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SOIL SCIENCE

C And N Stocks And Soil Organic Matter Dynamics In Succession Agroforestry Systems In Brazil

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Abstract: Soil organic matter is closely linked to the quality of Agroecosystems and directly influences the agricultural production and the environmental conditions. Understanding of soil organic matter dynamics in agroforestry systems requires studies with a temporal focus, since the changes in its chemical composition tend to follow a gradual behavior. The aim of this study was to investigate the dynamics of changes in stocks and chemical composition of soil organic matter under agroforestry, using systems in different stages of vegetation succession. The soil sampling was carried out from trenches, and litter fractions were also sampled. The samples were collected from different layers of the soil profile under the following conditions: Control; agroforestry with 1 year; agroforestry with 3 years; agroforestry with 7 years and Forest in natural regeneration. The following attributes/parameters were determined/calculated: i) C and N contents and stocks and C/N ratio; ii) C and N proportions in soil granulometric fractions and iii) kinetics of organic matter accumulation in soil with the time of systems evolution. The results showed: i) The C/N ratio tended to increase in depth but did not show a clear variation between the systems evaluated; ii) the adoption of successive agroforestry practices has the potential to increase the C and N stocks in soil; iii) the soil organic matter accumulation occurs gradually during the systems evolution and is mainly related to the particulate fraction (> 0.053 mm).

Key words: Agroecology, C and N kinetics accumulation, neossol, vegetation succession.

INTRODUCTION

Soil organic matter (SOM) is closely linked to the quality of Agroecosystems and directly influences the continuous flow of energy and matter within the system, as well as the interaction of this flow with the system's surroundings (Vezzani & Mielniczuk 2009). SOM is primarily regulated by the input and decomposition of organic residues (Maia et al. 2008) and is connected to the global balance of carbon (C) and nitrogen (N) cycles. In soils under natural vegetation, the levels/stocks of these elements tend to remain stable (Bortolon et al. 2009). Thus, when this balance is disrupted, the rates of C and N input and output tend to become imbalanced, generally resulting in the loss of these elements from the soil system (Cerri et al. 2008, Hanke & Dick 2019).

In tropical and subtropical environments, SOM plays a significant role as a nutrient source for organisms; nutrient retention; environmental filtering through heavy metal complexation and xenobiotic substances compartmentalizationin, soil aggregate stability; water hold capacity, soil hydraulic conductivity and aeration (Kaur et al. 2018, Hanke & Dick 2019). Furthermore, SOM can serve as a C and N source, as well as energy for heterotrophic microorganisms, making it a fundamental Agroecosystems component (Nelson & Cox 2002, Gliessman 2007).

Within the context of developing discussions about a new agricultural model for Brazil, the concept of Agroforestry Systems (SAFs) emerges. In essence, SAFs refers to specific arrangements of production systems that combine agricultural crops and forest species, and even animal components, into a systemic mechanism designed on a temporal scale (Souza & Piña-Rodrigues 2013). The adoption of SAFs technology is seen as a strategy for environmental regeneration, sought after due to degradation and declining quality of life, owing to its efficiency in restoring degraded areas.

In Brazil, numerous studies have highlighted the influence of SAFs on soil quality (Maia et al. 2006, Mamani-Pati et al. 2012, Nigussie & Kissi 2012, Notaro et al. 2014), nutrient redistribution in soil profile (Nogueira & Oliveira 2008, Nigussie & Kissi 2012), control of soil, water, and nutrient losses through erosive processes (Aguiar et al. 2010, Berhe et al. 2014), as well as their role as c and n reservoirs (Maia et al. 2007, 2008, Simões et al. 2010, Silva et al. 2014, Ribeiro et al. 2019), and their impact on soil structural quality (Silva et al. 2011, Tavares et al. 2018). These studies indicate that SAFs can serve as important alternatives for soil and water use, management and conservation. In Brazil south region, despite some initiatives focused on establishing SAFs as an alternative to the dominant agricultural model, significant gaps and information deficiencies persist regarding the diverse effects these systems can have on soil resources.

Understanding the SOM dynamics requires studies with a temporal focus, as changes in the SOM chemical composition tend to manifest gradually (Souza et al. 2009, Sacramento et al. 2013) in response to alterations in Agroecosystem management practices. The objective of this study was to investigate the dynamics of SOM stock changes under agroforests. To achieve this, systems in distinct stages of vegetational succession were examined.

The study aims to contribute to the broader understanding of how Agroforestry systems impact the C and N stocks, as well as the SOM dynamics, especially in varying ecological succession stages. Such investigations are essential for advancing sustainable agricultural practices and promoting the efficient and balanced use of soil and water resources, aligning with the overarching goal of achieving ecological resilience and agricultural productivity.

MATERIALS AND METHODS

The present study was conducted on an agroforestry farm located in the municipality of Pelotas, Rio Grande do Sul, Brazil (Agroforestry Property Schiavon). Soil samples were collected by opening trenches to an approximate depth of 0.5 meters. The soil was classified as Typical Dystrophic Regolithic Neosol with medium texture (approximately: clay = 33%; silt = 27%; sand = 41%) (Embrapa 2018). Soils in the region originate from the apparent crystalline basement of the Pelotas batholith and are developed from a framework of granitic rock (Physiographic Region of Southeastern Hills - RS). The relief area varies from undulating to strongly undulating, resulting in soils with low to intermediate degrees of pedogenic evolution (field observation).

The region climate was classified as Cfa in according to the Köppen climate classification system, with an average temperature of 18°C and an annual average precipitation of 1380 mm (Alvares et al. 2014). The property spans 9.3 hectares, distributed as follows: i) 6.2 hectares of productive area (successional agroforestry systems); ii) 3.1 hectares under permanent preservation conditions (regenerating natural forest). The property's coordinates are 31°

43’31” S 52°55’72” W. Further details regarding geographical, geomorphological information, as well as the model based on the Visible Atmospheric Resistance Index in the property’s Visible Region (Vegetation Index - VARI) can be observed in Figure 1.

The SAFs employed on farm operate in a successional manner, meaning they are organized in both time and space based on the processes involved in the natural ecosystem succession. This arrangement entails a variation in plant groups, transitioning from simpler levels (initial stage of the system - pioneering system) to more complex levels of organization (intermediate and late stages - secondary, primary, and climax systems). The initial stages involve the cultivation of annual crops (e.g., beans - *Phaseolus vulgari*s L.; cassava - *Manihot esculenta*, beetroot - *Beta vulgaris esculenta*, banana - *Musa sp*., zucchini - *Curcubita pepo*,

