Science of the Total Environment 851 (2022) 158185

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Determinants of carbon and nitrogen sequestration in multistrata agroforestry

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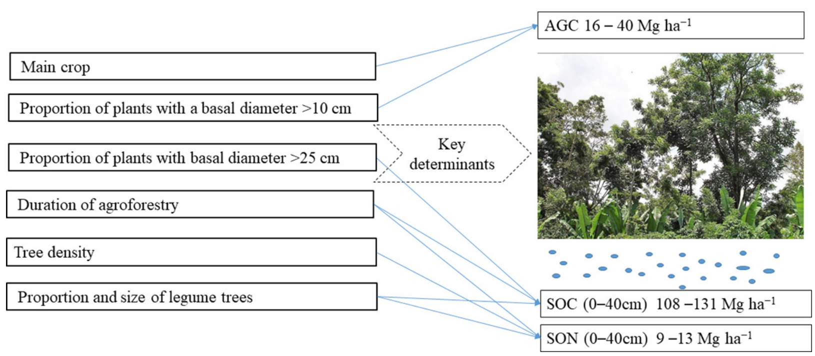
HIGHLIGHTS

- Management of agroforestry has a high potential to enhance C and N stocks.
- Duration, tree density and high basal diameter explained half of the SOC variation.
- Legume tree size and density, as well as duration, predicted N stock.
- Large legume trees reduced, and large non-legume trees increased C/N.
- Basal diameter > 10 cm, main crop and plant diversity predicted aboveground C.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Paulo Pereira

Keywords:
Agroforestry
Determinant
Management
Nitrogen
Carbon

ABSTRACT

Agroforestry has the potential to sequester carbon (C) and nitrogen (N), thereby counteracting climate change and soil degradation. However, the lack of empirical quantitative evidence on determinants of C and N stocks hampers the management of these stocks. The aim of this study was to identify the key determinants of the C and N stocks in multistrata agroforestry. We sampled 81 plots with broad variation in 12 hypothetical determinants of C and N stocks aboveground and in two soil layers, located in three Ethiopian regions with varied multistrata agroforestry traditions and characteristics. Above-ground stocks were assessed using an allometric equation, and soil stocks were assessed with the fixed-depth method. The hypothetical determinants, i.e., the duration of agroforestry practice, the tree density, the proportion of plants with a high basal diameter, legumes and native species, the species diversity, the main crops, soil texture and pH, and altitude, were tested using linear mixed models. The duration of agroforestry, tree density, and proportion of plants with a high basal diameter (>25 cm) explained half of the variation in the soil C stock, which represented nearly three quarters or more of the total C stock. Duration and tree density explained most of the soil N stock, although legumes also influenced soil N. A high proportion of large legume trees reduced C/N, whereas a high proportion of large non-legume trees increased C/N. The aboveground C stock was explained by species diversity or the proportion of stems with a basal diameter >10 cm, depending on the main crop. There is a high potential to manage C and N stocks and their persistence, as well as soil productivity, by managing the duration of agroforestry, the density of large trees, the proportion of legumes, and the main crops in multistrata agroforestry.

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http://dx.doi.org/10.1016/j.scitotenv.2022.158185
Received 5 May 2022; Received in revised form 17 August 2022; Accepted 17 August 2022
Available online 20 August 2022

1. Introduction

Forests play an important role in global carbon (C) and nitrogen (N) cycles. The increasing rate of deforestation and rising demand for food necessitate the search for land use strategies that can complement the declining role of forests (Tesfaye et al., 2016) while providing food and income. Agroforestry integrates trees within agricultural landscapes and counteracts climate change and soil degradation while providing food, income and multiple ecosystem services such as biodiversity conservation, carbon sinks, nutrient cycling and aesthetic values (Jose, 2009). In Ethiopia, coffee mostly integrated with agroforestry provides a livelihood for 15 million farmers, along with their families, and represents 25 % of the national export income (Moat et al., 2017). Agroforestry systems are, however, varied, with diverse impacts on climate and soil (Negash and Starr, 2015; Ma et al., 2020).

Agroforestry has the potential to reduce C and N losses caused by deforestation and to sequester C and N relative to cereal-based food production systems (Rimhanen et al., 2016). Enhancing agroforestry to increase C and N stocks may thus harbor significant potential to minimize land use-related net losses of C and N stocks. Currently, agricultural land with at least 10 % tree cover represents 43 % of the global agricultural area, in which 45.3 Pg (1 Pg = 10^{15} g) C is sequestered and trees contribute >75 % of C sequestration (Zomer et al., 2016). If 630 million ha of unproductive cropland and grassland were converted to agroforestry, an additional 586,000 Mg C yr^{-1} would be sequestered (Smith et al., 2007; Verchot et al., 2007). Accordingly, 40 % of developing countries have proposed agroforestry as a solution to combat forest decline in their nationally determined contributions to Paris Agreement on climate change mitigation (Rosenstock et al., 2019). Indeed, agroforestry is predicted to have the highest potential C sequestration of all land use types in developing countries by 2040 (IPCC, 2007; Smith et al., 2007; Verchot et al., 2007). An understanding of the determinants of C and N stocks is required to realize this potential. The aboveground and soil C and N stocks at various depths in tropical agroforestry systems may depend on several major variables, including the land use history and the duration of the agroforestry system (Ma et al., 2020; Rimhanen et al., 2016; Tesfaye et al., 2016); tree size and density (Fernańdez-Nuñẽz et al., 2010; Islam et al., 2015; Pan et al., 2013; Saha et al., 2009; Stephenson et al., 2017); species composition and diversity (Cong et al., 2014; Dawud et al., 2016; Jiang et al., 2011; Lange et al., 2015; Ma et al., 2020; Steinbeiss et al., 2008), including the legume proportion (Rosenstock et al., 2014; Wu et al., 2017); management practices (De Beenhouwer et al., 2016; Mafongoya et al., 1998); altitude and climate (Berg and Laskowski, 2006; Dossa et al., 2013; Hobley et al., 2015; Mehta et al., 2014); topography (Yimer et al., 2006), and soil type and properties (Hobley et al., 2015; Pan et al., 2013; Wang et al., 2012). These variables may determine the dynamics among the initial C and N stocks, plant growth, the quantity and quality of litter and root inputs, and decomposition and sequestration, and consequently the C and N stocks under multistrata agroforestry.

The major determinants of the C and N stocks of various agroforestry systems cannot be understood based only on the theory or impact of the system components. For example, an increase in legume proportion may increase N availability and thus biomass production, which will increase the organic matter input to the soil (Bell et al., 2005; Fornara and Tilman, 2008; Zak et al., 2003). However, more legumes may also lead to the more rapid decomposition of organic matter via increased microbial activity and may hence reduce the soil organic C (SOC) concentration (Lange et al., 2015). Therefore, the interactions and the relative importance of various hypothetical determinants must be empirically quantified, to enable the management of agroforestry systems for maintaining and enhancing these stocks. This understanding could aid in combining climate change mitigation and food and income provisioning, potentially triggered through appropriate policies, such as C payments (Kahiluoto et al., 2014; Pan et al., 2011; Rimhanen et al., 2016).

