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Amount and Vertical Distribution of Soil Organic Carbon and Total Nitrogen in a Dry Tropical Forest Ecosystem, Tanzania

George Bunyata Bulenga[1, 2, *], Salim Maliondo[1], Josiah Zephania Katani[3]

[1]Department of Ecosystems and Conservation, Sokoine University of Agriculture, Morogoro, Tanzania

[2]Department of Research and Publication, Tanzania Research and Conservation Organization (TRCO), Morogoro, Tanzania

[3]Department of Forest Resources Assessment and Management, Sokoine University of Agriculture, Morogoro, Tanzania

Email address:

bbunyata@gmail.com (George Bunyata Bulenga), salim.maliondo@gmail.com (Salim Maliondo), katani@sua.ac.tz (Josiah Zephania Katani), josiahkatani@gmail.com (Josiah Zephania Katani)

[*]Corresponding author

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Abstract: There is a growing interest in understanding soil organic carbon (SOC) and total nitrogen (TN) of various ecosystems worldwide, because they are important indicators of soil quality and soil fertility, especially on the availability of essential nutrients for plant growth; and climate change mitigation. We tested the hypothesis that the amount and vertical distribution of SOC and TN in 0-30cm and 30-100cm depths differ significantly in miombo woodland ecosystems. Soil samples were collected from 15m-radius circular plots (n=33). SOC was determined by Mid-Infrared (MIR) spectroscopy (ICRAF approach) and the Walkley and Black method (NAFORMA approach). The mean amount of SOC and TN at 30-100cm depth were significantly higher (p=0.003 and p=0.0001, respectively) than that within the 0-30cm depth. The amount of SOC at 20-40cm (39tCha^{-1}) was found to be significantly (p=0.0007) higher than at 0-20cm (32tCha^{-1}) followed by decreasing pattern to 100cm. On the other hand, TN decreased substantially from 0-20cm to 100cm depth. SOC was significantly (p<0.05) and positively correlated with TN. The NAFORMA approach estimated significantly ($P<0.05$) higher SOC than ICRAF approach. Clearing of forests for sesame cultivation invariably resulted in increased nitrogen in the top soil due to addition of ammonium fertilizers, but loss of SOC is due to removal of biomass (including slash burning) and a reduction in the quantity and quality of organic inputs added to the soil. Accurate estimation of SOC at national and regional scales should use the modern methods complimented by the standard methods in different ecosystems.

Keywords: Miombo Woodland, Vertical Distribution, Mid-Infrared, Walkley and Black

1. Introduction

Soil organic carbon (SOC) and total nitrogen (TN) are important indicators of soil quality and sustainable land use management [1]. They are also major components of the global carbon and nitrogen cycles [2-4, 60]. It is important to understand SOC and TN stocks, not only because these are often key variables determining soil quality and soil fertility, but also because of global climate change largely through the role soils can play as a source or sink for C and N from a local to global scale [5].

Previous studies focused on the amount and vertical distribution of SOC and TN in the topsoil layers, i.e., 0-30 cm or slightly more [6, 7, 61] because changes below this depth were often considered to be negligible [2, 8]. SOC and TN stored in the subsoil layer (30-100 cm) was ignored when limiting their estimates to the topsoil layer. However, Chai et al. [9] and Kafle [10] suggest that the subsoil layer contains significant quantities of SOC and TN than topsoil layer in typical Chinese terrestrial ecosystems and Kankali community forest located in the tropical region of Nepal, respectively. It is assumed that half of the SOC and TN is

located in subsoil layer [11] indicating the need of more studies to verify this assumption. Moreover, many studies have demonstrated that SOC and TN content in forest ecosystems generally decreases with increasing soil depth [12-14]. Others suggest that, the amount and vertical distribution patterns of SOC and TN at certain depths are less described [8, 15-17] especially in subsoil layers, indicating the need of further studies. In this context, this study aimed to quantify SOC and TN in different soil layers in Kibutuka miombo woodland ecosystem up to 100 cm soil depth.