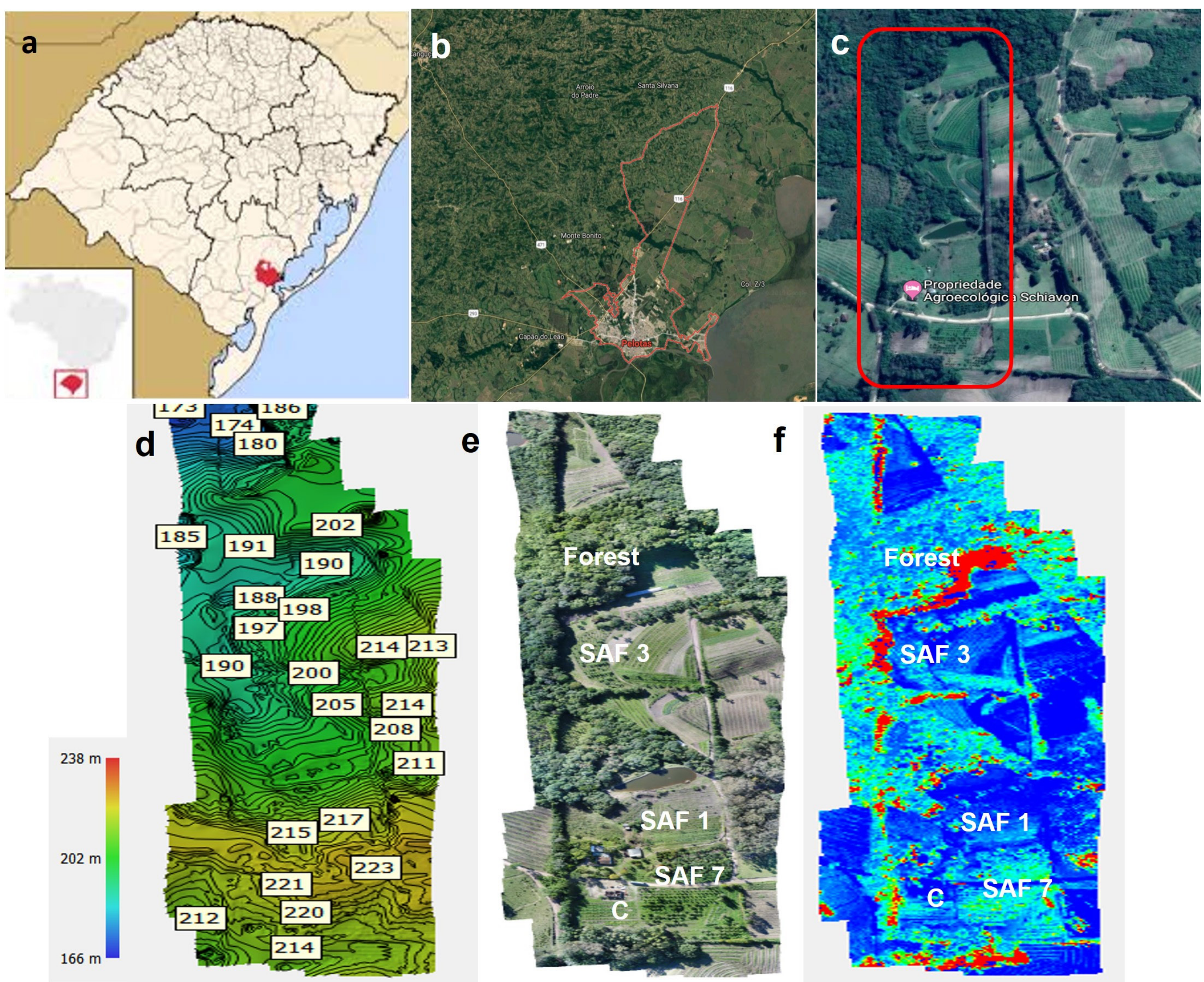

Figure 1. Geographic and Aerophotogrammetric Products of the Study Area: a) Location of Rio Grande do Sul State in Brazil and municipality of Pelotas position in state. b) Satellite image focusing on the municipality of Pelotas, showing the approximate location of study area - Serras do Sudeste / RS. c) Satellite image focusing on the “Agroforestry Farm Schiavon” - study area. d) 3D Digital Terrain Model of area, created by overlaying 292 high-resolution RGB aerial photographs captured by an unmanned aerial vehicle (UAV) - Phantom-4 PRO drone. e) RGB Orthophoto. f) VARI (Visible Atmospherically Resistant Index) indicating differences in vegetation density among the sampled areas (systems).

maize - *Zea mays*, and others). These crops are interspersed with tree seedlings selected for their fruit-bearing potential, wood extraction, and service provision (e.g., black wattle - *Acacia decurrens*, citrus - *Citrus sp*., persimmon - *Diospyros kaki*, palm heart - *Euterpe oleraceae*, Brazilian pepper tree - *Schinus terebinthifolius*, grape - *Vitis labrusca*, Japanese raisin tree - *Hovenia dulcis*, Paraná pine - *Araucaria angustifolia*, and others).

As the initial systems develop and fruit-bearing tree species grow, the intermediate and late stages emphasize the production of fruits and wood products. In these stages, these tree species expand their presence in the production system and coexist with a diverse array of understory species that can tolerate partial or total shading. Notably, among these understory plants are species of Poaceae and herbaceous plants considered Unconventional Food Plants (PANCs). In these later stages of the SAFs, the practice of pruning becomes essential for managing internal light conditions within the plant stand and replenishing nutrients to the soil. Consequently, SAFs differ over time primarily due to the growth stage and diversity of plants, aiming to mimic the natural processes that occur during vegetation succession in forest environments.

With increasing development time, agroforests tend to exhibit higher levels of net primary productivity (NPP) and greater organic material input into the soil through pruning and the natural shedding of above-ground tissues (leaves and branches). Furthermore, as an agroforest matures, a larger volume of soil tends to be explored by the roots of tree and shrub species.

Due to the areas of the evaluated situations being relatively small, in each study condition sampling was carried out at five (5) different points, in a grid-shaped sampling. The soil samples, both disturbed and undisturbed (monoliths and stainless steel cylinders of known volume), were collected from different soil profile layers (0-5; 5-10; 10-20; 20-40; and 40-50 cm - 5 replicates, n = 5) under the conditions described in Table I.

The disturbed samples were subsequently air-dried, ground, and sieved through a 2 mm mesh sieve, resulting in air-dried fine earth (TFSA). The undisturbed samples were used to determine soil physical attributes, which will be presented later. Additionally, litter samples were collected using squares templates with sides measuring 25 cm (area of 0.0625 m²) during the coldest (June) and hottest (December) months.

The litter fractions were visually separated into new litter (SN) - the uppermost layer composed of plant residues more similar to canopy plant tissues - and old litter (SV) - the layer directly above the mineral soil surface and

Table I. **Identification of the evaluated conditions and additional information.**

Condition evaluated	Condition identification	Description of condition
Control	C	Initial system: without agroforestry pratices
SAF1	SAF1	Agroforestry with 1 year of age
SAF3	SAF3	Agroforestry with 3 years of age
SAF7	SAF7	Agroforestry with 7 years of age
Forest	Forest	Reference system: natural regeneration forest with 30 years, with the use of agroforestry practices, analogous to other systems, in the first 15 years

consisting of plant residues in a more advanced stage of decomposition. The SN and SV fractions collected in winter and summer were dried, their mass determined, and subsequently stored for characterization purposes, as presented and discussed in detail in Hanke et al. (2024a). It's important to note that during the área conversion to agroforestry system (starter point), external organic cover materials (leaves, pruning residues, and dry pasture cuttings) were added to the soil to aid in its conservation. For informational purposes, the biomass input (cover) in each situation was approximately: T (1.84 kg biomass m^{-2} $year^{-1}$); SAF1 (1.43 kg biomass m^{-2} $year^{-1}$); SAF3 (2.27 kg biomass m^{-2} $year^{-1}$); SAF7 (2.86 kg biomass m^{-2} $year^{-1}$); and Forest (2.91 kg biomass m^{-2} $year^{-1}$), similar to observations by Selecky et al. (2017) in other agroforestry areas in tropical region of South America.

