Research and Full Length Article:

Soil Microbial Biomass Carbon and Nitrogen in Himalayan Rangeland of Eastern Nepal: A Comparison between Grazed and Non-grazed Rangelands

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Abstract. Soil microbial biomass plays an important role in nutrient transformation in terrestrial ecosystems. Microbial biomass is also an early indicator of changes in total soil organic carbon. Thus, the main objective of this study was to identify and quantify the present status of soil microbial biomass carbon and nitrogen with various management practices in Himalayan rangeland. To meet the aforementioned objectives, a field study was conducted in Tinjure Milke Jaljale (TMJ) eastern Himalaya Nepal in 2011-2013. Soil samples were collected from the depths of 0-15 cm at three soil cores in each quadrat. Quadrat size was 30*30 cm and core size was 4 cm in diameter and 15 cm deep. Composite soil sample was made while mixing all the samples of a quadrat. Five quadrats were taken from each subplot. Soil core was separated into three sections viz. 0-5, 5-10 and 10-15 cm profiles with 5 cm length of each slice. Soil sample analysis was carried out by the process of chloroform fumigation method. The Result showed that soil microbial biomass C ranged from 219.84 to 987.5 mg/kg. The soil microbial biomass C was increasing with decrease of grazing intensity of the rangeland and differences were significant. Similarly, the soil microbial biomass N with value of 207.72 mg/kg was significantly higher in occasional grazing plot than two other treatments. Both soil microbial biomass C and N values were in decreasing trend with increase of soil depth of the rangeland.

Key words: Rangeland, Carbon, Nitrogen, Grazed, Legume, Himalayan

Introduction

Soil Microbial Biomass (SMB) is the living portion of soil organic matter, constituted by archaea, bacteria and eukaryotes, excluding roots and animals smaller than 5x103 μm^3 (Jenkinson and Ladd, 1981). Soil microbial biomass plays a critical role in nutrient transformation in terrestrial ecosystems (Singh *et al*., 1989). Changing microbial biomass may affect the cycling of soil organic matter (Shahriari *et al.,* 2011 and Tajik *et al*., 2012). Soil microorganisms also process plant litter and residues into Soil Organic Matter (SOM), which improves soil quality by increasing soil aggregation and aeration and decreasing soil bulk density (Franzluebbers *et al*., 1999; Dominy and Haynes, 2002; Spaccini *et al*., 2002). Generally, up to 5% of the total organic carbon and N in soil are in the microbial biomass. When microorganisms die, these nutrients are released in such forms that can be taken up by plants. Microbial biomass is also an early indicator of changes in total soil organic Carbon (C) (Wiesmeier *et al*., 2019).

Overgrazing is a driver of desertification and thus poses a serious pressure in areas where vegetation cover and soil are unsuitable for intensive agriculture (Kairis *et al*., 2015). According to a meta-analysis conducted by Dlamini *et al.* (2016), overgrazing is the main factor of grassland degradation and associated loss of SOC (Soil Organic Carbon) stocks as it is detrimental to grass primary production and associated carbon inputs to soils and favor soil carbon erosion by wind and water. The effect is more pronounced under dry climate and low soil pH.

Low microbial activity in soil is an indication of an ecosystem under stress (Visser and Parkinson, 1992). Microbial biomass performs two important functions in soils, namely: (i) oxidation of carbonaceous materials and (ii) storage of C and mineral nutrients in the living biomass (Anderson and Domsch, 1980; Smith and Paul, 1990).

It is very important to improve and amend the grazing land of Himalayan rangeland for raising cattle. This crucial function is related to microbial activities. Microbial activities and density are governed by various factors such as climate, nutrient and disturbances. There are number of studies on the effect of grazing on soil microbial biomass dynamics in the different parts of the world (Tracy and Frank, 1998; Bardgett *et al*., 2001; Li *et al*., 2005; Wang *et al*., 2008; Ayoubi *et al.,* 2009; Qi *et al*., 2010; Ayoubi *et al.,* 2012;); there is limited information on the impact of grazing on the dynamics of soil microbial biomass in the grassland ecosystems in the Himalaya (Singh *et al*., 1991; Singh and Yadava, 2006; Srivastava, 1992). Furthermore, there is no published record on the influence of grazing intensity on soil microbial biomass in grassland ecosystems in the Himalaya.