Previous studies on the impacts of agroforestry characteristics on C and N stocks focused on single characteristics, such as plant diversity, management intensity or elevation, mainly in Asia (Islam et al., 2015; Saha et al., 2009), but also in Africa (De Beenhouwer et al., 2016), Latin America (Ma et al., 2020), and temperate regions (Dawud et al., 2016). However, limited quantitative empirical evidence is available on the relative importance and interactions of the main determinants of C and N stocks in multistrata agroforestry systems. This knowledge gap undermines the management potential of these stocks (Niether et al., 2020; Schneidewind et al., 2018). Consequently, the objective of this study was to identify the key biophysical determinants of C and N stocks in multistrata agroforestry systems. The hypothesis tested was that the main biophysical determinants of C and N stocks of an agroforestry system are duration; the density of trees and the proportion of thick stems, legumes and native species; species diversity; the main crops; soil texture and pH; and altitude.

2. Materials and methods

2.1. Study sites

The study was conducted in Haru-Gedeo (Gedeo) in southeastern Ethiopia, in Bokansa-Wonsho-Sidama (Sidama) in south-central Ethiopia, and in Menekus-Abdoguma-Fenote-Selam (Fenote-Selam) in northwestern Ethiopia. In these regions, multistrata agroforestry systems dominate as traditional cropping systems that provide livelihoods for rural communities. The multistrata systems in the study consist of three or more vertical canopy layers (Table 1). The agroforestry systems in Gedeo and Sidama evolved through gradually and selectively removing trees from natural forests and intensifying the land use (Negash et al., 2012; Asfaw and Ågren, 2007), while deliberately retaining native trees and shrubs to maintain multistrata agroforestry. In Fenote-Selam, the agroforestry systems were formed via the intensification of previous mixed farming that combined cereal-based cropping with grazing animals.

The sites selected for this study represented a relatively high agricultural productivity within each of the three regions and a broad range of variation within the multistrata agroforestry system in terms of the hypothetical determinants (refer to Section 2.4). The regions are characterized by moist to subhumid warm subtropical climates (Table 1). The southeastern Gedeo and south-central Sidama agroforestry sites are the most densely populated areas of Ethiopia (Mebrate, 2007; Abebe, 2005), which reflects the high carrying capacity of the agroforestry systems. The perennial, herbaceous, monocarpic, banana-like plant enset (*Ensete ventricosum* (Welw.) Cheesman) is the main crop and serves as a staple food in central, southwestern and southern Ethiopia. Coffee (*Coffea arabica* L.) is the main cash crop across the three study sites. Organic coffee grown under shade trees known by the names Yirgachefe (Gedeo) and Sidama coffee and is internationally recognized for its high quality. Farmers in Sidama also produce khat (*Catha edulis* Forskal), and farmers in Fenote-Selam produce pepper (*Capsicum* spp.) in monoculture to obtain additional income.

The plant species compositions of the vertical strata of the Gedeo agroforestry system were dominated (63 %) by N-fixing native trees, such as *Millettia ferruginea* (Hochst.) Baker and *Erythrina brucei* Schweinf. Conversely, in Sidama, non-N-fixing tree species, including *Cordia africana* and enset, accounted for 85 % of the species, and in Fenote-Selam, *C. africana* and *C. arabica* accounted for 71 % of the species. Farmers in Fenote-Selam also integrated exotic N-fixing species (*Sesbania sesban* (L.) Merr.) to a low extent. The tree density (stems ha^{-1}) in the agroforestry of Fenote-Selam was approximately two times higher than that in Gedeo and four times higher than that in Sidama (Table 1). Smallholder farmers in the three sites practice pruning, pollarding and thinning to reduce shading to coffee and other crops grown under tree cover. Particularly in Gedeo and Sidama, farmers use green manure from enset leaf litter for chopping and mulching adding organic matter to the soil to conserve soil moisture and enhance soil fertility (Negash and Starr, 2015; Negash et al., 2022). Mineral fertilizers were not applied at any of the study sites. Farmers in Gedeo practice smashing herbaceous weeds into mulch and enhancing the organic matter input to the farms, unlike farmers in Sidama and Fenote-Selam.

Table 1
Biophysical description of the study sites. The values show means, while ranges are shown in parentheses. SOC = soil organic carbon; SON = soil organic nitrogen; mth = mean tree height.

Biophysical variables	Southeastern (Gedeo)	South-central (Sidama)	Northwestern (Fenote-Selam)
Geographic coordinates	5° 50′–6° 12′ N, 38° 03′ –38° 18′ E	7° 00′–7° 06′ N, 38° 34′ –38° 37′ E	10° 39′ –10°42′N, 37°15′ –37°16′ E
Altitude, m	1941 [1910, 1963]	2043 [2000, 2063]	1891 [1885, 1899]
Slope, %	10–30	10–30	10–20
Annual rainfall, mm	800–1200	1200–1500	1200–1500
Mean monthly temperature, °C	13–28	15–20	16–20
Soils	Nitisols	Nitisols	Nitisols
Sand, %	2.8 [1.0, 6.0]	16.1 [10.0, 28.0]	4.5 [2.0, 11.0]
Silt, %	26.5 [16.0, 50.0]	32.0 [25.0, 46.0]	30.1 [19.0, 59.0]
Clay, %	70.7 [47.0, 83.0]	51.7 [29.0, 65.0]	65.4 [36.0, 79.0]
pH (1:25)	6.3 [5.8, 7.1]	6.8 [5.9, 7.6]	6.9 [6.3, 7.3]
SOC, %	3.2 [1.3, 6.2]	3.0 [1.6, 6.0]	2.7 [1.3, 4.7]
N, %	0.3 [0.1, 0.6]	0.3 [0.2, 0.6]	0.2 [0.1, 0.4]
Bulk density, g cm^{-3}	1.0 [0.8, 1.2]	1.1 [0.8, 1.5]	1.0 [0.9, 1.2]
Aboveground C, Mg ha^{-1}	50.3 [4.9, 114.9]	16.3 [0.8, 81.8]	45.1 [1.4, 196.9]
SOC (0–40 cm), Mg ha^{-1}	121.3 [84.5, 160.6]	130.9 [93.2, 221.3]	107.8 [88.2, 148.4]
SON (0–40 cm), Mg ha^{-1}	11.7 [7.7, 15.8]	12.5 [8.5, 20.6]	8.8 [6.3, 11.5]
C/N	10.5 [9.3, 11.4]	10.4 [9.3, 11.6]	12.5 [10.2, 14.3]
Smallholding mean size, ha	0.40	0.75	0.25
Agroforestry type	Multistrata rainfed	Multistrata rainfed	Multistrata rainfed + irrigated
Median agroforestry duration, yr	38 [10, 45]	45 [32, 54]	26 [19, 35]
Diversity of abundance	0.6 [0.0, 1.1]	0.5 [0.0, 0.9]	0.5 [0.0, 1.1]
Stems w basal diam >10 cm, %	75.9 [30.5, 99.7]	97.3 [74.2, 100]	68.0 [12.8, 98.1]
Stems w basal diam >25 cm, %	46.9 [0, 98.2]	40.9 [0, 100]	36.9 [0, 96.7]
Diversity of basal area	0.7 [0.1, 1.1]	0.4 [0.1, 0.9]	0.5 [0.0, 1.3]
Major canopy trees	*Millettia ferruginea* (mth ~14 m), *Erythrina* sp. (mth ~15 m), *Cordia africana* (mth ~18 m)	*C. africana* (mth ~9 m) *Croton macrostachyus*, *Persea americana*	*C. africana* (mth ~14 m) *Ficus* spp. *Sesbania sesban*
Legume trees (%)	10.7 [0.0, 100.0]	1.9 [0.0, 20.0]	13.5 [0.0, 100.0]
Tree density, stems ha^{-1}	367 [100,1200]	200 [100, 500]	785 [100,2300]
Coffee strata density, stems ha^{-1}	240 [30, 680]	10 [10,10]	263 [60, 470]
Basal area m^2 ha^{-1} (>2.5 cm)	62.9 [9.3, 261.1]	38.3 [7.7, 129.4]	21.8 [0.3, 74.2]
Main crop			
Enset (%)	53.1 [26.0, 74.0]	79.7 [43.0, 90.0]	–
Coffee (%)	71.0 [13.0, 95.0]	20.0 [20.0, 20.0]	77.8 [43.0, 100]
Tree spp. (%)	11.4 [5.0, 20.0]	20.1 [10.0, 57.0]	30.3 [8.0, 100.0]
Vertical structures	Upper layer: canopy tree spp., mid layer: enset and banana, lower layer: coffee, ground layer: root and herbaceous crops and weeds.	Upper layer: canopy tree spp., mid layer: enset, lower layer: coffee, ground layer: herbaceous crops and weeds.	Upper layer: canopy tree spp., mid layer: coffee, ground layer: weeds
Farm management practices	Pruning, lopping, pollarding, thinning, weeding. Enset leaves, herbaceous plants and foliage of Millettia for composting, mulching, shading coffee plants.	Pollarding and thinning of trees. Enset leaves used for composting and mulching.	Pollarding and thinning of trees
Main food and cash crops	Coffee, enset, banana, taro, yam	Coffee, enset	Coffee, wheat, barley, maize, green pepper, teff