The organic carbon (C) and total nitrogen (N) contents were determined through dry combustion using an elemental analyzer C/N (Vario El III). Soil density was determined using undisturbed samples, following the procedure described by Embrapa (1997). The C and N stocks were calculated based on the proportion of these elements in the soil mass. To calculate the soil mass were used the undisturbed samples collected. After obtained the soil mass it was calculated the C and N stocks using the followed equation: Ms = Ds x Ec x A, where Ms = soil mass; Ds = soil density; Ec = layer thickness; and A = area (m^2) (Hanke & Dick 2017a). The soil density values are showed and discussed in detail in Hanke et al. (2024b), but they ranged from 1.08 to 1.43 g cm^{-3}, decreasing over time of succession.

To understand the processes and mechanisms involved in C and N retention and accumulation in evaluated conditions, SOM was physically fractionated based on the samples particle size, following the method described by Cambardella & Ellioti (1992). In "snapcap" containers, 20 g of air-dried fine earth were weighed, and 60 mL of sodium hexametaphosphate solution (5 g L^{-1}) was added. The suspension was agitated for 15 hours on a horizontal shaker. Subsequently, the material was passed through a 53 µm sieve with the aid of water jet. The material retained on the sieve, constituting the particulate organic matter (POM) and soil sand fraction, was transferred to a plastic container and dried in an oven at 40°C. The dried sample was weighed, ground, and analyzed for C and N contents through dry combustion using an elemental analyzer C/N (Vario El III).

The suspension passing through the 53 µm sieve, containing the silt and clay fractions, was transferred to a 1000 mL graduated cylinder, being its volume adjusted with pH 10 water. Subsequently, these fractions were separated according to Stokes' Law. Successive clay extractions were performed through cycles of dispersion and siphon sedimentation until the solution became translucent. The clay fraction was transferred to a plastic container, leaving only the silt fraction in the graduated cylinder. The fractions were flocculated with 1 mol L^{-1} hydrochloric acid (HCl), and the supernatant was discarded. After drying at 60°C, the fractions were weighed, ground, and the C and N content was determined using an elemental analyzer C/N (Vario El III). The C/N ratio was calculated for each SOM fraction.

The data were statistically described (mean and standard deviation) and subsequently analyzed using: i) simple linear regression models; ii) exponential (kinetic) models to investigate SOM accumulation in bulk samples and particulate fractions (>0.053 mm): $f(x) = a(1-e^{-bx})$ - this exponential model assumes that the rate of C and N accumulation is decreasing and that the saturation of C and N tends toward the maximum, represented by the parameter "a" of the function, as the system's evolution time tends to infinity. The maximum time for C and N saturation in the system was assumed to be the value at 99% of the parameter "a."; iii) Principal Component analysis: Principal component analysis: using a correlation matrix as a measure of similarity, with the dispersion data being subjected to the bootstrap resampling test (p<0.05) - sampling with replacement - to evaluate the stability pattern of the ordering diagram.

RESULTS AND DISCUSSION

Soil organic matter atributes and parameters

The C and N content and C/N ratio of litter fractions samples varied as follows, respectively: i) C: SN = 378.1, 20.9 and 18; SV = 392.2, 23.7 and 17; ii) SAF1: SN = 382.3, 25.8 and 15; SV = 389.1, 26.8 and 14; iii) SAF3: SN = 411.5, 28.6 and 14; SV = 425.6, 27.9 and 15; iv) SAF7: SN = 417.8, 32.8 and 13; SV = 433.8, 33.4 and 13 e; v) Forest: SN = 455.7, 31.9 and 14; SV = 484.4, 36.2 and 14 (Table II). In general, the C and N contents tended to increase over time during the development of systems, especially N, since the C/N ratio tended to decrease over time (Table II). This fact is probably due to changes in the structure of vegetation over time, including, in late stages of agroforestry evolution, species richer in nitrogen compounds.

In bulk samples, the C and N contentes showed a clear increase in the following order: C < SAF1 < SAF3 < SAF7 < Forest (Table II). The agroforestry system with the shortest development time (SAF1) showed C and N contents more similar to those observed in the control group (C), while the other two agroforestry systems (SAF3 and SAF7) presented an intermediate situation, closely resembling the levels observed in the reference system (Forest), as expected. The organic C content ranged in surface (considering 0-20 cm) from 13.1 (C) to 35.1 g kg^{-1} (Forest), and in subsurface (considering 20-50 cm) from 6.2 to 13.0 g kg^{-1}, being the C content increase more pronounced in soil surface layer. On the other hand, in surface the total N content ranged from 0.90 to 3.11 g kg^{-1}, and in subsurface from 0.41 to 0.90 g kg^{-1} (Table II). This increase in SOM content is directly related to the input of organic material through litterfall and management of tree species pruning, over time of agroforestry systems (Souza & Piña-Rodrigues 2013).

The C/N ratio ranged from 9 to 43 (Table II) and, in general, showed a tendency to increase with depth, which could be associated with the percolation of more aromatic compounds in the subsurface and/or the aliphatic compounds selective preservation due to their higher biochemical recalcitrance (Kalbitz et al. 2000, Hanke & Dick 2017a, b). Furthermore, the high biogeochemical processes intensity in systems with high net primary productivity, as well as the high plant diversity may be responsible for the greater consumption of N in surface soil layers (Odum 1988, Gliessman 2007, Simões et al. 2010). This fact preventing this material, even of hydrophilic structure, from percolating through the soil profile and enriching subsurface horizons with nitrogenous groups. This hypothesis about the higher N consumption at the surface by systems can be supported by the higher C/N ratio observed in the subsurface layers of soils under Agroforestry (SAF1, SAF3, and SAF7) compared to C and Forest. Another aspect that could have led to the increase in the C/N ratio is the presence of pyrogenic carbon in the subsurface, as observed and decribed by other authors (Knicker et al. 2013, Santos et al. 2018, Hanke & Dick 2019, 2017a).