There is a limited number of studies and inconsistence of sampling and analytical methods focusing on the content of SOC in the soil [59, 62]. Different approaches have been used when sampling and analyzing SOC stock, resulting in a confusing array of choices when selecting a method to be used [18, 59]. In the context of carbon sequestration and greenhouse gases (GHGs) emission studies, carbon stock in the soil need to be quantified precisely [18]. In soil sample collection approaches, some use the soil auger and cylinders or cores while others excavate soil pits. In soil sample analysis, the dry combustion for total carbon, and calcimeter method for inorganic carbon and wet oxidation for SOC [19, 20], are conventional and standard procedures but are time-consuming, laborious and expensive. Several radiation-based methods are used as an alternative which include diffuse reflectance infrared spectroscopy (IR) like Mid-Infrared (MIR) [21, 22]. Comparing the amount of SOC collected and analyzed by two approaches namely International Centre for Research in Agroforestry (ICRAF) and National Forest Resource Monitoring and Assessment (NAFORMA) at Kibutuka miombo woodland ecosystem is crucial.

2. Materials and Methods

2.1. Description of Study Site

This study was conducted in Kibutuka division found in Liwale district, Lindi region, Tanzania [66] (Figure 1 upper left). Kibutuka miombo woodland ecosystem is located in six villages namely; Kibutuka, Ngumbu, Kitogoro, Kiangara, Kiperere and Mirui (Figure 1 lower right). The altitude of Liwale district ranges from 300 to 600 m.a.s.l and it lies between 36° 50’ and 38°48’ E, 8° and 10° 50’ S. The climate of Liwale district is influenced by the south-east winds in mid-year and the north-east winds during the turn of the year. The temperature ranges from 20°C to 30°C and the average temperature is 25°C per annum. The rainfall pattern is unimodal starting from mid-November and running until mid-April with a dry season from June to October [23]. The short rain period starts from late November to January, whereas long rains continue from March to May. According to Mtwara weather station, the annual rainfall for Liwale ranges from 600 to 900 mm. Vegetation is characterized by miombo woodlands and the predominant genera are *Brachystegia* and *Julbernardia* reaching a height of 15-20 m, while most of trees are in under storey at 5-10 m height including genus *Diplorhynchus* and *Combretum*. The soils of Liwale are mainly occupied by a sandy clayey soil that is deep [24]. The soils of the miombo woodlands are generally leached, sandy and poor in nutrients [25]. These soils include those classified as Hypoluvic Arenesol besides Profondic and Arenic Luvisols.