2.2. Sampling design

The three study sites of the multistrata agroforestry system were selected in May–June 2015 to ensure a broad range regarding the hypothetical determinants of C and N stocks across and within the sites (Fig. 1). The altitude, slope and soil texture of the selected sites were determined, and soil type was sourced from previous studies (Negash and Starr, 2015; Abebe, 2005). A total of 324 farms in the sites practiced enset and/or coffee-based agroforestry, and 25 % of the farms were randomly selected. The management history of the plots was determined by interviewing the owner of the farm, and local officials or trustees as ‘key informants’. The farms were preliminarily grouped into farms with a low, medium and high diversity of plant species and sizes to ensure representativeness of the plots and sub-plots (Fig. 1). On the farms with a low plant species diversity, one to two species were represented, while on farms with a mediocre diversity, three to four species and on farms with a high diversity more than four species were represented. A total of 81 agroforestry plots with dimensions of 10 m × 10 m were randomly selected following a stratified random approach; 27 plots were selected from each of the three sites; and each plot was located on a separate farm for the inventory of perennial plant species, including enset and coffee, and for soil sampling. The plots were selected to represent the available range within the hypothetical determinants. The individual sampled farms were located 15–75 m apart. To select the sampled plot location, each farm was visually divided into equal grid points (Fig. 1). The sample point was selected via lottery by assigning a random number to each grid point. The GPS location of each plot was recorded. The sampled agroforestry plots were 10 to 54 years old across the selected sites, with histories of forest in the southeastern Ethiopia (Gedeo) and south-central Ethiopia (Sidama) plots and mixed farming in the northwestern (Fenote-Selam) plots. An inventory of all the trees and shrubs on the plots with a breast-height diameter ≥2.5 cm (at 1.3 m) and height ≥1.5 m was performed. The number of saplings and seedlings with a breast-height diameter <2.5 cm and height <1.5 m was small, and were not included in the estimation of biomass C stocks. In the case of multistemmed shrubs, each stem was measured, and the equivalent diameter of the plant was calculated as the square root of the sum of the diameters of all stems per plant (Snowdon et al., 2002).

Five subplots (1 m × 1 m) were laid down at the corners and center of a larger plot (10 m × 10 m), and a random number was assigned to each subplot, among which three subplots were randomly selected using a lottery (Fig. 1). A wooden sample frame of 1 m × 1 m, which was composed of 10 by 10 unit square grids (10 cm × 10 cm each) was overlaid on selected subplots. Ten soil subsamples per subplot with depth ranges of 0–20 cm and 20–40 cm were randomly collected with an auger (3.8 cm diameter), and a

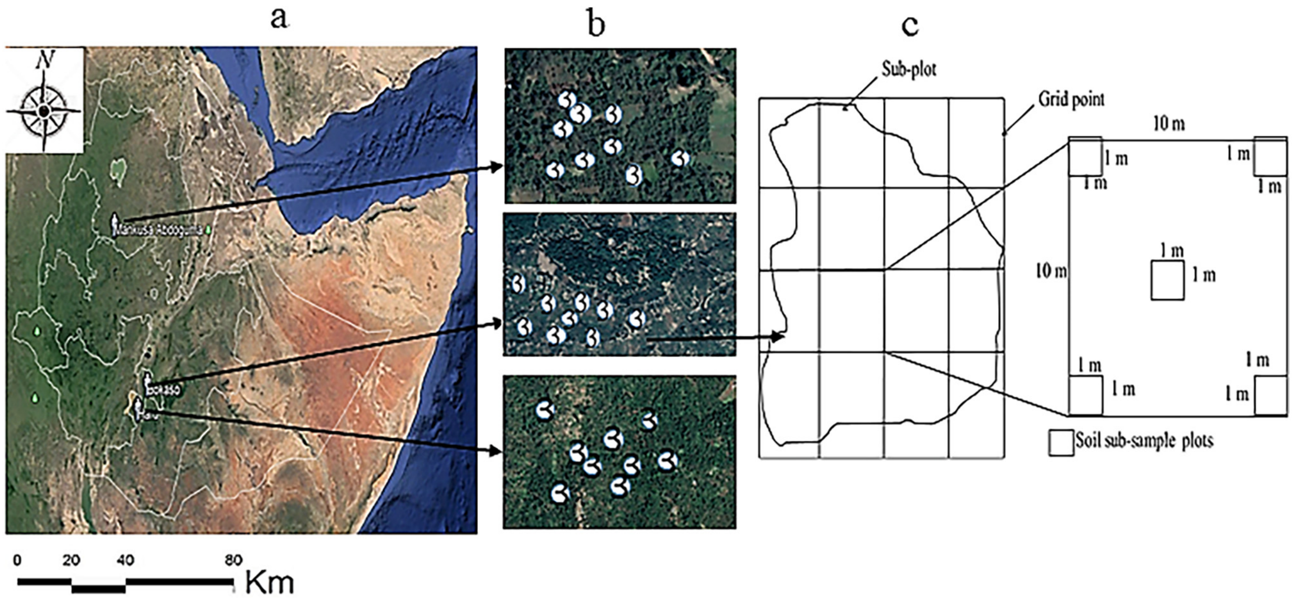

Fig. 1. Spatial design of the study. (**a**) Location of the northwestern (Mankusa-Abdogoma-Fenot-Selam), south-central (Bokaso-Wonsho-Sidama) and southeastern (Haru-Gedeo) study regions and the specific sites. (**b**) Agroforestry plots within the sites located at the distance of 15 to 75 m from each other are denoted by white circles, which indicate the number of subplots within each plot. (**c**) The layout within each subplot for the plant inventory (10 m × 10 m) and soil sub-sample plots (1 m × 1 m), each with 10 soil sub-samples forming one composite sample, with a total of five composite samples per sub-plot (Google Maps 2020).

composite sample was formed for C and N analyses. Six soil samples for bulk density analysis were separately collected from each subplot at both soil depths.