The stocks of C and N ranged from 6.32 to 12.60 and from 0.35 to 0.98 kg m^{-2}, respectively, showing a clear trend of increase from the conditions of C and SAF1 to the agroforests with

Table II. **Carbon (C) and Nitrogen (N) Contents, C/N Ratio, and Proportion of C and N in Litter farctions, Soil bulk samples and Soil granulometric physical fractions (sand, silt, and clay).**

Attribute / layer	C		N		C/N	C (% of total C)		N (% of total N)		C/N	C (% of total C)		N (% of total N)		C/N	C (% of total C)		N (% of total N)		C/N
	g kg^{-1}					Sand-particulate (>0.053 mm)					Silt (0.053-0.002 mm)					Clay (<0.002 mm)				
C SN	378.1	±10.2	20.9	±1.91	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C SV	392.2	±11.8	23.7	±1.61	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C 0-5 cm	13.1	±1.1	1.12	±0.11	12	12.7	±2.2	8.2	±0.8	18	26.4	±9.5	21.8	±8.8	15	60.9	±7.8	69.9	±8.1	10
C 5-10 cm	16	±0.8	1.01	±0.13	16	12	±1.8	9.8	±3.0	21	19.3	±12.3	20.7	±7.5	16	68.8	±10.5	69.5	±6.9	16
C 10-20 cm	10.1	±1.2	0.72	±0.10	14	11.4	±3.5	9.3	±4.6	18	9.7	±3.3	11.5	±3.0	12	78.9	±3.5	79.2	±5.4	14
C 20-40 cm	9.2	±1.2	0.42	±0.02	22	7.3	±3.6	7	±3.0	20	11.6	±4.0	13.2	±3.3	17	81.1	±2.9	79.7	±3.8	21
C 40-50 cm	6.1	±2.2	0.44	±0.07	14	4.2	±1.5	7.9	±1.9	37	9.2	±2.5	8.6	±3.2	14	86.6	±2.3	83.5	±2.6	14
SAF1 SN	382.3	±12.6	25.8	±1.32	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAF1 SV	389.1	±10.7	26.8	±1.51	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAF1 0-5 cm	19.3	±3.2	1.42	±0.21	14	14.3	±2.3	14.2	±4.6	14	18	±10.3	9.8	±4.9	28	67.8	±8.7	76	±6.0	12
SAF1 5-10 cm	15.9	±2.9	1.02	±0.18	16	15.7	±2.9	13.8	±1.1	17	15.4	±12	15.9	±6.8	16	68.9	±9.6	70.3	±6.9	15
SAF1 10-20 cm	10	±2.3	0.73	±0.04	14	9.9	±1.1	11.5	±3.8	12	10.2	±4.3	9.5	±6.0	16	79.9	±3.5	79	±3.0	15
SAF1 20-40 cm	7.2	±2.3	0.32	±0.04	23	8.4	±3.2	7.2	±2.9	25	8.9	±4.3	11.5	±3.3	17	82.7	±3.0	81.3	±3.8	26
SAF1 40-50 cm	4.9	±0.6	0.11	±0.01	45	4.4	±2.3	7.7	±1.1	22	6.6	±3.2	6.5	±2.7	49	89	±2.4	85.8	±2.7	15
SAF3 SN	411.5	±17.1	28.6	±2.11	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAF3 SV	425.6	±16.3	27.9	±2.33	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAF3 0-5 cm	20.1	±5.1	1.11	±0.13	18	22.1	±3.6	22	±7.1	18	15.6	±8.0	9.4	±7.5	30	62.3	±5.3	68.6	±11.1	17
SAF3 5-10 cm	20.4	±2.2	1.51	±0.31	14	24.3	±4.5	21.3	±1.7	15	13.1	±9.6	6.9	±4.4	26	62.6	±5.8	71.7	±4.4	12
SAF3 10-20 cm	13.8	±2.2	0.71	±0.17	19	15.3	±1.6	17.8	±5.9	19	8.9	±4.6	7.7	±2.9	23	75.8	±3.4	74.5	±4.6	21
SAF3 20-40 cm	9.3	±1.7	0.43	±0.09	22	13.1	±5.0	11.2	±4.6	28	8.5	±5.7	5.6	±3.5	39	78.4	±2.8	83.2	±4.4	14
SAF3 40-50 cm	6.7	±0.7	0.3	±0.04	22	6.8	±3.5	11.9	±1.7	10	8.8	±4.0	2.8	±1.3	65	84.4	±2.3	85.3	±1.9	18
SAF7 SN	417.8	±9.5	32.8	±1.87	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAF7 SV	433.8	±14,2	33.4	±1.92	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAF7 0-5 cm	31.2	±3.9	1.21	±0.11	26	24.5	±4.0	24.5	±7.9	25	18.1	±8.0	12.4	±6.9	35	57.4	±4.9	63.1	±10.2	13
SAF7 5-10 cm	24.2	±3.7	1.49	±0.19	16	27	±5.0	23.7	±1.9	28	15.3	±9.6	10.3	±4.2	24	57.7	±5.4	66.1	±4.0	14
SAF7 10-20 cm	23.8	±4.1	2.71	±0.23	9	17	±1.8	19.7	±6.5	38	13.2	±4.5	11.7	±3.5	10	69.8	±3.1	68.6	±4.2	9
SAF7 20-40 cm	14.1	±2.1	1.44	±0.13	10	14.5	±5.6	12.4	±5.1	11	13.3	±6.1	10.9	±3.7	12	72.2	±2.8	76.6	±4.1	9
SAF7 40-50 cm	7.8	±1.2	0.33	±0.11	24	7.5	±3.9	13.2	±1.8	18	14.7	±4.3	8.3	±1.3	50	77.8	±2.1	78.5	±1.7	19
Forest SN	455.7	±24.7	31.9	±1.81	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Forest SV	484.4	±25.8	36.2	±1.74	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Forest0-5 cm	40.2	±7.1	3.41	±0.29	12	26.5	±4.3	26.4	±8.5	12	20.2	±7.9	14.9	±6.8	16	53.3	±4.5	58.7	±9.5	11
Forest 5-10 cm	36.9	±5.3	3.6	±0.18	10	29.1	±5.4	25.6	±2.0	22	17.3	±9.7	13	±4.0	13	53.6	±5.0	61.4	±3.8	9
Forest 10-20 cm	29.1	±2.6	2.21	±0.33	13	18.4	±2.0	21.3	±7.1	11	16.8	±4.4	15	±4.1	15	64.9	±2.9	63.7	±3.9	13
Forest 20-40 cm	14	±1.4	0.91	±0.04	15	15.6	±6.0	13.4	±5.5	17	17.2	±6.4	15.4	±3.9	17	67.1	±2.4	71.2	±3.8	14
Forest 40-50 cm	11.3	±1.0	0.74	±0.07	15	6.9	±3.6	13.7	±1.9	28	20.9	±3.9	13.3	±1.4	24	72.2	±2.0	72.9	±1.6	15

***Obs: SN = new litter; SV = old litter; C = control (without agroforestry); SAF1 = agroforestry with 1 year age; SAF3 = agroforestry with 3 years age; SAF7 = agroforestry with 7 years age; Forest = naturally regenerating forest with 30 years age; C = soil organic carbon content of the sample; N = total sample nitrogen content; C/N = ratio between the C and N contents; C (% of total C) = proportion of total organic C in each granulometric fraction; N (% of total N) = proportion of total N present in each granulometric fraction; values followed by ± represent the standard deviation among the sample replicates.**

longer age (SAF3 and SAF7) and Forest (Figure 2). This result may also be related to the increase in C and N contents supplied via litter, as shown in Table II. These stocks are consistent with the C stocks observed by other authors under tropical and subtropical climate conditions (ranging from 3.4 to 41.8 kg m^{-2}) (Novaes-Filho et al. 2007, Scheer et al. 2011, Hergoualc'ha et al. 2012, Hanke & Dick 2017a). For organic C, it was found that, on average (considering all conditions evaluated),

