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# Temporal Variation of Soil Organic Carbon and Total Nitrogen Stock and Concentration along Land Use, Species and Elevation Gradient of Chilimo Dry Afromonate Forest and Adjacent Land Uses, Ethiopia

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## ABSTRACT

Forests play a vital role in the natural global carbon cycle by capturing carbon from the atmosphere through photosynthesis and converting it into forest biomass. Forests sequester and stores more carbon than any terrestrial ecosystem and act as sources as well as sinks of $CO_2$. However, the increasing rate of deforestation and the impact of changes in land use require a critical and updated look at what is happening in the tropics. This work emphasized the temporal variation of bulk density, carbon (C) and nitrogen (N) stock and concentration in four land-use categories: natural forest, tree plantations, crop-land and degraded soil along elevation gradient and soil depth. The study was conducted in the Central Highlands of Ethiopia, where deforestation and human pressure on native forests are exacerbated and erosion has caused extensive soil loss. We hypothesized that, there is temporal variation of C and N concentrations and stocks in native forest along elevation gradient, land use type, species and soil depth. Carbon and N concentrations and stock and bulk densities in mineral soil were analysed as repeated measures in an irregular vertical space ranging from 0–10 cm, 10–30 cm, 30–50 cm and 50–100 cm, using a linear mixed model approach in two-time scale period 2012 - 2017. Double observations in 2012 and 2017, were made from the forest floor were analysed by a general linear mixed model. There was significant variation in organic carbon and nitrogen stock along elevation gradient for forest floor. Results also indicated that soil depth was more important factor than elevation gradient in native forests, though C and N concentrations and stocks diminished near human settlements. Native forest stored on average more nitrogen than bare soil, cropland and plantations, respectively. Conversion of crop and degraded land into plantations ameliorated soil degradation conditions, but species selection did not affect carbon and nitrogen stocks. Thus, appropriate forest management options should be applied in order to increase productivity and carbon sink of Chilimo dryafromontane forest and adjacent land use. Temporal monitoring and reporting of carbon stock and concentration is also important to understand the role of Chilimo dryafromonate forest in climate change mitigation and adaptation agendas.

***Keywords*:** Forest Floor; Mineral Soil; Soil Depth; Mixed Model; Species Identity; Impact Assessment

## 1. Introduction

Soil is a major carbon (C) pool in terrestrial ecosystems containing nearly 1500 Pg of C as soil, with 11 % of SOC held in forest soils worldwide in the first 1m depth (Dey, 2005; Negi *et al*., 2013; Yuan *et al*., 2013). Forests play a vital role in the natural global carbon cycle by capturing carbon from the atmosphere through photosynthesis and converting it into forest biomass. Forests sequester and stores more carbon than any terrestrial ecosystem and act as sources as well as sinks of $CO_2$ (Jandl *et al*., 2006). Forests in general and forest soils in particular play a vital role in carbon balance. Forest soils are one of the major carbon sinks on earth, because of the high amounts of organic matter stored in forest soils. Terrestrial vegetation plays an important role in the global carbon cycle and in alleviating atmospheric $CO_2$

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elevation (Bonan, *et al*., 2008). The global terrestrial ecosystem's gross primary production is 123 ± 8 peta grams of carbon per year Pg C year$^{-1}$ and forests account 80 % of this production (Christian *et al*., 2010). In addition, forests play Reducing Emissions from Deforestation and Forest Degradation (REDD). Nitrogen is a constituent of soil organic matter (SOM) that directly influences SOC accumulation via net primary productivity (NPP). N - Fixing plant species can substantially add to the amount of available N in the soil via biological N - fixation (Resh *et al*., 2002; Binkley, 2005). This increase in N can decrease microbial respiration rates, facilitate C- sequestration and improve soil fertility (Bowder *et al*., 2004; Wang *et al*., 2010).

Soil organic carbon (SOC) is affected by environmental factors such as topography, parent material or soil depth (Fu *et al*., 2004; Johnson *et al*., 2000). The key relationships between environmental factors and soil depth are often indirect and complex. Topography influences precipitation, temperature, solar radiation and relative humidity (Tsui *et al*., 2004). Land use and plant species also significantly influence SOC estimations. SOC stocks varied across landscapes and land-use practices. In the tropics, deforestation and changes in land use are significantly impacting the global carbon cycle by increasing the rate of carbon emissions (Silver *et al*., 2000). Conversion of forest ecosystem into agricultural ecosystems negatively affects SOC concentration and stock by 20–50 % (Solomon *et al*., 2002; Lemenih and Itanna, 2004; Lal, 2005). In tropical forests, deforestation accounts 20 % of total anthropogenic $CO_2$ emissions into the atmosphere (Baccini *et al*., 2008). Mitigation strategies to reduce the impact of climate change by augmenting carbon sequestration and reducing $CO_2$ emissions from soils include proper forest management, afforestation and/or reforestation programs (FAO, 2006). Quantification and continuous assessment changes in C and N pool sizes and fluxes are fundamental to understanding the effects of changes in land use/land cover on ecosystem functioning and limiting greenhouse gas emissions (Jaramillo *et al*., 2003; Lemma *et al*., 2006).

Afforestation is one of the cost-effective strategies for climate change mitigation. It also protects soil against wind and water erosion (Chang *et al*., 2011; Jarecki and Lal, 2003). However, both the magnitude and direction of soil C dynamics following afforestation are poorly studied. It is important to evaluate the biomass carbon stock and sequestration potential across land use, elevation gradient and species (McKinley *et al*., 2011).

The Highlands account 45 % of the country's total area, 85 % of the human population and 75 % of the livestock population and forest cover in these areas are dominantly by dry and moist afromontane forest. However, much of the Highland forests were disappeared due to reckless cutting of trees for fuelwood, charcoal, construction wood and farmlands (Teketay, 2001). In Ethiopia deforestation, overharvesting and permanent conversion to other forms of land use is leading to shrinkage of forest resources in these areas. As a result, forest cover has been declining rapidly and only remnant forests are confined in the south and south-western parts of the country (Bekele, 2002). Deforestation is one of the main causes of the prevailing land degradation in the country and forests and woody vegetation are disappearing at a rate of 150,000 to 200,000 ha annually. The reduction of forests in the tropics impairs important atmospheric functions as carbon sinks and the combustion of forest biomass releases atmospheric $CO_2$, contributing to the build-up of GHGs and global warming. The climate of Ethiopia has been changing due to global and local effects of vegetation degradation (Teketay, 2001). Today, forest management activities are increasingly taking into consideration the role of forests as carbon sinks and information on factors that determine the forest carbon stock is given concern (McEwan, 2011).

Chilimo forest is one of the few remnants of dry afro-montane forest located in Central Highlands, Ethiopia. The forest is a small enclave in western direction of a chain of hills and ridges that stretch 200 km from north of Addis Ababa. Native coniferous and broad-leaf species such as: *Juniperus procera, Podocarpus falcatus, Olea europaea ssp. cuspidiata, Scolopia theifolia, Rhus glutinosa, Olinia rochetiana and Allophylus abyssinicus* are dominante in the forest (Bekele, 2003; Kelbessa and Soromessa, 2004; Kassa *et al*., 2008; Tesfaye, 2015). Tesfaye (2015) reported 33 different native species (22 tree species and 11 shrub species) in three forest patches of Chilimo dry afromontane forest. Soromessa and Kelbessa (2014) reported 213 different plant species categorized into 83 families and 17 plant species are unique to the Chilimo forest. The forest is found in the nearby the capital city Addis Ababa, easily accessible through all-weather road and having old historical palaces inside it. Adjacent to the natural forest there is plantation forest around 400 hectares of *Eucalyptus saligna, Pinus patula, Cupressus lusitanica* used for cash income for forest user groups (Kassa *et al*., 2008; Tesfaye, 2015).

Due to continuous deforestation, the Chilimo forest cover has declined from 22,000 ha in 1982 to 6000 ha in 1991 and 4500 ha in 2016 (Shumi, 2009; Teshome, 2015). Consequently, some plant species are becoming endangered (Soromessa and Kelbessa, 2014) as the need for fuel wood, arable land and timber drive forest degradation (Soromessa and Kelbessa, 2013). This forest has been categorized into one of Ethiopia's 58 national priority forest protection areas for better management and serves as a carbon sink. Alternative strategies to reduce the pressure on the native forest by alleviating the fuel wood shortage fast-growing tree plantations should be planted at homesteads, farm boundaries and woodlots (Alebachew, 2012). Temporal carbon assessment activities in the forest floor and mineral soil is generating vital information regarding the importance of the forest for carbon exchange and climate change mitigation at local, regional and international levels. The history, topography, stewardship and intense transformation in land use of the Chilimo forest make it an optimal case study. On these premises, we hypothesized that there will be temporal variation in soil organic carbon (SOC) and soil organic nitrogen (SON) stock in the forest floor and in mineral soil along an elevation gradient in native forest. Likewise, there will be temporal variation SOC and SON stocks at different depths in the forest and adjacent land use. The specific research questions to be addressed in this study are (1–5): (i). Is there temporal variation of carbon and nitrogen concentration and stock in the forest floor along an elevation gradient? (ii). Is there temporal variation of soil bulk density across land use and/or soil depths? (iii). Is there temporal variation in carbon and nitrogen concentrations and stocks in mineral soil at different soil depths along an elevation gradient in native dry afromontane forests? (iv). How does the temporal variation of intensive land use change soil carbon and nitrogen concentrations and stocks at different soil depths?

# 2. Material and Methods

## 2.1 Study site location and description

The experimental site is located in the Chilimo–Gaji dry afromontane forest of the Western Shewa zone of the Dendi district in the central Highlands of Ethiopia. The forest is surrounded by crop land, degraded areas and plantation of *Eucalyptus saligna*, *C. lusitanica* and *Pinus patula*. Geographically it is located from 38° 07′ E to 38° 10′ E longitude and 9° 30′ to 9° 50′ N′ latitude, at an elevation of 2170 to 3054 m above sea level (**Figure 1** and **2**, **Table 1**). The mean annual temperature of the area ranges between 15 °C and 20 °C and the mean annual precipitation is 1264 mm.

