



Impact of changes in land use, species and elevation on soil organic carbon and total nitrogen in Ethiopian Central Highlands



Mehari A. Tesfaye^{a,c,1}, Felipe Bravo^{a,c}, R. Ruiz-Peinado^{b,c}, Valentín Pando^{a,c}, Andrés Bravo-Oviedo^{b,c,*}

^a Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid (Campus de Palencia), Avda. Madrid, 44, 34071 Palencia, Spain

^b INIA-Forest Research Center, Ctra A. Coruña, km. 7.5, Madrid, Spain

^c Sustainable Forest Management Research Institute, UVA-INIA, Spain

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ABSTRACT

African tropical forests are thought to play an important role in global carbon sequestration. However, the increasing rate of deforestation and the impact of changes in land use require a critical and updated look at what is happening. This work emphasizes the role of bulk density as a main driver in carbon (C) and nitrogen (N) stock in four land-use categories: natural forest, tree plantations, crop land and degraded soil. 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. The methodological approach consisted of evaluating the confounding effect of bulk density and then estimating C and N stocks based on a fixed-mass method rather than the usual fixed-depth method, in order to compare differences across land use categories. We hypothesized that elevation gradient would play a determining role in C and N concentrations and stocks in native forest, whereas tree species would be the main factor in plantations. C and N concentrations and bulk densities in mineral soil were analyzed 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. Single observations from the forest floor were analyzed by a general linear model. Results indicated that soil depth is a 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 84.4%, 26.4% and 33.7% more carbon and 82.4%, 51.8% and 27.1% more nitrogen than bare soil, crop land and plantations, respectively. Conversion of crop and degraded land to plantations ameliorated soil degradation conditions, but species selection did not affect carbon and nitrogen stocks.

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1. Introduction

Forests in general and forest soils in particular play a vital role in carbon balance. The global soil carbon pool has been estimated to contain more than 3.3 times the atmospheric carbon pool and 4.5 times the biotic pool (Lal, 2004). Forest soils also account for 54% of stored carbon in old-growth forests (Luyssaert et al., 2008). Pan et al. (2011) quantified global forest carbon sinks and estimated the total stock to be 861 Pg, of which 383 Pg (45%) is in soil (to a depth of 1 m), 363 Pg (42%) in above and belowground biomass, 73 Pg (8%) in deadwood and 43 Pg (5%) in litter. One-third of the world's soil carbon is stored in the tropics (Lemma et al., 2006).

In forest ecosystems, biomass and soil carbon are stored in dynamic equilibrium with the environment. 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 potentially complex. Topography influences precipitation, temperature, solar radiation and relative humidity (Tsui et al., 2004); aspect determines length of exposure to sunlight and can influence soil weathering and vegetation (Rech et al., 2001; Sidari et al., 2008; Yimer et al., 2006).

Land use and plant species also significantly influence SOC estimations. 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 into agricultural ecosystems negatively affects SOC concentration and stock by 20–50% (Solomon et al., 2002; Lal, 2005; Lemenih and Itanna, 2004). In tropical forests, which serve as powerful carbon sinks, deforestation accounts for 20% of total anthropogenic CO₂ emissions into the atmosphere (Baccini et al., 2008).

Mitigation strategies to reduce the impact of climate change (FAO, 2006) by augmenting carbon sequestration and reducing CO₂ emissions from soils include proper forest management and afforestation or reforestation programs. Quantification and continuous assessment of changes in C and N pool sizes and fluxes are fundamental to understanding

* Corresponding author at: INIA-Forest Research Center, Ctra A. Coruña, km. 7.5, Madrid, Spain.

E-mail address: bravo@inia.es (A. Bravo-Oviedo).

¹ Present address: Ethiopian Environment and Forest Research Institute (EEFRI), P.O. Box 25436, code 1000, Addis Ababa, Ethiopia.

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).

Deforestation is a continuous process in Ethiopia (Nyssen et al., 2004) although reliable data on forest cover change is scarce (Pankhurst, 1995). Tree plantations cover approximately 500,000 ha (WBISPP, 2005), of which 133,041 ha was established as public plantations between 1978 and 1989. The most common species are *Eucalyptus spp.* (58%), *Cupressus lusitanica* (29%), *Juniperus procera* (4%) and *Pinus spp.* (2%) (Moges et al., 2010). The Highlands account for 45% of the country's total area, supporting about 85% of the human population and 75% of the livestock population. Forest cover can be broadly separated into dry or moist montane forest. Dry montane forests are dominated by sclerophyll evergreen, while moist montane forests are characterized by large broadleaf and soft-leaf species (Gatzweiler, 2007). However, much of the Highland forest is disappearing or being converted into agricultural land (Teketay, 2001). Annual deforestation in the Highlands is estimated at 150,000 to 200,000 ha, fertile topsoil loss is estimated at 1.9 billion Mg of soil yr⁻¹, and an average of 42 Mg ha⁻¹ is eroded annually (UNEP, 2002; World Bank, 2001). Ethiopia also has one of the highest rates of soil nutrient depletion (Lemma et al., 2006).