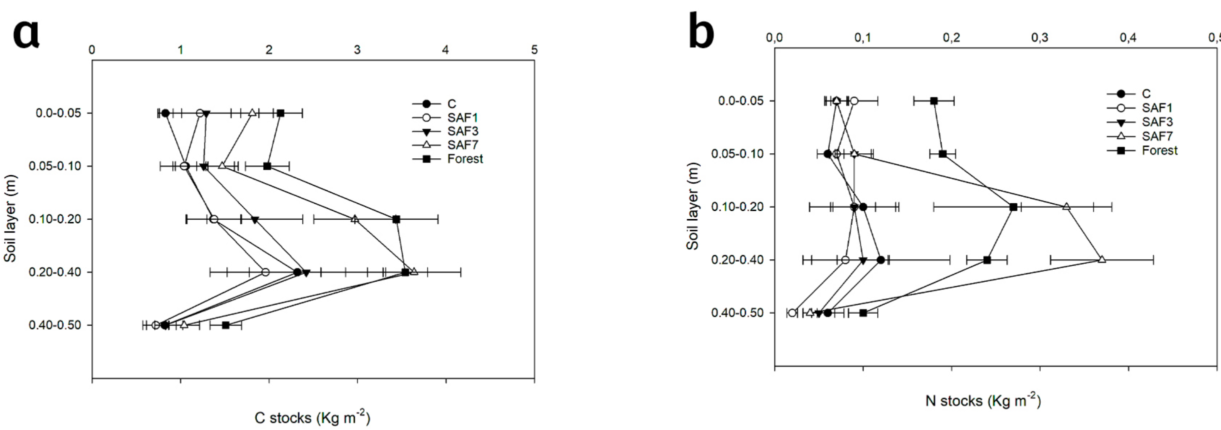

Figure 2. Soil C and N Stocks in evaluated conditions. (*Note: C = control; SAF1 = agroforestry with 1 year age; SAF3 = agroforestry with 3 years age; SAF7 = agroforestry with 7 years age; Forest = naturally regenerating forest with 30 years age).

56.6% of the stocks of this element are located in the surface layer of the soil (0-20 cm).

However, the amount of C stored in the 0-20 cm layer was not the same in all analyzed situations, being lower in C (50.9%), higher in the reference system (Forest) (59.9%), and intermediate in the Agroforests (57.2, 57.5, and 57.6%). In the subsurface (20-50 cm), the observed trend was different, as expected, where the % of the total C stock was higher in C (49.1%), lower in Forest (40.1%), and intermediate in the Agroforests (42.4, 42.5, and 42.8%). Although the stocks in surface layer were higher than those observed in the subsurface layers, this difference was not very significant and highlights the importance of the subsurface environments in C stock. Other authors have also previously highlighted the importance of diagnostic horizons for SOM storage in tropical and subtropical environments (Benites et al. 2005, Novaes-Filho et al. 2007, Scheer et al. 2011, Hanke & Dick 2017a).

The surface layer contribution to the N stock was also more important than the subsurface, with an average of 62.8% of the total N stock stored in 0-20 cm. However, significant differences were observed between the analyzed conditions, with the percentage of the total N stock in profile (contained in 0-20 cm) increasing in following order: SAF7 (54.4%) < C (56.1%) < SAF3 (62.5%) < Forest (65.3%) < SAF1 (71.4%). In 20-50 cm layer, the proportion of N stocks varied in the following order: SAF1 (28.6%) < Forest (34.7%) < SAF3 (37.5%) < C (45.9%) < SAF7 (45.6%). The N stocks compartmentalization did not show a clear trend as was observed for C. Although these elements are strongly associated in organic structure synthesis and cell reproduction, as well as decomposition-related processes, there may be differences in terms of microbiological demand and chemical mobility, which could have caused these observed differences. In other words: the change in the composition of the litter itself, in terms of C and N content, can contribute to explaining this process.

In general, the adoption of agroforestry practices lead to a significant increase in SOM stocks. These results demonstrate that, in addition to the enhancement of nutrient content (especially bases interacting with mineral exchange sites and SOM) and the reduction of acidity componentes (Hanke et al. 2024b), the establishment of agroforestry production systems can serve as important sinks for atmospheric carbon. Similar findings were also observed by Selecky et al. (2017).

SOM dynamics and Mechanisms of C and N Accumulation

To investigate the SOM accumulation kinetics, the C and N stock values were used in relation to the years of evolution of the evaluated systems, considering: i) C as the starting point (0 years); ii) SAF1 (1 year of evolution); iii) SAF3 (3 years of evolution); iv) SAF7 (7 years of evolution); and Forest (30 years of evolution). Thus, the accumulated C and N stock values in the entire soil profile, in the 0-20 cm (surface) and 20-50 cm (subsurface) layers, were plotted (variable y) against time (variable x), where an exponential first-order model (approaching a maximum) was fitted. Figure 3 shows the behavior of C and N stocks of the samples (entire soil profile, surface - 0-20 cm - and subsurface - 20-50 cm) over time. The stock values were standardized by the difference from the smallest value (the smallest value was considered equal to zero (0) at time zero (0), meaning zero at the origin of the scatter plot. The other values were adjusted by the difference from the smallest value of the origin, thus maintaining the symmetry of the distribution of the original data).

Based on the mathematical models, the time required for the soil (entire profile) to reach the maximum C and N stocks was 36.7 and 35.6 years, respectively (Figure 3). However, a difference was observed between the time required for saturation of these stocks between the surface layer (0-20 cm) and subsurface layer (20-50 cm), varying for C from 34.6 to 30.8 years, respectively. For N, this difference was more pronounced, ranging from 54.0 years (0-20 cm) to 22.3 years (20-50 cm). As mentioned earlier, these differences are likely due to the distinct dynamics that these elements exhibit in complex production systems (high diversity of organisms and high input of organic material). Based on the “a” parameter, readjusted by the smallest value (which for the model application was considered = “0”), the maximum C stock that the soil can reach was: i) 12.9 kg m^{-2} (entire soil profile); ii) 7.7 kg m^{-2} (0-20 cm); and iii) 5.6 kg m^{-2} (20-50 cm) (Figure 3).

