



# No-till systems restore soil organic carbon stock in Brazilian biomes and contribute to the climate solution

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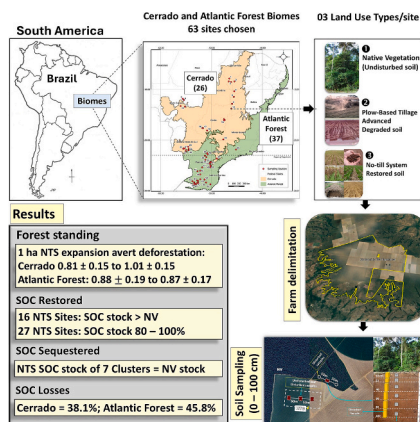
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## HIGHLIGHTS

- SOC stocks assessed in 63 sites to 1-m depth for three land-uses in two biomes
- Depletion of SOC stocks under PBT compared to NV was 38.1 % and 45.8 % in two biomes
- SOC stocks in 16 NTS sites exceeded those under NV and another 27 restored 80 to 100 %
- SOC stock at seven of 13 edaphoclimatic zones was comparable to that under NV
- NTS restored the SOC stock (1-m depth) to the same level of NV in 36.4 to 55.0 years

## GRAPHICAL ABSTRACT



## ARTICLE INFO

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**Abbreviations:** SOC, Soil organic carbon; LUT, Land use types; NV, Native vegetation; NTS, No-till system; PBT, Plow-based tillage; LU, Land use; LUC, Land use change; MAT, Mean annual temperature; MAP, Mean annual precipitation; Pg, Petagrama (1 billion ton); BMPs, Best management practices; ESM, equivalent soil mass;  $\rho_b$ , bulk density; GHG, global greenhouse gas; CA, Conservation agriculture; Bha, billion hectares; Mha, million hectares; ABC, agriculture-based low C-CO<sub>2</sub> emissions.

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## ABSTRACT

No-till systems grounded in the principles of conservation agriculture can restore the soil organic carbon (SOC) stock and environmental sustainability. Here, we assessed the SOC stocks to 1-m depth for three land-uses (i.e.,

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Land use types

Climatic zones, Cerrado biome

Atlantic Forest biome

Soil organic carbon stock

native vegetation - NV, no-till system - NTS, and plow-based tillage - PBT) across 26 sites in the Cerrado and 37 sites in the Atlantic Forest biomes of Brazil for 3402 soil samples. The depletion of SOC stocks under PBT compared to NV was equivalent to a loss of 38.1 % and 45.8 % of the original NV SOC stock for Cerrado and Atlantic Forest biomes, respectively. The SOC stocks of 16 NTS sites exhibited levels that exceeded those under NV, and SOC stock was restored from 80 to 100 % of its NV levels in 27 other NTS sites across the Brazilian biomes. The SOC stock at seven of 13 edaphoclimatic zones (Clusters) was comparable to or more than that under NV. The duration of NTS to restore SOC stock to that under NV ranged from 36.4 to 55.0 years for the Cerrado and Atlantic Forest biomes, respectively. The NTS/NV SOC stock ratio indicated that one hectare of land under NTS has the potential to avert deforestation for food production of  $0.81 \pm 0.18$  to  $1.01 \pm 0.15$  ha of NV in the Brazilian biomes. In essence, NTS has been demonstrated to effectively restore SOC stocks in Brazil's biomes and play a pivotal role in integrating agriculture as a part of the solution for mitigation strategies for climate change.

## 1. Introduction

The global challenge is to develop a food production system that can simultaneously prevent deforestation, meet the food needs of 9.8 billion people by 2050, and make agriculture part of the solution to mitigating anthropogenic climate change. Food production is responsible for approximately 25 % of global greenhouse gas (GHG) emissions, (Crippa et al., 2021) and increasing to  $\approx 33$  % when all agricultural products are considered (Poore and Nemecek, 2018).

Furthermore, soils account for approximately 15 % of the total global warming increase (radiative forcing) due to net anthropogenic emissions (Kopittke et al., 2024). As demonstrated by Ballantyne et al. (2012), the loss of 1 gigaton of soil organic carbon (SOC) to the atmosphere results in an increase of 2.13 ppm in carbon dioxide (CO<sub>2</sub>) emissions, thereby contributing to the climate change.

Estimates of historical C loss due to land use (LU) and land-use change (LUC) range from 45 to 114 Pg C (mean = 79.5 Pg C) for the pre-1870 period (Anderson et al., 2020) and from 108 to 188 Pg C (mean = 148 Pg C) for 1870 to 2014. The global SOC stock loss is estimated at 116 Pg C due to cultivation (Lal, 2004; Anderson et al., 2020), which represents  $\approx 8.0$  % of the currently total SOC stored in the world's soils to 1-m depth. The historical GHG emissions, including deforestation and burning of NV, estimated at 620 Pg CO<sub>2</sub>eq, have strong impacts on atmospheric composition, and represent 10.8 % of the C stock of terrestrial vegetation (Houghton, 2014).

In Brazil, the onset of soil degradation triggered by the conversion of NV to pastureland, has been aggravated by overgrazing and the abandonment of degraded pastures. Historical SOC losses include two primary categories: (a) C emitted into the atmosphere by the burning of NV during the conversion of natural ecosystems to agricultural land, estimated at 7.3 Pg C, and (b) oxidation of SOC by plowing, equivalent to a loss of 6.5 Pg C (Sanderman et al., 2017). Historically, South America has been a relatively minor emitter of GHGs from fossil fuel combustion, contributing 0.25 Pg C yr<sup>-1</sup> (Sá et al., 2017). However, emissions from LU and LUC, particularly from deforestation in the Amazon and Cerrado biomes, amount to 0.34 Pg C yr<sup>-1</sup> (Gloor et al., 2012). These emissions have had a significant impact on the increase in atmospheric concentration of carbon dioxide (CO<sub>2</sub>) (Groppo et al., 2015). However, adoption of best management practices (BMPs) has the potential to offset GHG emissions by 0.30 to 1.17 Pg C yr<sup>-1</sup> globally (Neufeldt et al., 2015; Lal et al., 2018), which represents 2.7 to 10.4 % of global GHG emissions (Le Quéré et al., 2015; Friedlingstein et al., 2024).

The role of agricultural sector to anthropogenic climate change is a contentious issue due to diverse effects on crop performance and biomass-C production (Sloat et al., 2020; Grigorieva et al., 2023). However, the debate often neglects to differentiate between agricultural systems that use BMPs associated with soil conservation principles and those that rely on plow-based tillage (PBT) (Abdo et al., 2024). The challenge is to establish an agricultural system that can ensure resilience to precipitation and temperature fluctuations (Abbass et al., 2022; WMO, 2025; Xiang et al., 2024) to reduce the negative impact on crop performance, which can aggravate the uncertainty in annual crop production, return of biomass-C to the soil, and transformation of

agricultural land into an effective SOC sink (Zomer et al., 2017; Frank et al., 2024).