## 2.2 Forest floor sampling

The Chilimo forest site was stratified into 3 major natural forest patches : Chilimo, Gallessa, and Gaji. Thirty – five, 20 × 20 m plots were laid out following a top-down gradient, from the top edge of the mountain to the bottom and approximately 150 m away from the outer ridge in order to avoid edge effects. The distance between one plot edge to the next plot was 100 m and plot location was determined using measuring tape, GPS, altimeter and compass. Two times forest floor samples were sampled in 2012 and in 2017 in the already established sample plots within a 0.25 × 0.25 m (0.0625 $m^2$) metallic frame in the center of the main plot.

## 2.3 Mineral soil sampling

Primarily, mineral soil samples were taken below the forest floor up to a nominative depth of 1 m in 2012 then resampling was done within the same sampling plot in 2017. In both cases pit sampling method were used for data collection. Firstly, sample pits (1 m long × 60 cm wide) were dug at the center of the main plot in every other plot. A total of 28 pits (13 in natural forest, 9 in plantations, 3 in cultivated land and 3 in degradad lands) plots were dug for soil collection per year. Then the same amount of sampled plots were taken after five years and samples were taken from four soil depth categories (0–10 cm, 10–30 cm, 30–50 cm and 50–100 cm). Soil bulk density was calculated with a 5 - cm high cylinder that was introduced vertically in one sampling point for each depth interval. A total of 224 mineral soil samplings and other 224 cores were collected for analyzing organic C %, total N % and bulk density, respectively in two times.

## 2.4 Laboratory analysis

Similar lab analysis procedure were used in both periods. Forest floor sample layers were air - dried and homogenized prior to analysis. All samples were weighed and sub-samples were oven-dried for 24 h at 65 °C to constant weight. The chemical analysis for organic carbon in the forest floor was done by drying samples at 105 °C and subsequently, burning using the loss-on-ignition method at 400 °C (Ben-Dar and Banin, 1989). Then soil organic matter was converted into organic carbon according to Eq. (2).

$$\% \, SOC = \frac{w105 - w400}{w105} * 100$$

$$\% \, C = \% \, SOM * 0.58$$

where, C: the organic carbon concentration, SOM: soil organic matter; w105: weight of dry soil sample at 105 °C, w400: weight of ground soil sample at 400 °C and 0.58 is the carbon concentration in the soil organic matter which has been found to be the most conveniente conversion factor from organic matter to carbon content in forest floor (deVos *et al*., 2005). Although, Pribyl (2010) recomendad a value of 0.5 we retained the 0.58 value in forest floor as it has been commonly used and it allows comparisons with other studies. Mineral soil sampled was air dried and passed into less than 2 mm sieve size to

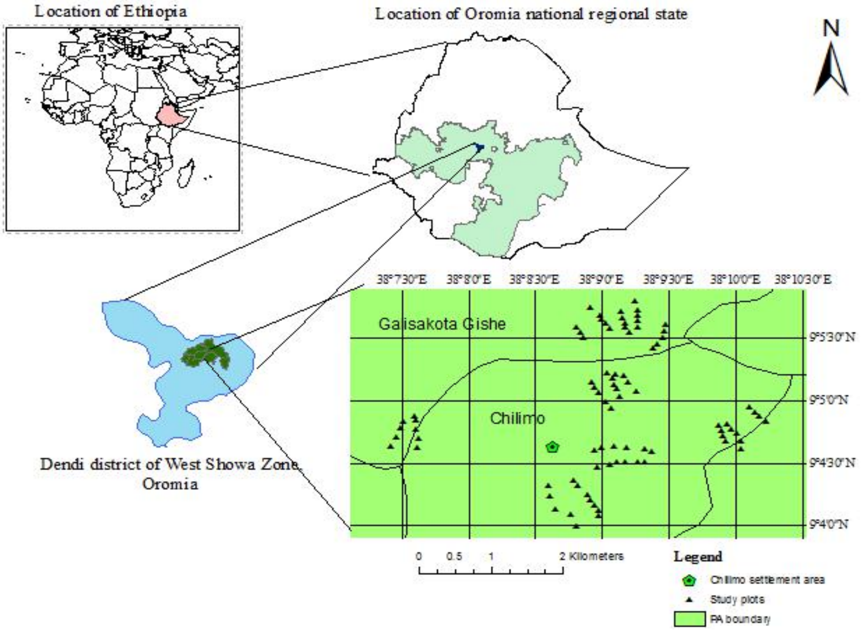

**Figure 1**; Location map of Chilimo dry afro-montane forest**.**

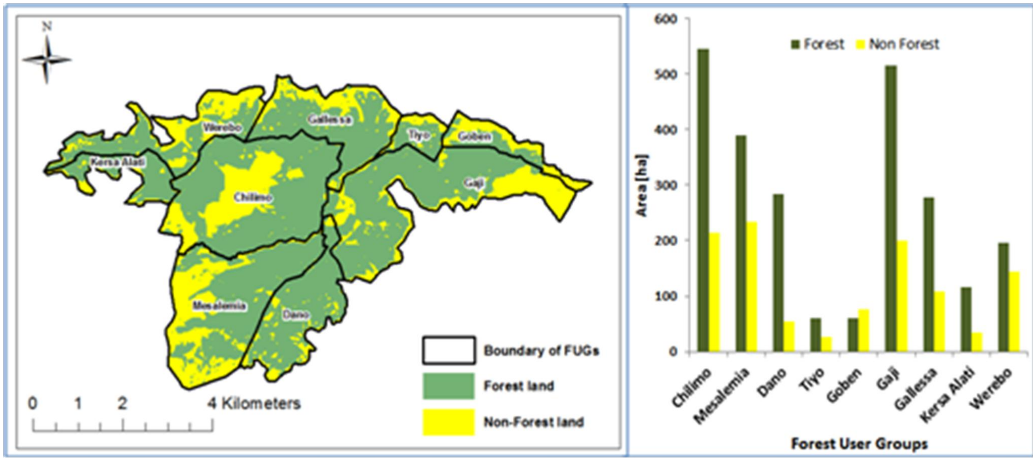

**Figure 2**; The total forest area and non forest area coverage in each forest user group compartment of Chilimo dry afromontane forest**.**

obtain the fine fraction for chemical analysis. The coarse rock fragments (2 mm) sieved sizes were removed from the ample and their percentage (% of stoniness and or rockiness) were calculated by oven dried samples at 67 °C for 24 h for each soil depth

$$CFW\,\% = \frac{Weight\ not\ passing\ a\ 2\ mm\ sieve}{weight\ of\ total\ soil * 100}$$

where CFw is the percentage of coarse fragments by weight (Page-Dumroese *et al.*, 1995).

| Land use type | Forest patch | Latitude | Longitude | Altitude range (m) | Aspect (%) | No. sample plots | No soil samples | Density (N ha$^{-1}$) | Dg (cm) | G (m$^2$ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Native forest | Chilimo | N09°04´013´´-<br>N09°04’857’’ | E038°08´557´´-<br>E038°09´960’’ | 2470-2770 | 8-70 % | 20 | 40 | 2533±28 | 26.12±5.3 | 18.9±1.92 |
| Native forest | Gallessa | N09°05´162´´-<br>N09°05’765’’ | E038°09´847´´-<br>E038°10´283’’ | 2700-2921 | 25-70 % | 11 | 20 | 848±10 | 19.88±2.5 | 18.18±1.91 |
| Native forest | Gaji | N09°04´269´´-<br>N09°04’340’’ | E038°09´861´´-<br>E038°10´025’’ | 2680-2793 | 45-50 % | 4 | 12 | 1638±20 | 23.45±4.4 | 13.81±1.40 |
| Plantation | *Cupressus* | N09°04´115´´-<br>N09°04’297’’ | E038°07´808´´-<br>E038°07´849’’ | 2370-2420 | 3-12 % | 3 | 12 | 575±8 | 23.42±4.4 | 25.5±2.60 |
| Plantation | *Eucalyptus* | N09°04´155´´-<br>N09°04’298’’ | E038°03´0011´´-<br>E038°08´0011’’ | 2360-2400 | 3-10% | 3 | 12 | 1000±13 | 12.79±2.2 | 14.67±1.50 |
| Plantation | *Pinus* | N09°03´514´´-<br>N09°03’676’’ | E038°08´260´´-<br>E038°08´329’’ | 2396-2405 | 6-20 % | 3 | 12 | 1167±15 | 14.52±3.2 | 21.25±2.16 |
| Crop | Chilimo | N09°04´48´´-<br>N09°03’532’’ | E038°08´559’’-<br>E038°08´612’’ | 2406-2423 | 5-15 % | 3 | 12 | | | |
| Degraded land | Chilimo | N09°03´805´´-<br>N09°04’266’’ | E038°07´703’’-<br>E038°07´793’’ | 2350-2425 | 8-30 % | 3 | 12 | | | |

**Table 1.** General description of Chilimo natural forest and adjacent land use types**.**

Then total organic carbon (%) was analyzed according to Walkley–Black's method following the procedure described in Anderson and gram (1996). Bulk density for each soil depth was the ratio of mass of core sampled oven dry weight of dry soil to volume of 5 cm diameter and 5 cm height steel-cylinder following the procedure of Blake (1965). Total N was determined using Kjeldahl method, following the procedure in Keeny and Nelson (1982).

## 2.5 Data analysis approach

Elevation was converted into three discrete classes in order to analyze the effect of the altitudinal gradient: Class 1 (low elevation): ≤ 2600 m, Class 2 (middle elevation): 2600–2700 m and Class 3 (high elevation): ≥ 2700 m. A preliminary analysis of normality and equal variances among groups was performed before selecting the most suitable statistical analysis. Elevation was converted into three discrete classes in order to analyze the effect of the altitudinal gradient: Class 1 (low elevation): ≤ 2599 m, Class 2 (middle elevation): 2600-2700 m and Class 3 (high elevation): ≥ 2701 m. A preliminary analysis of normality and equal variances among groups was performed before selecting the most suitable statistical analysis.

### 2.5.1. Carbon and nitrogen concentration in the forest floor

Data for carbon and nitrogen concentrations and stocks in the forest floor were analysed using the SAS PROC GLM method (SAS Inst. Inc., 1999). To analyse equality of means, we used a Tukey–Kramer test for multiple comparisons among elevation classes at α = 0.05. Data for carbon and nitrogen concentrations and stocks in the forest floor were analysed using the SAS PROC GLM method (SAS Inst. Inc. 1999). To analyse equality of means, we used a Tukey-Kramer test for multiple comparisons among elevation classes at α=0.05.