2.3. Stock analysis

The aboveground biomass (stem plus bark, branches, and foliage) C stocks for each plot (Mg ha^{-1}) were calculated based on the product of the biomass dry matter and C content. The aboveground biomass for trees and shrubs was determined following the allometric equation developed by Kuyaha et al. (2012): $AGB = 0.225 \times d^{2.341} \times \rho^{0.73}$ ($R^2 = 0.98, n = 72$), where AGB is the aboveground biomass (kg dry matter plant^{-1}), d is the breast height diameter (cm), and ρ is the basic wood density (g cm^{-3}). To estimate the aboveground biomass of coffee and enset plants, the allometric equations that were developed from onsite harvested plants in a previous study were used (Negash et al., 2013a, 2013b): $AGB_{coffee} = 0.147 \times d_{40}^2$ ($R^2 = 0.80, n = 31$), and $\ln(AGB_{enset}) = -6.57 + 2.316\ln(d_{10}) + 0.124\ln(h)$; ($R^2 = 0.91, n = 40$), where d_{40} is the stem diameter (cm) of the coffee plant at a height of 40 cm, d_{10} is the basal diameter (cm) of the enset pseudostem at a height of 10 cm and h is total height (m). A biomass C content of 48 % was applied for trees and shrubs grown in agroforestry (Kuyaha et al., 2012), a biomass C content of 49 % was applied for coffee grown in agroforestry (Negash et al., 2013a), and a biomass C content of 47 % was applied for ensets grown in agroforestry systems (Negash et al., 2013b).

The soil samples were air-dried and ground (<2 mm). The total C and N concentrations were analyzed by dry combustion at 1100 °C using the Leco CN-2000 analyzer (Leco Corporation, St. Joseph, MI, USA) in the laboratory of the Natural Resources Institute of Finland. The analysis was carried out for the original samples and those treated with 6 M HCl to remove carbonate carbon (0–0.40 %, median 0.13 %). In this study, the organic C that remained after the HCl treatment was used to determine the SOC stocks. The soil pH was measured with electrodes in a 1:2.5 soil/water suspension, and particle size fractions were measured using the hydrometer method after dispersion with sodium hexametaphosphate solution. The bulk density samples were oven-dried at 105 °C for 24 h and weighed, and the weights of the >2 mm and <2 mm fractions were recorded. The bulk density (ρ_b) (g cm^{-3}) was calculated as the oven-dried weight of the soil divided by the volume of the soil. The SOC and soil organic N (SON) stocks (Mg ha^{-1}) were primarily calculated using the fixed-depth method by multiplying the concentrations (%) of soil C or N by the bulk density (ρ, g cm^{-3}) and the depth of the sampled soil (z, 0–20 or 20–40 cm) (Solomon et al., 2002); in other words, for SOC or SON, Mg ha^{-1} = C or N (%) × ρ × z × 100.

2.4. Hypothetical determinants

Twelve hypothetical determinants, i.e., hypothetical explanatory variables, of the C and N stocks and the distribution between the soil layers, as well as those of soil C/N, were tested. The hypothetical variables were as follows: (1) the duration of agroforestry practice on the sampled plot of each farm in years; (2) the tree density (number of stems ha^{-1} regardless of the species); (3) the proportion of legume trees in percentages; (4) the proportion of stems with basal diameter >10 cm or (5) >25 cm (%); (6) the proportion of native trees based on plant basal area (diameter <2.5 cm excluded); (7) the species diversity of plant individuals; (8) the species diversity based on plant basal diameter ($H_b = -\Sigma P_i (P_i)\ i = 1$, where P_i is the proportion of basal diameter of the ith species, with diameters <2.5 cm excluded); (9) the main crops (enset, coffee, and other crops); (10) the soil texture; (11) the pH; and (12) the altitude. The Shannon-Wiener diversity index (Shannon and Weaver, 1949) was applied in calculating diversities. The data with a correlation matrix for the hypothetical determinants and response variables are shown in Fig. A.1.

2.5. Statistical analysis

Linear mixed models (LMMs) were employed in the analysis of the SOC and SON stocks, the C/N and the aboveground C stock. Log-transformation was utilized for all stocks because of skewed distributions. Combined depths (0–40 cm) were employed in the analysis, but for SOC and SON stocks, the depths were analyzed separately. The models were fitted using the residual maximum likelihood (REML) or residual pseudolikelihood (REPL) estimation method with depth (0–20 cm and 20–40 cm); the duration of agroforestry in years; the tree density (number of stems ha^{-1} regardless of the species); the proportion of legumes in percentages; the main crops; the proportion of native trees based on plant basal area in percentages; proportion of stems with basal diameter >10 cm and >25 cm; the species diversity of plant individuals; and the species diversity based on basal diameter excluding plants with basal diameter <2.5 cm, calculated using the Shannon-Wiener diversity index with their two-way interactions denoted as fixed effects. All the main effects of the selected predictors were considered to be relevant predictors and were included in the models. This method was used to control them, even if they were not statistically significant. All possible interactions were added to the models and either kept or separately removed based on their statistical significance. In addition, the effects of the composite samples (10 subsamples) with soil chemical analysis (pH and the proportions of sand, silt, and clay) were tested, but were found to be statistically significant only for the aboveground C stock.

The plots within each site were assumed to be independent with normally distributed random effects. Each plot consisted of three replicates, and each replicate was a composite of 10 subsamples. The correlation between each replicate at both depths was considered in the model with a heterogeneous compound symmetry covariance structure that allowed unequal variances for each depth (Gbur et al., 2012).

The residuals were checked for normality using a boxplot and plotted against the fitted values. These plots indicated that the assumptions of the models were adequate. The Kolmogorov-Smirnov test for normality was also used to support the interpretations. Only for SOC (Model 3; $p = 0.03$) the assumption of normality was found to be questionable, due to a few relatively small outliers at both tails of distribution, and thus this violation was interpreted as minor. The degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 2009). A significance level of $\alpha = 0.10$ was applied in the model selection. The marginal and conditional R^2-values were calculated based on the variance of fixed, random, and residual effects using SAS Macro %GOF (Vonesh and Chinchilli, 1997). The former measures a reduction in the residual variance explained solely by the fixed effects, while the latter measures a reduction in the explained variance due to the fitting of both the fixed effects and the random effects versus the explained variance due to the fitting of an overall mean. The conditional R^2-values were calculated only for full models, while marginal R^2-values were separately calculated for each fixed effect to evaluate their explanatory power.