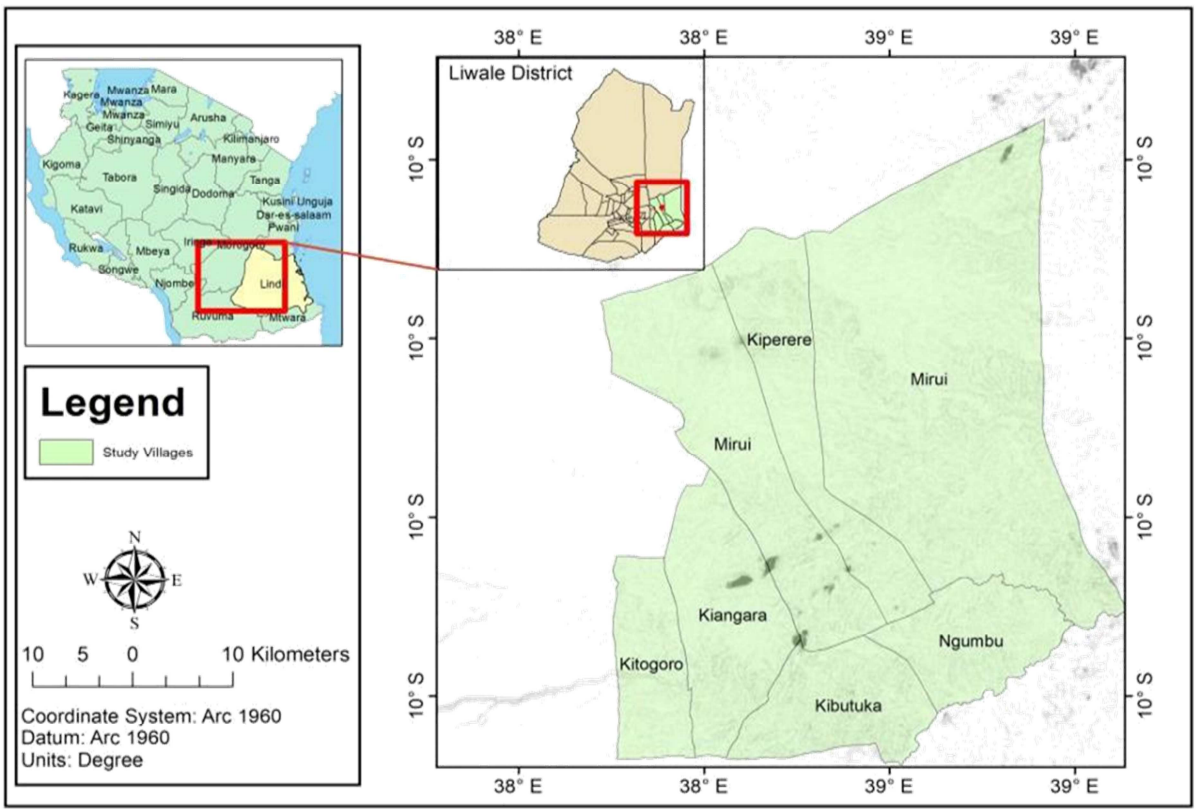

Figure 1. *A map of Tanzania showing the study site of Kibutuka miombo Woodland Ecosystem.*

2.2. Sampling Design

The NAFORMA forest inventory exercise conducted between 2009 and 2013 established a number of sampling clusters in Liwale district which are characterized by Miombo woodlands. During this study only four NAFORMA clusters were used (grey in color), and seven clusters were added (black in color) in order to improve the reliability of the estimates (Figure 2 right). The clusters and their locations in respective villages are shown in Figure 2 left. Each cluster had ten circular plots of 15 m radius spaced at intervals of 250 m (Figure 3). Five plots were laid towards north while the other five were laid towards east (Figure 3). In this study only three plots in each cluster (plot 4, 7 and 10) were chosen systematically for soil samples collection.

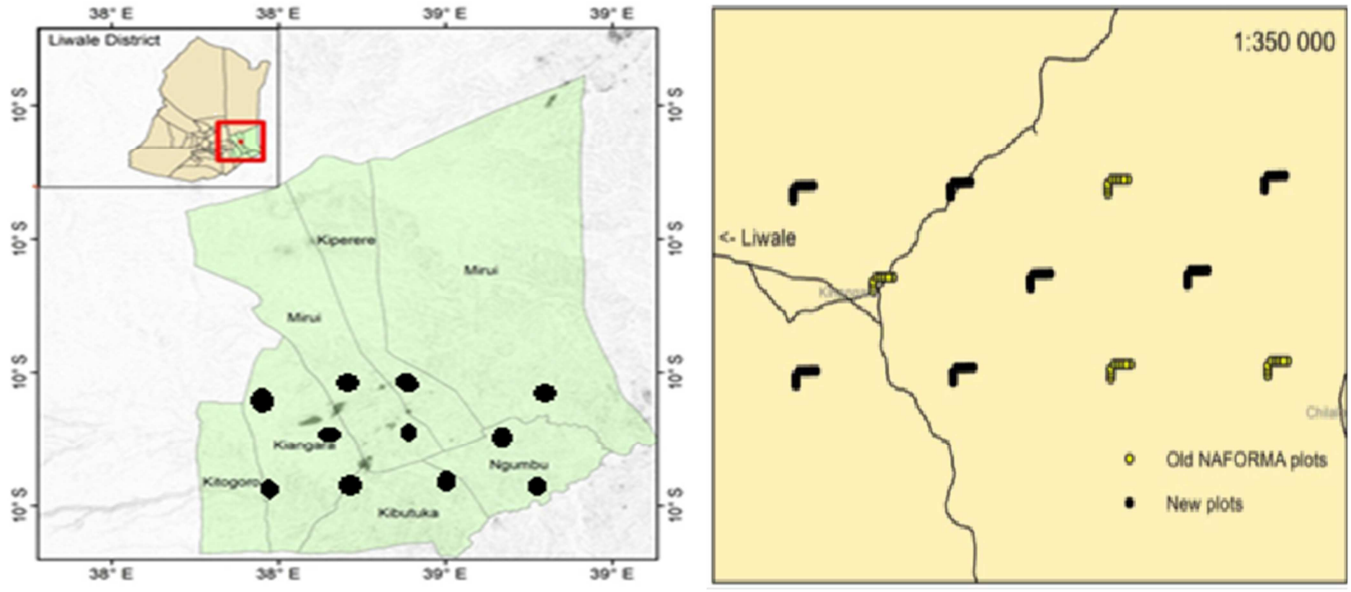

Figure 2. *Distribution of clusters in Kibutuka miombo woodland ecosystem and their locations in different villages of Kibutuka Division in Liwale District, Tanzania.*