### 2.5.2 Bulk density and carbon and nitrogen concentration in mineral soil

Samplings were taken two times in five-year interval in the same plot. The results are presented as net change in a treatment in relation to other treatments which means a temporal change in SOC and SON due to differences in treatments assuming that concentration and stocks were similar in time or not 0, i.e. all land uses were the same (native forest) in the past and after five years' time.

The C and N concentrations and bulk densities in mineral soil were analyzed as repeated measurements in an irregular vertical space ranging from 0–10 cm, 10–30 cm, 30–50 cm and 50–100 cm. A subject specific approach was used with the SAS PROC MIXED method along with a Toeplitz Heterogeneous Variance Structure (SAS Inst. Inc., 1999); four variance parameters and three correlation coefficients, which were estimated using the restricted maximum likelihood method (REML). We considered one between-subjects factor at a time (species, land use type or elevation) and one within-subjects factor (depth at four levels) according to the mathematical model:

The C and N concentration and bulk density in mineral soil were analyzed as repeated measurements in an irregular vertical space ranging from 0 -10 cm, 10 - 30 cm, 30 - 50 cm and 50 -100 cm. Results from a previous analysis of bulk density differences among treatments (elevation classes, land use and species planted) indicated that, the most appropriate method for estimation of carbon and nitrogen stock (fixed - mass vs fixed - depth). For these analyses, the SAS PROC MIXED method was used with a Toeplitz Heterogeneous Variance Structure (SAS Inst. Inc. 2015). We used a linear mixed model analysis of variance with repeated measurements, considering one between - subjects factor (species, land use type or elevation) and one within - subjects factor (depth at four levels) according to the mathematical model:

$$Y_{ij;k} = \mu + \alpha_i + \beta_k + \alpha\beta_{ik} + \varepsilon_{ij;k} \qquad \text{(eq.4)}$$

where *I* = 1,…,n for the between-subjects factor (n = 3 for species and elevation, n = 4 for land use type), *j* = 1,…,n for the replicates and *k* = 1, 2, 3, 4 for the within-subject factor (depths), $Y_{ij;k} =$ observed value of the dependent variable for the plot *j* of level *i* in the between-subject factor at depth *k*; $\mu$ is the general mean effect, $\alpha_i$ is the main effect of the $i^{th}$ level for the between-subject factor; $\beta_k$ is the main effect of the $k^{th}$ depth; $\alpha\beta_{ik}$ is the interaction effect of the $i^{th}$ level for the between-subject factor and the $k^{th}$ depth; $\varepsilon_{ij;k}$ is the random error in the dependent variable for the plot *j* of level *i* in the between-subject factor at depth *k*.

The assumptions for the errors in the linear mixed model were:

$\varepsilon_{ij;k} \backsim N\left(0,\sigma_k^2\right)$ , with = random variance for the errors at depth *k*.

$$Cov\left(\varepsilon_{ij;k},\varepsilon_{i'j';k'}\right) = \begin{cases} \sigma_k\sigma_{k'}\rho_{|k-k'|} & \text{if } i = i', j = j' \ and \ \ k \neq k' \\ 0 & \text{if } i \neq i' \ or \ \ j \neq j' \end{cases},$$

Where $\rho_{|k-k'|}$ is the correlation coefficient for the errors at consecutive depths?

Carbon and nitrogen stock in the mineral soil were calculated by layers and depths using carbon concentrations, thickness of each layer, percentage of stoniness (rockiness) and soil bulk density at each depth, on a fixed-depth basis (Ellert *et al.*, 2008).

$$YFD = \sum D_{CS}C_{CS}L_{CS}(1 - CFW)0.1 \qquad \text{(eq.5)}$$

where yFD is the soil organic carbon (SOCFD) stock or nitrogen stock (SONFD) to a fixed depth (Mg C $ha^{-1}$ to the specified depth), DCS is the bulk density of core segment (g $cm^{-3}$), CCS is the organic C concentration of core segment (mg C $g^{-1}$ dry soil), and LCS is the length of core segment (cm) and CFW is the percentage of coarse fragments weight. The statistical analysis approach for comparing C and N stocks at different depths (0–10 cm; 10–30 cm; 30–50 cm and 50–100 cm) was similar to the mixed model approach already described. However, calculating the element stock with Eq. (1) can lead to biased comparisons if bulk density is significantly different between land uses or treatments (Ellert *et al.*, 2008). As an alternative, SOC stock to fixed mass was calculated if differences in bulk density were detected (research question 3), using the following equation:

$$y_{FM} = YFD - MexCsn/1000 \qquad \text{(eq.6)}$$

where yFM is the soil organic carbon (SOCFM) or nitrogen (SONFM) stock for a fixed mass of Mref (the lowest soil mass at a specified depth), Mex is the soil mass subtracted to equalize soil mass among treatments and Csn is the stock concentration in the deepest soil core segments (mg C $g^{-1}$dry soil) (core segment = n) (Ellert *et al.*, 2008). For analysing stock calculated at fixed mass, we selected an SAS PROC GLM general linear model (SAS Inst. Inc., 1999) that compared species (3 levels), elevation (3 levels) and land use (4 levels) as main factors at different soil sampling depths (0–10 cm, 0–30 cm, 0–50 cm and 0–100 cm). The mathematical formulation of the model was:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \tag{eq. 7}$$

with i=1,…,n for the levels of the factor (n=3 for species and elevation, n=4 for land use type) and j=1,…,n for the replicates; is the observed value of the dependent variable for the plot j in the level i of the factor; is the general mean effect; is the main effect of the level i of the factor; is the random error in the dependent variable for the plot j in the level i of the factor. Errors were assumed to be independent and equally distributed with normal distribution;, and is the random variance for the errors.

Finally, the Tukey-Kramer test was used for comparisons of least squares means. Values are reported as mean ± standard error of the mean.

# 3. Results

## 3.1 Is there temporal variation of carbon and nitrogen concentrations and stocks in the forest floor vary along an elevation gradient?

The minimum and maximum forest floor carbon concentrations of 2012 ranged from 319.2 mg C g$^{-1}$ to 666 mg C g$^{-1}$ of soil, whereas the nitrogen concentration ranged from 9.6 to 19.8 mg N g$^{-1}$ of the soil. However, the minimum and maximum forest floor carbon concentrations ranged from 244.13 mg C g$^{-1}$ to 251.15 mg C g$^{-1}$ of soil, whereas the nitrogen concentration was ranged from 13.12 to 14.20 mg N g$^{-1}$ of the soil after five years in 2017. There was significant reduction in carbon and nitrogen concentration in the forest floor in the last five years. Increasing carbon concentrations were found in the upper part of the elevation gradient in the middle elevation gradient (Table 2). However, after five years the concentration of carbon and nitrogen were higher in the middle elevation gradient and lower in the lowest elevation gradient.

The general linear model revealed no association of carbon and nitrogen concentrations with elevation gradient in natural forest (F - test p - value < 0.05) in both time intervals. The same was occurred for carbon and nitrogen stocks, where there was no significant variation with elevation (F - test P - value < 0.05 in both cases). The mean carbon and nitrogen stocks for the forest floor were 9.36 ± 1.17 Mg C ha$^{-1}$ and 0.25 ± 0.03 Mg N ha$^{-1}$, respectively.

| **Altitude class** | **Depth (cm)** | **2012** | | **2017** | |
|---|---|---|---|---|---|
| | | **C (mg g$^{-1}$)** | **N (mg g$^{-1}$)** | **C (mg g$^{-1}$)** | **N (mg g$^{-1}$)** |
| 1 | Forest floor | 424.5 ± 34.8 | 11.16 ± 0.5 | 246.92±54.45 | 19.75±4.36 |
| | 0-10 | 80.5 ± 13.5 | 4.06 ± 0.94 | 125.90±27.31 | 7.07±2.52 |
| | 10-30 | 50.13 ± 15.12 | 2.96 ± 1.22 | 77.47±16.61 | 6.50±1.91 |
| | 30-50 | 24.17 ± 13.95 | 2.17 ± 1.25 | 36.83±9.31 | 3.07±1.63 |
| | 50-100 | 18.16 ± 5.33 | 1.56 ± 0.37 | 23.83±6.84 | 2.03±0.72 |
| | 0-100 | 46.5 ± 8.7 | 2.8 ± 0.5 | 66.01±15.02 | 4.67±1.70 |
| **2** | **Forest floor** | 517.02 ± 31.5 | 14.63 ± 1.05 | 251.15±49.97 | 20.09±3.99 |
| | 0-10 | 98.98 ± 9.95 | 6.5 ± 0.68 | 100.53±16.72 | 7.25±1.54 |
| | 10-30 | 70.23 ± 11.29 | 2.23 ± 0.91 | 52.61±10.17 | 3.84±1.17 |
| | 30-50 | 35.35 ± 13.68 | 2.58 ±0.46 | 37.06±5.70 | 3.34±0.99 |
| | 50-100 | 17.33 ± 3.33 | 1.63 ± 0.29 | 22.68±4.19 | 2.16±0.44 |
| | 0-100 | 55.6 ± 7.6 | 3.9 ± 0.5 | | |

| | | | | | |
|---|---|---|---|---|---|
| **3** | **Forest floor** | 524.15 ± 36.44 | 13.85 ± 0.94 | 244.13±48.53 | 19.53±3.85 |
| | 0-10 | 114.2 ± 13.64 | 8.1 ± 0.94 | 81.83±17.88 | 12.13±1.65 |
| | 10-30 | 62.35 ± 19.34 | 4.42 ± 1.41 | 41.37±10.87 | 5.46±1.25 |
| | 30-50 | 30.7 ± 11.28 | 2.55 ± 0.99 | 20.04±6.10 | 3.47±1.06 |
| | 50-100 | 17.75 ± 7.02 | 1.42 ± 0.61 | 17.29±4.48 | 2.14±0.47 |
| | 0-100 | 56.2 ± 11.4 | 4.1 ± 0.79 | 40.13±9.83 | 5.8±1.11 |