Diversity indices were calculated and presented in logarithmic form, but when back-transformed to the original scale, the more interpretive exponential form, also known as true diversity, was employed (Jost, 2007). The analyses were performed using the GLIMMIX procedure in the SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA). The correlation matrix was plotted using R (R Core Team, Version 4.0.2).

3. Results

3.1. Determinants of aboveground carbon

The proportion of stems with basal diameters >10 and >25 cm and the main crop were the most important predictors of aboveground C stocks, and the proportion of stems with basal diameters >10 cm was the single most important predictor (Table 2 Model 1). Including their interactions, these determinants explained 55.6 % of the total variation in the aboveground C stock.

Relative to coffee or other crops, when the main crop was enset, the aboveground C stock was explained more by species diversity based on basal diameter but less by the proportion of plants with a basal diameter >10 cm (Fig. 2). With coffee as the main crop, the aboveground C stock increased rapidly when the proportion of stems with a basal diameter >10 cm was higher than 50 %. The association of aboveground C with tree density was quadratic in Model 1 (Table 2), which meant that an increase in tree density caused an increase in aboveground C until a density of 1000 trees per hectare was achieved, after which aboveground C started to decrease.

3.2. Determinants of soil carbon and nitrogen

The duration of agroforestry was clearly the most significant predictor of the soil C stock (Table 2 Model 3, Fig. 3). The duration resulted in a quadratic increase in SOC on a logarithmic scale from 90 to 165 with time, from 10 to 50 years. The growth in tree density was associated with an increase in SOC in all sites, with the largest increase in south-central Ethiopia (Sidama). Additionally, a basal diameter >25 cm caused an increase in the SOC.

Soil carbon stocks were similar in all three sites, and only slightly greater in Sidama than in the other sites (Table 1). The trees were larger in Sidama than in the north (slightly smaller than in Gedeo) as shown by the nearly double basal area relative to that in the North even though the density of trees was clearly the lowest in Sidama. Consequently, the increase in the low mean density of the larger trees in Sidama outweighed the increase in SOC by increasing the tree density in the other two sites (Fig. 3). More than 60% of the total variation was explained by fixed effects. When the two depths were considered separately (Table A.2 Model 5), the marginal R^2 was improved by approximately 15 %. An increase in duration enhanced the SOC to a greater extent at the depth of 0–20 cm than at the depth of 20–40 cm.

For the N stock, the duration of agroforestry was the most important predictor, and explained >30 % of the total variation (Table 2 Model 4). An increase in tree density was associated with an increase in the N stock, whereas an increase in the proportion of legume trees was associated with a decrease in the N stock; a one-unit percentage increase in the proportion of legume trees would decrease the N stock by 0.25 %. Thus, if the proportion of legume trees increases from 50 % to 100 %, the N stock will decrease by 12 %. However, this result was strongly influenced by a small number of plots, composed of 100 % legumes and with relatively low N stocks. The exclusion of these observations ($N = 81$ to $N = 77$) would change the slope to a slightly positive one; a one-unit increase in the proportion of legume trees would then increase the N stock by 0.1 %. A limited number of observations with the proportion of legume trees between 20 and 80 % makes conclusions about this range uncertain. Furthermore, the dependence of N stocks on C stocks and on the size of the trees (Fig. 4) can explain the unexpected results regarding the dependence of N stocks on legume trees alone.

Over 70 % of the total variation was explained by the fixed effects. When SON stocks at the two depths were considered separately (Table A.2 Model 6), the marginal R^2 improved by 8 %. At the depth of 0–20 cm, increasing diversity increased the N stock, whereas in the deeper soil layer the contrary was true. Higher species diversity of plant individuals tended to decrease the N stock if the trees were mainly native but tended to increase the N stock in the presence of many non-native trees.

3.3. Determinants of soil C/N

With a high proportion of legume trees, an increase in the proportion of stems with a basal diameter >25 cm led to a lower C/N (Table 2 Model 2, Fig. 4). Conversely, with a low proportion of legume trees, such an increase in the proportion of larger trees increased the C/N. Almost two-thirds of the total variation was explained by fixed effects, but when random variation in plots was taken into account, the explanatory power was almost 95 %.

4. Discussion

The duration of agroforestry was clearly the main determinant of soil C and N stocks. The proportion of plants with a high basal diameter (>25 cm) for the C stock and tree density and proportion of legume trees for the N stock were also important. A high proportion of large legume trees reduced C/N, whereas a high proportion of large non-legume trees increased C/N. The aboveground C stock was determined by the proportion of plants with moderately-sized or high (>10 cm) basal diameter and the main crop.

4.1. Potential to manage carbon and nitrogen stocks in multistrata agroforestry

The C and N stocks of the native forest also decline under agroforestry, even if they decline at a substantially slower rate than in monocropping (Demessie et al., 2013; Negash et al., 2022; Rimhanen et al., 2016). Therefore, the identification of the manageable determinants of the C and N stocks in multistrata agroforestry increases the potential of agroforestry to

Table 2

Four models for aboveground C stock, C/N, and SOC and SON stock (1 to 4), including all main effects of predictors and statistically significant second-order interactions of predictors. All dependent variables, except C/N, were log-transformed, and the effects of predictors are therefore shown on a logarithmic scale. For the categorical variables, two estimates are shown when the number of categories is three (the last category is zero). As the diversity indices are measured in logarithmic form, when transformed to the original scale, their more interpretive exponential form, also known as their true diversity, is employed in the interpretation. The change in percent in the dependent variable due to every 10 % increase in true diversity, can be calculated as $(=1.1^{estimate} - 1) * 100$ %. For other predictors, the change in percent in the dependent variable caused by a one-unit increase in the independent variable can be calculated as $(=\mathbf{e}^{estimate} - 1) * 100$. C/N back-transformation differs as it is not log-transformed. The estimate divided by 100 indicates the change in units in the C/N ratio due to every 1 % increase in true diversity. For other predictors, the estimate reveals the change in units in the C/N ratio caused by a one-unit increase in the independent variable. The variances explained for each variable indicate the most significant predictors and sum up to marginal R^2values. In addition, conditional R^2values including random effects are shown for full models. C = carbon; C/N = ratio of SOC and SON; SOC = soil organic carbon; SON = soil organic nitrogen; Ln AG C = natural logarithm for aboveground carbon; Ln SOC = natural logarithm for SOC, and Ln SON = natural logarithm for SON.