The Chilimo forest is one of the few remnants of native dry afro-montane forest, located in the central highland plateau of Ethiopia. Native coniferous species predominate in this mixed broad-leaf and coniferous forest, where the main species include *J. procera*, *Podocarpus falcatus*, *Prunus africana*, *Olea europaea ssp. cuspidata*, *Scolopia theifolia*, *Rhus glutinosa*, *Olinia rochetiana*, *Allophylus abyssinicus* (Kelbessa and Soromessa, 2004). A center of biodiversity and endemism, the Chilimo forest is also home to over 180 bird species, 21 mammal species and several precinctive sub-species such as the Menelik's bushbuck, vervet monkey, Colobus monkey, Anubis baboon and leopard (Woldemariam, 1998). Soromessa and Kelbessa (2014) reported a total of 213 different plant species categorized into 83 families, including 17 plant species that are unique to the Chilimo forest. Due to continuous deforestation, the Chilimo forest cover has declined from 22,000 ha in 1982 to 6000 ha in 1991 (Shumi, 2009). 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). In order to minimize deforestation, the forest has been categorized as one of Ethiopia's 58 national priority forest protection areas and receives more attention due to its potential as a carbon sink. Alternative strategies to reduce the pressure on the native forest by alleviating the fuel wood shortage include fast-growing tree and shrub plantations around homesteads, establishment of clear farm boundaries and wood lots in nearby rural communities (Alebachew, 2012). At the same time, carbon assessment of 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 soil organic carbon (SOC) and soil organic nitrogen (SON) stock in the forest floor and in mineral soil would vary along an elevation gradient in native forest. Likewise, land use and tree species would also determine SOC and SON stocks at different depths. The specific research questions to be addressed in this study are (1–5):

1. Do carbon and nitrogen concentration and stock in the forest floor vary along an elevation gradient?
2. Does soil bulk density significantly vary across land use categories and/or soil depths?
3. Do carbon and nitrogen concentrations and stocks in mineral soil change at different soil depths along an elevation gradient in native dry afro-montane forests?
4. How does intensive land use change soil carbon and nitrogen concentrations and stocks at different soil depths?

5. Does species selection have any effect on carbon and nitrogen concentrations and stocks at different soil depths in plantations?

2. Materials and methods

2.1. Study site location and description

The experimental site is located in the Chilimo–Gaji dry afro-montane forest of the Western Shewa zone of the Dendi district in the central Highlands of Ethiopia. The forest is surrounded by crop land (mainly teff, *Eragrostis tef*), degraded areas and three 28 year old plantations 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 (Fig. 1, Table 1). The mean annual temperature of the area ranges between 15 and 20 °C and the mean annual precipitation is 1264 mm. A total of 33 different native species (22 tree and 11 shrub species) were recorded in the forest. The quadratic mean diameter, i.e. the square root of the ratio of square of diameter at breast height to number of stems, of the sampled plantation and natural forest ranged from 12.79 to 26.12 cm and the basal area for the sample plots studied ranged from 13.81 to 25.5 m² ha⁻¹ (Table 1).

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. Twenty-one forest floor samples were collected within a 0.25 × 0.25 m (0.0625 m²) metallic frame in the center of the main plot.

2.3. Mineral soil sampling

Mineral soil samples were taken below the forest floor up to a non-invasive depth of 1 m. 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 cultivated land and 3 in degraded lands) were dug for soil collection. 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 112 mineral soil samplings and other 112 cores were collected for analyzing organic C %, total N % and bulk density respectively.

2.4. Laboratory analysis

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)

$$\%SOM = \frac{w_{105} - w_{400}}{w_{105}} \times 100 \quad (1)$$

$$\%C = \%SOM \times 0.58 \quad (2)$$

where, C: the organic carbon concentration, SOM: soil organic matter; w105: weight of dry soil sample at 105 °C, w400: weight of ground