**Table 2.** Carbon (C) and nitrogen (N) concentration (mg $g^{-1}$) in forest floor and mineral soil at different depths (cm) by altitude classes

| **Altitudes class** | **Depth (cm)** | **2012** | | **2017** | |
|---|---|---|---|---|---|
| | | **SOC (Mg $ha^{-1}$)** | **SON (Mg $ha^{-1}$)** | **SOC (Mg $ha^{-1}$)** | **SON (Mg $ha^{-1}$)** |
| **1** | 0-10 | 40.3 ± 6.77 | $2.06^{a}$ ± 0.48 | 28.56±6.17 | 2.55±0.43 |
| | 0-30 | 105 ± 18.73 | 5.73 ± 1.80 | 79.93±19.73 | 7.05±2.05 |
| | 0-50 | 154 ± 33.21 | 5.62 ± 3.24 | 107.10±30.21 | 10.23±5.30 |
| | 0-100 | 198.33 ± 44.16 | 12.4 ± 4.19 | 143.09±22.15 | 14.06±6.24 |
| **2** | 0-10 | 49.52 ± 4.98 | $3.26^{ab}$ ± 0.34 | 50.29±655 | 3.63±0.36 |
| | 0-30 | 136.12 ± 15.63 | 9.3 ± 1.27 | 128.01±12.12 | 9.27±1.26 |
| | 0-50 | 190.97 ± 23.33 | 13.27 ± 1.93 | 182.08±20.12 | 13.65±1.94 |
| | 0-100 | 233.58 ± 29.42 | 16.8 ± 2.47 | 230.55±27.42 | 18.59±2.90 |
| **3** | 0-10 | 57.12 ± 6.81 | $4.07^{b}$ ± 0.46 | 40.91±5.45 | 6.07±1.46 |
| | 0-30 | 137.07 ± 23.71 | 9.78 ± 1.88 | 100.19±20.21 | 13.91±2.06 |
| | 0-50 | 189.25 ± 41.6 | 13.72 ± 3.33 | 149.89±35.6 | 20.70±5.23 |
| | 0-100 | 232.22 ± 57.71 | 17.2 ± 4.78 | 186.69±45.5 | 25.63±5.55 |

Different letters in the upper 10 cm of mineral soil indicate significant differences ($p < 0.05$)

**Table 3.** Soil organic carbon SOC (Mg C ha-$^{1}$) and SON (Mg N ha-$^{1}$) in natural forests by altitude classes and soil depths.

## 3.2 Is there temporal variation of soil bulk density across land use and/or soil depths?

The bulk density was showed an increment in the last five years. For example, the bulk density of mineral soil was ranged from a minimum value of 0.5 g $cm^{-3}$ dry soil to a maximum value of 1.40 g $cm^{-3}$ dry soil in 2012, while it was increased slightly from a minimum value of 0.86 g $cm^{-3}$ to a maximum value of 1.47 g $cm^{-3}$ in 2017. The bulk density was showed an increasing trend along with increasing soil depth. The bulk density of deepest soil was always higher than shallowest soil depth.

The bulk density of cultivated land, degraded land and plantation forest was non - significant among different soil depth within and among land use types. However, there was a significant difference in bulk density between plantation forest in the top soil depth and in the deepest soil. In addition, bulk density was significantly varied among land use types and soil depth and the interaction was significant in 2012, however, the interaction effect was non-significant in 2017 (Table 4). Studentized residuals followed a normal distribution in both cases ($p < 0.3693$) (2012 and 2017).

The bulk density of degraded land was always higher than other land types in all soil depth. On the contrary the bulk density of the natural forest was always lower than other land use types. The values of bulk density was varied among land use types and significantly lower in the natural forest as compared to other land uses in the upper 10 cm in 2012, while it was lower as compared to other land use types in all soil depth in 2017. Highest bulk density was found in plantation forest in 2012 and in degraded land in all soil depth in 2017. There was significant different of bulk density between the upper and the lower layer in natural forest. In the meantime, the bulk density of 10-30 and 30-50 cm of the plantation forest was higher than cultivated land.

Results from the bulk density analysis confirmed that the appropriateness of using the fixed-mass approach to analyze carbon and nitrogen stock changes along an altitudinal gradient in natural forests. There was no strong departure from normality and the general linear model for carbon stock showed no significant variation along the gradient at the same soil depth in 2012 this was true for cultivated land and degraded land in 2017, while there was significant variation among the upper and deepest depth of the natural forest (Table, 5 and **Figure 3**). This indicated that the soil storing capacity was quite homogenous across the studied elevation gradient. For nitrogen stock, however, significant variation appeared in the first 10 cm (Table 6) between the upper part of the gradient (4.07 ± 0.46 Mg C ha$^{-1}$) and the lower part of the gradient (2.06 ± 0.48 Mg C ha$^{-1}$). The soil carbon and nitrogen stock were showed a slight reduction along altitudinal gradient in the last five years. The SOC stock of the altitudinal gradient was showed a slight reduction in the last five years it was ranged from 198.33 to 233.58 Mg C ha$^{-1}$ whereas SON stock of the natural forest was showed a slight increment from 12.4 Mg N ha$^{-1}$ to 17.2 Mg N ha$^{-1}$ in 2012 while the carbon stock of the natural forest (**Table 6**).