The objective of the present study was to estimate microbial biomass carbon and nitrogen in various management practices in Himalayan rangeland. A grazing experiment was conducted in a temperate grassland of Eastern Nepal to 1) evaluate the influence of different grazing intensities on soil microbial biomass C and N, 2) explore the distribution of soil microbial biomass C and N on various soil profiles and 3) test the hypothesis that grazing intensity alter the soil microbial biomass C and N in Himalayan rangeland.

Materials and Methods

Study Area

The study was conducted in the Tinjure-Milke-Jaljale (TMJ) Mountain ridge-political border of three districts, i.e., Taplejung, Tehrathum and Sankhuwasabha of Eastern Nepal. Geographically, the area lies between 27°6'57" to 27°30'28" Northern latitude and 87°19' 46" to 87°38'14" Eastern longitude (Fig. 1). The study area falls under the lesser Himalaya ranging from 2400 m to 3000 m asl. The climate of the study area is moist

temperate, which receives moderate snowfall from December to February. Average climatic detail (2011-2013) of the study area is given in Fig. 2. Mean annual maximum temperature was $23.65 \pm 4.95^\circ$C whereas mean annual minimum temperature was $4.12 \pm 5.24^\circ$C. Mean annual rainfall was 2,274 mm.
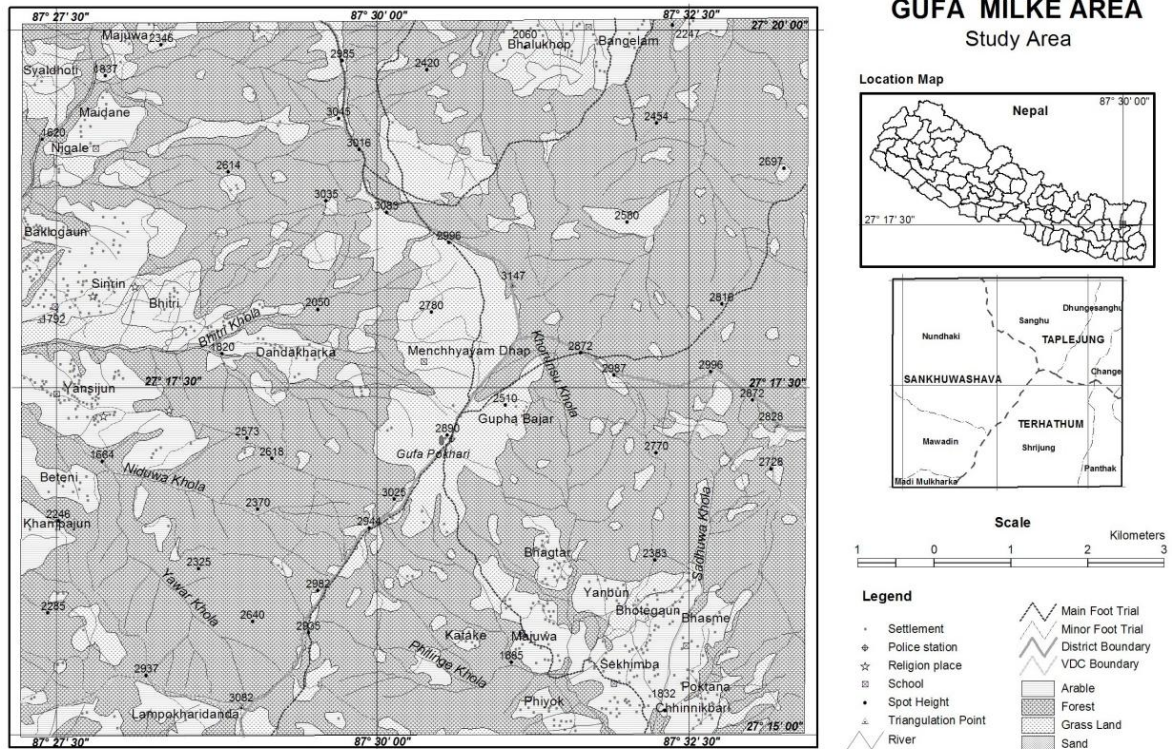