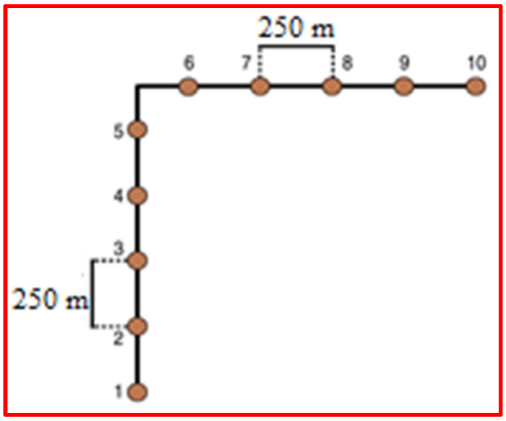

Figure 3. *Cluster and plot design in Kibutuka Miombo woodland ecosystem.*

3. Data Collection

3.1. Soil Samples Collection Through Soil Mini-Pit (NAFORMA Approach)

At each soil sampling point, four points located systematically at the main compass directions (east, south, west and north) were identified. At each point, a soil mini-pit was excavated to 30 cm depth, with at least one vertical surface that was used for soil sampling. Where the soil was too hard to dig a 30 cm deep pit, sampling was limited to the upper 20 cm layer; and this was noted in the field forms. On the vertical face of the soil mini-pit, three layers were marked at each 10 cm depth interval: 0-10 cm, 10-20 cm and 20-30 cm where the volumetric soil samples were taken. Soil sampling cylinders with inner diameter of 7 cm and height of 4.8 cm were used to collect soil samples. From each sampling plot, soil samples collected at the same sampling depth were placed into a clearly labeled paper bag to form a composite soil sample of the plot at the particular depth. Therefore, there were three composite soil samples from each sampling plot making a total of 99 soil samples.

3.2. Soil Sample Collection Using a Soil Auger (ICRAF Approach)

A soil auger was used to collect soil samples from the center of the plot about 3 m towards east. The rationale was to avoid the compaction of soil, which was done at the center during plot layout. At this point, three randomly selected sites were chosen for soil sample collection at a depth interval of 0-10 cm to 100 cm. Maximum sampling depth was noted together with the reason, whether it was due to hardpan, stones, ground water or large surface roots. Soil samples from three sites were mixed to form a composite sample. The total weight of the composite soil sample was measured by using a digital weighing scale to the nearest gram.

3.3. Data Analysis

Soil organic carbon from the soil samples collected using NAFORMA approach were determined by the Walkley and Black dichromate wet oxidation method [26], whereby the oxidizable matter in the soil is oxidized by 1 N $K_2Cr_2O_7$ solution. The following formula was used: SOC (%) = (meq. $K_2Cr_2O_7$ – meq. $FeSO_4$) × 0.003 × 100 × f × MCF; Where, MCF = moisture correction factor, f = correction factor of the organic carbon not oxidized by the treatment (normally approximately 1.3). SOC and TN from soil samples collected using ICRAF approach was determined by MIR spectroscopy. The amount of SOC in tones per hectare was obtained by: SOC ($tCha^{-1}$) = BD (g/cm^3) × OC (%) × depth (cm) × 10 000 $m^2/100$; Where, BD = bulk density, OC = organic carbon. The mean of SOC collected by ICRAF approach were estimated by MIR spectroscopy from depth interval of 0-10 and 10-20 cm, 20-30 and 30-40 cm to 100 cm were calculated to get five intervals of 0-20, 20-40, 40-60, 60-80 and 80-100 cm for determination of vertical distribution of SOC and TN. The analysis of SOC through Walkley and Black dichromate wet oxidation method [65] as done in the Department of Ecosystems and Conservation Laboratory at Sokoine University of Agriculture (SUA) in Tanzania, while analysis of SOC and TN through MIR spectroscopy was done in Nairobi Kenya at ICRAF soil Laboratory. Pearson correlation analyses between paired samples in R-software version 3.5.1 [27] were used to identify the relationships between SOC, TN and BD at $P \leq 0.05$. A comparison of SOC at 0-30 cm depths from two different approaches were then analyzed based on student *t*-test using R software to compare their means at $P \leq 0.05$. SOC and TN data were reported for the topsoil (0-30 cm) and subsoil (30-100 cm) according to IPCC guideline requirements [28].