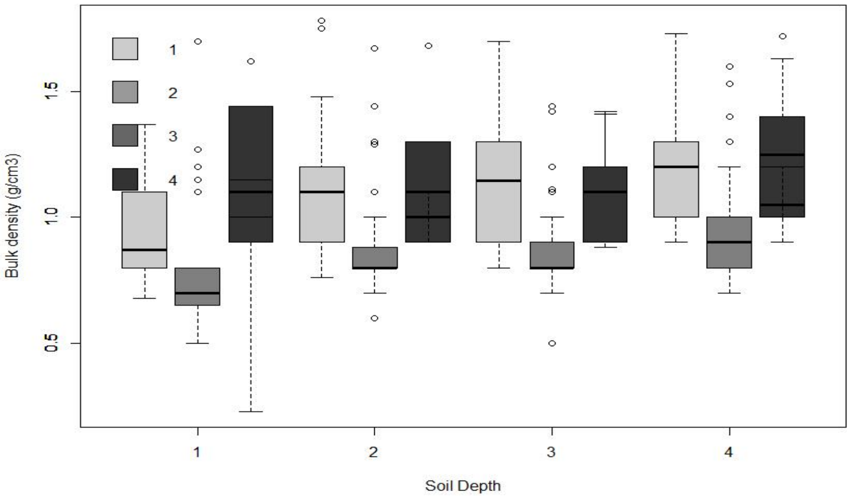

**Figure 3**; Bulk density (g cm-$^{3}$) at different depths by land use type**.**

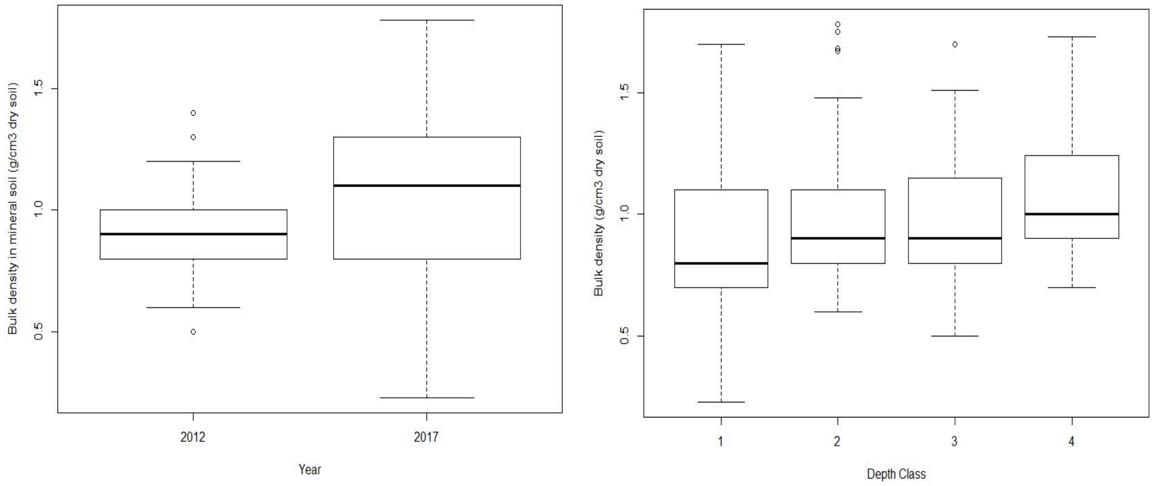

**Figure 4**; Bulk density across altitudinal gradient and slope class.

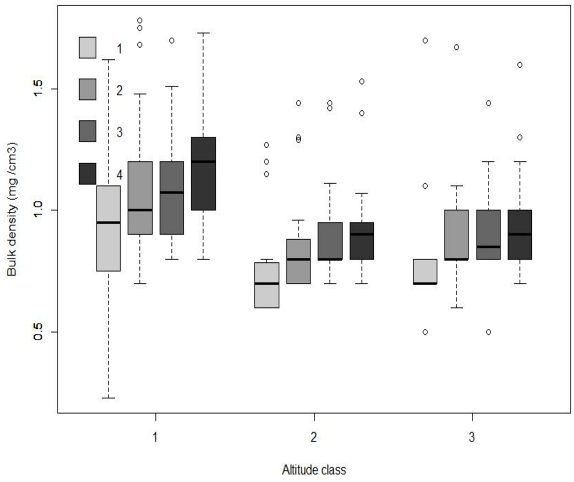

**Figure 4a**; bulk density (g cm-$^{3}$) along altitude class.

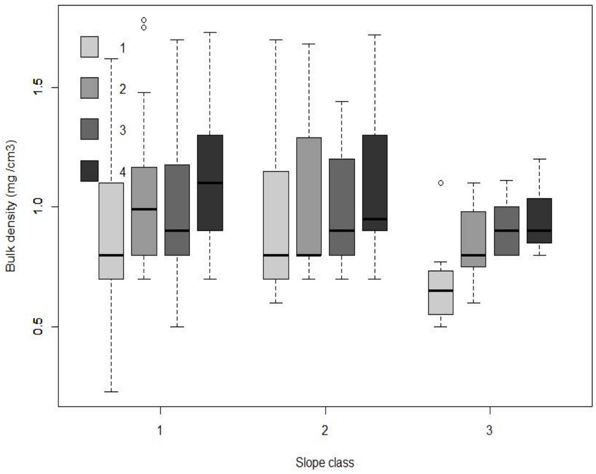

**Figure 4B**; bulk density (g cm-$^{3}$) along slope class.

## 3.3 How does land use change soil carbon and nitrogen concentrations and stocks at different soil depths?

In mineral soil, carbon concentration ranged from 7 to 129.4 mg C g$^{-1}$ of soil, whereas nitrogen concentration ranged from 0.6 to 10 mg N g$^{-1}$ of soil in 2012, however, the carbon concentration of the mineral soil was showed a slight reduction in the last five years and it was ranged from 17.29 to 125.90 g C kg$^{-1}$ dry. While the nitrogen concentration showed a slight increase in concentration and it was ranged from 2.14 to 20.09 g N kg$^{-1}$ dry soil in 2017. In the upper part of the gradient there were higher average C and N concentration values (114.2 mg C g$^{-1}$ and 8.1 mg N g$^{-1}$ in 2012 and 51.22 mg C g$^{-1}$ and 21 mg N g$^{-1}$ in 2017, **Table 3**). The results showed that the carbon and nitrogen concentrations were highly influenced by land use type and soil depth (**Table 4**). Analysis of studentized residuals showed that the normality assumption was not met for carbon concentration $p < 0.0047$; however, it was met for nitrogen concentration $p < 0.0001$. Among the four land use types, carbon and nitrogen concentration in native forest was always higher than other land use types at all soil depths.

Non - parametric comparison of least squares means indicated that significant differences (**Figure 4a**, **Table 4**) in carbon concentration, whereas native forest and plantations showed differences according to depth. Nitrogen concentration analysis showed differences in natural forest and plantations according to soil depth, whereas crop and degraded land were quite homogenous (**Figure 4b** and **Table 4**). Nitrogen concentration showed similar trends in crop and degraded land, whereas natural forest and plantations showed higher values in the upper 30 cm.

The SOC and SON stock of natural forest were significantly varied with other land use types and always higher in all soil depth. On the contrary the SOC and SON stock for degraded land was the lowest in all soil depth. The SOC and

SON between CL, DL and Pln were non-significant in most land use types, although, the value of plantation forest was higher. The mean carbon stock was higher in natural forest than in all other land use categories and at all depths in 2012 and 2017 (225.03 ± 22.7 Mg C ha$^{-1}$ at one-meter depth in 2012 and 221 Mg ha$^{-1}$ in 2017), whereas the SON for the NF was 21.73 (Mg C ha$^{-1}$) (**Table 6**). In plantations, carbon stock at the same depth was one-third less than in natural forest but 35 % higher in crop land and 77 % higher in degraded land. The first 10 cm of mineral soil plantations had significantly more carbon content than crop land and degraded land (**Table 7a,b**), though, the differences vanished at depths below 50 cm.

| | | **2012** | | | | **2017** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Response variable** | **Effect** | **F-test** | **p-value** | **Covariance parameters** | | **F-test** | **p-value** | **Covariance parameters** | |
| Bulk density | Land use | 13.47 | <0.0001 | $\sigma_1^2$ | 0.0138 | 3.17 | 0.0381 | $\sigma_1^2$ | 0.11500 |
| | Depth | 6.86 | 0.0004 | $\sigma_2^2$ | 0.01348 | 6.57 | 0.0004 | $\sigma_2^2$ | 0.09016 |
| | Land use x depth | 2.53 | 0.0062 | $\sigma_3^2$ | 0.01989 | 1.11 | 0.3605 | $\sigma_3^2$ | 0.07896 |
| | | | | $\sigma_4^2$ | 0.01177 | | | $\sigma_4^2$ | 0.0584 |
| | | | | Toeph 1 | 0.7029 | | | Toeph 1 | 0.5934 |
| | | | | Toeph 2 | 0.508 | | | Toeph 2 | 0.6569 |
| | | | | Toeph 3 | 0.4119 | | | Toeph 3 | 0.6357 |
| Carbon concentration | Land use | 11.33 | <0.0001 | $\sigma_1^2$ | 810.52 | 7.62 | 0.0006 | $\sigma_1^2$ | 1536.15 |
| | Depth | 14.75 | <0.0001 | $\sigma_2^2$ | 507.75 | 6.08 | 0.0008 | $\sigma_2^2$ | 533.41 |
| | Land use x depth | 3.57 | 0.0009 | $\sigma_3^2$ | 167.566 | 1.83 | 0.0735 | $\sigma_3^2$ | 226.36 |
| | | | | $\sigma_4^2$ | 43.23 | | | $\sigma_4^2$ | 86.051 |
| | | | | Toeph 1 | 0.643 | | | Toeph 1 | 0.5867 |
| | | | | Toeph 2 | 0.54 | | | Toeph 2 | 0.4065 |
| | | | | Toeph 3 | 0.3481 | | | Toeph 3 | 0.1369 |
| Nitrogen concentration | Land use | 6.23 | 0.0025 | $\sigma_1^2$ | 4.5237 | 5.37 | 0.0043 | $\sigma_1^2$ | 15.2396 |
| | Depth | 10.91 | <0.0001 | $\sigma_2^2$ | 4.5619 | 5.18 | 0.0023 | $\sigma_2^2$ | 7.8446 |
| | Land use x depth | 2.31 | 0.0231 | $\sigma_3^2$ | 1.2349 | 1.13 | 0.3518 | $\sigma_3^2$ | 4.2477 |
| | | | | $\sigma_4^2$ | 0.3353 | | | $\sigma_4^2$ | 0.8764 |
| | | | | Toeph 1 | 0.7866 | | | Toeph 1 | 0.5577 |
| | | | | Toeph 2 | 0.6454 | | | Toeph 2 | 0.2278 |
| | | | | Toeph 3 | 0.4226 | | | Toeph 3 | 0.03420 |

**Table 4.** Mixed effects model for bulk density (g cm-$^3$) carbon and nitrogen concentration (mg g-$^1$).

| Soil parameters | Soil depth | Land use type | | | |
|---|---|---|---|---|---|
| | | Cultivated land | Degraded land | Natural forest | Plantation forest |
| C- con (g C / Kg DM) | 0-10 | $29.47^{Aa}$ ± 22.63 | $22.73^{Aa}$ ± 22.63 | $97.48^{Aa}$±9.24 | $55.20^{Aa}$±11.82 |
| | 10-30 | $12.23^{Aa}$ ± 13.33 | $15.30^{Aa}$ ± 13.33 | $52.38^{Ba}$±5.44 | $25.81^{Ba}$±6.96 |
| | 30-50 | $12.77^{Aa}$± 8.61 | $9.47^{Aa}$ ± 8.61 | $30.41^{Ca}$±3.52 | $19.36^{Ba}$±4.50 |
| | 50-100 | $15.53^{Aa}$ ± 5.36 | $5.33^{Aa}$ ± 5.36 | $20.78^{Ca}$±2.19 | $13.51^{Ba}$±2.80 |
| N-con (g N / Kg DM)) | 0-10 | $2.60^{Aa}$ ± 2.25 | $2.37^{Aa}$±2.25 | $9.12^{Aa}$±0.92 | $5.91^{Aa}$±1.18 |
| | 10-30 | $2.13^{Aa}$± 1.62 | $1.37^{Aa}$ ± 1.62 | $4.91^{Ba}$±0.66 | $3.16^{ABa}$±0.85 |
| | 30-50 | $2.27^{Aa}$ ± 1.19 | $0.77^{Aa}$± 1.19 | $3.34^{BCa}$±0.48 | $1.89^{ABa}$±0.62 |
| | 50-100 | $2.03^{Aa}$ ± 0.54 | $0.27^{Aa}$ ± 0.27 | $2.13^{Ca}$±0.22 | $1.38^{Ba}$±0.28 |
| Bulk density (g / cm3) | 0-10 | $1.00^{Aa}$ ± 0.20 | $1.27^{Aa}$ ± 0.20 | $0.86^{Aa}$±0.08 | $0.97^{Aa}$±0.10 |
| | 10-30 | $1.13^{Aa}$± 0.17 | $1.37^{Aa}$ ± 0.17 | $0.99^{Aa}$±0.07 | $1.22^{ABa}$±0.09 |
| | 30-50 | $1.20^{Aa}$± 0.16 | $1.17^{Aa}$ ± 0.16 | $0.96^{Aa}$±0.07 | $1.26^{Ba}$±0.09 |
| | 50-100 | $1.37^{Aa}$ ± 0.14 | $1.47^{Aa}$ ± 0.16 | $1.03^{Aa}$±0.06 | $1.27^{Ba}$±0.07 |

Different letters indicate significant differences in the response variable within the same sampling depth ($p < 0.05$).