Fixed effect	Ln AG C			C/N (depth 0–40 cm)			Ln SOC (depth 0–40 cm)			Ln SON (depth 0–40 cm)		
	Model 1 (interactions included)			Model 2 (interactions included)			Model 3 (interactions included)			Model 4 (interactions included)		
	Effect (log scale)	P value	Variance explained	Effect (orig. scale)	P value	Variance explained	Effect (log scale)	P value	Variance explained	Effect (log scale)	P value	Variance explained
Intercept	2.6228			11.8975			4.3352			1.7260		
Site	2.879/1.874	**0.004**	19.0 %	−0.450/1.194	**0.005**	52.4 %	0.238/0.377	**0.015**	13.1 %	0.129/0.012	**0.071**	34.3 %
Main crop	−2.470/4.916	**0.000**	4.9 %	−0.054/0.078	0.908	0.0 %	−0.116/0.044	0.135	0.4 %	−0.122/−0.027	0.348	0.4 %
Native trees (%)	−0.4783	0.105	0.3 %	−0.3253	0.285	0.0 %	−0.0323	0.666	1.0 %	0.0461	0.575	0.5 %
Duration (years)	0.0059	0.424	1.4 %	−0.0275	0.176	3.6 %	−0.0055	0.550	37.3 %	0.0178	**0.000**	30.7 %
Legume trees (%)	−0.0005	0.916	4.2 %	0.0144	**0.089**	1.1 %	−0.0019	0.121	0.4 %	−0.0025	***0.060***	2.6 %
Species diversity of plant individuals	0.0925	0.844	1.3 %	0.1379	0.712	0.2 %	−0.0759	0.408	0.0 %	−0.0661	0.502	0.1 %
Tree density	0.0035	**0.000**	2.1 %	0.0001	0.592	0.0 %	0.0007	**0.003**	1.6 %	0.0001	**0.026**	2.7 %
Species diversity based on plant basal diameter	−0.4475	**0.001**	1.2 %	0.3868	0.237	0.7 %	0.0795	0.303	0.1 %	0.0384	0.649	0.0 %
Basal diameter > 10 cm (%)	−0.0457	**0.000**	26.9 %	−0.0071	0.198	2.0 %	−0.0017	0.242	0.0 %	−0.0611	0.711	0.3 %
Basal diameter > 25 cm (%)	0.0257	**0.000**	9.5 %	0.0047	0.122	0.1 %	0.0012	***0.064***	3.0 %	0.0981	0.161	1.1 %
Duration ^{2} (years)							0.0003	**0.023**	3.4 %			
Site * tree density							−0.001/−0.001	***0.057***	3.5 %			
Legume trees (%) * basal diameter > 25 cm (%)				−0.0004	**0.086**	5.5 %						
Species diversity based on plant basal diameter * Main crop	0.537/5.209	**0.000**	8.5 %									
Basal diameter > 10 cm (%) * main crop	0.028/−0.041	**0.000**	3.2 %									
Tree density^2	−0.0000	**0.001**	1.3 %									
Diversity of individuals * basal diameter > 25 cm (%)	−0.0209	**0.018**	3.3 %									
Sand (%)	−0.5254	**0.000**	0.0 %									
Sand (%) * site	−0.541/−0.375	**0.029**	0.3 %									
Sand (%) * main crop	0.265/−0.503	**0.000**	1.6 %									
Sand (%) * tree density	−0.0003	**0.008**	0.5 %									
Sand * basal diameter > 10 cm (%)	0.0107	**0.000**	3.0 %									
Variance explained ($R^2_{marginal}/R^2_{conditional}$)		92.5 %/93.7 %			65.0 %/94.8 %			63.9 %/69.5 %			72.6 %/78.4	

Statistically significant *P* values are bolded.

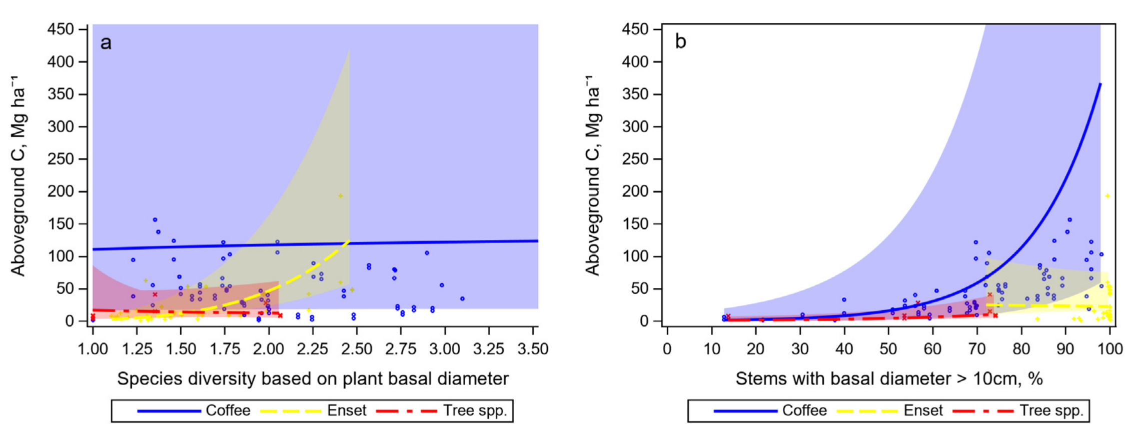

Fig. 2. Aboveground carbon determined by species diversity and stem area. Aboveground carbon (C, t ha^{-1}) was determined by (a) species diversity and (b) proportion of middle-sized or thick stems, in interaction with main crop. When the main crop was enset, an increase in species diversity increased aboveground carbon, but when the main crop was coffee, a high proportion of thick stems was more effective. The estimates of aboveground carbon and species diversity were transformed to the original scale. The 95 % confidence limits for the estimates are indicated by the shaded area.