The area of earth's land surface is about 13 billion hectares (Bha), of which 1.9 Bha is allocated to cultivation of annual crops for food production (FAO, 2017), and 0.2 Bha or 11.5 % of the land area uses conservation agriculture (CA) (Kassam et al., 2022; Rezaei et al., 2023). The CA-based NTS management can make production systems less vulnerable and more resilient to anthropogenic climate change (Rezaei et al., 2023), improve crop performance (Teng et al., 2024), and increase profitability while protecting soil, water, and air resources (Jayaraman et al., 2021). Brazil has the second largest land area under NTS in the world and estimated at 43 million ha (Mha) in 2020 (Kassam et al., 2022). It represents 20.5 % of the global food production (Kassam et al., 2022) and has a pronounced impact in the Brazilian grain production (Fig. S1). In 2020, the total CO<sub>2</sub> emissions from LU and LUC in Brazil were 0.59 Pg C (Tsai et al., 2024). The Brazilian agriculture-based low C-CO<sub>2</sub> emissions (ABC) plan show that contribution of NTS to C mitigation estimated at 0.14 Pg was equivalent to 22.8 % of agricultural emissions in 2020 (Tsai et al., 2024).

Several studies have been conducted since 1970 to assess sequestration of SOC by a range of strategies such as using a comparative approach, and by comparing soil analyses from plots managed by conventional tillage with those by NTS in long-term tillage experiments. These studies have been conducted in the northern (West and Post, 2002; Lal et al., 2018; Sperow, 2020; Stroud, 2020; Mondal et al., 2023) and southern (Bayer et al., 2006; Sá et al., 2014; Sá et al., 2022) hemispheres, and thus, have a wide range in rate of SOC sequestration. Some experiments resulted in negative or neutral (West and Post, 2002) rates, while others showed positive and high rates under soil/site specific situations (Sá et al., 2006; Sá et al., 2022). Such a differential response is attributed to several factors that impact C storage in soil, including soil type, mineralogy, texture, sampling depth, landscape position, and climatic conditions, particularly mean annual temperature (MAT) and precipitation (MAP), which directly affect crop growth, potential biomass-C input, and the rate of SOC decomposition and sequestration (Davidson and Janssens, 2006; Conant et al., 2011).

The effectiveness of SOC sequestration through NTS depends on the use of CA principles such as eliminating soil disturbance (restricted only to the sowing line), maintaining permanent soil cover, and diversifying crop rotations (Derpsch et al., 2014). Therefore, the present study is designed to test the hypothesis that NTS based on its principles, would lead to the restoration of SOC stock irrespective of the edaphoclimatic zones. Thus, the aim of this study is to demonstrate if an agricultural model based on the long-term adoption of NTS, in association with input of biomass-C through production systems, can replenish the depleted SOC stocks and environmental sustainability in Brazilian biomes and make agriculture a part of the solution to climate change, alongside other sectors.

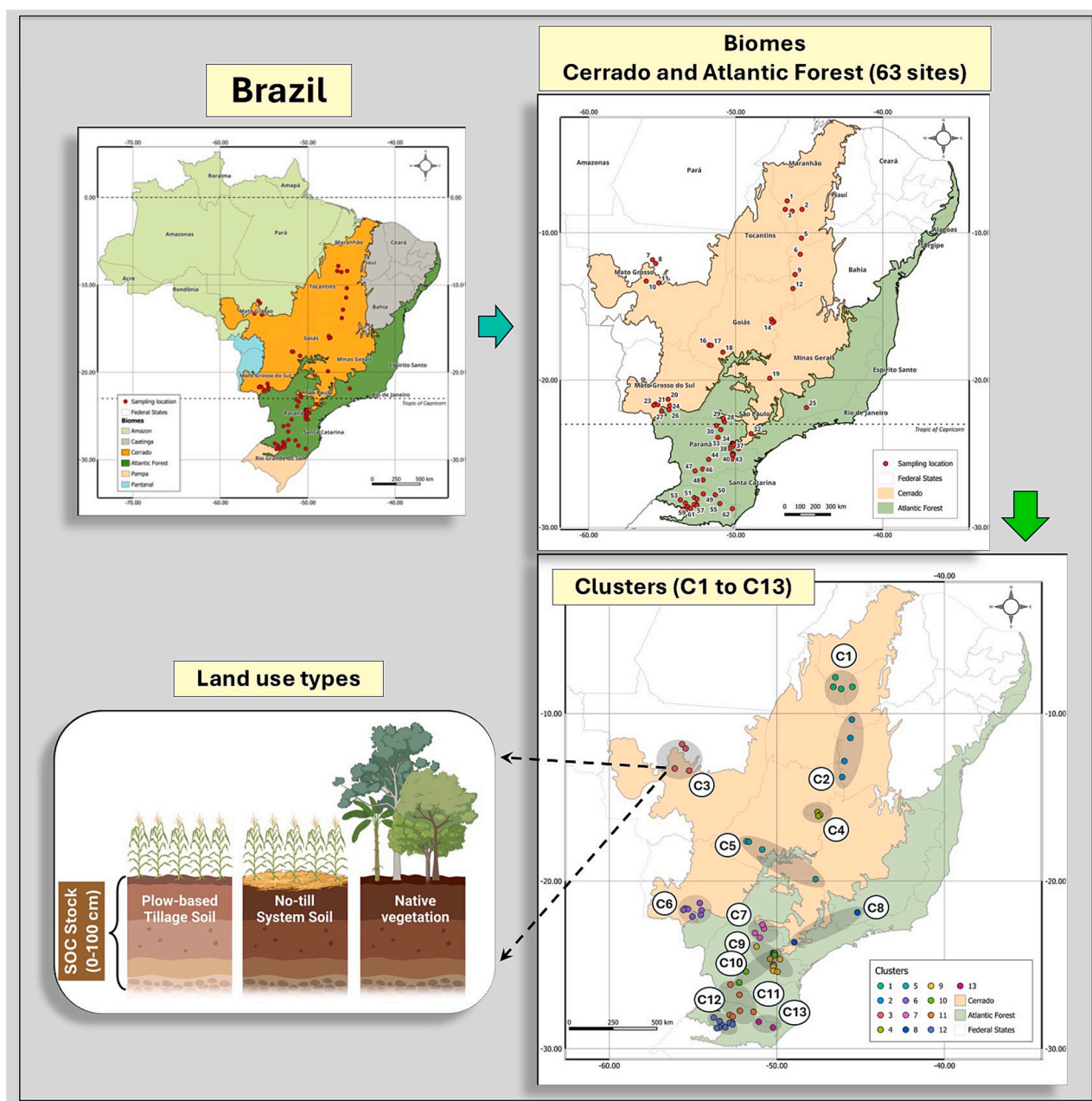


Fig. 1. Brazil map and sites distribution in the Cerrado and Atlantic Forest biomes, Cluster map and the land use types.

## 2. Material and methods

### 2.1. Study sites across biomes

Brazil's land area of 8.51 Mkm<sup>2</sup> comprises of six biomes: the Amazon, Cerrado, Atlantic Forest, Caatinga, Pantanal, and Pampa. The country is divided into five climatic zones: Equatorial, Tropical Equatorial, Tropical Central, Tropical Northeast, and Subtropical humid-temperate.

A total of 63 sites chosen for the present study are distributed across two biomes (Fig. 1): 26 sites in the Cerrado and 37 sites in the Atlantic Forest and the surface area of these two biomes (3.14 million Km<sup>2</sup>) is equivalent to 31 % of the Europe continent and contribute to 75 % of Brazilian food production (CONAB, 2024). The sites were selected to represent a range of latitudes within Brazil, spanning from  $-7$  to  $-28^{\circ}$  South. Each site consisted of three Land Use Types (LUT), which were compared to assess losses, gains and restoration of SOC stocks.

### 2.2. Cerrado and Atlantic Forest biomes - brief description

The Cerrado biome, which is characterized by a tropical savanna, covers an area of 2.036.448 km<sup>2</sup> and accounts for approximately 60 % of Brazil's food production (Fig. 1). The mean annual precipitation (MAP) ranges from 600 to 2000 mm, increasing from east to west (Cardoso Da Silva and Bates, 2002), with a mean of 1430 mm. About half of the biome receives rainfall amounts ranging from 1400 to 1600 mm. However, a major part of the Cerrado region experiences a rainless period from April to September.