4. Results

4.1. Amount and Vertical Distribution of SOC and TN in 0 to 100 cm Soil Depth

The mean amount of SOC and TN at 30-100 cm depth was significantly ($p = 0.003$ and $p = 0.0001$ respectively**)** higher than that contained within the 0-30 cm depth (Table 1). The percentage contribution of SOC at 0-30 cm and 30-100 cm depth was about 42% and 58%, respectively. While TN had 43% at 0-30 cm depth and 57% at 30-100 cm depth. There was a significant mean difference at $p < 0.05$ of SOC from 0-20 cm to 60-80 cm and TN from 0-20 cm to 40-60 cm (Table 2). The amount of SOC at 20-40 cm (39 $tCha^{-1}$) was found to be significant ($p = 0.0007$) higher than at 0-20 cm (32 $tCha^{-1}$); then it decreased with depth to 100 cm (Figure 4) while TN was found to decrease substantially from 0-20 cm to 100 cm depth (Figure 5). But, soil bulk density (g/cm^3) increased from 0-20 cm to 100 cm depth though the increase was not significant (Figure 6).

Table 1. *Amount and percentage contribution of SOC and TN in 0-30 cm and 30-100 cm.*

Measured parameter	0-30 cm	30-100 cm	0-100 cm	Subsoil/Topsoil	t-ratios	p-value
Amount of SOC ($tCha^{-1}$)	65.91	89.54	155.45			
Percentage contribution of SOC (%)	42.4	57.6	100	1.36	-3.145	**0.003**
Amount of TN (%)	0.124	0.165	0.289			
Percentage contribution of TN (%)	42.91	57.09	100	1.33	-7.015	**0.0001**

Table 2. *Significant tests of SOC and TN in different depths from 0-100 cm.*

Test parameters	Soil organic carbon		Total nitrogen	
	t-ratio	Significant codes	t-ratio	Significant codes
0-20 Vs 20-40	-7.24	***	5.68	**
20-40 Vs 40-60	9.11	***	2.26	*
20-60 Vs 60-80	5.68	***	1.01	NS
60-80 Vs 80-100	0.29	NS	1.24	NS