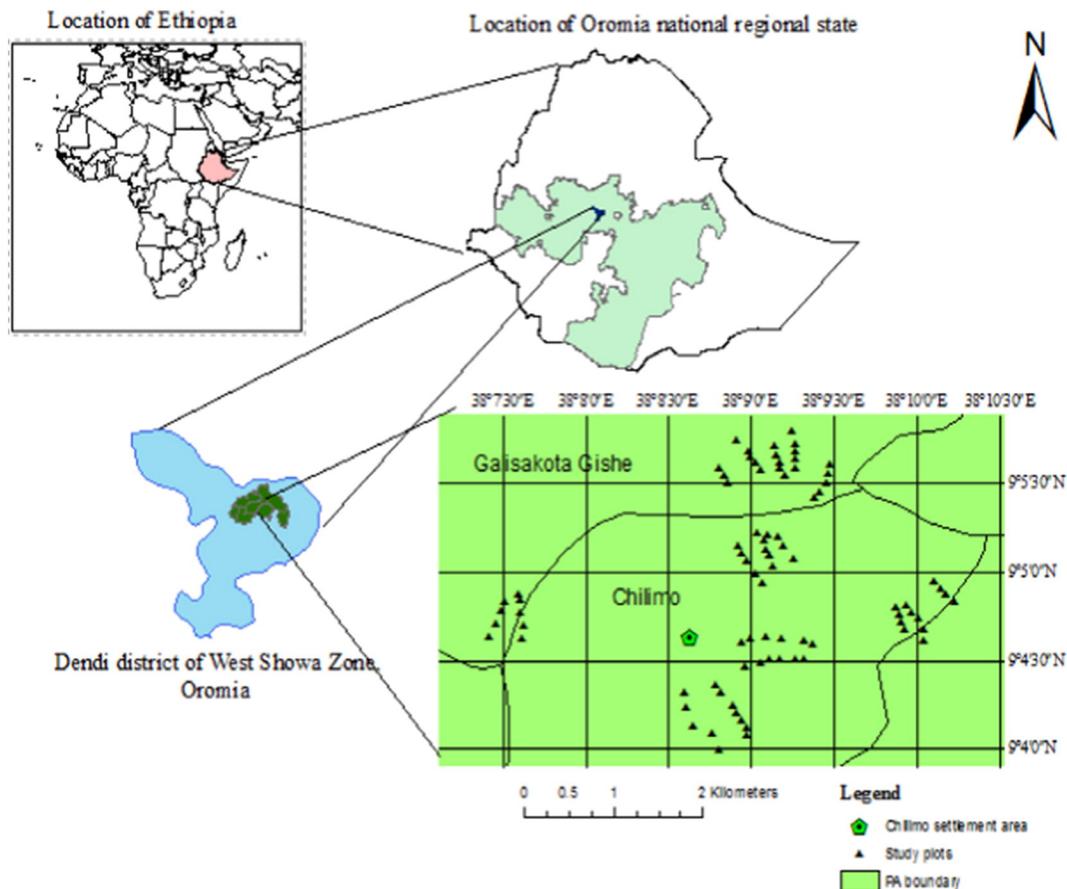


Fig. 1. Location map of Chilimo dry afro montane forest.

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 convenient conversion factor from organic matter to carbon content in forest floor (de Vos et al., 2005). Although Pribyl (2010) recommended 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 obtain the fine fraction for chemical analysis. The coarse rock fragments (>2mm) sieved sizes were removed from the sample

and their percentage (% of stoniness and or rockiness) were calculated by oven dried samples at 67 °C for 24 h for each soil depth

$$CFw (\%) = \frac{(\text{Weight not passing a 2 mm sieve}) \times 100}{\text{Weight of total soil}} \quad (3)$$

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

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

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)
Native forest	Chilimo	N09°04'013"–N09°04'857"	E038°08'557"–E038°09'960"	2470–2770	8–70%	20	40	662 ± 222	26.12 ± 5.3	18.9 ± 1.92
Native forest	Gallessa	N09°05'162"–N09°05'765"	E038°09'847"–E038°10'283"	2700–2921	25–70%	11	20	600 ± 148	19.88 ± 2.5	18.18 ± 1.91
Native forest	Gaji	N09°04'269"–N09°04'340"	E038°09'861"–E038°10'025"	2680–2793	45–50%	4	12	475 ± 196	23.45 ± 4.4	13.81 ± 1.40
Plantation	<i>Cupressus</i>	N09°04'115"–N09°04'297"	E038°07'808"–E038°07'849"	2370–2420	3–12%	3	12	575 ± 8	23.42 ± 4.4	25.5 ± 2.60
Plantation	<i>Eucalyptus</i>	N09°04'155"–N09°04'298"	E038°03'0011"–E038°08'0011"	2360–2400	3–10%	3	12	1000 ± 13	12.79 ± 2.2	14.67 ± 1.50
Plantation	<i>Pinus</i>	N09°03'514"–N09°03'676"	E038°08'260"–E038°08'329"	2396–2405	6–20%	3	12	1167 ± 15	14.52 ± 3.2	21.25 ± 2.16
Crop	Chilimo	N09°04'48"–N09°03'532"	E038°08'559"–E038°08'612"	2406–2423	5–15%	3	12			
Degraded land	Chilimo	N09°03'805"–N09°04'266"	E038°07'703"–E038°07'793"	2350–2425	8–30%	3	12			

Dg: quadratic mean diameter, G: basal area.

Then total organic carbon (%) was analyzed according to Walkley–Black’s method following the procedure described in Anderson and Ingram (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’s method, following the procedure in Keeny and Nelsson (1982).

2.5. Data analysis approach

Elevation was converted to 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.

2.5.1. Carbon and nitrogen concentrations in the forest floor

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

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

Samplings were taken in a single point in time. 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 0, i.e. all land uses were the same (native forest) in the past.

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:

$$Y_{ij,k} = \mu + \alpha_i + \beta_k + \gamma_{ik} + \varepsilon_{ij,k} \tag{4}$$

where $i = 1, \dots, n_f$ for the levels of the between-subjects factor ($n_f = 3$ for species and elevation, $n_f = 4$ for land use type), $j = 1, \dots, n_{pi}$ for the plots within the levels of the factor 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 ; μ is the general mean effect, α_i is the main effect of the i^{th} level for the between-subject factor; β_k is the main effect of the k^{th} depth; γ_{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: $\varepsilon_{ij,k} \rightarrow N(0, \sigma_k^2)$, with σ_k^2 = random variance for the errors at depth k .

