
Silt (%)	21.3±2.7	20.8±2.3	21.2±1.1	21.1±1.7
Clay (%)	34.1±2.9	31.7±3.1	31.1±6.1	32.3±2.6
Texture class: Sandy Clay Loam				
Soil moisture (%)	17.0±1.6	14.7 ±0.8	13.8±0.6	15.2±0.6

Texture class of soil was similar in all soil depths of grassland and it was sandy clay loam. The sand % was higher in all soil depths with a mean value of 46.5% and the clay % was second dominant (Table 2). Soil moisture in grassland decreased with increasing depth.

Water holding capacity was relatively higher in macroaggregate than microaggregate (Table 3). This may be due to higher value of soil microbial biomass observed in macroaggregate component of soil. Moreover, at 10-15 cm depth, WHC was higher than 0-5 cm depth in both micro and macroaggregate. At this depth, organic matter is added by the fine roots from the below ground part, so may be the reason of higher WHC.

A high rate of vegetation coverage ensures enrichment in organic matter. Organic matter enhances water-retention ability by changing the soil structure and strengthening the adsorption of nutrients into the soil. It is suggested that water retention of soil is determined by the distribution and connectivity of pores in the soil medium, which is largely regulated by soil texture, combined with structural characteristics (aggregation) and soil organic matter content [21]. pH in micro and macroaggregate both slightly increased with depth. However, pH value did not show significant variation between the two soil aggregates.

Table 3: Water holding capacity (WHC) and pH in soil aggregates of grassland community of Biratnagar, eastern Nepal (x ± S.E)

Soil Depth (cm)	Soil Aggregates	
	Microaggregate	Macroaggregate
	WHC (%)	
0-5	60.9±6.1	76.0±4.4
5-10	65.9±9.8	75.8±7.5
10-15	71.2±9.1	85.8±1.5
	pH	
0-5	5.68±0.4	5.61±0.4
5-10	5.88±0.2	6.04±0.3
10-15	6.02±0.1	6.08±0.1

Soil organic carbon and total nitrogen concentrations were maximum in upper 0-5cm layer in both microaggregate and macroaggregate (Table 4). The concentrations decreased depth wise in both aggregates. In comparison to the lower depth (10-15 cm) the organic carbon was higher in upper layer (0-5 cm) by 75% in microaggregate and 77% in macroaggregate. Difference in the concentrations of soil organic carbon between microaggregate and macroaggregate were significant in 0-5 cm and 10-15 cm depths ($p<0.005$) while not significant in 5-10 cm depth ($p>0.05$). It may be due to the higher inputs of organic matter in top soils compared to lower soil depths. Similar findings were also reported in selected forests of China where greatest concentrations of soil organic carbon and nitrogen were in the top soil and the concentrations decreased depth wise [22] The result of the present study is also consistent with the findings of many other previous studies of the grassland ecosystem [23, 24, 25]

The total nitrogen concentration in the upper layer (0-5 cm) was higher by 112% in comparison to lower depth (10-15 cm) in microaggregate and by 62 % in macroaggregate exhibiting a narrow variation in soil profile in this case. Difference in the concentrations of total nitrogen between microaggregate and macroaggregate was significant in 0-5 cm and 5-10 cm depth. Regarding the C:N ratio it was wide in microaggregate than in macroaggregate. It showed that accumulation of nitrogen is relatively more in macroaggregate. Further, C:N increased in lower depth (10-15 cm) in microaggregate whereas it decreased in

Table 4: Soil organic carbon (SOC), total nitrogen (TN) and their ratio in soil aggregates of grassland community of Biratnagar, eastern Nepal (% ± S.E)

Soil Depth (cm)	Soil Aggregates							
	Microaggregate			Macroaggregate			SOC ratio in Macro and micro	Total N ratio in Macro and Micro
	Soil Organic carbon	Total Nitrogen	C:N	Soil Organic	Total Nitrogen	C:N		
0-5	2.07±0.36	0.189±0.006	10.9	1.38±0.16	0.154±0.009	8.9	0.67	0.81
5-10	0.98±0.11	0.098±0.01	10.0	0.95±0.11	0.103±0.004	9.2	0.97	1.05
10-15	1.18±0.21	0.089±0.01	13.3	0.78±0.17	0.095±0.014	8.2	0.66	1.06
Mean	1.41±0.23	0.125±0.008	11.4	1.04±0.15	0.117±0.009	8.8	0.73	0.93

macroaggregate which showed that in the lower depth microaggregate accumulated more carbon while macroaggregate retained more nitrogen. Total nitrogen was higher in topsoil than in the subsoil probably due to loss in N- mineralization in the subsoil. Significantly greater soil organic matter and total nitrogen concentrations were also reported in top soil layer than in the sub soil layers among the highlands resources of Northeast Wollega, Ethiopia [26].