The mean annual temperature (MAT) is  $>24^{\circ}\text{C}$  in the north and  $<18^{\circ}\text{C}$  in the extreme south. The Cerrado biome is home to the most expansive agricultural regions in Brazil and is currently undergoing substantial alterations in land use and land tenure-ship (Velazco et al., 2019).

The Atlantic Forest biome (Fig. 1) encompasses an area of 1.1 million km<sup>2</sup> and accounts for 15 % of the country's food production. The climate

is influenced by maritime conditions, with high rainfall and mild temperatures throughout the year. The annual rainfall is <700 mm in the northeast arid areas, compared with up to 2500 mm in the subtropical southern region of the country. The central humid subtropical region receives rainfall of 1000 and 1600 mm (Alvares et al., 2013a). The mean annual temperature is greatly influenced by longitude and altitude, with extremes ranging from 8 to 29 °C. Microclimatic patterns in several regions of the country are determined by elevation and relief. In general, higher elevations (> 800 masl) are located on the extreme eastern flank, while lower elevations (Alvares et al., 2013b) are found on the western part of the country. The natural vegetation in the region is characterized by the presence of more than six distinct forest formations, including dense ombrophilous forests, mixed ombrophilous forests, open ombrophilous forests, semideciduous seasonal forests, and deciduous seasonal forests. In addition to these forests, the ecosystem is further diversified by the presence of mangroves, restinga vegetation, high-altitude fields, inland marshes, and forest enclaves, particularly in the Northeastern region.

### 2.3. Land use types

Soil samples were obtained from each site for three categories of LUT, as described briefly below:

1. Native vegetation (NV): The LUT native vegetation is an undisturbed soil associated with a NV that characterizes each site. These soils are under diverse plant species, have a thick layer of organic litter on the surface, and are characterized by a continuous flow of carbon (C), nitrogen (N), and nutrients into the soil, which lead to development of SOC pools. This LUT represents the steady-state dynamic equilibrium of SOC.
2. Plow-based tillage (PBT): Soil under this LUT has undergone significant degradation by the repeated soil disturbance due to the use of disking and harrowing or chisel plow prior to the seeding of crops. This disturbance causes disruptions of soil structure and macroaggregates, and results in the release of organic compounds and cementing agents due to decomposition by microbiota and emission of CO<sub>2</sub> and leading to the soil degradation (Table S1).
3. No-till system (NTS): This LUT involves a systemic approach that is characterized by the principles of the CA based on the absence of soil disturbance (restricted to the sowing line), the maintenance of permanent soil cover, and the diversification of crop rotations based on improving biomass-C input. NTS approach ensures soil input of C, N, and nutrients, which results in the recovery of the C stocks, re-aggregation and formation of new soil structure, and restoration of soil functions. The selected farms were managed according to the principles of CA and use of NTS for a minimum of 18 years (Table S1).

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### 2.4. Soil sampling protocol

Soil samples were obtained in five steps as follows.

1. Characterization of the farms: A template was developed for collecting data on a range of environmental factors, including elevation, landscape characteristics, slope percentage, rainfall patterns, and temperature trends and soil type. These data compiled through satellite imagery were used to integrate the farm information and generate a map to identify the sampling location.
2. NTS plot selection: It involved identification of NTS plot within each farm (Fig. S2 a,b) which accurately represented the farm's performance and was achieved through an evaluation of each farm's plots, which were maintained under a long-term NTS. The selected NTS plot was situated near an area under NV and had the same soil type and textural class as that of the NTS. The plot under PBT was selected according to followings criteria: (1) proximity to NTS and NV and maximum distance up to 10-km within the same microclimate; (2) the same soil type of NTS and NV; (3) the same or similar textural class compared to NTS and NV LUTs; and (4) the frequency and intensity of plow-tillage.

NTS plot selection: It involved identification of NTS plot within each farm (Fig. S2 a,b) which accurately represented the farm's performance and was achieved through an evaluation of each farm's plots, which were maintained under a long-term NTS. The selected NTS plot was situated near an area under NV and had the same soil type and textural class as that of the NTS. The plot under PBT was selected according to followings criteria: (1) proximity to NTS and NV and maximum distance up to 10-km within the same microclimate; (2) the same soil type of NTS and NV; (3) the same or similar textural class compared to NTS and NV LUTs; and (4) the frequency and intensity of plow-tillage.

3. Benchmark and excavation of trenches: The benchmark for soil sampling were chosen based on three criteria: a) variations in the percentage of slope, and b) the soil type present within a given field, and c) textural class. Each benchmark represented a pseudo-replicate and hereinafter designated as Trench 1, 2 and 3.

The benchmark was established at the center of the plot by defining a transect comprising of three equidistant points at 50 m distance from one another (Fig. S2b). Trenches were dug at each benchmark to a specific depth (1.5 m wide, 1.5 m deep and 1.5 m long) using a retro digger. The trench 1, 2 and 3 were dug to obtain soil samples for NTS and PBT LUTs. Soil sampling under NV was conducted in accordance with the following protocol: a trench under NV was dug 50 m within NV to ensure that the sampling points were not influenced by any boundaries (Fig. S2b) and two equi-distant 50 m locations were defined as a benchmark. The protocol for NV involved a trench that was excavated manually to obtain soil core samples for measuring bulk density ( $\rho_b$ ) (Fig. S2c).

4. Soil sampling: Geographic coordinates of the collection points were recorded, marked with a barcode on the plastic bags containing soil samples obtained from each trench, transported to the laboratory, and stored until analyses

A total of six teams from precision agriculture companies were selected and trained for standardization of the soil sampling protocol to ensure the consistency and accuracy of the sampling collection. Soil sampling was done between April 2023 and May 2024 in accordance with the crop calendar and precipitation patterns. Disturbed and undisturbed samples were obtained from each trench at six depths (0–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm), comprising 3402 bulk samples and 3402 undisturbed core samples (i.e., 03 LUT × 03 pseudo-replicates × 06 depths × 63 sites = 3402 samples). Disturbed samples were obtained from each layer, and ≈ 1 kg soil was obtained by a trough



as bulk soil (Fig. S2c). Four samples were obtained from each layer and for each trench face. A total of 3402 cores (5 cm height and 5 cm diameter) were obtained by a core sampler for determination of soil bulk density ( $\rho_b$ ). Two soil cores were obtained from the center of each layer. Each core sample was wrapped in a plastic film, transported to the laboratory, the plastic film removed, soil trimmed, cores dried in an oven at 105 °C, and weighed to compute soil  $\rho_b$ . All samples were shipped to the IBRA Megalab (Sumaré, SP, Brazil) for the soil carbon analyses. The flowchart delineates and synthesizes the context of the present study (Fig. S3).

## 2.5. Soil carbon analysis

Bulk soil samples were stored in a cold room (i.e.,  $\pm 7$  °C) at the IBRA laboratory pending processing and analysis done between November 2023 and July 2024. Prior C analysis, samples were sieved through a 2-mm mesh sieve, homogenized and dried in an oven at 40 °C. Each sample was arranged in a plastic tray and quartered it. From each quarter, a sub-sample of 20 g was collected and ground to pass through a 150- $\mu$ m sieve. This process ensured the desired level of fineness, avoided the biases and represented the original sample collected from the field. A portion of 250-mg of grinding soil was weighed and placed in a combustion boat for analysis. The C concentration was determined using the dry combustion method with a CN elemental analyzer apparatus (LECO 832 Series Combustion, St. Joseph, MI 49085, USA). Being Oxisols ( $\approx 80$  % of the total samples), of acidic pH, soil inorganic C (SIC) was not considered. The remaining soil samples belonged to Argisols (Ultisols) and Cambisols (Inceptisols) according to the U.S. Soil Taxonomy and have an SIC content of  $<0.25$  % (Sá et al., 2013).