**Table 5.** Carbon and nitrogen stock (Mg C $ha\text{-}^{1}$) and SON (Mg N $ha\text{-}^{1}$) in mineral soil at different sampling depths by land use type (2017 data analysis result)

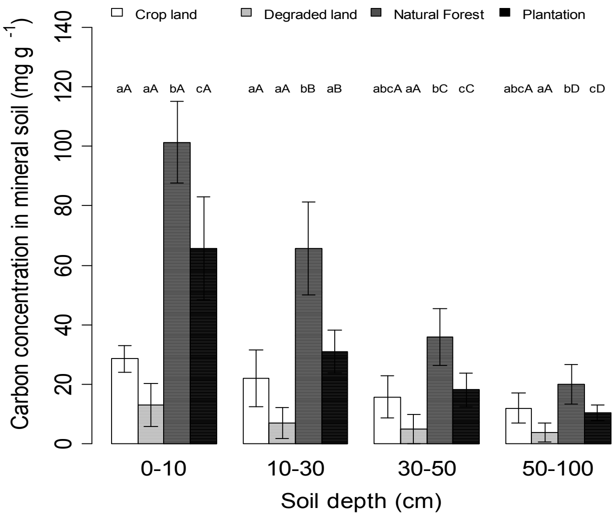

**Figure 5a**; Carbon concentration (mg $g\text{-}^{1}$) at different depths by land use type. Different letters indicate**.**

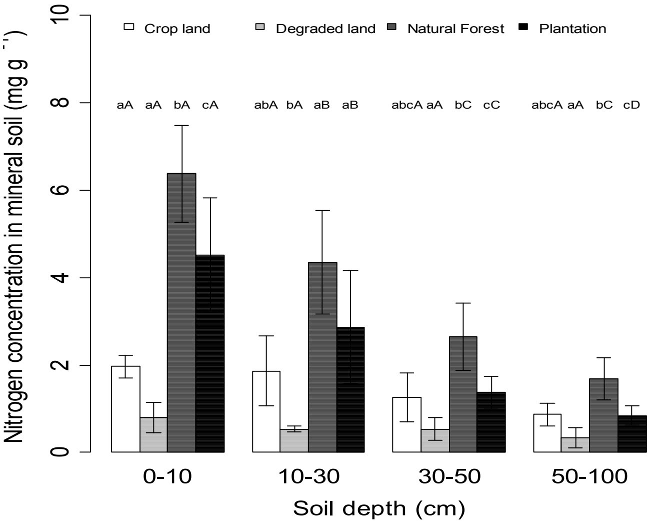

**Figure 5b**; Nitrogen concentration (mg $g\text{-}^{1}$) at different depths by land use type. Different letters indicate**.**

| Land use type | Land use type | Soil depth (cm) | | | |
|---|---|---|---|---|---|
| | | 0-10 | 0-30 | 0-50 | 0-100 |
| **SOC (Mg ha-1)** | CL | $14.76±14.67^{B}$ | $35.73±35.89^{B}$ | $48.73±51.01^{B}$ | $80.03±58.96^{B}$ |
| | DL | $11.36±14.67^{B}$ | $33.83±35.89^{B}$ | $55.70±51.01^{B}$ | $76.07±58.96^{B}$ |
| | NF | $48.75±14.67^{A}$ | $122.43±35.89^{A}$ | $176.38±51.01^{A}$ | $221.46±58.96^{A}$ |
| | Pln | $27.67±14.67^{B}$ | $70.15±35.89^{B}$ | $105.41±51.01^{B}$ | $141.68±58.96^{B}$ |
| **SON (Mg ha-1)** | CL | $1.33±1.37^{B}$ | $3.90±3.38^{B}$ | $6.20±4.68^{A}$ | $11.17±5.44^{AB}$ |
| | DL | $1.20±1.37^{B}$ | $2.90±3.38^{B}$ | $4.96±4.68^{A}$ | $6.93±5.44^{B}$ |
| | NF | $4.56±1.37^{A}$ | $11.41±3.38^{A}$ | $16.85±4.68^{A}$ | $21.73±5.44^{A}$ |
| | Pln | $2.97±1.37^{AB}$ | $7.88±3.38^{AB}$ | $11.07±4.68^{A}$ | $14.90±5.44^{AB}$ |

Different upper-case letters of mineral soil indicate significant differences among land use types with the same soil depth ($p < 0.05$)

**Table 6.** Soil organic carbon SOC (Mg C ha-$^1$) and SON (Mg N ha-$^1$) land use type soil depth 2017 data analysis result

## 3.5 Does species selection have significant temporal variation of carbon and nitrogen concentration and stock at different soil depths in plantations?

The carbon and nitrogen stock and concentration were non-significant among the species in all the measured time. The nitrogen concentration of the top soil was the highest in all species in all soil depth. To a depth of 1 m, total carbon stored in plantations was ranged from 112.43 ± 4.32 to 185.83 ± 29.9 Mg C ha$^{-1}$ for *Pinus patula* and *Eucalyptus saligna*, respectively in 2012 (Table 8 and 9), whereas total nitrogen stock was ranged from 8.50 ± 0.44 to 12.26 ± 1.9 Mg N ha$^{-1}$ for the same species in the same period. *Cupressus lusitanica* plantations presented intermediate values for carbon stock 126.1 ± 32.2 Mg C ha$^{-1}$ and nitrogen stock 9.1 ± 1.8 Mg N ha$^{-1}$. After five years, the SOC stock slightly decreased; on the contrary the SON stock was slight increased in all the species in all soil depth. In line with, the SOC stock was ranged from 148.32 to 15.87 Mg C ha$^{-1}$ while the SON stock of the plantation was ranged from 14.97 to 1.97 Mg N ha$^{-1}$. The highest SOC stock was recorded for *Eucalyptus saligna* in all soil depth except topsoil, while the SON stock was highest in *Cupressus lusitanica* in all soil depth.

The carbon concentration of the mineral soil after five years was ranged from 9.57 g N kg-1 dry soil for *Eucalyptus saligna* to 51.63 g C kg-1 dry soil, whereas the nitrogen concentration was ranged from 0.8 g N kg-1 dry soil of *Eucalyptus saligna* to 7.10 g N kg-1 dry soil for *Cupressus lusitanica* plantation. The carbon and nitrogen concentration of the top soil is the highest in all the species, while the lowest carbon and nitrogen concentration was found in the deepest soil depth. The highest carbon concentration 25.83 g C kg-1 dry soil was found in *Cupressus lusitanica*. The N content of deepest soil Pinus patula was the highest

The species effect was significant on bulk density values (Table 8 and 9). Soil bulk density in *Eucalyptus saligna* plantations was 21 %, significantly higher than in *Pinus patula* plantations (Figure 4) in 2012, while the bulk density of *Pinus patula* was higher in 2017. However, species did not influence carbon and nitrogen stock calculated with the fixed-mass method. The bulk density of *Pinus patula* plantation was higher in 2012, while after five years the bulk density of *Pinus patula* plantation was reduced. *Eucalyptus saligna* has higher bulk density in all the times.

| | | 2012 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Response variable** | **Effect** | **F-test** | **p-value** | **Covariance parameters** | | **F-test** | **p-value** | **Covariance parameters** | |
| C (mg g$^{-1}$) | Species | 0.06 | 0.274 | $\sigma_1^2$ | 508.620 | 0.06 | 0.9403 | $\sigma_1^2$ | 318.32 |
| | Depth | 22.35 | <0.0001 | $\sigma_2^2$ | 139.290 | 10.09 | 0.0004 | $\sigma_2^2$ | 77.58 |
| | Species x Depth | 0.8 | 0.5835 | $\sigma_3^2$ | 18.130 | 0.94 | 0.4913 | $\sigma_3^2$ | 138.93 |
| | | | | $\sigma_4^2$ | 7.700 | | | $\sigma_4^2$ | 35.67 |
| | | | | Toeph 1 | 0.420 | | | Toeph 1 | 0.2447 |
| | | | | Toeph 2 | -0.050 | | | Toeph 2 | 0.2698 |
| | | | | Toeph 3 | 0.025 | | | Toeph 3 | -0.00759 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| N (mg $g^{-1}$) | Species | 1.15 | 0.3784 | $\sigma_1^2$ | 1.748 | 1.31 | 0.3376 | $\sigma_1^2$ | 4.3315 |
| | Depth | 27.22 | <0.0001 | $\sigma_2^2$ | 0.433 | 21.21 | <0.0001 | $\sigma_2^2$ | 2.5809 |
| | Species x Depth | 0.42 | 0.8555 | $\sigma_3^2$ | 0.095 | 1.13 | 0.3849 | $\sigma_3^2$ | 0.4360 |
| | | | | $\sigma_4^2$ | 0.041 | | | $\sigma_4^2$ | 0.4109 |
| | | | | Toeph 1 | 0.382 | | | Toeph 1 | 0.4099 |
| | | | | Toeph 2 | -0.205 | | | Toeph 2 | -0.07038 |
| | | | | Toeph 3 | -0.040 | | | Toeph 3 | 0.2715 |
| Bulk density (g $cm^{-3}$) | Species | 12.2 | 0.0077 | $\sigma_1^2$ | 0.015 | 0.68 | 0.5404 | $\sigma_1^2$ | 0.06215 |
| | Depth | 11.3 | 0.0002 | $\sigma_2^2$ | 0.006 | 5.13 | 0.0097 | $\sigma_2^2$ | 0.1284 |
| | Species x Depth | 4.03 | 0.0099 | $\sigma_3^2$ | 0.024 | 0.70 | 0.6517 | $\sigma_3^2$ | 0.07792 |
| | | | | $\sigma_4^2$ | 0.001 | | | $\sigma_4^2$ | 0.02792 |
| | | | | Toeph 1 | 0.525 | | | Toeph 1 | 0.002651 |
| | | | | Toeph 2 | -0.060 | | | Toeph 2 | 0.1910 |
| | | | | Toeph 3 | -0.301 | | | Toeph 3 | 0.2842 |

**Table 7.** Mixed effects model of carbon, nitrogen concentration (mg g-$^{1}$) and bulk density (g cm-$^{3}$) in plantations.

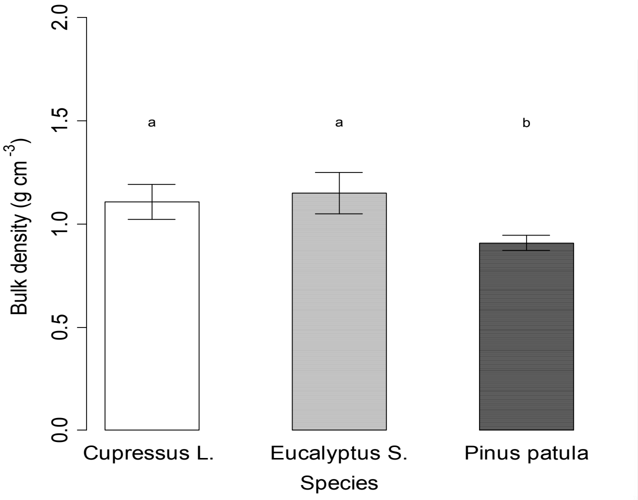

**Figure 6a**; Bulk density (g cm$^{-3}$) in plantations by species. Different letters indicate significant differences ($p < 0.05$) (2012).