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

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

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

$$y_{FD} = \sum D_{CS} C_{CS} L_{CS} (1 - CFw) 0.1 \tag{5}$$

where y_{FD} is the soil organic carbon (SOC_{FD}) stock or nitrogen stock (SON_{FD}) to a fixed depth (Mg C ha⁻¹ to the specified depth), D_{CS} is the bulk density of core segment (g cm⁻³), C_{CS} is the organic C concentration of core segment (mg C g⁻¹ dry soil), and L_{CS} is the length of core segment (cm) and CFw is the percentage of coarse fragments. 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:

$$y_{FM} = y_{FD} - M_{ex} C_{sn} / 1000 \tag{6}$$

where y_{FM} is the soil organic carbon (SOC_{FM}) or nitrogen (SON_{FM}) stock for a fixed mass of M_{ref} (the lowest soil mass at a specified depth), M_{ex} is the soil mass subtracted to equalize soil mass among treatments and C_{sn} is the stock concentration in the deepest soil core segments (mg C g⁻¹ dry soil) (core segment = n) (Ellert et al., 2008). For analyzing 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:

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

with $i = 1, \dots, n$ for the levels of the factor ($n = 3$ for species and elevation, $n = 4$ for land use type) and $j = 1, \dots, n$ for the replicates; Y_{ij} is the observed value of the dependent variable for the plot j in the level i of the factor; μ is the general mean effect; α_i is the main effect of the level i of the factor; ε_{ij} 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; $\varepsilon_{ij} \rightarrow N(0, \sigma^2)$, and σ^2 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 \pm standard error of the mean.

3. Results

3.1. Do carbon and nitrogen concentrations and stocks in the forest floor vary along an elevation gradient?

The minimum and maximum forest floor carbon concentrations 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. Increasing concentrations were found in the upper part of the elevation gradient and increasing mean nitrogen concentrations in the middle part (Table 2). The general linear model revealed no association of carbon and nitrogen concentrations with elevation in natural forest (F-test p-value > 0.05 in both cases). The same occurred for carbon and nitrogen stocks, 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 \pm 1.17 Mg C ha⁻¹ and 0.25 \pm 0.03 Mg N ha⁻¹, respectively.

3.2. Does soil bulk density significantly vary across land uses and soil depths?

The bulk density of mineral soil 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. Bulk density significantly varied among land use types and soil depth and the interaction of both (Table 3). Studentized residuals followed a

Table 2

Carbon and nitrogen concentrations \pm standard error (mg g^{-1}) in the forest floor and mineral soil at different depths (cm) by elevation classes in native forest.

Altitude class	Depth (cm)	C (mg g^{-1})	N (mg g^{-1})
1	Forest floor	424.5 \pm 34.8	11.16 \pm 0.5
	0–10	80.5 \pm 13.5	4.06 \pm 0.94
	10–30	50.13 \pm 15.12	2.96 \pm 1.22
	30–50	24.17 \pm 13.95	2.17 \pm 1.25
	50–100	18.16 \pm 5.33	1.56 \pm 0.37
	0–100	46.5 \pm 8.7	2.8 \pm 0.5
2	Forest floor	517.02 \pm 31.5	14.63 \pm 1.05
	0–10	98.98 \pm 9.95	6.5 \pm 0.68
	10–30	70.23 \pm 11.29	2.23 \pm 0.91
	30–50	35.35 \pm 13.68	2.58 \pm 0.46
	50–100	17.33 \pm 3.33	1.63 \pm 0.29
	0–100	55.6 \pm 7.6	3.9 \pm 0.5
3	Forest floor	524.15 \pm 36.44	13.85 \pm 0.94
	0–10	114.2 \pm 13.64	8.1 \pm 0.94
	10–30	62.35 \pm 19.34	4.42 \pm 1.41
	30–50	30.7 \pm 11.28	2.55 \pm 0.99
	50–100	17.75 \pm 7.02	1.42 \pm 0.61
	0–100	56.2 \pm 11.4	4.1 \pm 0.79

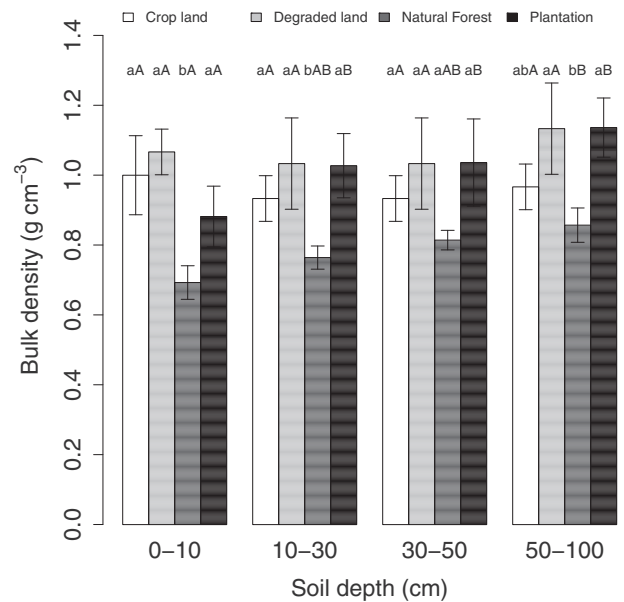


Fig. 2. Bulk density (g cm^{-3}) at different depths by land use type. Different letters indicate significant differences among land use types within the same soil depths whereas different capital letters indicate significant differences among soil depths within the same land use type.

normal distribution ($p < 0.3693$). Bulk density was significantly lower in natural forest compared to other land use categories in the upper 10 cm. Values were higher in crop land and degraded land (Fig. 2). Bulk density was only significantly different between the upper and the lower layer in natural forest soils with lower values in the upper layer than in the deepest (Fig. 2, capital letters); whereas bulk density in the first 10 cm of plantation soils was significantly lower than in the other profiles. For crop land and degraded soils, bulk density was rather constant across soil depths; there were no significant differences among these two land use categories or across depths in the same category (Fig. 2).