## 2.6. Basis and protocol for calculating soil organic C stocks

The SOC stock for each soil layer was calculated by the equivalent soil mass (ESM) method proposed by Ellert and Bethany (Ellert and Bettany, 1995; Ellert et al., 2001), and the equivalent soil mass (ESM) approach has been discussed since 1960s by Nye and Greenland (Nye and Greenland, 1964). Several studies have evaluated soil C storage in terms of mass of C per unit area to a fixed depth (Tiessen et al., 1982; Aguilar et al., 1988), and this approach enables quantitative comparisons among different treatments, but it overlooks the influence of variable soil mass as indicated in the timeline of ESM evolution (Fig. S4).

$$\text{NTS ESL for 0–10 cm} = ((\text{Average } (1098 \text{ Mg ha}^{-1}; 1221 \text{ Mg ha}^{-1}; 1163 \text{ Mg ha}^{-1})) / (1292 \text{ Mg ha}^{-1}) \times 10 = 8.97 \text{ cm}$$

In this study, the rationale was to utilize the soil layers under NV of each site as these represent the natural and undisturbed soil condition and the baseline or natural state of soil as the reference for accounting for the ESM in the PBT and NTS.

In summary, the following three steps were undertaken:

(i) The soil mass was calculated by multiplying the bulk density, expressed in  $\text{Mg m}^{-3}$  by the volume of the soil layer ( $\text{m}^3$ ) for each of the layers within each LUT; (ii) The equivalent soil layer (ESL) for PBT and

NTS, in terms of their relation to NV, was calculated. This was achieved by dividing the soil mass under NV by the PBT or NTS soil mass, and resulting product then multiplied by the corresponding soil depth; iii) The equivalent soil mass was calculated by multiplying the bulk density ( $\text{Mg m}^{-3}$ ) of each layer of PBT and NTS by the equivalent soil layer mass.

### 2.6.1. Example of SOC stock calculation

Calculation of soil mass.

$$\text{NV soil mass (0–10 cm)} = \rho_b \times 1000 \text{ m}^3 \text{ ha}^{-1}$$

Where  $\rho_b$  is the bulk density ( $\text{Mg m}^{-3}$ ) of each soil layer and  $1000 \text{ m}^3 \text{ ha}^{-1}$  is the volume of 0–10 cm layer for 1 ha.

$$\text{Pit 1 : NV } \rho_b \text{ (0–10 cm)} = 1.098 \text{ Mg m}^{-3}$$

$$\text{NV soil mass (0–10 cm)} = 1.098 \text{ Mg m}^{-3} \times 1000 \text{ m}^3 = 1098 \text{ Mg ha}^{-1}$$

$$\text{Pit 2 : NV } \rho_b \text{ (0–10 cm)} = 1.221 \text{ Mg m}^{-3}$$

$$\text{NV soil mass (0–10 cm)} = 1.221 \text{ Mg m}^{-3} \times 1000 \text{ m}^3 = 1221 \text{ Mg ha}^{-1}$$

$$\text{Pit 3 : NV } \rho_b \text{ (0–10 cm)} = 1.163 \text{ Mg m}^{-3}$$

$$\text{NV soil mass (0–10 cm)} = 1.163 \text{ Mg m}^{-3} \times 1000 \text{ m}^3 = 1163 \text{ Mg ha}^{-1}$$

Where, 1.098, 1.221 and 1.163 are the bulk densities value for the 0–10 cm layer for Pit 1, 2 and 3, respectively. Multiplying  $\rho_b$  by the volume of 0–10 cm layer of 1 ha to obtain soil mass.

$$\text{Pit 1 NTS } \rho_b \text{ (0–10 cm)} = 1.292 \text{ Mg m}^{-3}$$

$$\begin{aligned} \text{NTS soil mass (0–10 cm)} &= 1.292 \text{ Mg m}^{-3} \times 1000 \text{ m}^3 \text{ ha}^{-1} \\ &= 1292 \text{ Mg ha}^{-1} \end{aligned}$$

### 2.6.2. Calculation of equivalent soil layer (ESL) for NTS or PBT

The equivalent soil layer of NTS or PBT to NV was calculated by dividing the average of the soil mass value of NV by value of corresponding soil layer of NTS or PBT.

$$\text{NTS ESL} = ((\text{NV soil mass}) / (\text{NTS or PBT soil mass}) \times \text{NV thickness})$$

Thus, 10 cm under NV is equivalent to 8.97 cm under NTS for this specific example.

### 2.6.3. Calculation of ESM SOC stock for NTS or PBT

$$\text{NTS soil layer volume} = 0.0897 \text{ m} \times 100 \text{ m} \times 100 \text{ m} = 897 \text{ m}^3$$

$$\text{NTS ESM (0 – 10 cm)} = \text{NTS soil layer volume (897 m}^3) \times \text{NTS } \rho_b \text{ for 0–10 cm (1.292 Mg m}^{-3}) = 1158 \text{ Mg of soil per 0–10 cm layer}$$

**Table 1**  
Clusters (Edaphoclimatic Zones) characterization.

Clusters	Cluster	Sites per	Latitude	Clusters Composition							
Designation	ID	Cluster	(° South)	Elevation (masl)	Climate classification, Köppen	Mean annual temperature (°C)	Mean annual precipitation (mm)	Dry period per year (months)	Summer	Winter	Soil textural class
Tropical Equatorial Oriental	C1	4	7 to 8	< 300	Af, Cwb, Aw	26.4	1096	6	Hot	Hot	Sand-Clay
Tropical Equatorial Zone	C2	4	10 to 13	770–953	Aw	25.4	1201	6 to 7	Hot	Mild	Sand-Clay
Equatorial/Tropical Center	C3	4	11 to 13	352–472	Aw	25.5–26.1	1850 to 2150	4 to 6	Hot	Hot	Clayey
Tropical Center	C4	3	15.9–16.1	885–1023	Aw	22.6	1028	6 to 7	Hot	Mild	Clayey to Very Clay
Tropical Center	C5	4	17.6–19.9	680–870	Aw	22.9	1158 to 1600	6	Hot	Mild	Clayey
Tropical Center	C6	7	21 to 22	300–587	Aw	23.9	1110 to 1400	5 to 6	Hot	Mild/Cold	Clayey
Subtropical Humid	C7	4	21 to 23	374–476	Aw	22.9 to 23.4	1261 to 1400	5 to 6	Warmer	Cold	Clayey
Subtropical Humid	C8	3	22 to 23	108–873	Cwb, Aw	22.5	1215 to 1400	No Dry period	Warmer	Cold	Clayey
Subtropical Humid	C9	7	23 to 25	820–1040	Cfa	17.6 to 18.3	1440 to 1490	No Dry period	Mild	Cold	Sand-Clay To Clayey
Subtropical Humid	C10	6	24 to 26	960–1040	Cfb	18.8	1450–1700	No Dry period	Mild	Cold	Clayey
Subtropical Humid	C11	6	26 to 28	650–870	Cfb	17.9–19.8	1700–1850	No Dry period	Mild Cold	Cold	Sand-Clay to Clayey
Subtropical/ Temperate	C12	9	28	380–510	Cfb, Cfa	18.34	1600–1850	No Dry period	Mild Cold	Cold	Clayey
Subtropical/Temperate	C13	2	28	900–1080	Cfb	17.9	1600 to 1800	No Dry period	Mild Cold	Cold	Clayey