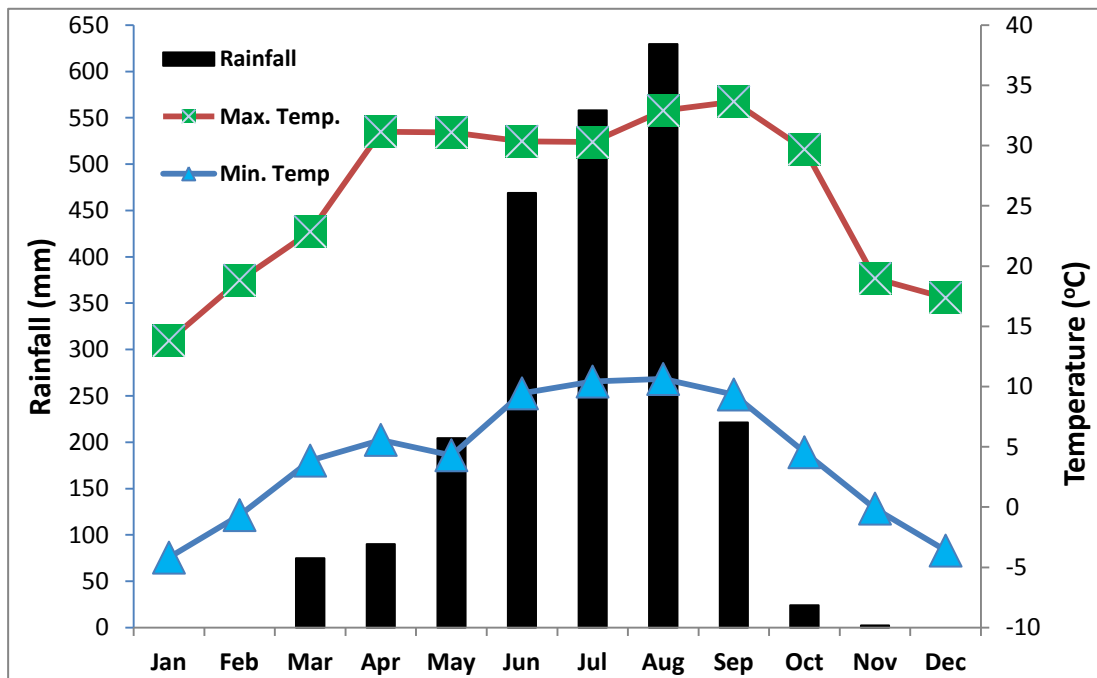**Fig. 1.** Tinjure milke jaljale (TMJ) study area map

Fig. 2. Climatic detail of the study area (2011-2013) Source: Field study, 2013

The study area was established in 2011. Three sites were: a) rangeland with heavily grazed (Heavily season-long Grazing-HG), b) rangeland with Occasional Grazed, OG and c) Non-Grazed enclosures rangeland, NG.

Heavy grazing implies the continuous and undisturbed season-long traditional grazing as practiced by the natives. Occasional grazing implies intermittent grazing for 1 year (15 days continuous grazing followed by a non-grazed period of another 15 days). Non-grazed enclosures mean the rangeland area kept enclosed and not grazed for 3 years. At the end of the 3-year grazing (late September 2013), ten sampling points were established in two parallel transect lines. One quadrat (30×30 cm) was established at each sampling point. Within each quadrat, three soil cores were collected at depths of 0–5 cm (upper soil profile), 5-10 cm (second soil profile) and 10–15 cm (third soil profile). Similarly, soil bulk density was determined for three different strata using the core method (Blake and Hartge, 1986).