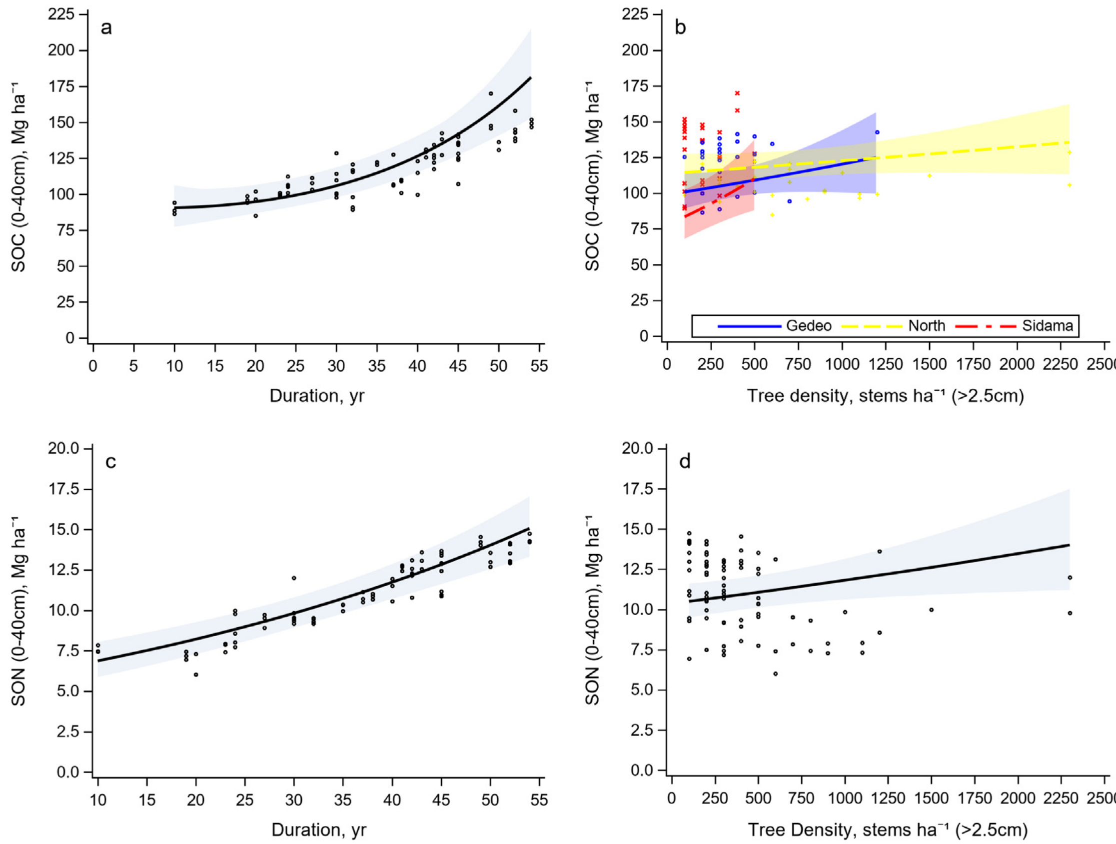

Fig. 3. Soil organic carbon and nitrogen stocks determined by the duration of agroforestry and tree density. Both soil organic carbon (SOC) and nitrogen (SON) stocks continuously increased along with the increase in the duration, whereas the size of the increase of soil carbon stock under increased tree density varied among the three regions. The estimates of SOC and SON were back-transformed to the original scale. The 95 % confidence limits for the estimates are shown as the shaded area.

maintain the stocks. The soil stock of C, which represented the majority (71 to 89 %) of the total C stock, as well as the SON stock, were highly affected by factors that farmers could manage, such as the duration of agroforestry, tree density and the proportion of legumes. Via managing species composition and diversity, rotation age and stem density farmers can also manage the basal area, which appeared as an important determinant for both soil and aboveground stocks. For example, an addition of 2803, 3928 and 5145 kg C ha^{-1} yr^{-1} through litterfall was observed with a tree density of 625, 1240 and 1505 stems ha^{-1} in enset, coffee and fruit-coffee agroforestry systems, respectively, in southeastern Ethiopia (Negash and Starr, 2013). Furthermore, including common legume tree species such as *Erythrina brucei* and *Millettia ferruginea* added up to 1428 kg N and 37–61 kg N ha^{-1} yr^{-1} with a tree density of 110 and 225 stems ha^{-1}, respectively.

4.2. Duration of agroforestry and tree density

The duration of agroforestry was not directly related to the aboveground C stock. This is because trees are also harvested, and the age of individual trees is managed for shade effects and the production of fodder and fuelwood. As the duration of agroforestry increases, the canopy cover of individual trees increases, and farmers reduce the number of shade trees through thinning, in order to reduce excessive shade for coffee plants to secure coffee yields. This may also explain the observed negative correlation between tree density and duration.

In accordance with the findings of the present study, an increase in SOC with the duration of a broad range of multistrata agroforestry systems was also observed by Rimhanen et al. (2016) in a smaller set of agroforestry plots in a southern-central region, and by Tesfaye et al. (2016) in the central highlands of Ethiopia. The volumes of trees which are reflected in the basal diameter that appeared as a determinant for both aboveground C stock and soil C stock in this study, increase with age, thereby enhancing the leaf and fine root biomass sources of C and N (Saha et al., 2009) and further increasing the SOC stock (Stephenson et al., 2017). The increase in the lowest density of the larger trees in Sidama compared to that in the North, as shown by the nearly doubled basal area, outweighed the increase in SOC at the other two sites, despite the highest proportion of sand in soil found in Sidama; sandy soil tends to reduce SOC accrual relative to soils with a greater proportion of clay (Kätterer and Andren, 1999). Similarly to our findings, a high SOC was also attributed to a high tree density by Islam et al. (2015) and Saha et al. (2009), both in tropical home garden agroforestry and in temperate agroforestry (Fernańdez-Nuńẽz et al., 2010). The slightly smaller SON stock in the north compared to that in the other sites likely was due to the abundant but small legume trees of the north which had accumulated less nitrogen at the time of the study. Accordingly, the increase in the size of legume trees increased the SON stock relative to the SOC stock, and the C/N decreased.

The important role of tree density in determining SOC and SON stocks implies that increasing tree cover is a practical means of simultaneously enhancing climate change mitigation and soil productivity. Maintaining high tree density by using species with great longevity (Pan et al., 2013) enhances deep rooting and the effective capture of nutrients and moisture thus supplying a large soil volume with fine root litter and root exudates. While the integration of a tree component in agricultural systems may enhance climate resilience and provide nutritional benefits through fruit harvest in addition to improving soil C and N stocks, there may be significant trade-offs associated with high tree cover depending on the farming system and climatic conditions, such as trade-offs concerning productivity, food security or the hydrologic balance (Zomer et al., 2016).

The greater proportion of near-surface SOC under higher tree density found in this study is explained by litterfall and fine root C inputs (Freier et al., 2010), whereas the N distribution was more impacted by tree species,

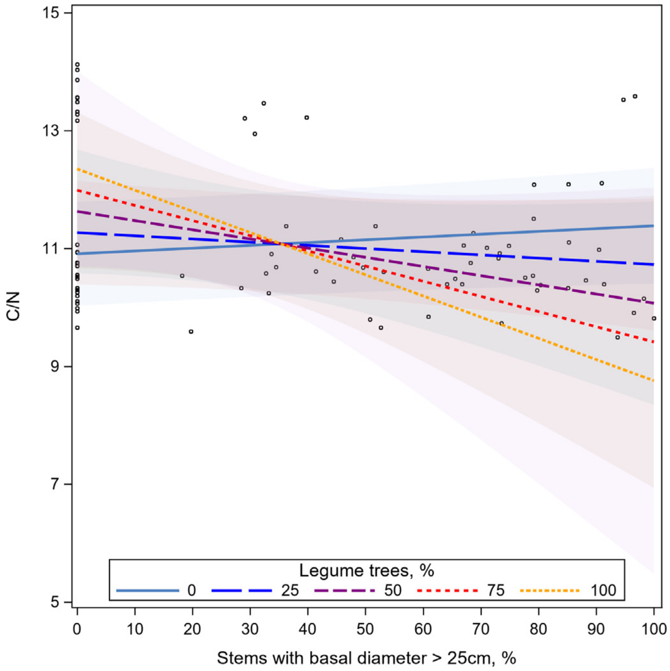

Fig. 4. Ratio between soil organic carbon and soil organic nitrogen (C/N) determined by the proportion of legume trees. C/N depended on the proportion of legume trees with a basal diameter >25 cm. A high proportion of legumes and stems with a high diameter reduced the predicted response of C/N, whereas a small proportion of legumes and stems with a small diameter increased the predicted response of C/N. Legume tree proportions were fixed at five evenly spaced percentages. The 95 % confidence limits for the estimates are shown as the shaded area.

especially legume composition, than density. Additionally, the turnover rate of organic matter decreases with an increase in depth, which together with the contribution of aged trees leads to prolonged SOC accumulation in the deeper layers where geology also affects the stocks more than at the surface (Hobley et al., 2015).