For N, the maximum stocks that can be achieved were: i) 1.08 kg m^{-2} (entire soil profile); ii) 0.68 kg m^{-2} (0-20 cm); and iii) 0.45 kg m^{-2} (20-50 cm) (Figure 3). Based on the “b” parameter of the model (a determinant parameter in the slope structure of the model - the rate of C and N accumulation), it can be observed that, in general, the rate of SOM accumulation is higher in the subsurface (Figure 3). However, this subsurface compartment is smaller, and precisely because

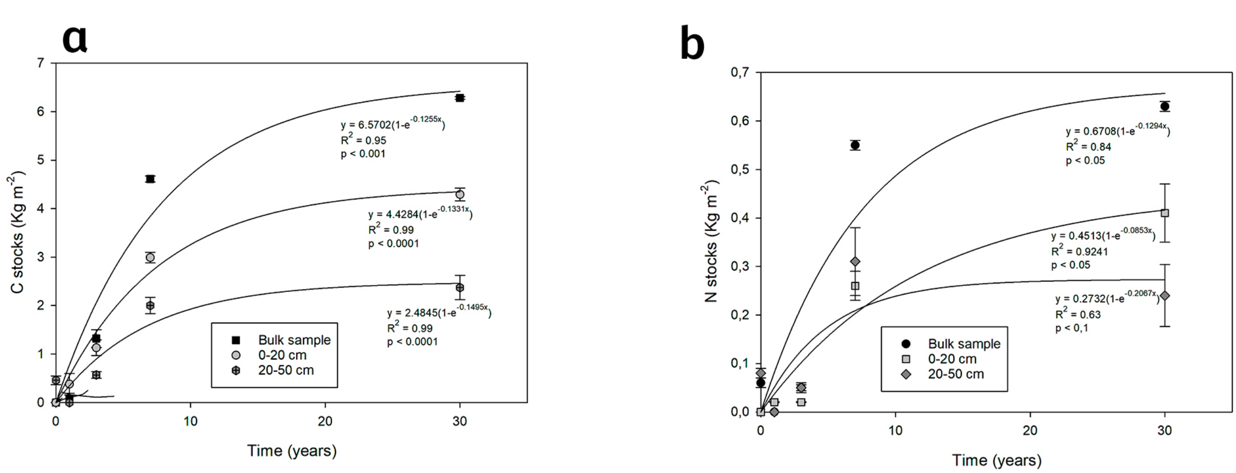

Figure 3. Soil C and N accumulation in evaluated systems.

of this, saturation occurs more quickly (shorter time to reach the maximum). Additionally, the greater inaccessibility of the subsurface layers may result in a virtually faster saturation of this C and N compartment. On the other hand, at the surface, the C and N accumulation rate is lower, even though the compartment is larger and more accessible (contact with litter and with higher root and organism density). As a result, the time to reach the maximum C and N stocks is longer in the 0-20 cm compartment than in the 20-50 cm layer.

The C and N accumulation dynamics were significantly different, with the N accumulation rate being lower at the surface than in the deeper layer. This fact may be related to the greater N consumption in surface environments due to higher organism activity and interaction among them.

To better understanding of the possible mechanisms involved in C and N accumulation in this soil, SOM was granulometrically fractionated, as presented earlier. The proportion of C and N in the granulometric fractions was generally higher in the clay fraction (<0.002 mm) (71.0% and 73.5%, respectively), followed by the fraction >0.053 mm (sand) (14.7% and 15.0%, respectively), and the silt fraction (0.053 to 0.002 mm) (14.3% and 11.5%, respectively) (Table I). The percentage of particulate C and N (>0.053 mm) varied from 4.2 to 29.1% and 7.0 to 26.4%, respectively (Table I). The percentage of C and N in the silt fraction (0.053 to 0.002 mm) ranged from 6.6 to 26.4% and from 2.8 to 21.8%, respectively. Meanwhile, the proportion of C and N in the clay fraction ranged from 53.3 to 89.0% and from 58.7 to 85.8%, respectively (Table II). These results highlight the importance of the clay fraction for SOM retention and it was supported by the results observed by Barreto et al. (2011).

The C/N ratio was higher in the silt fraction (0.053 - 0.002 mm) (24.0), intermediate in the particulate fraction (>0.053 mm) (20.2), and lower in the clay fraction (<0.002 mm) (14.6), suggesting that the stabilized compounds in the finer fraction are in a more advanced stage of decomposition (Table II).

Between the control condition (C) and the reference system (Forest), an increase in the percentage of C and N in the particulate fraction (>0.053 mm) was observed, with intermediate values for the Agroforestry systems (SAF1, SAF3, and SAF7) (Table II). This increase in the proportion of C and N content in the particulate fraction was accompanied, as expected, by a decrease in the percentage of total C and N in the clay fraction. This result suggests that the increase in C and N content and stocks over the development time of (agro)forestry systems primarily occurs through the particulate fraction. This result is supported by the existence of significant positive correlations between C and N stocks and the percentage of these elements in the particulate fraction (>0.053 mm) (Figure 4). This does not necessarily mean that the mechanisms of interaction between SOM and clay fraction minerals (phyllosilicates and Fe and Al oxides) are not important for SOM retention and stability, but rather that the high inputs of organic material in these systems are the main drivers of increase C and N stocks (Hanke et al. 2015). The decrease in the percentage of total C and N in the clay fraction suggests that the organo-mineral interaction mechanism may be more important for maintaining SOM stocks under conditions of low C and N input. Thus, when the rates of SOM input increase, the maintenance of C and N stocks in these systems depends on the high and frequent influx of organic material into the system. Similar results were also observed by Fontaine et al. (2007). In this regard, the conversion of these (agro) forestry practices to less efficient ones in terms of net primary productivity and organic material

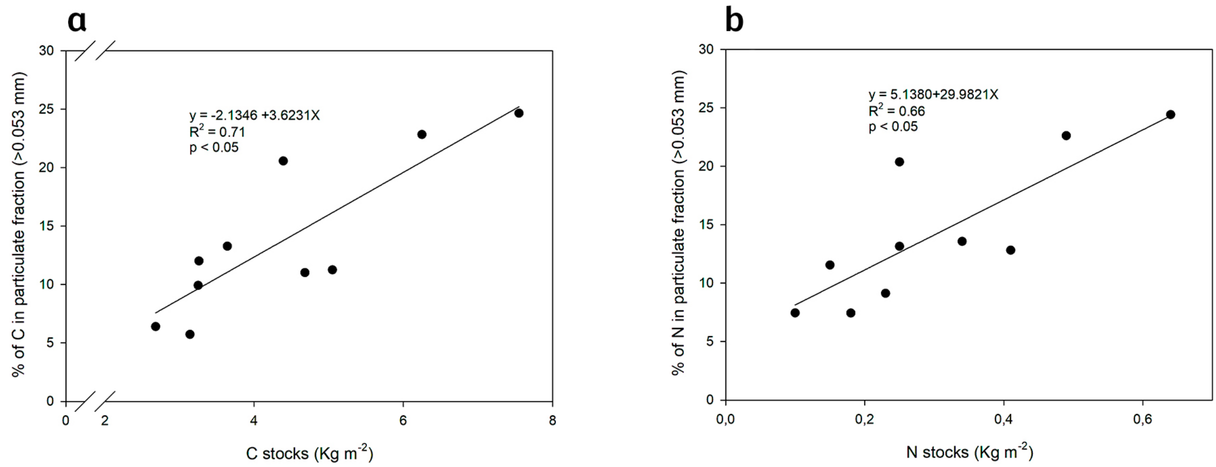

Figure 4. Linear regression between: a) Carbon stocks and % of Carbon in particulate fraction; b) Nitrogen stocks and % of Nitrogen in particulate fraction.

input to the soil could result in rapid and drastic reductions in SOM content. Similar results were also observed by Hergoualc'ha et al. (2012).