Soil Sampling

Soil samples were collected in September 2013 from depths of 0-15 cm at three soil cores in each quadrat. Quadrat size was 30×30 cm and core size was 4 cm in diameter and 15 cm deep. Composite soil sample was made with a mix of all samples of a quadrat. Five quadrats were taken from each subplot. Soil core was separated into three sections viz. 0-5, 5-10 and 10-15 cm slice with 5 cm length of each slice. Each layer of the soil was packed in separate zipped polythene bag and brought to laboratory. Soil samples were immediately placed in ice bank for transporting to the laboratory of central campus of technology (a constituent campus of Tribhuvan University, Nepal) and subsequently stored at 4°C until analysis. Samples were homogenously mixed prior to laboratory analysis. A 30 g field-moist soil subsample was brought to

50% water holding capacity and analyzed for Soil Microbial Biomass Carbon (SMBC) and N using the chloroform fumigation–incubation method (Horwath and Paul, 1994; Franzluebbers *et al.*, 1999).

Laboratory Analysis of Sample

Microbial biomass C and N were determined using the fumigation extraction methods (Brookes *et al.*, 1985; Vance *et al.*, 1987). The filtered soil extracts of both fumigated and non-fumigated samples were analyzed for organic C using the acid dichromate method (Vance *et al.*, 1987). Total nitrogen in K_2SO_4 soil extract was determined by acid digestion and Kjeldahl distillation (Brookes *et al.*, 1985).

Then, fumigation-extraction method was used to measure microbial biomass Carbon and Nitrogen. For this purpose, 50 g of oven dried soil sieved through <2 mm sieve was weighed in triplicate into glass screw-top jars (100 ml). These jars were placed in a desiccator, having moistened tissue paper at the bottom together with a 25 ml vial of soda lime and a 50 ml beaker containing 30 ml $CHCl_3$ and 2-3 anti-bumping granules. The desiccator was evacuated using air pump until $CHCl_3$ was boiling vigorously. It was continued for 2 minutes. The valve was then closed and the pump was detached. The desiccator was placed in 25°C in a dark room for 24 hrs.

The soil samples, fumigated as well as non-fumigated, were transferred separately to 350 ml plastic screw-top bottles. Two hundred ml of 0.5 M K_2SO_4 was added and shaken for 30 min on a reciprocating shaker (200 strokes min-1). The bottles were removed from shaker and filtered through Whatman 42 filter papers. The quantity of extraction was noted. Three blanks were prepared in the same way.

Microbial Biomass Carbon Measurement

8 ml of the filtered extract was placed with 2 ml of 66.7 mM $K_2Cr_2O_7$, 70 mg HgO

and 15 ml of a mixture of 2 parts H_2SO_4 and 1 part H_3PO_4 in a round bottomed flask. The mixture was boiled gently under reflux for 30 min. Cold blank was not heated. It was then cooled and diluted with 20 ml water. The residual dichromate was

$$C (\mu g\ ml^{-1}) = (Hbl - S)/Cbl \times N \times Q/A \times B \times 1000 \qquad \text{(Equation 1)}$$

Where:
Hbl = titration solution consumed by hot blank
S = titration solution consumed by sample
Cbl = titration solution consumed by cold blank
N = normality of $K_2Cr_2O_7$ =0.4
Q = quantity of $K_2Cr_2O_7$ =2 ml
A = aliquot quantity = 8 ml
B = 3 = conversion of Cr VI to Cr III
1000 = to change into µg