*** Significant at $p < 0.001$, ** Significant at $p < 0.01$, * Significant at $p < 0.05$ and **NS** Not Significant.

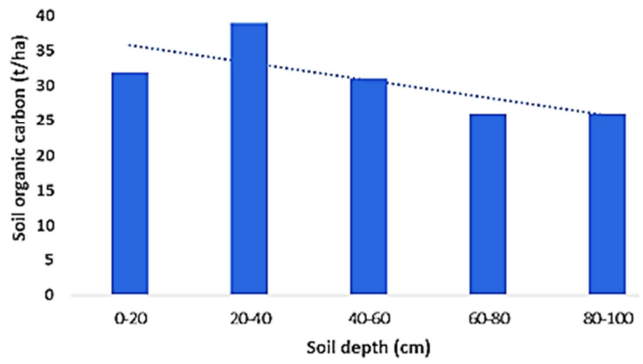

Figure 4. *Vertical distribution of soil organic carbon in 0-100 cm depth.*

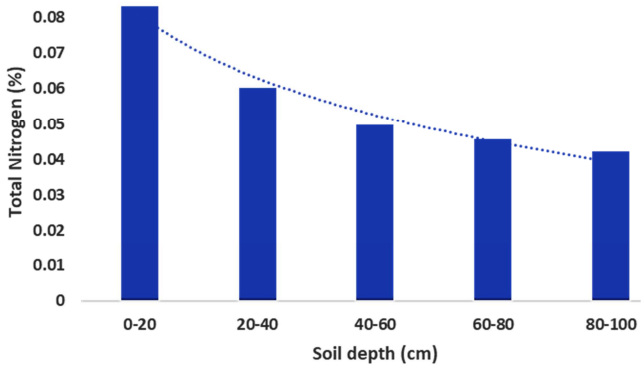

Figure 5. *Vertical distribution of total nitrogen in 0-100 cm depth.*

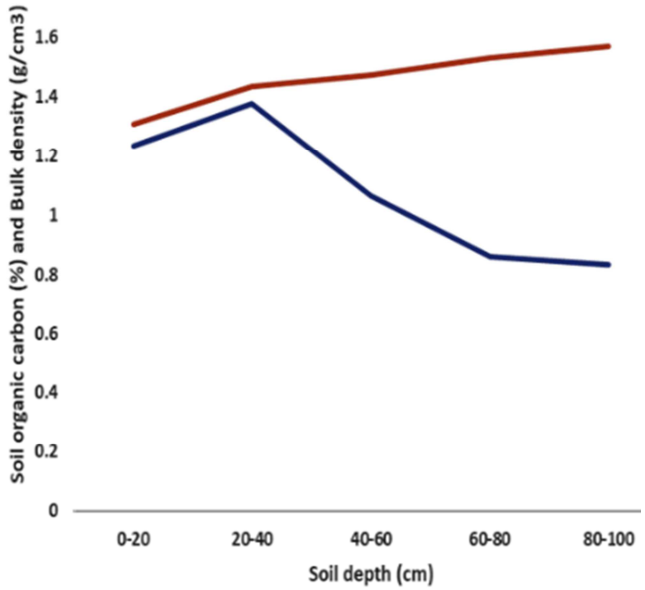

Figure 6. *Distribution trend of amount of soc (blue line) and bulk density (red line) to 0-100cm depth.*

4.2. Relationships Between Soil Organic Carbon, Total Nitrogen and Bulk Density

Soil organic carbon was positively correlated with total nitrogen (Figure 7) and the correlation was significant at $p < 0.05$ (Table 3). However, it was found that SOC and TN were negatively correlated with soil BD [64] though it was not significant at $p < 0.05$ (Table 3).

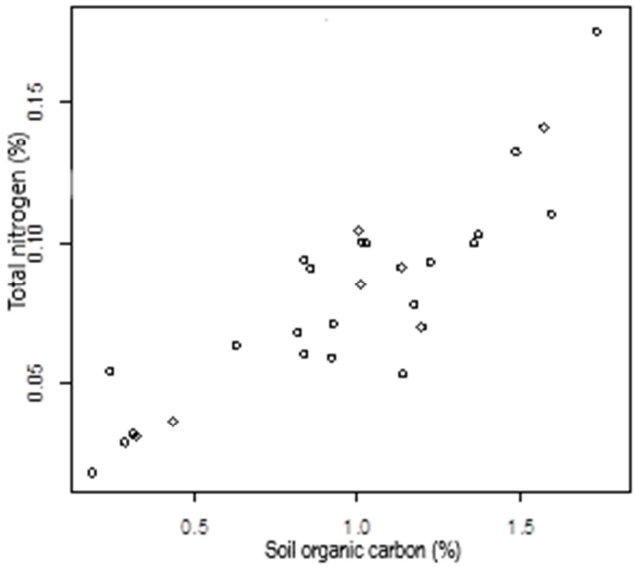

Figure 7. *Pearson's product-moment correlation between soil organic carbon and total nitrogen.*

Table 3. *Pearson's product-moment correlation between soil variables.*

Test parameters	r-values	*p*-values
SOC Vs TN	0.51	**0.003**
SOC Vs BD	-0.08	0.66
TN Vs BD	-0.06	0.75

4.3. Amount of SOC Collected and Analyzed Using ICRAF and NAFORMA Approaches

The mean proportions of SOC determined using ICRAF and NAFORMA approaches at Kibutuka Miombo woodland shows that, the mean SOC determined using NAFORMA approach was found to be significantly higher ($P < 0.05$) than that determined using the ICRAF approach (Table 4).