4.3. Plant species diversity and composition

The previously observed increase in the aboveground and soil C stocks via an increase in the plant diversity (Lange et al., 2015; Ma et al., 2020) of the perennial species used in agroforestry (Cong et al., 2014) was not observed in the present study. This was likely caused by interference due to the plant composition, such as the main crops and legumes, as well as by the tree size, as indicated by their statistically significant interactions. A high proportion of native species often with a high wood density may contribute to aboveground C accumulation (Bastin et al., 2015; Pan et al., 2013), whereas the broad diversity of the mostly dominant native species did not produce a difference. While species diversity may increase photosynthetic efficiency through diversified rooting niches and thus increased soil exploration and nutrient uptake as well as the C distribution in the soil, the shift in species composition through diversification can either slow or enhance C and N accumulation depending on factors such as, how decomposable the litter inputs are. The soluble forms of C (including polyphenols) compared to the available N in organic materials influence microbial growth and activity, as well as net nutrient mineralization or immobilization (Mafongoya et al., 1998).

Legumes support microbial N_2 fixation from the atmosphere to the biosphere, fixing 5 to 300 kg N ha^{-1} yr^{-1} depending on the species, which increases the soil N availability via root and leaf litter decomposition (Rosenstock et al., 2014). Modelling has suggested that legumes enhance the soil C sink, especially at early growth stages of a tree (Levy-Varon et al., 2019). However, according to the empirical findings of the present study, legumes achieved a dual effect by simultaneously contributing to lowering the C/N and, when the proportion of larger legume trees was high, probably enhancing the decomposition, which increased N stocks and productivity as well as the priming effect (Kuzyakov, 2010). This implied decomposition of the N-rich organic matter and release of NO_2 and CO_2 (Mutuo et al., 2005; Kirkby et al., 2013), with a tendency to reduce SOC. In accordance with this result, a low proportion of larger legume trees increased the C/N. Through well-informed management of the determinants of C/N in agroforestry identified in this study — the legume proportion, in addition to the tree size, species composition, and diversity — it is possible to counteract trade-offs (Rosenstock et al., 2014) and achieve synergy between economic benefits and environmental objectives in tropical developing countries.

4.4. Generality of the findings

The altitude did not influence either the SOC or SON stock or their distribution in the present study in Ethiopian highlands in three regions with different agroforestry traditions but all within the range of 1890 to 2050 m.a.s., in climatic zones where this cropping system has a high potential. Altitude may, however, be important via its influence on climatic factors such as precipitation and temperature (Hobley et al., 2015; Mehta et al., 2014; Wang et al., 2012) and on plant diversity and biomass production (Berg and Laskowski, 2006; Dossa et al., 2013).

The studied multistrata agroforestry systems represented varied agroforestry traditions but all with a relatively high diversity and degree of integration, and thus a high potential for C sequestration. Consequently, the SOC stocks in the study regions were higher than in many tropical agroforestry systems (Oelbermann et al., 2006; Kirby and Potvin, 2007; Dossa et al., 2008; Dube et al., 2011). While the aboveground C stocks were somewhat lower than those reported for coffee agroforestry in southwestern Ethiopia (De Beenhouwer et al., 2016), they were within the range reported elsewhere in the tropics (Montagnini and Nair, 2004; Mutuo et al., 2005; Henry et al., 2009; Luedeling and Neufeldt, 2012; Schmitt-Harsh et al., 2012).

The three distinct regions and 81 separate agroforestry farms likely ensure the generality of the findings on the differences in the C and N stocks for east Africa, while the identified key determinants of the stocks can be generally applied for multistrata agroforestry systems.

4.5. Implications for management

Multistrata agroforestry is not a uniform system. The implementation of the great potential of multistrata agroforestry depends not only on the climatic and topography-related factors which a smallholder farmer hardly can affect. Instead, as shown by the findings of the current study, the potential benefits of multistrata agroforestry on C and N stocks, on their relation C/N and distribution in soil, and thus on climate change mitigation and adaptation, biodiversity, and long-term productivity as well as on food and nutrition security, depend greatly on, how the system is designed and maintained. Current demand for this understanding is high, because effective agroforestry helps countries to accomplish their national determined contributions (NDCs) to implement the Paris Agreement on climate; 40 % of the countries that developed NDCs explicitly propose agroforestry as a solution and 50 % of the developing countries having strategies to reduce deforestation and forest degradation identified agroforestry as the way to combat the decline in forests (Rosenstock et al., 2019).

The findings of the current study highlight the importance of the determinants of soil stocks, because they represent major part of the entire stocks of multistrata agroforestry also regarding C. The exploitation of the aboveground vegetation in building or for food, fodder, energy etc. is decisive and that prevents the overall impact from being measured in the above-ground vegetation on-place. The duration of agroforestry found as the key determinant as well as the proportion of plants with a high basal diameter as another key determinant of C and N stocks emphasizes the importance of the long-term maintenance of each multistrata agroforestry plot to exploit the full gains of the continuously increasing impact on the stocks and thus on climate, soil conservation and productivity. For the above-ground C stock and its preservation, the main crop and further use of the C stock in wood and leaves, and the properties and effects of the energy source replaced by the firewood (Kuisma et al., 2013), are decisive.

Since tree density and proportion of legume trees were most important for N stock and a high proportion of large legume trees reduced C/N whereas a high proportion of large non-legume trees increased C/N, ensuring a long-term maintenance of growing legume trees is essential for the productivity. The delicate balance between C sink and productivity is best managed through the proportion of the large legume and non-legume trees, and the context-dependence and quantification of the management of this balance deserve further attention, modelling and empirical research.

5. Conclusions

C and N stocks in agroforestry systems appear to be highly manageable. The determinants of soil stocks clearly have a higher potential for management than the determinants of aboveground stocks as the size of the soil stock is considerably larger. Consequently, the duration and tree density that are critical for soil stocks appear to be the main determinants, while the legume proportion is also important for the persistence of soil C and for soil productivity via C/N. Regarding management of the delicate balance between soil C and N, more empirical in-depth research is needed. In addition, the potential of practices such as pruning and soil amendment which currently vary little among farms, deserve further research.

As a conclusion, there is great potential to manage the stocks of C and N and their persistence, as well as their distribution in soil, through understanding their biophysical determinants in multistrata agroforestry. Such management practices can employ the synergy between supporting, regulating, and provisioning ecosystem services to counteract climate change, and can simultaneously increase soil productivity and food security there where they are under the highest pressure.

CRediT authorship contribution statement

Mesele Negash: Conceptualization; Data curation; Investigation; Methodology; Resources; Visualization; Roles/Writing – original draft.

Janne Kaseva: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing – review & editing.

Helena Kahiluoto: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

We are grateful to the farmers who allowed the collection of data from their farms. Special thanks are extended to Hanna Mäkinen and Zebene Asfaw for their help in facilitating the collection of soil samples. We also thank the staff at the soil laboratory of Natural Resources Institute Finland for the soil analyses. This work was supported by the Academy of Finland (SOILMAN project, Decision No. 270106/294135).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158185.

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