3.3. Do carbon and nitrogen concentrations and stocks in mineral soil change at different depths along an elevation gradient in native dry afro-montane forests?

In mineral soil, carbon concentration ranged from 7 mg C g^{-1} to $129.4 \text{ mg C g}^{-1}$ of soil, whereas nitrogen concentration ranged from 0.6 to 10 mg N g^{-1} of soil. In the upper part of the gradient there were higher average C and N concentration values ($114.2 \text{ mg C g}^{-1}$ and 8.1 mg N g^{-1} , Table 3), though the mixed model suggested that these differences were not significant (Table 4).

Table 3

Mixed effects model for bulk density (g cm^{-3}) and carbon and nitrogen concentrations (mg g^{-1}).

Response variable	Effect	F-test	p-value	Covariance parameters	
Bulk density	Land use	13.47	<0.0001	σ_1^2	0.0138
	Depth	6.86	0.0004	σ_2^2	0.01348
	Land use \times Depth	2.53	0.0062	σ_3^2	0.01989
				σ_4^2	0.01177
				Toeoph 1	0.7029
				Toeoph 2	0.508
				Toeoph 3	0.4119
Carbon concentration	Land use	11.33	<0.0001	σ_1^2	810.52
	Depth	14.75	<0.0001	σ_2^2	507.75
	Land use \times Depth	3.57	0.0009	σ_3^2	167.566
				σ_4^2	43.23
				Toeoph 1	0.643
				Toeoph 2	0.54
				Toeoph 3	0.3481
Nitrogen concentration	Land use	6.23	0.0025	σ_1^2	4.5237
	Depth	10.91	<0.0001	σ_2^2	4.5619
	Land use \times Depth	2.31	0.0231	σ_3^2	1.2349
				σ_4^2	0.3353
				Toeoph 1	0.7866
				Toeoph 2	0.6454
				Toeoph 3	0.4226

Results from the bulk density analysis (research question 1) confirm 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 (Table 5). This indicated that the soil storing capacity was quite homogenous across the elevation gradient studied. For nitrogen stock, however, significant variation appeared in the first 10 cm (Table 5) between the upper part of the gradient ($4.07 \pm 0.46 \text{ Mg C ha}^{-1}$) and the lower part ($2.06 \pm 0.48 \text{ Mg C ha}^{-1}$).

3.4. How does land use change soil carbon and nitrogen concentrations and stocks at different soil depths?

The results showed that the carbon and nitrogen concentrations were highly influenced by land use and soil depth (Table 2). Analysis

Table 4

Mixed effects model of carbon and nitrogen concentrations (mg g^{-1}) in native forest, along the altitudinal gradient and by sampling depths.

Response variable	Effect	F-test	p-value	Covariance parameters		
Carbon concentration	Altitude	0.29	0.7559	σ_1^2	825.91	
	Depth	35.94	0.0001	σ_2^2	1007.22	
	Altitude \times Depth		1.12	0.3755	σ_3^2	336.94
					σ_4^2	86.94
					Toeoph 1	0.658
					Toeoph 2	0.5983
			Toeoph 3	0.3704		
Nitrogen concentration	Altitude	0.74	0.502	σ_1^2	3.281	
	Depth	45.13	0.0001	σ_2^2	5.799	
	Altitude \times Depth		3.97	0.0048	σ_3^2	2.707
					σ_4^2	0.6917
					Toeoph 1	0.8052
					Toeoph 2	0.7715
			Toeoph 3	0.5455		

of studentized residuals showed that the normality assumption was not met for carbon concentration ($p < 0.0047$) or nitrogen concentration ($p < 0.0001$). Among the four land use types, carbon and nitrogen concentrations in native forest were always higher than other land use types at all soil depths. Non-parametric comparison of least squares means indicated significant differences (Fig. 3a) 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 land and degraded land were quite homogenous (Fig. 3b). Nitrogen concentration was similar in crop land and degraded land, whereas natural forest and plantations showed higher values in the upper 30 cm.

Mean carbon stock was higher in natural forest than in all other land use categories and at all depths ($225.03 \pm 22.7 \text{ Mg C ha}^{-1}$ at one meter depth) (Table 6). In plantations, carbon stock at the same depth was one-third less than in natural forest but 35% more than in crop land and 77% more than in degraded land. The first 10 cm of mineral soil plantations had significantly more carbon content than crop land and degraded land (Table 6), though the differences vanished at depths below 50 cm.

Native forest stored more nitrogen per hectare but the differences were only significant compared to crop land and degraded land in the upper 10 cm. The total nitrogen confidence interval in native forest to 1 meter depth was $15.90 \pm 1.98 \text{ Mg N ha}^{-1}$, which was 82%, 52% and 27% more than in degraded land, crop land and plantations, respectively.

3.5. Does species selection have any effect on carbon and nitrogen concentration and stock at different soil depths in plantations?

Sampling depth had a strong effect on carbon and nitrogen concentrations. The species effect was significant on bulk density values

Table 5

Soil organic carbon and nitrogen stocks \pm standard error (Mg ha^{-1}) in native forests by altitude classes and soil depths.