Microbial Biomass Nitrogen Measurement

30 ml of K_2SO_4 extracts (both fumigated and non-fumigated) were pipetted into digestion tubes containing some anti-bumping granules. To this, 0.6ml of $CuSO_4$ (0.19M) and 10 ml of conc. H_2SO_4 were added and refluxed for 3 hrs. It was then cooled and diluted with 20 ml water. To these tubes, 25ml 10M NaOH was added and mixed. The tubes were attached

measured by back titration with 0.4 M ferrous ammonium sulphate solution using 25 mM 1, 10 phenanthrone ferrous sulphate complex as an indicator. Extractable C is calculated using the following relation (Grace et al., 2003):

to the steam distillation unit and 25ml more NaOH was added in order to render the solution alkaline. It was then steam-distilled into a titration vessel containing 5ml 2% boric acid which absorbed the evolved NH_3 until 40ml of distillate was collected. The solution was titrated to pH 4.7 with 50mM H_2SO_4 using a standard burette. Total N extracted was determined using the relation:

$$N (\mu g\ g^{-1}\ od\ Soil) = \frac{(Vs - Vb) \times M \times At \times 100 \times 0.15}{W} \qquad \text{(Equation 2)}$$

Where:
Vs = volume H_2SO_4 used to titrate the sample
Vb= volume H_2SO_4 used to titrate the blank
M= the molarity of H_2SO_4 = 0.05
At = Atomic weight of Nitrogen = 14
1000 =to convert into microgram
0.15 = the fraction of extract used for the titration (i.e.) 30/200

$$W = \frac{K2SO4\ extractant + Soil\ moisture\ content}{Oven\ dried\ weight\ of\ soil}$$

Statistical Analysis

Statistical analyses were carried out using SPSS statistics software version 20 (IBM-SPSS, 2011). The effect of grazing intensity and soil profile on soil microbial biomass Carbon (C) and Nitrogen (N) were

determined by Analysis Of Variance (ANOVA). A 95% confidence limit ($P<0.05$) was chosen to indicate differences between samples. Least Significant Differences (LSD) were

calculated when samples were significantly different.

Results
Soil microbial biomass carbon (SMBC) of the study area revealed the range from 219.84 ± 1.6 to 987.5 ± 1.93 (mean $\pm$ S.E) mg/kg (Table 1). Usually, the SMBC was in increasing trend with the decrease of grazing intensity of the rangeland. The enclosed non-grazed plot and occasionally grazed plot had 10.58% and 9.95% more SMBC value than heavily grazed (heavy grazed) plot, respectively (Fig. 2A). Thus, the differences were significant ($p<0.01$). On the contrary, the difference between the SMBC value of occasionally grazed and enclosed non-grazed plot was not significant ($p= 0.61$). There was a significant main effect for grazing intensity ($p<0.01$) on soil microbial biomass carbon.

Soil microbial biomass nitrogen was observed from 15.11 ± 0.89 to 44.1 ± 1.99 (mean $\pm$ S.E.) mg/kg (Table 1) in the study area. Occasionally, grazed plot had significantly higher ($p<0.01$) Soil Microbial Biomass Nitrogen SMBN than other grazing intensities. It had 10.4% and 11.9% higher SMBN than heavily grazed and enclosed non-grazed plots, respectively (Fig. 2B). When observing the main effect of independent variable of the analysis, it was significant for grazing intensity ($p<0.01$) on soil microbial biomass nitrogen.

Table 1. Mean and standard error of the soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) of the study area (Gupha rangeland)