NTS SOC stock based on ESM (0 – 10 cm)

$$= (26.5 \text{ kg C Mg}^{-1} \times 1158 \text{ Mg of soil}) / 1000 = 30.69 \text{ Mg C ha}^{-1}$$

## 2.7. Soil carbon budget

The soil C budget was computed as follows:

- 1) The historic SOC stock losses for each layer were calculated with the following equation:  
NV SOC stock - PBT SOC stock, in  $\text{Mg C ha}^{-1}$ . The sum of SOC loss for all depths represented the total loss to 1-m depth.
- 2) The historic SOC gains or total SOC sequestered (in  $\text{Mg C ha}^{-1}$ ) were calculated as follows:

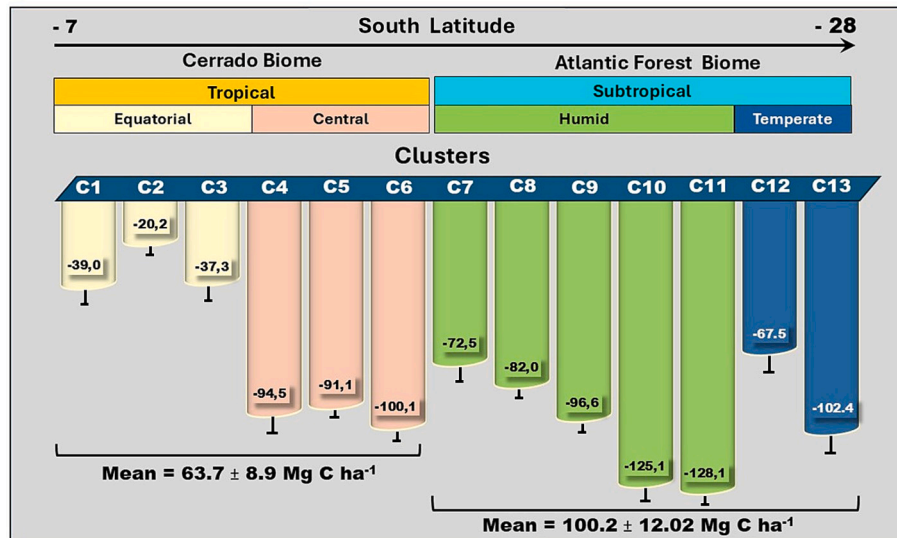
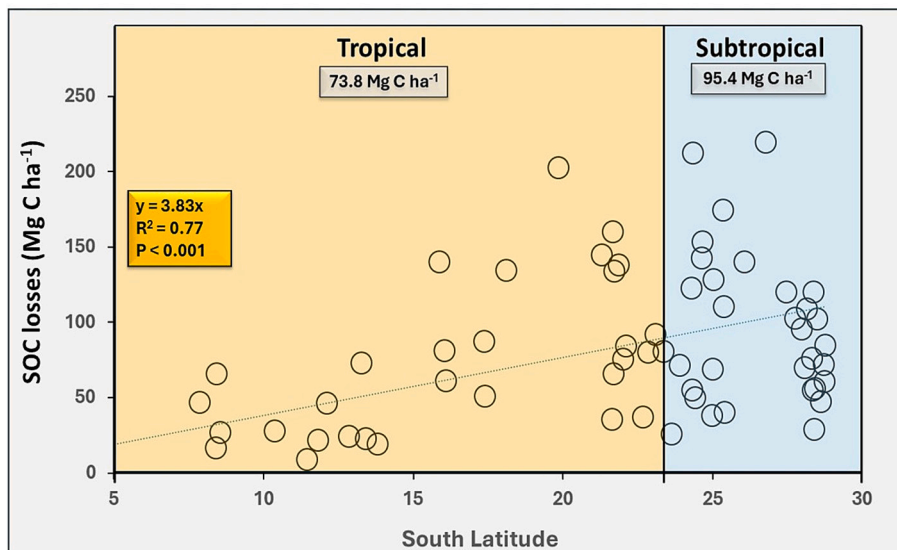
NTS SOC stock – PBT SOC stock for each layer was computed for each site. The SOC to 1-m depth was computed by summing the value for each depth sampled.

- 3) SOC sequestration rates were assessed from the relationship between the NTS duration and the total SOC stock sequestered for each site to 1-m depth. The sequestration rate was assessed from the linear regression between the NTS duration (years) as an independent variable (X-axis) and the total SOC stock sequestered as a dependent variable (Y-axis) for 1-m depth. Four graphical representations were generated: (i) a linear regression including all 63 sites, (ii) a tropical zone-specific representation, (iii) a subtropical zone-specific representation, and (IV) a linear regression using the mean NTS duration of each cluster as the independent variable (X-axis) and the weighted mean total SOC sequestered of each cluster as the dependent variable (Fig. S5 a,b,c). The mean SOC stock for the 1-m depth interval, duration time, and sequestration rates were calculated for pooled data of all sites, representing 13 clusters.

SOC sequestration rates were assessed from the relationship

between the NTS duration and the total SOC stock sequestered for each site to 1-m depth. The sequestration rate was assessed from the linear regression between the NTS duration (years) as an independent variable (X-axis) and the total SOC stock sequestered as a dependent variable (Y-axis) for 1-m depth. Four graphical representations were generated: (i) a linear regression including all 63 sites, (ii) a tropical zone-specific representation, (iii) a subtropical zone-specific representation, and (IV) a linear regression using the mean NTS duration of each cluster as the independent variable (X-axis) and the weighted mean total SOC sequestered of each cluster as the dependent variable (Fig. S5 a,b,c). The mean SOC stock for the 1-m depth interval, duration time, and sequestration rates were calculated for pooled data of all sites, representing 13 clusters.

- 4) Restoration of SOC stock was computed based on the potential of NTS to replenish the SOC stock in comparison to the NV stock for each site was calculated as follows.: a) SOC stock restored = NV SOC stock – NTS SOC stock, in  $\text{Mg C ha}^{-1}$ , for 1-m depth; b) Percentage of SOC stock restored by NTS in relation to NV for 1-m depth was calculated as follows: % SOC restored =  $(100 - (\text{NV SOC stock} - \text{NTS SOC stock}) \times 100 / \text{NV SOC stock})$ .
- 5) NTS SOC stock equivalent to NV SOC stock refers to the minimum area (in hectares) of NTS land use type that is required to maintain one hectare of NV SOC stock. This interpretation is supported by the concept of land sparing (Phalan et al., 2011; Gomiero, 2016), which involves setting aside of less productive agricultural land areas based on the intensification of agricultural practices on more productive land under NTS, for the preservation of NV. The objective is to reduce the necessity for the conversion of NV for agricultural production by increasing yields, biomass-C input, and C storage in the soil through NTS on farmland. The calculation was based on the ratio of SOC stock to 1-m depth between the NV and the NTS according to the expression:  $\text{Ratio NV/NTS} = \text{NV SOC stock} / \text{NTS SOC stock} = x \text{ ha of preserved from deforestation by employing } 1 \text{ ha of NTS}$ .