Altitude class	Depth (cm)	SOC (Mg ha^{-1})	SON (Mg ha^{-1})
1	0–10	40.3 ± 6.77	$2.06^a \pm 0.48$
	0–30	105 ± 18.73	5.73 ± 1.80
	0–50	154 ± 33.21	5.62 ± 3.24
	0–100	198.33 ± 44.16	12.4 ± 4.19
2	0–10	49.52 ± 4.98	$3.26^{ab} \pm 0.34$
	0–30	136.12 ± 15.63	9.3 ± 1.27
	0–50	190.97 ± 23.33	13.27 ± 1.93
	0–100	233.58 ± 29.42	16.8 ± 2.47
3	0–10	57.12 ± 6.81	$4.07^b \pm 0.46$
	0–30	137.07 ± 23.71	9.78 ± 1.88
	0–50	189.25 ± 41.6	13.72 ± 3.33
	0–100	232.22 ± 57.71	17.2 ± 4.78

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

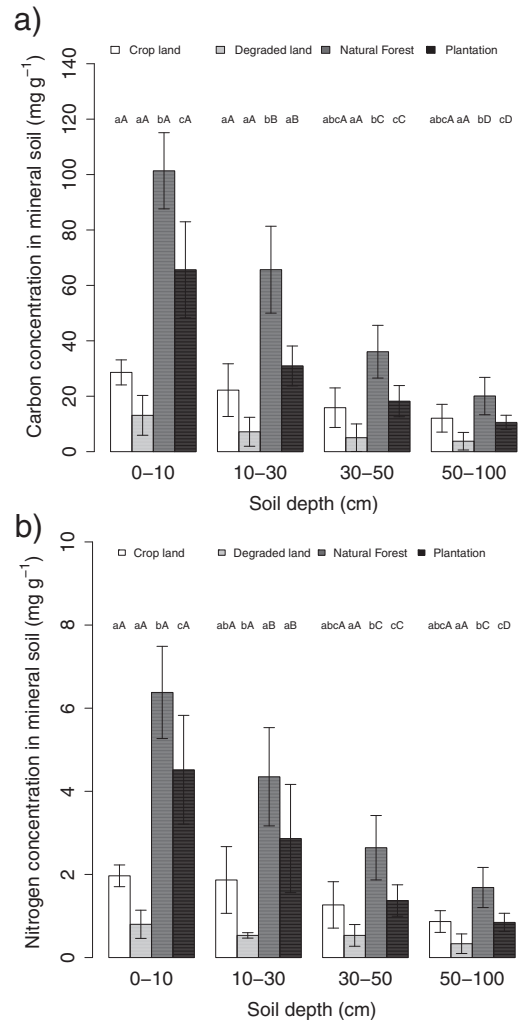


Fig. 3. a. Carbon concentration (mg g^{-1}) at different depths by land use type. Different letters indicate significant differences among land use types within the same soil depths whereas different capital letters indicate significant differences among soil depths within the same land use type ($p < 0.05$) using a Wilcoxon test ($p < 0.05$). b. Nitrogen concentration (mg g^{-1}) at different depths by land use type. Different letters indicate significant differences among land use types within the same soil depths whereas different capital letters indicate significant differences among soil depths within the same land use type ($p < 0.05$) using a Wilcoxon test ($p < 0.05$).

(Table 7). Soil bulk density in *Eucalyptus* plantations was 21%, significantly higher than in *P. patula* plantations (Fig. 4). However, species did not influence carbon and nitrogen stocks calculated with the fixed-mass method. To a depth of 1 m, total carbon stored in plantations ranged from 112.43 ± 4.32 to $185.83 \pm 29.9 \text{ Mg C ha}^{-1}$ for *P. patula* and *E. saligna*, respectively (Table 8), whereas total nitrogen stock ranged from 8.50 ± 0.44 to $12.26 \pm 1.9 \text{ Mg N ha}^{-1}$ for the same species. *C. lusitanica* plantations presented intermediate values for carbon storage ($126.1 \pm 32.2 \text{ Mg C ha}^{-1} \pm$ standard error) and nitrogen stock ($9.1 \pm 1.8 \text{ Mg N ha}^{-1}$).

4. Discussion

The effect of changes in land use on carbon and nitrogen stocks can be exacerbated if differences in bulk density are not taken into account (Wendt and Hauser, 2013). In this study, we analyzed bulk density to see if it was significantly different among treatments (land use type, elevation classes in native forests and tree species in plantations) and applied the fixed-mass method when necessary (Ellert et al., 2008). By modifying the type of statistical analysis used, we obtained more

Table 6Carbon and nitrogen stocks \pm standard error (Mg ha^{-1}) in mineral soil calculated with the fixed-mass method at different sampling depths by land use type.