Grazing intensity	treatment	Soil depth (cm)	SMBC Mean± S.E (mg/kg)	SMBN Mean± S.E (mg/kg)
Heavily Grazed	Legume	00-05	987.50± 1.92	40.73±2.55
		05-10	683.52±25.42	34.68±2.49
		10-15	349.26± 2.34	21.18±1.62
	Non-legume	00-05	560.46± 2.60	33.74±1.68
		05-10	281.73±15.11	31.78±2.39
		10-15	219.84±1.59	26.05±2.01
Occasionally Grazed	Legume	00-05	829.05±17.09	40.23±1.98
		05-10	729.00± 4.50	29.93±1.58
		10-15	412.04±16.02	27.21±0.85
	Non-legume	00-05	616.63± 8.12	44.10±1.99
		05-10	477.36± 7.44	36.28±1.61
		10-15	324.41±11.77	29.97±1.10
Un-grazed	Legume	00-05	903.19±14.22	30.96±0.98
		05-10	780.89± 2.60	22.95±1.42
		10-15	584.34± 4.75	26.63±1.03
	Non-legume	00-05	536.00±10.08	22.3±0.73
		05-10	352.47±12.62	18.41±1.13
		10-15	251.59±8.50	15.11±0.89
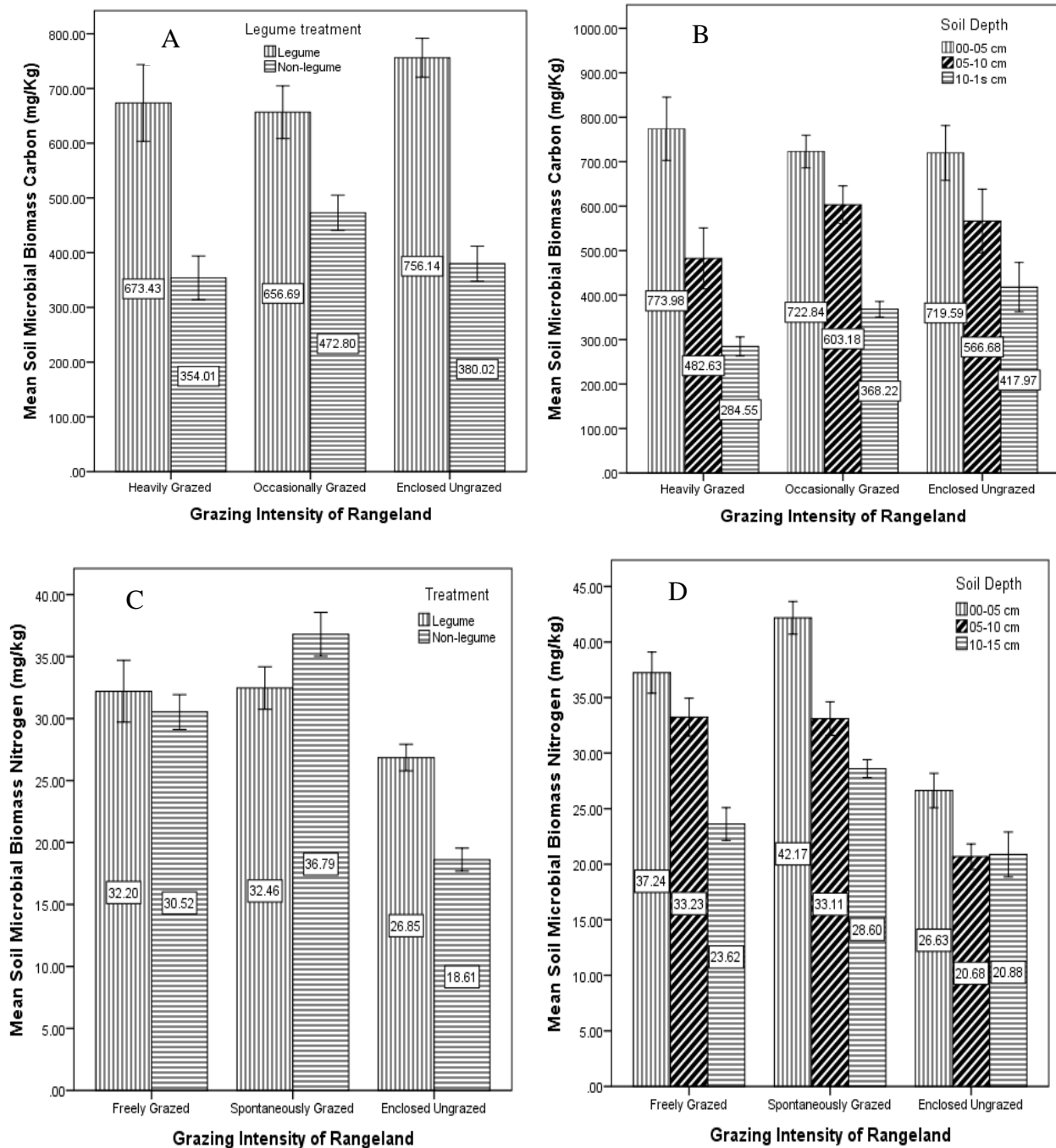
Fig. 3. Soil microbial biomass Carbon (values are means and bars represent standard errors) (A) Interaction effects of grazing intensity and legume treatment (B) Interaction effects of grazing intensity and soil depth on SMBC, and Soil microbial biomass Nitrogen (values are means and bars representing standard errors) (C) Interaction effects of grazing intensity and legume treatment. (D) Interaction effects of grazing intensity and soil depth on SMBN