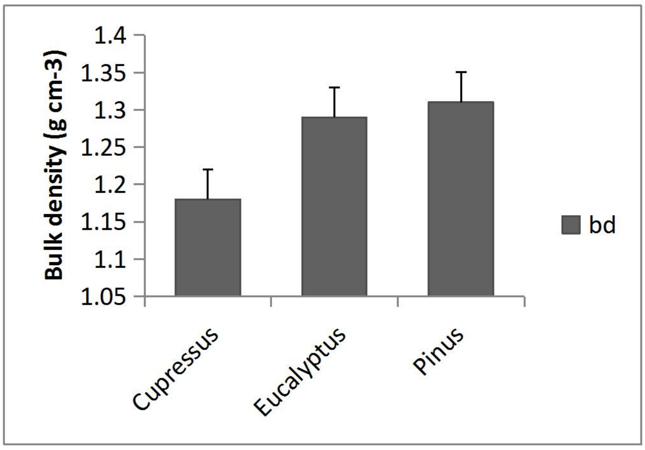

**Figure 6b**; Bulk density (g cm-3) in plantations by species. Different letters indicate significant differences ($p < 0.05$)(2012).

| **Species** | **Depth (cm)** | **2012** | | **2017** | |
|---|---|---|---|---|---|
| | | **SOC (Mg ha$^{-1}$)** | **SON (Mg ha$^{-1}$)** | **SOC (Mg ha$^{-1}$)** | **SON (Mg ha$^{-1}$)** |
| ***Eucaliptus saligna*** | 0-10 | 33.53 ± 5.56 | 2.1 ± 0.21 | 24.50±5.56 | 2.77±0.21 |
| | 0-30 | 90.80 ± 10.34 | 5.83 ± 0.47 | 69.03±10.34 | 7.57±0.47 |
| | 0-50 | 142.96 ± 21.78 | 12.12 ± 1.23 | 106.90±21.78 | 10.67±1.23 |
| | 0-100 | 185.83 ± 29.94 | 12.26 ± 1.89 | 148.37±29.94 | 14.97±1.89 |
| ***Cupressus lusitanica*** | 0-10 | 26.8 ± 10.75 | 1.86 ± 0.68 | 25.83±10.75 | 3.57±0.68 |
| | 0-30 | 66.70 ± 22.50 | 4.63 ± 1.34 | 66.50±22.50 | 9.07±1.34 |
| | 0-50 | 98.46 ± 32.82 | 6.93 ± 1.87 | 92.03±32.82 | 12.57±1.87 |
| | 0-100 | 126.1± 32.20 | 9.10 ± 1.76 | 123.17±32.20 | 16.53±1.76 |
| ***Pinus patula*** | 0-10 | 24.96 ± 1.03 | 1.80 ± 0.1 | 15.87±1.03 | 1.97±0.1 |
| | 0-30 | 62.9 ± 1.80 | 4.67 ± 0.12 | 51.83±1.80 | 4.90±0.12 |
| | 0-50 | 89.00 ± 1.80 | 6.76 ± 0.26 | 92.37±1.80 | 6.97±0.26 |
| | 0-100 | 112.43 ± 4.32 | 8.50 ± 0.44 | 125.20±4.32 | 10.47±0.44 |

**Table 8.** Carbon and Nitrogen stock (Mg ha-$^{1}$) in plantations at different sampling depths (2017)

| **Species** | **Soil depth** | **C-con** (*g C / Kg dry matter* ) | **N-con** (*g C / Kg dry matter* ) | **Bulk density (g cm$^{-3}$)** |
|---|---|---|---|---|
| ***Eucaliptus saligna*** | 0-10 | 48.97$^{A}$±10.30 | 5.50$^{A}$±1.20 | 1.10$^{A}$±0.14 |
| | 10-30 | 26.30$^{A}$±5.09 | 2.67$^{AB}$±0.92 | 1.37$^{A}$±0.21 |
| | 30-50 | 16.87$^{A}$±6.81 | 1.63$^{AB}$±0.38 | 1.43$^{A}$±0.16 |
| | 0-100 | 9.57$^{A}$±3.45 | 0.80$^{B}$±0.37 | 1.27$^{A}$±0.10 |
| ***Cupressus lusitanica*** | 0-10 | 51.63$^{A}$±10.30 | 7.10$^{A}$±1.20 | 1.00$^{A}$± 0.14 |
| | 10-30 | 23.10$^{AB}$±5.09 | 3.13$^{AB}$±0.93 | 1.10$^{A}$±0.21 |
| | 30-50 | 14.40$^{AB}$±6.81 | 1.93$^{B}$±0.38 | 1.33$^{A}$±0.16 |
| | 50-100 | 10.17$^{B}$±3.45 | 1.60$^{B}$±0.37 | 1.27$^{A}$±0.10 |

| | | | | |
|---|---|---|---|---|
| ***Pinus patula*** | 0-10 | 31.67$^{A}$±10.30 | 3.87$^{A}$±1.20 | 1.00$^{A}$±0.14 |
| | 10-30 | 23.17$^{A}$±5.09 | 1.90$^{A}$±0.93 | 1.43$^{A}$±0.21 |
| | 30-50 | 19.97$^{A}$±6.81 | 1.87$^{A}$±0.38 | 1.30$^{A}$±0.16 |
| | 50-100 | 18.63$^{A}$±3.45 | 1.67$^{A}$±0.37 | 1.50$^{A}$±0.10 |

**Table 9.** Carbon and Nitrogen concentration (g C / Kg dry matter) and bulk density (g cm-3) in plantations at different sampling depths

## 4. Discussion

This carbon and nitrogen stock study is the first of its kind for understanding the temporal variation of carbon and nitrogen stock and concentration in Chilimo Dryafromonate forest for two consecutive periods and covered an estimate of carbon and nitrogen stock and concentration and bulk density across altitudinal gradient, land use type and species for the last five years. In our study, carbon stock did not vary significantly along altitudinal gradient as suggested by other studies in African forests (Zewdu *et al*., 2004; Twongyirwe *et al*., 2013). Because elevation gradient is one of the environmental factors that affect the carbon stock of the forests in different carbon pools and thus, it can be used as a useful variable tool to predict the forest carbon and nitrogen stock in different carbon pools (Bayaux, 2007). Results of the present study revealed that higher carbon and nitrogen stock was found in the middle altitudinal gradient than in the top and bottom altitudinal gradient this was might be due to low disturbance level. However, the carbon stock and concentration in the forest floor showed a reduction in the last five years. This is might be due to illegal cuttings of trees in the study area and frequent removal of litter fall and twigs by fuelwood collectors. In line with a lot of illegally cut new stumps have been recorded during field survey. The low carbon and nitrogen concentration in the top and bottom elevation gradient was might be due to the impact of anthropogenic conditions such as many farming communities living in these areas and their livelihoods is mostly depending on Chilimo forest. In addition, there is continuous removal of fallen litter, dead wood and twigs, by fuelwood collectors. Tree cutting for firewood, charcoal making, lumber logging for construction wood, forest clearing for agricultural land and free livestock grazing are also frequently occurred in these areas. In line with this a study done by Adugna *et al.* (2017) found that the carbon stock in above ground biomass, below ground biomass, litter biomass and soil organic carbon exhibited distinct pattern along environmental gradients in one of the dryafromonate forest in Ethiopia.

Bulk density was significantly influenced by type of land use, soil depth and time. Higher bulk densities were observed in degraded land and subsoil, due to higher soil compaction, higher erosion rate, lack of inputs and low soil fertility. This finding is in consistent with other studies on the impact of changes in land use (Gebremariam and Kebede, 2010; Michel *et al*., 2010; Awotoye *et al*., 2013; Sierra *et al*., 2013). The bulk density of the natural forest was low (ranging between 0.86 to 1.03 g cm$^{-3}$) as compared to other land use types, which indicated the natural forest has high organic matter content than other land use types.

In our study the separation among plots (100 m) and the irregular mixture in each plot are considered enough to assume that there is a negligible horizontal spatial autocorrelation. However, the vertical spatial autocorrelation within a soil profile is explicitly modelled. The results displayed that depth is an important factor in C, N concentrations and bulk densities and that there is strong correlation between 0–10 and 10–30 cm layers (**Table 7**) that otherwise could not have been overlooked.

The carbon and nitrogen allocation among soil depth is varied timely. **Figure 7a**, b showed that the distribution of carbon and nitrogen stocks by sampling layers. Remarkably, around 80 % of both elements to 1 m depth are stored in the upper 50 cm of soil in 2012 where as 90 % of nitrogen and carbon concentration was stored in 2017. Sampling effort would be drastically reduced if the nominal 1m sampling pit depth found in local studies can be reduced by half. Soil tillage in crop - land can be reduce if the amount of total carbon stored in the upper 10 cm. Figure 5a indicated that sampling depth should be greater for crop land than for natural forests, whereas, most of the carbon is stored in the upper-most part of the soil in 2012, on the contrary sampling depth should be greater for plantation and natural forest, where most of the carbon is stored in the upper-most parts of the soil after five years in 2017 (Murty *et al.*, 2002).

Land use is a major factor in carbon and nitrogen stocks, among the four land use types studied in Chilimo dryafromontane forest and adjacent land uses. In this study in both cases the carbon and nitrogen stock and concentration in the natural and plantation forest was higher than cultivated and degraded land, this is might be due to higher litter fall, decomposition rate and species composition. The low erosion rate in the natural and plantation forest was might be due to interception of the raindrops by plants. The carbon and nitrogen concentration were slightly reduced in the last five years due to lack of appropriate land management practices to improve the productivity of the land and continuous illegal cutting in the natural forest more serious than previous years. In addition, it was found that, there is no adequate forest management practice applied in the natural forest to improve its productivity and growth. The low carbon and nitrogen stock and concentration in the degraded land and cultivated land was might be due to low nutrient cycling, continuous tillage and crop residue removal for livestock feed in the cultivated land. In addition, in the degraded land there is over sealing and surface crusting effect, which reduced microbial activity and leads to high run off and soil erosion. In general there is slight increase in carbon and nitrogen stock in degraded land in the last five years; this is might be due to some exclosure activities conducted in these areas. Similar results were also reported by several authors, Girmay *et al.* (2008) reported that, the carbon stock in the topsoil (0–10 cm) in Ethiopia was decreased after conversion of native forest into crop lands (− 63 %) and plantations (− 83 %). Solomon *et al.* (2002) indicated that conversion of humid tropical forests for maize (Zea mays) cultivation in Southern Ethiopia resulted in a 55–60 % reduction in SOC stock, 58.3– 63.9 Mg C ha$^{-1}$ in forest soil to 33.9–39.7 Mg C ha$^{-1}$ in cultivated land. Mohammed and Bekele (2014) in Gera moist afromontane forest found that the total carbon stock in the native forest is greater than coffee-based agroforestry practice which showed much greater difference than annual crop field. Ashagrie *et al.* (2005) also reported losses of 13 Mg C ha$^{-1}$ over a period of 21 years in southern Ethiopia when natural forest was converted to Eucalyptus plantation. In Brazil, Zinn *et al.* (2002) reported a 23–48 % loss in SOC after a native wooded savannah was converted into Eucalyptus plantation. Rhoades *et al.* (2000) reported a 70 % reduction in SOC in Ecuador in the upper 30 cm of top soil when original forest was converted to sugarcane plantation (Saccharum spp).