3.2 Soil microbial biomass carbon (MB-C) in soil aggregates

Soil microbial biomass carbon (MB-C) showed significant difference between macroaggregate and microaggregate ($p<0.005$) in each layer of soil profile (Table 5). In the upper 0-5 cm layer, concentration of MB-C in macroaggregate was higher by 78 % than microaggregate. Greater value of microbial biomass carbon in the macroaggregate may reflect a differential species composition of soil microbial biomass in different aggregate sizes. Fungi rich in C having wide C:N ratio (10-12) dominate in macroaggregates while bacteria poor in C and rich in N having narrow C:N ratio (3-5) dominate in microaggregates [6, 27]. Bacterial domination in microaggregate exhibit high turnover and C loses while fungal domination in macroaggregate may favor fungal based food web organisms and lead to greater retention of microbial biomass carbon [28].

Greater value of microbial biomass carbon in the macroaggregates than microaggregates size class was also reported in Savanna ecosystem of a seasonally dry tropical region, India (13). MB-C in both microaggregate and macroaggregate decreased from upper to lower depth as also reported by Dong et al. [29]. The higher concentration of MB-C in upper layer may be due to high value of soil organic carbon which may be congenial to soil microbial breeding. Soil microorganisms provide nutrients for plants through the process of decomposition of organic matter, which can effectively avoid ineffective nutrient loss and caused changes in soil microbial biomass [30]. Soil microbial biomass carbon as percentage of soil organic carbon was comparatively higher in macroaggregate than microaggregate even in the soil profile. It represents that proportion of soil organic C is immobilized in the microbial biomass. Macroaggregate, which was poor in soil organic C exhibited greater C immobilization as evident by greater value of MB-C as percentage of soil organic C (Table 5). Similar result was also reported by Singh and Singh [13].

Macroaggregate soil showed increasing trend in MB-C as percentage of soil SOC towards lower depth and surprisingly it was maximum (3.82%) in 10-15cm depth. At this depth microbial biomass carbon accumulated at high rate which may be due to addition of organic carbon through fine roots. Oades [31] suggested that, the macroaggregates united together by the temporary bonding agents, mainly roots and fungal hyphae. After short time span, the bonding agents decompose into tiny particles, coated with mucilage and encrusted with clay particles resulting in microaggregates within the macroaggregates. On the other hand, Jastrow

Table 5: Microbial biomass carbon (MB-C: μgCg-1 dry soil) in soil aggregates of grassland community of Biratnagar, eastern Nepal (x ±S.E)

Soil Depth (cm)	Soil Aggregates				MB-C ratio in Macro: Microaggregate
	Microaggregate		Macroaggregate		
	MB-C	MB-C as % of Organic C	MB-C	MB-C as % of Organic C	
0-5	193±23	0.93	343 ±48	2.50	1.7
5-10	183±25	1.87	258±41	2.71	1.4
10-15	184 ±14	1.56	298±52	3.82	1.6
Mean ($\bar{x}$)	187±21	1.45	300 ±46	3.01	1.6

et al. [32] proposed that the recently incorporated organic matter bind microaggregates into larger fraction i.e., macroaggregates.

Ratio of soil organic carbon (SOC) between macroaggregate and microaggregate was less than 1 (0.66 to 0.97) while the ratio of MB-C between macro and microaggregate was more than 1 (1.40 to 1.77) in all soil depths. It showed that in case of soil organic carbon, microaggregate was dominant over macroaggregate where as in the case of MB-C the case was reverse i.e. macroaggregate was dominant over microaggregate.

For the crop development, macroaggregate may represent more suitable component of soil as there is high level of soil microbial biomass which is active fraction and functional component of soil. Soil microbial biomass carbon (MB-C) in grassland is mainly affected by the litter decomposition, and also with abundant root exudates which promotes the mass production of microbial biomass [29].

4. Conclusion

It is concluded that in the grassland community, lower concentration of soil organic carbon in macroaggregate enhances to more C immobilization as reflected in the high value of MB-C in macroaggregate. It means, the functional factor of soil (i.e. MB-C) is comparatively more in macroaggregate. Consequently, macroaggregate component of soil may play vital role for the development of grassland as it is dominant in soil structure with rich microbial biomass carbon.

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