Discussion

Findings of this study confirm that grazing intensity and soil depth (profile) of study area significantly influenced soil microbial biomass carbon and nitrogen. The grazing intensity and the concentration of SMBC are negatively correlated but grazing intensity and the concentration of SMBN are not correlated, occasionally grazed showed high SMBN.

These results support its initial hypothesis regarding soil microbial biomass carbon (SMBC) that heavy grazing alters the SMBC value (Heavily grazed has low value of SMBC). On the other hand, SMBN rejected the hypothesis because grazing activities did not clearly change the amount of SMBN of study area. The likely cause of the declines in Soil Microbial Biomass on these grazed plots was the slightly lower amounts of ground cover, and concomitant reductions in plant litter on the soil surface. Minor declines are likely in rooting activity and associated biomass of the plants (Engel *et al*., 1998).

Holt (1997) has also reported that reduction in microbial biomass in the heavily grazed treatment may have been influenced by lower rate of organic matter input as a result of reduction in herbage biomass. He noted 20-40% reduction of microbial activities with grazing pressure. Soil microbial biomass does not respond uniformly to grazing by livestock or other large animals, and has been observed to increase or decrease in response to grazing of the plant community (Bardgett and Wardle, 2003).

The findings of mean soil microbial biomass C and soil microbial biomass N were 628 and 35.23 mg/kg in heavily (heavy) grazing plot, 663.01 and 37.63 mg/kg in occasional (light) grazing plot and 643.13 and 23.65 mg/kg in enclosed (non-grazing) plot at 0-10 cm soil depth, respectively. Devi *et al*. (2014) reported that grassland soil microbial biomass C of temperate grassland of Northeast, India were 258.5, 347.8 and 309.2 mg/kg C at 0-10 cm soil depth of heavily, lightly and non-grazed plot, respectively. Soil microbial biomass N was reported by the same scholars as 38.3, 45.4 and 42.6 mg/kg in heavily, occasionally and non-grazed grassland, respectively.

The aforementioned compared values of SMBC and SMBN and showed that Gupha-Milke rangeland has higher value than temperate grassland of Northeast, India. It is considered that Gupha–Milke rangeland study area is colder and located at higher altitude than temperate grassland of Northeast India. Nutrition dynamic and decomposition rate of biomass is slow in colder region, Gupha–Milke rangeland, as a result of SMBC and SMBN stored longer. The decrease in temperature with increasing altitude has a strong effect on soil microbial biomass (Heaney and Proctor, 1989; Pabst *et al*., 2013). Pabst *et al*. (2013) reported that the SMBC in grassland of Mountain Kilimanjaro at 0–10 cm was 1221 mg/kg. The data of the Microbial Biomass C of independent three sites (Manipur, Northeast, India; Mt. Kilimanjaro, Tanzania and study area, Nepal) revealed that this finding is two times more than Northeast Indian's report but it is half time less than report of Mt. Kilimanjaro. Microbial biomass is very sensitive and its results depend on various condition. The microbial activities and abundance is determined with extreme climatic condition, topographic condition, soil type and biotic availability (Killham, 1990; Wilhelmi and Rothe, 1990; Ingram and Fernandes, 2001; King et al., 2008).