Response variables	Depth (cm)	Crop	Degraded land	Natural forest	Plantation
Carbon stock (Mg ha^{-1})	0–10	14.3 ^{ac} \pm 1.15	6.56 ^a \pm 1.84	49.73 ^b \pm 3.63	32.83 ^c \pm 4.42
	0–30	43.60 ^{ac} \pm 4.97	17.73 ^a \pm 4.19	129.27 ^b \pm 10.87	79.15 ^c \pm 8.29
	0–50	69.26 ^a \pm 10.09	26.26 ^a \pm 6.26	182.02 ^b \pm 17.28	116.08 ^a \pm 12.92
	0–100	98.10 ^a \pm 16.09	35.10 ^a \pm 9.89	225.03 ^b \pm 22.7	149.21 ^a \pm 16.10
Nitrogen stock (Mg ha^{-1})	0–10	1.03 ^{ac} \pm 0.07	0.43 ^a \pm 0.09	3.23 ^b \pm 0.30	2.28 ^{bc} \pm 0.33
	0–30	3.23 ^{ab} \pm 0.38	1.13 ^b \pm 0.20	8.62 ^a \pm 0.96	6.13 ^a \pm 1.05
	0–50	5.40 ^{ab} \pm 0.79	1.80 ^b \pm 0.26	12.42 ^a \pm 1.51	9.10 ^a \pm 1.19
	0–100	7.66 ^{ab} \pm 1.25	2.80 ^b \pm 0.40	15.90 ^a \pm 1.98	11.59 ^a \pm 1.70

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

accurate results. Assad et al. (2013) applied the fixed-mass method and found differences in carbon stocks among different land uses, to a maximum depth of 60 cm. We extended the sampling depth to 1 m and include nitrogen stock in the analysis.

Bulk density was significantly influenced by type of land use and soil depth. 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 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 strong confounding effect of soil bulk density may lead to overestimation of soil carbon storage capacity (Murty et al., 2002) and misleading conclusions in assessments of the impact of changes in land use. For example, SOC variation after forest conversion was non-significant using the fixed-depth method (Twongyirwe et al., 2013), due to large site-to-site variation. We argue that accurate C and N stock estimation can only be performed when the bulk density effect is discounted, and that the fixed-mass method is more appropriate. However, much debate continues regarding which is the best estimation method of bulk density (Lee et al., 2009; Wendt and Hauser, 2013).

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 modeled. The results displayed that depth is an important factor in C, N concentrations and bulk densities and that there is strong correlation between the 0–10 and 10–30 cm layers (Table 7) that otherwise could have been overlooked.

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

Response variable	Effect	F-test	p-value	Covariance parameters	
C (mg g^{-1})	Species	1.64	0.274	σ_1^2	508.620
	Depth	22.35	<0.0001	σ_2^2	139.290
	Species \times Depth	0.8	0.5835	σ_3^2	18.130
				σ_4^2	7.700
				Toeoph 1	0.420
				Toeoph 2	-0.050
N (mg g^{-1})	Species	1.15	0.3784	σ_1^2	1.748
	Depth	27.22	<0.0001	σ_2^2	0.433
	Species \times Depth	0.42	0.8555	σ_3^2	0.095
				σ_4^2	0.041
				Toeoph 1	0.382
				Toeoph 2	-0.205
Bulk density (g cm^{-3})	Species	12.2	0.0077	σ_1^2	0.015
	Depth	11.3	0.0002	σ_2^2	0.006
	Species \times Depth	4.03	0.0099	σ_3^2	0.024
				σ_4^2	0.001
				Toeoph 1	0.525
				Toeoph 2	-0.060
			Toeoph 3	-0.301	

The fixed-mass method of calculating soil carbon and nitrogen stocks provides the added advantage of facilitating comparison of the percentage of carbon/nitrogen stored at different depths. Fig. 5a and b shows 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. The implication of this finding is clear for large-scale evaluation of carbon stocks in dry afro-montane forests. Sampling effort would be drastically reduced if the nominal 1 m sampling pit depth found in local studies can be reduced by half. Soil tillage in crop land can reduce the amount of total carbon stored in the upper 10 cm. Fig. 5a indicates that sampling depth should be greater for crop land than for natural forests, where most of the carbon is stored in the upper-most part of the soil (Murty et al., 2002).

In our study, carbon stock did not vary significantly with elevation as suggested by other studies in African forests (Zewdu et al., 2004; Twongyirwe et al., 2013). However, Saby et al. (2008) found that elevation was a controlling factor on SOC in a French region of pasture and arable land. The pattern of SOC with elevation indicated lower carbon and nitrogen stock at lower elevation, which might be due to the higher impact of anthropogenic factors. Greater numbers of farming communities live in or around the forest at this end of the elevation gradient; and their livelihoods depend on the forest. This implies continuous removal of fallen litter, dead wood and twigs, collection of firewood, charcoal making, logging for construction wood, forest clearing for agricultural land and livestock overgrazing. Human pressure might be a confounding factor when analyzing the effect of elevation on SOC in forests.

Land use is a major factor in carbon and nitrogen stocks. Girmay et al. (2008) reviewed the carbon stock in topsoil (0–10 cm) in Ethiopia and

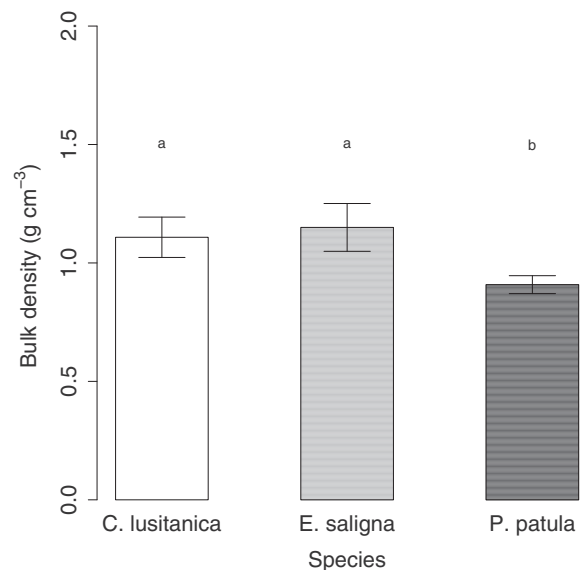
**Fig. 4.** Bulk density (g cm^{-3}) in plantations by species. Different letters indicate significant differences ($p < 0.05$).