**(a) SOC stock losses by clusters****(b) Linear regression for SOC losses as function of latitude**

**Fig. 2.** (a) Total soil organic carbon (SOC) stock losses by plow-based tillage (PBT) in the Cerrado and Atlantic Forest biomes for each cluster; (b) linear model for SOC losses in function of latitude for 1-m depth.

## 2.8. Rationale of clusters (Edaphoclimatic zones) and environmental covariates

The cluster approach is conceptually defined as a group of similar items that occur together (Fig. S6). The clustering algorithm chosen for this study is referred to as Spectral Clustering (von Luxburg, 2007) due to its higher efficiency in identifying complex patterns within the datasets. Spectral algorithm package (von Luxburg, 2007) was used for its ability to analyze the spatial variability of environmental covariates, including soil type, soil texture, MAP and MAT patterns, elevation, slope, and land cover coded as C1 to C13 (Table 1). It operates by transforming the data into a lower dimensional space by applying dimensionality reduction methods such as Laplacian Eigenmaps where the clusters are more discernible and differ from the traditional methods such as K-means or hierarchical clustering. In this study, a clustering approach was used to assess environmental variables data spanning 63 distinct geolocations over a seven-year period (Fig. S6). The objective was to infer similarities and groupings among these geolocations based on their environmental profiles, along with geographic information such

as coordinates and elevation to define edaphoclimatic zones. Additionally, the clay content (kg Mg<sup>-1</sup>) measurements obtained from soil sampling data were used for the generation of clusters. The data were subjected to normalization to ensure comparability of variables. The normalization was achieved with the Standard Scalar (von Luxburg, 2007) function from the scikit-learn Python library, which standardizes the scales across all features. Following the completion of the cluster analysis, each geolocation was assigned to a specific cluster based on the degree of similarity observed among its environmental and geological attributes. Furthermore, the integration of geographical attributes enabled the examination of potential correlations or higher-order shared mutual information among environmental patterns and geological factors.

The present study identified thirteen clusters, of which six were located within the Cerrado biome (Table 1). Of these six, three (C1 to C3) were situated within the tropical-equatorial climate zone, and the remaining three (C4 to C6) were located within the Tropical Central zone. Furthermore, seven clusters have been identified in the Atlantic Forest biome, with five located within the subtropical humid zone (C7 to

**Table 2**

Analysis of variance (ANOVA) with values of test statistics for two-tailed analysis for soil organic carbon stock in the Cerrado and Atlantic Forest biomes in function of depth intervals (0–20, 0–40, 40–100, 0–100 cm) in four climatic zones (Tropical Equatorial, Tropical Central, Subtropical Humid and Subtropical Temperate) and in 13 clusters (C1 to C13).

Climatic Zones	Clusters	Depth interval and test statistics															
		0–20				0–40				40–100				0–100			
		<i>n</i>	<i>df</i>	<i>F</i> / $\chi^2$	P value	<i>n</i>	<i>df</i>	<i>F</i>	P value	<i>n</i>	<i>df</i>	<i>F</i>	P value	<i>n</i>	<i>df</i>	<i>F</i>	P value
Cerrado/ Tropical Equatorial	C1	36	2	30.35	0.000	36 <sup>§</sup>	2	25.88	0.000	36	2	18.08	0.000	36	2	21.40	0.000
	C2	36 <sup>‡</sup>	2	22.47	0.000	36	2	34.00	0.000	36 <sup>§</sup>	2	2.21	0.126	36	2	14.58	0.000
	C3	45	2	24.53	0.000	45	2	16.34	0.000	45	2	16.34	0.000	45	2	10.15	0.000
Cerrado/ Tropical Central	C4	36 <sup>§</sup>	2	40.95	0.000	36 <sup>§</sup>	2	29.62	0.000	36	2	26.89	0.000	36 <sup>§</sup>	2	40.38	0.000
	C5	36	2	50.43	0.000	36	2	38.07	0.000	36 <sup>§</sup>	2	23.67	0.000	36 <sup>§</sup>	2	39.57	0.000
	C6	63 <sup>§</sup>	2	13.35	0.000	63 <sup>§</sup>	2	16.01	0.000	63 <sup>§</sup>	2	19.25	0.000	63 <sup>§</sup>	2	23.88	0.000
Atlantic Forest/ Subtropical Humid	C7	36	2	47.07	0.000	36	2	40.95	0.000	36 <sup>§</sup>	2	4.67	0.016	36	2	15.22	0.000
	C8	18 <sup>§</sup>	2	2.75	0.096	18 <sup>§</sup>	2	4.30	0.033	18 <sup>§</sup>	2	8.21	0.004	18	2	6.41	0.010
	C9	81	2	18.17	0.000	81	2	19.96	0.000	81	2	12.93	0.000	81	2	17.15	0.000
A. Forest/ Subtropical Humid	C10	45	2	16.66	0.000	45 <sup>§</sup>	2	20.04	0.000	45 <sup>§</sup>	2	5.20	0.010	45	2	12.17	0.000
	C11	36 <sup>§</sup>	2	12.31	0.000	36 <sup>§</sup>	2	12.78	0.000	36 <sup>§</sup>	2	4.09	0.026	36 <sup>§</sup>	2	8.27	0.001
	C12	81	2	30.54	0.000	81 <sup>§</sup>	2	26.08	0.000	81	2	22.15	0.000	81	2	29.11	0.000
	C13	18 <sup>§</sup>	2	11.71	0.000	18	2	7.71	0.005	18	2	7.71	0.005	18	2	6.77	0.008

<sup>‡</sup> Kruskal-Wallis test of ranks.

<sup>§</sup> Box-Cox transformed.

C11) and two within the subtropical temperate zone (C12 and C13) (Fig. S6).

## 2.9. Statistical analysis

The SOC stock responses of the sites were based on the observed differences among these clusters. The SOC stock data distribution was analysed for four depth intervals (0–20, 0–40, 40–100, and 0–100 cm) across 13 clusters. Statistical analyses were conducted using the Statistical Tool for Agricultural Research (STAR) v.2.0.1 (Gulles et al., 2014), a 2014 package developed by the International Rice Research Institute (IRRI) in the Philippines. Normality of the data was assessed using the Shapiro-Wilk test. In instances where the data did not satisfy the requisite normality assumptions for analysis of variance (ANOVA), a Box-Cox transformation (Box and Cox, 1964) was used in Excel to approximate a normalized distribution. The plot of residuals versus fitted values was used to assess the equality of variances. To test the effects of land use types on the SOC stock (the response variable), the data were subjected to ANOVA, except for 0–20 cm in C2 (Kruskal-Wallis test followed by Dunn post hoc). In the context of the ANOVA model, the LUT were conceptualised as treatments, while the sampling pits or trenches within a site were considered as pseudo-replications and the sites within a cluster as replications. A *p*-value of <0.05 was considered statistically significant. The means that were found to be statistically significant were then segregated using the Least Significant Difference (LSD) post hoc technique in the STAR v.2.0.1 (Gulles et al., 2014) package. To illustrate the data distribution at 0–100 cm depth interval in 13 clusters, boxplots were generated using the STAR package and presented with post-hoc results by the LSD technique. Linear regression analyses were performed between NTS duration versus total SOC sequestered, clay content versus SOC content, latitude versus SOC sequestered, and latitude versus total SOC losses. In the regression models, the first terms represented the independent variables and the second terms the dependent variables.