The soil microbial biomass C was found to be higher in the surface soil layer than the sub-surface soil layer. Maithani et al. (1998) reported that higher accumulation of microbial biomass C at the surface soil layer could be due to higher microbial populations (fungi and bacteria). The concentration of organic matter was also higher in the surface soil layer than in the sub-surface soil layer. Surface soil contains large pool of organic matter that supports a uniquely large and active soil microbial community (Arunachalam and Arunachalam, 2000). Because of the high nutrients concentration in the topsoil, soil microbial biomass increased at the surface layer and decreased with the increase in depth. It was observed that the concentration decreased with increase in depth in study area too.

Conclusion

The soil microbial biomass carbon was in increasing trend with decrease of grazing intensity but it was high in surface soil and decreasing trend with increase of soil depth of the Himalayan rangeland. Because low rate of organic matter input and low rooting activities in heavily grazed area and deep soil have low SMBC. Similarly, soil microbial biomass N did not respond to grazing intensities but showed response with soil depth that surface soil had high value of SMBN in Himalayan rangeland.

Acknowledgements

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مقایسه کربن زیست توده خاکی و نیتروژن در مراتع چرا شده و قرق در منطقه هیمالیا از بخش نپال شرقی

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چکیده. زیست توده خاکی نقش مهمی در تغییر مواد مغذی در اکوسیستم‌های زمینی دارد. زیست توده عناصر پرمصرف همچنین نشانگر اولیه تغییر در کل کربن آلی خاک است. بنابراین، هدف اصلی این مطالعه شناسایی و تعیین کمیت وضعیت موجود کربن زیست توده خاک و نیتروژن با روش‌های مختلف مدیریتی در مراتع هیمالیا بود. برای تحقق این هدف، مطالعه میدانی در سال ۱۳۹۱-۱۳۸۹ در منطقه تنجورمیک جلجلی در شرق هیمالیا از کشور نپال انجام شد. نمونه‌های خاک از عمق ۱۵-۰ سانتی‌متر در سه منطقه خاکی در هر چهار گروه جمع‌آوری شدند. اندازه قاب استفاده شده ۳۰×۳۰ سانتی‌متر و اندازه قطر هسته ۴ سانتی‌متر به عمق ۱۵ سانتی‌متر بود. نمونه خاک مرکب از مخلوط کردن تمام نمونه‌های کوادرات ساخته شد. از هر زیر مجموعه پنج قاب اطلاعات برداشت شد. هسته خاک به سه بخش تقسیم شد. پروفایل‌های ۰-۵ ، ۵-۱۰ و ۱۰-۱۵ سانتی‌متری با طول ۵ سانتی‌متر از هر برش مشخص شدند. تجزیه و تحلیل نمونه خاک با استفاده از روش بخار کلروفرم انجام شد. نتایج نشان داد که کربن خاک مراتع در دامنه ۲۱۹/۸۴ الی ۹۸۷/۵ میلی‌گرم در کیلوگرم بود. همچنین نتایج نشان داد که با کاهش شدت چرا در مرتع، کربن آلی خاک افزایش یافته و اختلاف معنی‌داری با مناطق چرا شده داشت. به طور مشابه، نیتروژن خاک به میزان ۲۰۷/۷۲ میلی‌گرم بر کیلوگرم در طرح چرای تناوبی به طور قابل توجهی بالاتر از دو تیمار دیگر (چرا شده و قرق) بود. در مجموع نتایج نشان داد که این دو عنصر پر مصرف در خاک (کربن آلی و نیتروژن) با افزایش عمق خاک در حال کاهش بودند.

کلمات کلیدی: مراتع، کربن، نیتروژن، چرا شده، لگوم، هیمالیا