By applying Principal Component Analysis (PCA) to the dataset, it was possible to observe that approximately 70% of the total variance was explained by the first two principal components (Figure 5). Principal Component 1 (PC1 - "x" axis) contained 56.2% of the variance, while Principal Component 2 (PC2 - "y" axis) explained approximately 14.4% of the total variance (Figure 5). The pattern of data dispersion in the ordination diagram was considered significant through the application of the bootstrap resampling test ($p < 0.05$), allowing the use of the diagram for interpreting the results.

The variables most strongly correlated with PC1 were: i) C (bulk sample); ii) N (bulk sample); iii) C/N (bulk sample); iv) Particulate C (> 0.053 mm); v) Particulate N (> 0.053 mm); vi) C - Clay (<0.002 mm); vii) N - Clay (<0.002 mm); and viii) C/N - Clay (<0.002 mm), as can be seen from the ordination scores (Table III). Thus, PC1 is a variable produced by compiling most of SOM attributes/ parameters used in this study, highlighting the observed inverse correlation between C and N in bulk soil samples and particulate fractions with the C and N associated to clay minerals (Table III and Figure 5). This reinforces the earlier discussion that the enrichment of C and N in the evaluated systems is possibly more related to the input of new particulate coarse materials than organic compounds interacting with the clay surfaces. On the other hand, PC2 is a variable produced by compiling the following MOS variables: i) Particulate C/N (> 0.053 mm); ii) C - Silt (0.053-0.002 mm); iii) N - Silt (0.053-0.002 mm); and iv) C/N - Silt (0.053-0.002 mm) (Table III). Thus, PC2 - which explains a smaller proportion of total data variance - includes variables that are apparently more related to N in the particulate fraction and the percentual of C and N in silt fraction, being less important to describe the pattern of data dispersion than the variables more strongly correlated with PC1.

Regarding PC1, a clear separation between surface layers (to the right of the origin) and subsurface layers (to the left of the origin) of the evaluated conditions was observed (Figure 5). By applying the minimum spanning tree algorithm - which establishes connections between sample units based on their degree of similarity - it was generally observed that the sample units form a gradient of transition in SOM content and the proportion of C and N in particulate fraction from deeper soil layers and systems with shorter

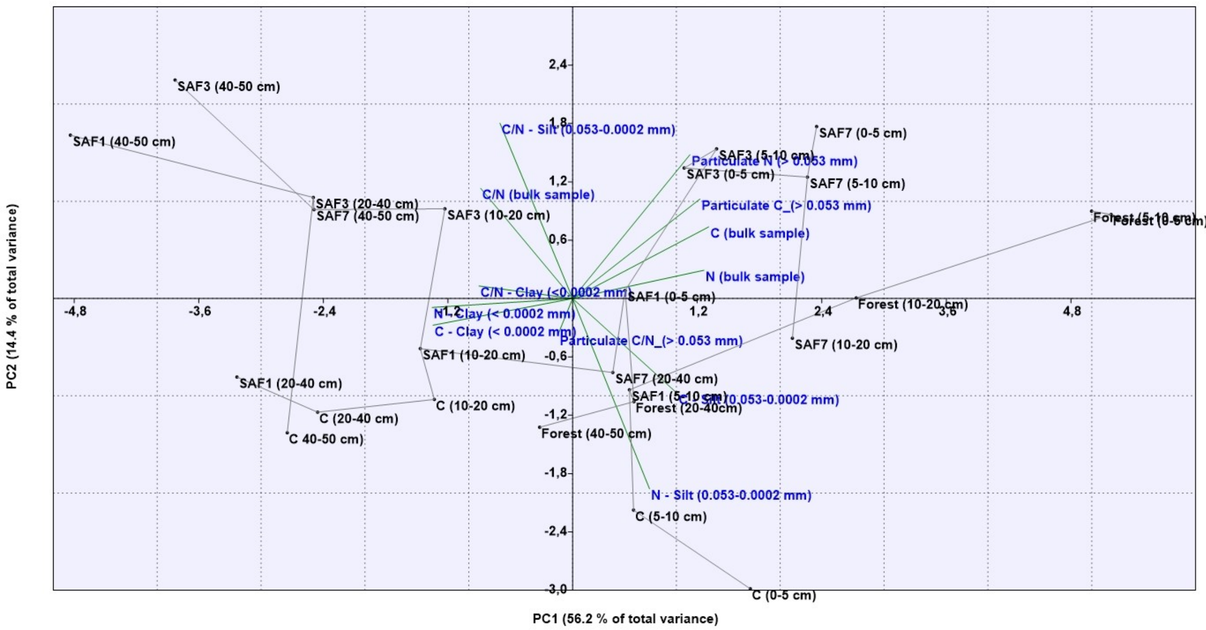

Figure 5. **Ordering diagram by Principal Component Analysis involving SOM variables and sampling units of evaluated conditions. *Note: C = control (without agroforestry); SAF1 = agroforestry with 1 year age; SAF3 = agroforestry with 3 years age; SAF7 = agroforestry with 7 years age; Forest = naturally regenerating forest with 30 years age; C (bulk sample) = soil organic carbon content; N (bulk sample) = total soil nitrogen content; C/N (bulk sample) = ratio between the C and N contentes of soil; Particulate C (>0.053 mm) = particulate soil organic C in sand fraction; Particulate N (>0.053 mm) = particulate soil N in sand fraction; Particulate C/N (>0.053 mm) = C/N ratio organc of particulate material in sand fraction; C – Silt (0.053 – 0.002 mm) = organic C associated to silt fraction; N – Silt (0.053 – 0.002 mm) = total N associated to silt fraction; C/N – Silt (0.053 – 0.002 mm) = C/N ratio of SOM associated to silt fraction; C – Clay (<0.002 mm) = organic C associated to clay fraction; N – Clay (<0.002 mm) = total N associated to clay fraction; C/N – Clay (<0.002 mm) = C/N ratio associated to SOM in clay fraction.**

development time (units farther and to the left of the origin) to the surface layers of systems with longer evolution time.

Concerning PC2, sample units appearing below the origin tend to have a higher proportion of C and N in silt fraction, with emphasis on sample units belonging to the control condition (Figure 5). It is possible that, in the initial stages of system evolution, due to the lower input of particulate organic materials into the soil (SOM >0.053 mm) - because of the lower stage of plants development and lower deposition of vegetal residues on litter - the silt fraction may concentrate a higher SOM relative proportion. Over time, the input of coarse particulate materials increases, and the relative proportion of C and N in the intermediate fraction (0.053 – 0.002 mm) decreases. Thus, a transitional gradient in relation to PC2 can also be observed, however in a distinct manner from that seen for PC1.