## 3. Results

### 3.1. Soil organic carbon stock distribution on the profile

A substantial degree of variation in C stocks was found in both the Cerrado and Atlantic Forest biomes (Table 2). The subtropical climate zone exhibited the most substantial variation, with an approximate 100 Mg C ha<sup>-1</sup> disparity in SOC stocks. The tropical equatorial, tropical

central, and subtropical temperate zones exhibited similar patterns of variability. This variation was influenced by factors such as land use type and sampling stratum. At all depths and in all clusters and climate zones, SOC stocks in the PBT were lower than SOC stocks in the NTS and NV (Table 3). The percentage of SOC stocks distribution patterns in the Cerrado biome for 0–40 and 40–100 cm layers were 53.7 and 46.3 % for NV, 51.5 and 48.5 % for PBT, and 56.0 and 44.0 % for NTS. For the Atlantic Forest biome, results for NV, PBT, and NTS were 54.9 and 45.1 %, 53.0 and 47.0 %, and 53.6 and 46.4 %, respectively (Table S2). These trends suggest that approximately 46 % of the SOC stocks are stored in 40–100 cm depth (Table S2).

### 3.2. Soil organic carbon stock losses owing to plow-based tillage

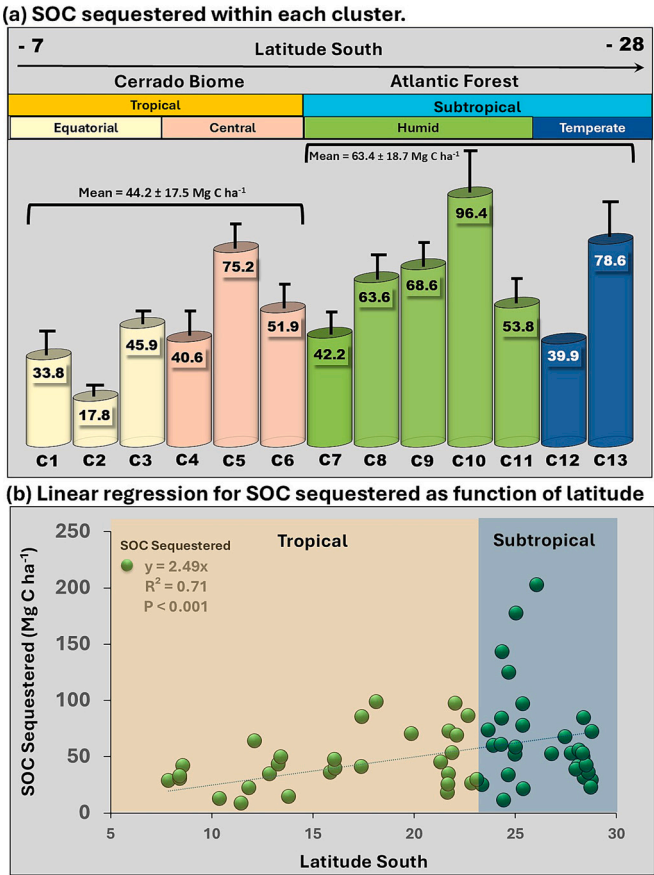
Conversion of NV to agricultural land and the subsequent use of PBT resulted in a marked depletion in SOC stocks from  $20.2 \pm 3.7$  to  $100.1 \pm 9.3$  Mg C ha<sup>-1</sup> for the 1-m depth in the Cerrado biome, with an average loss of  $63.7 \pm 8.9$  Mg C ha<sup>-1</sup> (Fig. 2a), and the observed loss is comparable to those reported before for the Cerrado biome (Gonçalves et al., 2024). The decline in SOC stock represents a reduction of 38.0 % of the original SOC stock (1-m depth). Losses of SOC stock in the clusters C1, C2 and C3 (Fig. 2a) ranged from  $20.2 \pm 3.7$  to  $39.0 \pm 5.9$  Mg C ha<sup>-1</sup> (mean =  $29.6$  Mg C ha<sup>-1</sup>) and are equivalent to 21.8 to 35.1 % of the original SOC stock lost from the NV for 1-m depth. These losses occurred in all sampled layers, with over 40 % of losses occurring in the 40 to 100 cm interval. Clusters 1 and 2 have the sand-clay textural class, with clay content of 170 to 270 g kg<sup>-1</sup> of soil, and the SOC stock under NV for 1-m depth ranged from  $82.2 \pm 7.4$  to  $132.4 \pm 9.5$  Mg C ha<sup>-1</sup>. In this context, the SOC loss may be more pronounced due to a weaker bond between SOC and other soil constituents, which may be a consequence of a higher MAT and thus aggravating SOC decomposition (Gonçalves et al., 2024). Additionally, clusters 1 and 2 are subject to an extended period of drought, marked by a deficit in precipitation (Table S3) and an evapotranspiration rate which may surpass that observed in cluster 3. During the drought period, an unmulched soil is prone to a high evaporation loss through the process of upward capillary action (Wang et al., 2024). The upward movement of water from deeper soil layers into the atmosphere, predominantly by vapor movement, stimulates microbial activity, depletes labile SOC and emits CO<sub>2</sub> into the atmosphere (Fang et al., 2005). The kinetic energy of water vapor accelerates CO<sub>2</sub> flux when labile SOC fractions are oxidized and emitted into atmosphere. About 40 % of the C accumulated in unprotected soil during the previous year may be lost through this process (Fang et al., 2005). The relative air



**Table 3**  
Comparisons of means of soil organic carbon (SOC) stock values based on Least Significant Difference (LSD) at four depth intervals (0–20, 0–40, 0–100, and 0–100 cm) among land use types (Native vegetation - NV; No-till System - NTS; Plow-based tillage - PBT) within each cluster (C1 to C13) in the climatic zones (Tropical Equatorial, Tropical Central, Subtropical Humid and Subtropical Temperate) of Brazilian Cerrado and Atlantic Forest biomes.

Biome/ Climatic Zones	Clusters	Depth interval (cm) and Land use types												Mg Cha <sup>-1</sup>
		0–20			0–40			40–100			0–100			
		NV	NTS	PBT	NV	NTS	PBT	NV	NTS	PBT	NV	NTS	PBT	
Cerrado/ Tropical Equatorial	C1	34.1 a <sup>§</sup>	34.1 a	21.5 b	62.9 a	62.4 a	39.6 b	48.4 a	43.7 a	32.6 b	111.3 a	106.0 a	72.2 b	
	C2	34.5 a	33.1 a	20.4 b	51.9 a	54.2 a	37.3 b	40.9 a	37.5 a	35.2 a	92.7 a	90.3 a	72.5 b	
	C3	47.3 a	50.7 a	30.5 b	79.6 a	83.7 a	55.4 b	68.1 a	72.6 a	55.0b	147.7 a	156.3 a	110.4 b	
	C4	70.0 a	54.5 b	39.3 c	123.5 a	95.3 b	73.9 c	113.9 a	88.3 b	70.7 c	237.4 a	183.5 b	142.9 c	
	C5	74.0 a	56.5 b	34.8 c	130.8 a	109.4 b	64.8 c	122.2 a	91.9 b	62.4 c	219.4 a	203.5 b	128.3 c	
Atlantic Forest/ Subtropical Humid	C6	61.2 a	47.6 b	28.2 c	104.4 a	80.6 b	48.9 c	91.0 a	68.8 b	49.2 c	196.9 a	148.8 b	96.8 c	
	C7	68.6 a	52.9 b	36.4 c	108.1 a	86.7 b	61.9 c	78.3 a	69.5 a	53.8 b	187.6 a	157.3 b	115.1 c	
	C8	57.9 a	46.5 b	30.6 c	85.0 a	82.5 a	52.5 b	99.2 a	83.2 b	49.7 c	184.2 a	165.7 a	102.1 b	
	C9	62.5 a	60.0 a	34.1 b	112.5 a	103.3 a	60.7 b	97.3 a	78.7 b	52.5 c	209.8 a	181.9 a	113.2 b	
Atlantic Forest/ Subtropical Temperate	C10	85.4 a	80.0 a	42.4 b	150.0 a	142.6 a	76.8 b	129.2 a	118.5 a	72.2 b	264.9 a	236.2 a	139.8 b	
	C11	85.9 a	53.0 b	35.54 c	143.2 a	97.77 b	61.4 c	129.5 a	100.6 b	83.3 c	272.7 a	198.4 b	144.6 c	
	C12	59.4 a	49.2 b	31.9 c	96.9 a	83.8 b	58.2 c	84.0 a	69.5 b	56.2 c	180.9 a	153.3 b	113.4 c	
	C13	70.6 a	47.2 b	30.7 c	118.8 a	90.8 a	55.8 b	82.5 a	86.6 a	43.2 b	201.3 a	177.4 ab	98.9 c	

<sup>§</sup> The same lower-case letter in the same line of each cluster means it is not significantly different within the same soil depth and same land use type.