Table 4. *Mean Soil Organic Carbon Estimated using ICRAF and NAFORMA approaches.*

Approach	Mean ± SE SOC (%)	Mean ± SE SOC ($tCha^{-1}$)	t-Ratio	P-value
ICRAF	0.74 ± 0.62	33.35 ± 3.2		
NAFORMA	0.98 ± 0.41	41.89 ± 2.1	2.59	0.01

5. Discussion

5.1. Amount of Soil Organic Carbon and Total Nitrogen to 100 cm Soil Depth

The results from this study support the widely held contention that subsoil (30-100 cm) contributes a substantial proportion of SOC and TN stocks [29, 30]. The subsoil layer has been shown to contribute approximately 58% and 57% which is more than half of the SOC and TN stocks, respectively. Gray et al. [31] working in the dry climate zones of eastern Australia demonstrated that SOC is stored in the subsoil with an average 54% while the global estimates found 52.4% of TN stored in the subsoil [2] which is nearly the same with our findings. Kunlanit et al. [32] reported that 30% of 1-m depth soil was in the subsoil across forest, cassava and rice paddy land uses in the North eastern Thailand and recommended that soil C estimates should be based on 100 cm soil. Estimation of SOC and TN to the deeper soil layers in different ecosystems should not be ignored, because it helps to know the amount and contribution of carbon and nitrogen sequestered in the topsoil and subsoil layers. However, the amount of SOC and TN at 30-100 cm depth relative to that at 0-30 cm in this study was higher by a mean factor of approximately 1.4 and 1.3 respectively (Table 2). Brahim et al. [33] reported that the soils of Tunisia stored 0.405 Pg C in the topsoil layer (0-30 cm) and 0.601 Pg C in the subsoil layer (30-100 cm); implying that the sub soil horizon stored about 1.48 times higher SOC than the top 0-30 cm, which is nearly the same with the findings of this study. Results from other scholars and our findings support our assumption that the subsoil layer contributes larger SOC and TN stock than the topsoil layer, regardless of the vegetation type. Thus, improved knowledge of amount of SOC and TN across different vegetation types is essential to determine whether carbon and nitrogen in deep soil layers will react to global change and accelerate the increase in atmospheric carbon dioxide (CO_2) and nitrogen concentration.

5.2. Vertical Distribution of Soil Organic Carbon and Total Nitrogen to 100 cm Soil Depth

Many studies have demonstrated that soil C and N content decreases with increasing soil depth [9, 13, 34] irrespective of vegetation types or climate zones [9]. Kafle [10] found a significant difference of SOC and TN between different soil layers from 0-100 at an interval of 20 cm in Kankali community forest located in the tropical region of Nepal. Hua et al., 2015 reported that soil C and N content significantly decreased with increased soil depth in agriculture, forest, grassland, and desert ecosystems. Jobbágy and Jackson [8] and Yang et al. [35] found that the vertical distribution of SOC in forest ecosystems at 20 cm intervals decreases as soil depth increases. Similar findings on the decline of stocks of SOC with the increase in soil depth were reported by Pandey and Bhusal [12] and Ghimire et al. [14] in tropical forests of Nepal.

The above findings agree with the findings of this study for the vertical distribution of total nitrogen and deviates for the vertical distribution of SOC [8]. Total nitrogen showed a decreased pattern as soil depth increased because some plots were located in cultivated woodlands which may have been amended with N fertilizers as the forest is currently in on going degradation for sesame cultivation. But, SOC contents increased from 0-20 cm to 20-40 cm depths followed by a decreasing pattern to 100 cm. Similar decrease in SOC in the topsoil was reported when forest land was converted to cultivated land in the tropics [36]. Clearing of forests for sesame cultivation in Kibutuka miombo woodland ecosystem invariably resulted in loss of SOC in the topsoil. This may be due to removal of biomass (including slash burning) during land clearing, and a reduction in the quantity and quality of organic inputs added to the soil. Also, a large part of Kibutuka Miombo woodland ecosystem is managed by the village government making it subjected to higher encroachment pressure. Kalbitz and Kaiser [37] found that the vertical distribution of soil organic carbon was affected by cultivation practices within the forest. Riezebos and Loerts [38] on the other hand, suggested that soil mixing in tillage systems can completely translocate surface SOC to lower depths. Similarly, a transition from forest to agricultural land use leads to a significant decrease of SOC in the topsoil [38].