To analyze the dynamics of POM (>0.053 mm), the same mathematical model applied to the previous C and N stock data was applied to the % of C and N in this fraction (Figure 6), using the same criteria applied before (standardization by the difference from the smallest value, meaning “0” value at time “0” - origin of the scatter plot). Based on the exponential model, the time required for the POM (considering the entire profile) to reach the maximum C and N stock was 15.5 and 13.2 years, respectively. Similar to the

Table III. **Correlation between SOM variables and ordering axes (PCA - principal components 1 and 2).**

Soil variables / Principal component	PC1	PC2
C (bulk sample)	0,93	0,27
N (bulk sample)	0,90	0,11
C/N (bulk sample)	-0,63	0,42
Particulate C (> 0.053 mm)	0,87	0,38
Particulate N (> 0.053 mm)	0,80	0,55
Particulate C/N (> 0.053 mm)	-0,10	-0,14
C - Silt (0.053-0.002 mm)	0,71	-0,76
N - Silt (0.053-0.002 mm)	0,53	-0,73
C/N - Silt (0.053-0.002 mm)	-0,50	0,67
C - Clay (< 0.002 mm)	-0,95	-0,10
N - Clay (< 0.002 mm)	-0,96	-0,03
C/N - Clay (<0.002 mm)	-0,64	0,05

***Note: PC1 = principal componente 1; PC2 = principal componente 2; C (bulk sample) = soil organic carbon content; N (bulk sample) = total soil nitrogen content; C/N (bulk sample) = ratio between the C and N contentes of soil; Particulate C (>0.053 mm) = particulate soil organic C in sand fraction; Particulate N (>0.053 mm) = particulate soil N in sand fraction; Particulate C/N (>0.053 mm) = C/N ratio organc of particulate material in sand fraction; C – Silt (0.053 – 0.002 mm) = organic C associated to silt fraction; N – Silt (0.053 – 0.002 mm) = total N associated to silt fraction; C/N – Silt (0.053 – 0.002 mm) = C/N ratio of SOM associated to silt fraction; C – Clay (<0.002 mm) = organic C associated to clay fraction; N – Clay (<0.002 mm) = total N associated to clay fraction; C/N – Clay (<0.002 mm) = C/N ratio associated to SOM in clay fraction.**

stocks in the whole soil samples, a difference was observed between the time required for saturation of this fraction between the surface layer (0-20 cm) and subsurface layer (20-50 cm), ranging for C from 16.2 to 13.7 years, respectively.

For N, this difference ranged from 11.9 years (0-20 cm) to 17.8 years (20-50 cm) (Figure 6). Thus, the results suggest that C exhibits faster saturation in the subsurface, while N shows faster saturation, in this fraction (>0.053 mm), at the surface. These differences are likely due to the fact that N is demanded in larger quantities and consumed more rapidly, especially in surface environments with higher microbial activity in more complex production systems. On the other hand, N losses in agroforestry systems with longer development times may be more pronounced at the surface. Based on the "a" parameter, readjusted by the smallest value (which for the model application was considered = "0"), it was observed that for C, the proportion of the maximum stock in this fraction that can be reached was: i) 21.9% (entire soil profile); ii) 18.4% (0-20 cm); and iii) 15.2% (20-50 cm).

For N in POM (>0.053 mm), the proportion of maximum stocks that can be achieved were: i) 20.7% (entire soil profile); ii) 22.5% (0-20 cm); and iii) 14.7% (20-50 cm). Based on the "b" parameter of the model (a determinant parameter in the slope structure of the model - the rate of C and N accumulation), it can be observed that, in general, the rate of C accumulation in this fraction is higher in the subsurface, as expected, since fewer losses occur through mineralization in the subsurface environment compared to the surface. However, this subsurface compartment of the particulate fraction is smaller, and precisely because of this, its saturation is faster (shorter time to reach the maximum). The inaccessibility of subsurface layers, as well as in the stocks in the whole soil samples, can result in a quicker saturation of this C and N compartment. On the other hand, at the surface, the rate of C accumulation is lower, even though the compartment is larger and more accessible (contact with litter and higher root and organism density). This is why the time required to reach the maximum proportion of C stock in the particulate fraction is longer in the 0-20 cm compartment than in the 20-50 cm layer. Conversely, N exhibited the opposite behavior, with the time required to reach the maximum proportion of this element's stock in this fraction being shorter at the surface than in depth (Figure 6). This is possibly due to the higher N input at the surface, which, although virtually constituting a larger compartment (22.5%) than

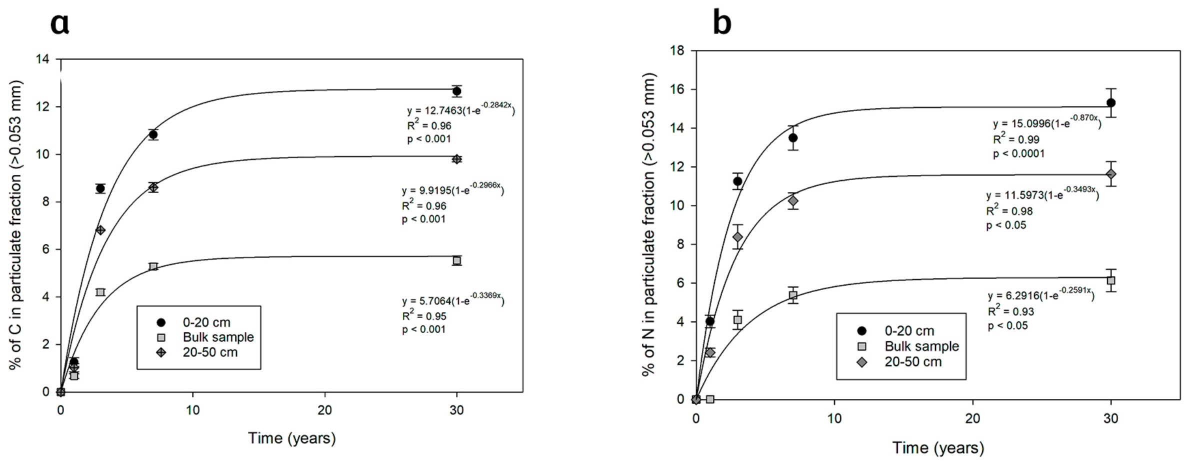

Figure 6. **C and N accumulation curves in the particulate fraction during the Systems' Evolution.**

in the subsurface (14.7%), shows greater N production (litter deposition, biological nitrogen fixation and cycling by microbiota).

These results can contribute as support and reinforcement for policies that promote the transition from conventional agricultural practices to ecologically based agroforestry practices. It is important to emphasize that actions related to the Carbon agenda in Brazil require information from various productive forms on how organic matter can be efficiently managed. The analyzed agroforestry systems demonstrate a great capacity to optimize the dynamics of SOM accumulation in agricultural systems, as well as the potential use of these technologies in the recovery of degraded agroecosystems. Furthermore, these results can contribute to a better understanding and systematization of the potential ecosystem services offered by ecologically based systems, addressing aspects of social, economic, and environmental sustainability. It is expected that these systems, with substantial SOM accumulation capacity, can influence many other attributes/parameters related to the recovery of local biodiversity. However, further studies - in related subjects - are necessary to ensure more information on this topic.

CONCLUSIONS

The adoption of successional agroforestry practices showed potential to increase soil C and N stocks. The accumulation of SOM occurs progressively during the system's evolution and it is primarily related to the particulate fraction (>0.053 mm).

The interruption of C and N input rates into the system can lead to a rapid decrease in SOM due to the virtual absence of mechanisms for stabilizing particulate organic matter in the studied soil.

The adoption of agroforestry practices can contribute to the sustainability of diversified food production agricultural systems, enhancing the potential for C and N stock expansion in soil profiles developed from granitic rock in the Southern region of Brazil.

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