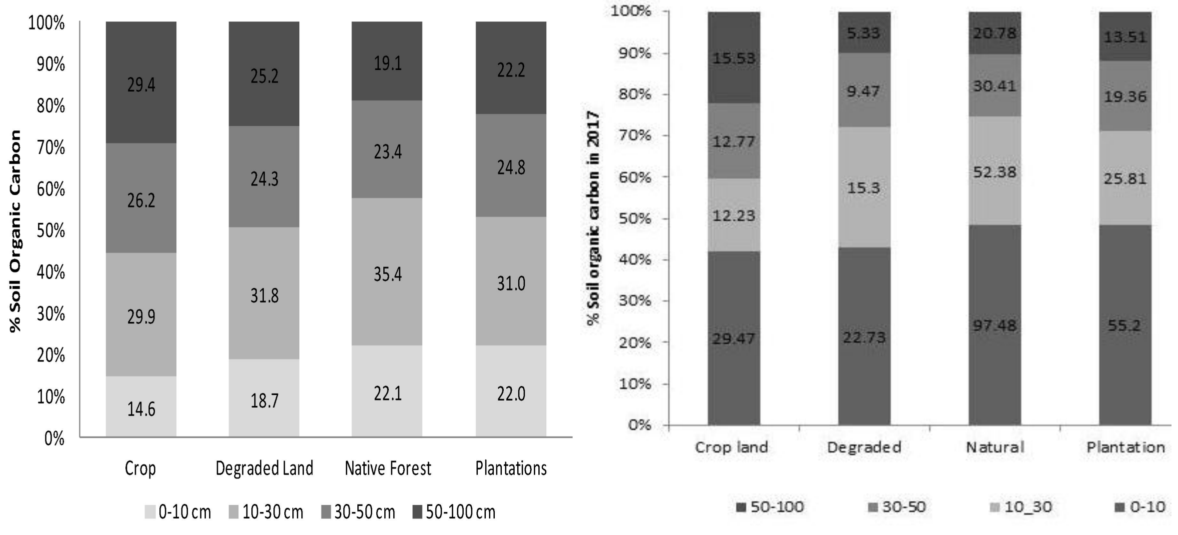

**Figure 7a**; Percentage of soil organic carbon distribution at sampling depths in 2012 and 2017**.**

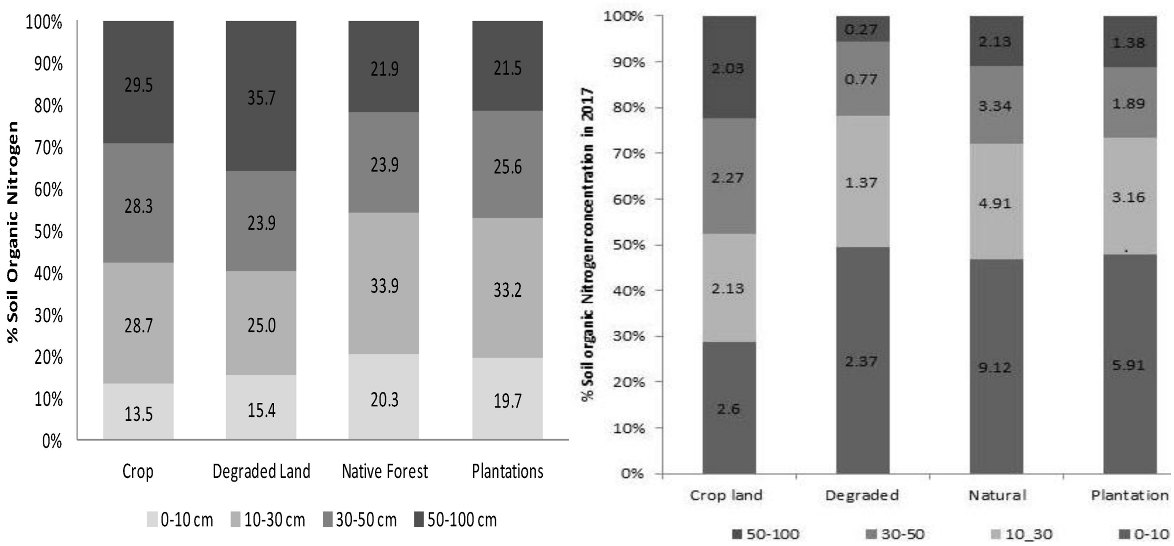

**Figure 7b**; Percentage of soil organic nitrogen distribution at sampling depths**.**

Berhangaray *et al.* (2013) investigated the impact of changes in land use on soil carbon and higher nitrogen stock and concentration was found under trees than under pasture and agricultural lands. In our study, tree plantations stored 34 % less carbon than native forest, but the land use change sequence was different. Plantations were originally planted outside the forest on bare or degraded land. In this situation, tree plantations stored 80 % more carbon than degraded land and 56.4 % more than crop land.

The nitrogen concentration and stock were higher in these plantations might be explained by a recovery of soil conditions 28 years after plantation establishment. The exotic species selected by local communities might have diminished the potential recovery effect of plantations, as native species have been observed to improve soil conditions to a greater extent than exotic species do (Tesfaye *et al.*, 2014). However, more studies on the species selection effect in restoration plantations should be performed to confirm this. The carbon concentration of Chilimo dry afromonate forest 185.83 ± 29.94 (2012) and 148.37 ± 29.94 Mg ha$^{-1}$ (2017) our results are in consist with a study done by Hu Du *et al.* (2015) on carbon storage in a five-year Eucalyptus plantation 162.7 Mg C ha$^{-1}$. The carbon stock was slightly decreasing for Eucalyptus and Cupressus species, while increased for *Pinus patula* plantation. However, the nitrogen stock was increased for all the species. The slight reduction of carbon concentration into species was might due to increasing of disturbance (illegal cutting) and litter fall removal. Whereas, such activities are not widely observed in Pinus patula plantation. The positive impact of plantations on degraded land and the negative impact of substitution of native forest with plantations is inconsistent with findings by other authors. In a similar way, carbon isotope analysis, Lemma *et al.* (2006) in South - western Ethiopia, found higher amounts of total SOC in the soil under *E. grandis* than under *C. lusitanica* and *P. patula* plantation. Solomon *et al.* (2002) in southern Ethiopia found land converted from mixed native species to *C. lusitanica* plantation showed a 27 % loss in SOC stock over a period of 25 years. In contrast, Zerfu (2002) indicated increased SOC stock under Eucalyptus plantation established on degraded land. Similarly, in south-western Ethiopia Lemma *et al.* (2006) reported a net SOC increase of 69.9 Mg ha$^{-1}$ under *C. lusitanica* and 29.3 Mg ha$^{-1}$ under P. patula 20 years after plantation establishment. The soil carbon pool is affected by soil properties, forest management practices, litter fall and root turnover (Jandl *et al.*, 2008; Zeng *et al.*, 2013). Soil C storage observed in the upper 100 cm was lower than the average value for carbon storage in forest soils in China (193.6 Mg ha$^{-1}$) (Zhou *et al.*, 2000). Among the five plantation development stages, soil C storage was highest at 0–10 cm and decreased with increasing soil depth. Soil organic matter content is the main source of soil C and is higher in topsoil (Seely *et al.*, 2010). Our soil C values in the upper 50-cm were much higher than those for the soil C pool stored in *Pinus koraiensis* plantations across all age classes (Li *et al.*, 2011).

Finally, our results showed that C and N concentration and stock under native natural forest and plantation forest in Chilimo dry afromontane forest was generally higher than those reported in other regions in all cases (Beets *et al.*, 2002;

Harms *et al*., 2005; Twongyirwe *et al*., 2013) and suggest two management strategies for improving soil conditions in the Central Highlands. The first is to maintain and preserve the Chilimo natural forest to maintain carbon storage in the future as other African tropical forests do (Lewis *et al*., 2009). The second is to recover abandoned crop-land and degraded-lands by establishing tree plantations to avoid overharvesting in natural forests. Monitoring of carbon and nitrogen concentration should be made in a continuous manner

## 5. Conclusion

The analysis of carbon and nitrogen stock and concentration of the study forest showed significant different of carbon storage among elevation gradient, land use type, soil depth and species with time. The results also found that the carbon and nitrogen stock of the forest floor was significantly reduced in the last five years, although, there was slight reduction in carbon concentration in all land use types with time. Chilimo natural forest stored more carbon and nitrogen stock and concentration than adjacent land use, but crop and degraded land stored less carbon and nitrogen stock and concentration in all the times. Hence, for adhering of higher carbon and nitrogen stock in these land use types it should be converted to plantations. The nitrogen concertation and bulk density was varied with time among the different introduced species for example significantly lower bulk density values were found under *P. patula* plantations in 2012 and under Eucalyptus plantation for 2017. The results found that there is lack of appropriate forest management practices to increase productivity and yield of the Chilimo dry afromontane forest, thus, appropriate forest management practices and options should be devised in these regards. Analysis of variance for carbon and nitrogen stock and concentration in different carbon pools of the forest area responded differently along different elevation gradients. Overall, the present study showed distinct patterns of carbon and nitrogen stock along elevation gradients, land use types, species and soil depth with time bound. We recommend that forest carbon related awareness creation for local people and promotion of the local knowledge can be regarded as a possible option for sustainable forest management. This will enhance the capacity of the existing forest for climate change mitigation and adaptation and other provision from the forest.

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