However, in natural ecosystems, soil forming factors, such as climate, topography, parent material, biology, and time are important for soil C and N vertical distributions [9, 39, 40]. Thus, distributions of SOC and TN in vertical dimension are necessary to investigate stocks and controls of SOC and TN [41, 60], and also important for understanding the role of SOC and TN in the global C and N cycles [42, 43, 60].

5.3. Relationships Between Soil Organic Carbon, Total Nitrogen and Bulk Density

A positive relationship found in our study in miombo woodland between soil organic carbon and total nitrogen indicates the interdependence between soil carbon and nitrogen in ecosystem processes and functions [63]. Studies by Reich et al. [44] and Liu and Greaver [45] reported that nitrogen plays a crucial role through the interaction with carbon in the ecosystem productivity and carbon sequestration. And the dynamics of soil N not only determine whether the terrestrial ecosystem functions as a N sink or source [46], but can even alter the biogeochemical cycles of other elements [47, 48]. Kafle [10] found a positive correlation between SOC and nitrogen in Kankali community forest in Chitwan district located in the tropical region of Nepal. Gautam and Mandal [49] also reported positive correlation between SOC and nitrogen in a tropical moist forest in eastern Nepal. However, nitrogen is a significant stimulant of plant growth [50, 51] and an important limiting element in terrestrial ecosystems [52]. This indicates that, while quantifying nutrients found in the soil and their interactions, nitrogen should not be ignored. Therefore, estimations of soil N stocks will facilitate assessments of the role of soil in terrestrial ecosystems in terms of N and C cycles. Rossi et al. [53] found a negative correlation between SOC and BD in miombo woodlands of Southeastern Tanzania which is similar to our results regardless of their insignificance. Kafle [10] also reported a negatively correlation of SOC and nitrogen with bulk density of the soil in Kankali community forest in Chitwan district located in the tropical region of Nepal. Negative correlation between SOC and bulk density of soil was also reported by Ali et al. [54] and Ghimire et al. [14]. The negative relationship might be attributed by soil compaction which gave higher estimates of bulk density [54] and this is true when the ecosystem is exposed to higher grazing pressures and the presence of hard pan [10].

5.4. Amount of Soil Organic Carbon Estimated Using ICRAF and NAFORMA Approaches

Results in this study showed that the Walkley and Black method (NAFORMA approach) estimated SOC significantly ($P < 0.05$) higher than the Mid-Infrared method (ICRAF approach). These results are similar to those reported by Stevens et al. [55] whereby the Walkley and Black laboratory values were higher than those determined by the radiation method. Wang et al. [56] also reported that the Walkley and Black method provided an accurate estimate of SOC with 100% recovery for most soil samples compared to the radiation methods. Lettens et al. [57] also demonstrated that compared with the automated CNS technique, the Walkley and Black method yielded almost 100% recoveries for SOC in the calcareous soils. Owing to its low cost and minimal requirements in laboratory equipment, the Walkley and Black procedure is still used widely to measure SOC content [58]. This could be due to its reliable estimation of SOC when compared with other analytical approaches. Janik et al. [19] suggested that if the sources of laboratory error can be identified while using the Walkley and Black method, the infrared method may in fact be a better tool for interpretation than the standard methods. Soil samples collection using the ICRAF approach was challenged by presence of a hardpan and time consuming but there were no difficulties when

sampling soil using the NAFORMA approach. Our assumptions are that sampling approaches for determining SOC at any location can be influenced by, soil type or parent material, topography, climatic condition, site management and soil sampling tools used which could also give out different estimates depending on the analytical method used.

6. Conclusions

The amount of SOC and TN in the subsoil layer (30-100 cm) is greater than the topsoil layer (0-30 cm) in miombo woodland ecosystem. Clearing of forests for sesame cultivation invariably resulted in increased N due to addition of Ammonium fertilizers and loss of SOC in the topsoil due to removal of biomass (including slash burning) during land clearing, and a reduction in the quantity and quality of organic inputs added to the soil. Accurate estimation of SOC at national and regional scales should use the modern methods complimented by the standard methods particularly when determining SOC in different ecosystems. Management actions are needed to combat the ongoing forest degradation which impact ecosystem processes like N and C cycles. Finally, further studies are also encouraged for greater understanding of the appropriate sampling and analytical methods to estimate SOC stocks in different ecosystems.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of Interests

The authors declare that they have no competing interests.

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