Table 8

Carbon and nitrogen stock \pm standard error (Mg ha^{-1}) in plantations calculated with the fixed-mass method at different sampling depths.

Species	Depth (cm)	SOC (Mg ha^{-1})	SON (Mg ha^{-1})
<i>Eucalyptus saligna</i>	0–10	33.53 \pm 5.56	2.1 \pm 0.21
	0–30	90.80 \pm 10.34	5.83 \pm 0.47
	0–50	142.96 \pm 21.78	2.12 \pm 1.23
	0–100	185.83 \pm 29.94	12.26 \pm 1.89
<i>Cupressus lusitanica</i>	0–10	26.8 \pm 10.75	1.86 \pm 0.68
	0–30	66.70 \pm 22.50	4.63 \pm 1.34
	0–50	98.46 \pm 32.82	6.93 \pm 1.87
	0–100	126.1 \pm 32.20	9.10 \pm 1.76
<i>Pinus patula</i>	0–10	24.96 \pm 1.03	1.80 \pm 0.1
	0–30	62.9 \pm 1.80	4.67 \pm 0.12
	0–50	89.00 \pm 1.80	6.76 \pm 0.26
	0–100	112.43 \pm 4.32	8.50 \pm 0.44

found it 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. In Brazil, Zinn et al. (2002) reported a 23–48% loss in SOC after a native wooded savannah was converted to *Eucalyptus* plantation. 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. 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* sp.). Berhangaray et al.

(2013) investigated the impact of changes in land use on soil carbon and found higher SOC 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.

Plantations were made in similar crops and degraded lands than current ones and the finding that nitrogen concentration and stock was 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 positive impact of plantations on degraded land and the negative impact of substitution of native forest with plantations is consistent with findings by other authors. In a similar 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*. 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 a *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.

Finally, our results showed that C and N concentrations and stocks under native natural forest and plantation forest in Chilimo were generally higher than those reported in other regions (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 in order 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.

5. Conclusion

This study has successfully answered the research questions presented in the Introduction and yields the following conclusions: (i) Bulk density can have an important confounding effect on soil condition assessment and an efficient estimation method for soil carbon and nitrogen must be applied accordingly. (ii) Soil depth is a more important factor than elevation in the study area, though C and N concentrations and stocks diminished near human settlements located in the lowest part of the elevation gradient. (iii) Chilimo natural forest stored more carbon and nitrogen than adjacent land use categories, but crop land and degraded land converted to plantations ameliorated soil degradation. (iv) Species selection did not affect carbon and nitrogen stock, despite the significantly lower bulk density values found in *P. patula* plantations.

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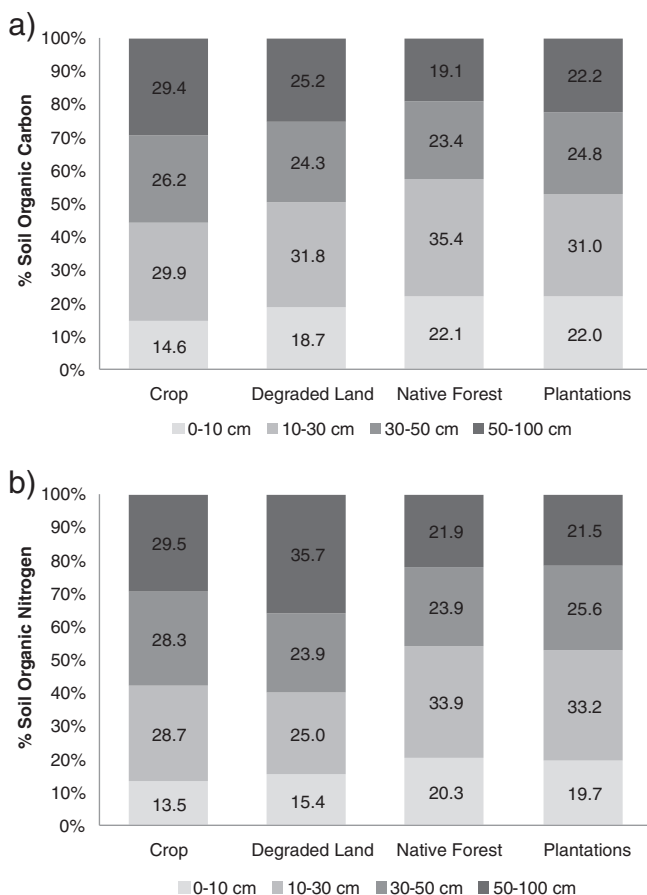


Fig. 5. a. Percentage of soil organic carbon distribution at sampling depths. b. Percentage of soil organic nitrogen distribution at sampling depths.

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