**Fig. 3.** (a) Total soil organic carbon (SOC) sequestered by no-till systems (NTS) in the Cerrado and Atlantic Forest biomes for each cluster; (b) linear model for SOC sequestered as function of latitude for 1-m depth.

humidity, during the driest period, especially in the Clusters 1 and 2 (Cerrado Biome) can be as low as 9 to 11 % during the driest period (Alvares et al., 2013a; Alvares et al., 2013b) and increases soil evaporation loss. Additionally, MATs for this geographical region range from 25.4 to 26.6 °C (Table 1). Furthermore, SOC losses in Cluster 3 with predominantly equatorial climate, were up to  $37.3 \pm 3.1$  Mg C ha<sup>-1</sup> or 25.3 % of the original SOC stock in NV to 1-m depth. Despite the large variation in clay content ( $460$  to  $720$  g kg<sup>-1</sup>) among sites within Cluster 3, the clay-SOC interactions are robust, and enhance SOC protection within aggregates (Gonçalves et al., 2017). The high MAT (26.6 °C) and MAP (1950–2150 mm year<sup>-1</sup>) cause breakdown of macro-aggregates under PBT (de Oliveira Ferreira et al., 2018), exposure of SOC to microbial activity, and acceleration of the decomposition process. Thus, lower percentage of SOC losses in cluster 3 in comparison to those in clusters 1 and 2 with predominantly sand-clay soils, may be attributed to the higher clay content, which creates a protective barrier of the clay-humus complex within the aggregates (de Oliveira Ferreira et al., 2018). MAP and clay content are among important environmental variables contributing to SOC losses in cluster 3 (Table S3).

Soils in Clusters 4, 5 and 6 have clayey to very clayey textural class, with SOC losses of  $91.1 \pm 8.3$  to  $100.1 \pm 7.9$  Mg C ha<sup>-1</sup> (Fig. 2a) representing 38.6 to 50.8 % of SOC lost from original SOC stocks in soils under NV to 1-m depth. The patterns of SOC loss observed in the 0–40 cm layer were comparable to those observed in the 40–100 cm layer. In soils with an oxidic composition, the predominant clay type is kaolinitic with a high content of iron and aluminum oxides (Jia et al., 2024), and thus abundance of positive charges on the exchange complex of the clay minerals because of the pH at the point of net zero charge within the range of 7.4 to 8.2. Conversely, the current pH measured below 40 cm

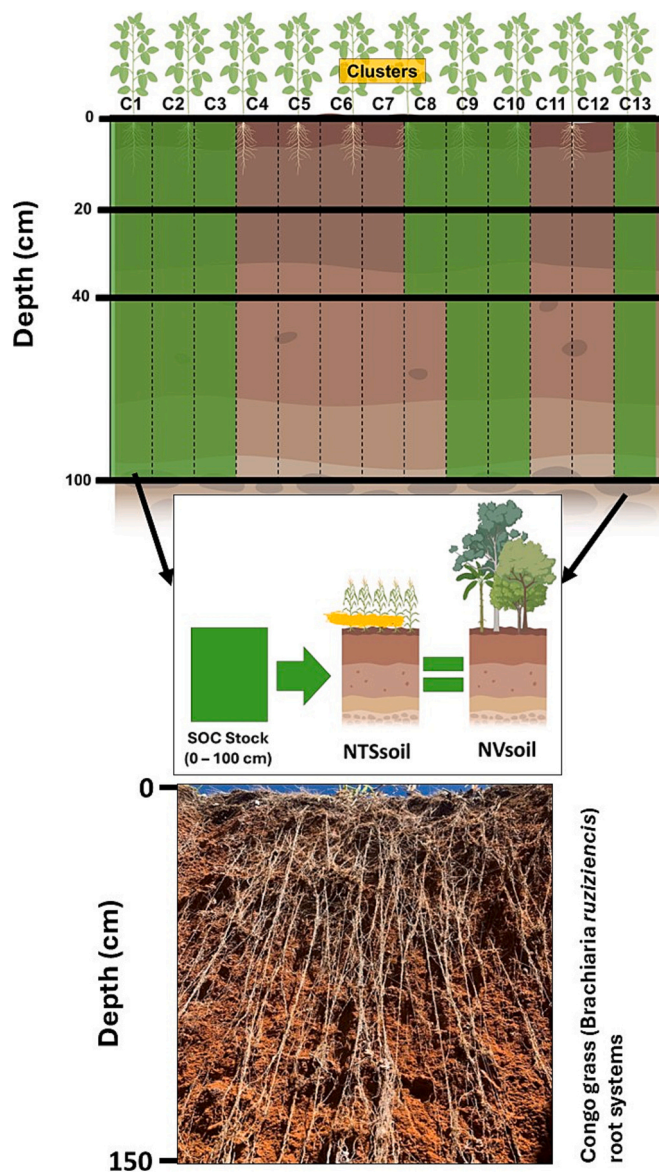


Fig. 4. Soil organic carbon (SOC) stock restoration by no-till systems (NTS) for each cluster in function of depth. Illustration of root system of Congo grass (*Brachiaria ruziziensis*) acting as the main contribution to recover SOC in the profile for 1-m depth.

depth in NV soil being  $\approx 4.5$  leads to the prevalence of positive charge and thus the collapse of the organic radicals around the positive charges (Jia et al., 2024). The binding energy between positive charges and organic radicals, which is based on electrostatic force, is relatively weak, particularly in sandy and sandy-clay soils. The data show a high  $R^2$  of linear regression between clay and SOC content ( $R^2 = 0.84$  to  $0.82$ ,  $n = 640$ , for each depth interval and  $p < 0.0001$ ). The high coefficient of determination ( $R^2$ ) indicates that, regardless of LUT, the most prevalent relationship is that between clay and SOC content and both are strongly linked (Fig. S7). Furthermore, the variation of low to elevation and solar radiation intensity (Cluster 4 and 5) were identified as key determinants of SOC losses (Table 1; Table S3) and indicate the significance of environmental variables in moderating C losses in a tropical center climatic zone in the Cerrado biome.

In the Atlantic Forest biome, the loss of SOC stock under PBT management ranged from  $67.5 \pm 6.2$  to  $128.1 \pm 9.2$  Mg C ha $^{-1}$  for 1-m depth. The weighted average loss of  $96.3 \pm 12.0$  Mg C ha $^{-1}$  was significantly higher than that of  $63.7$  Mg C ha $^{-1}$  loss observed for the

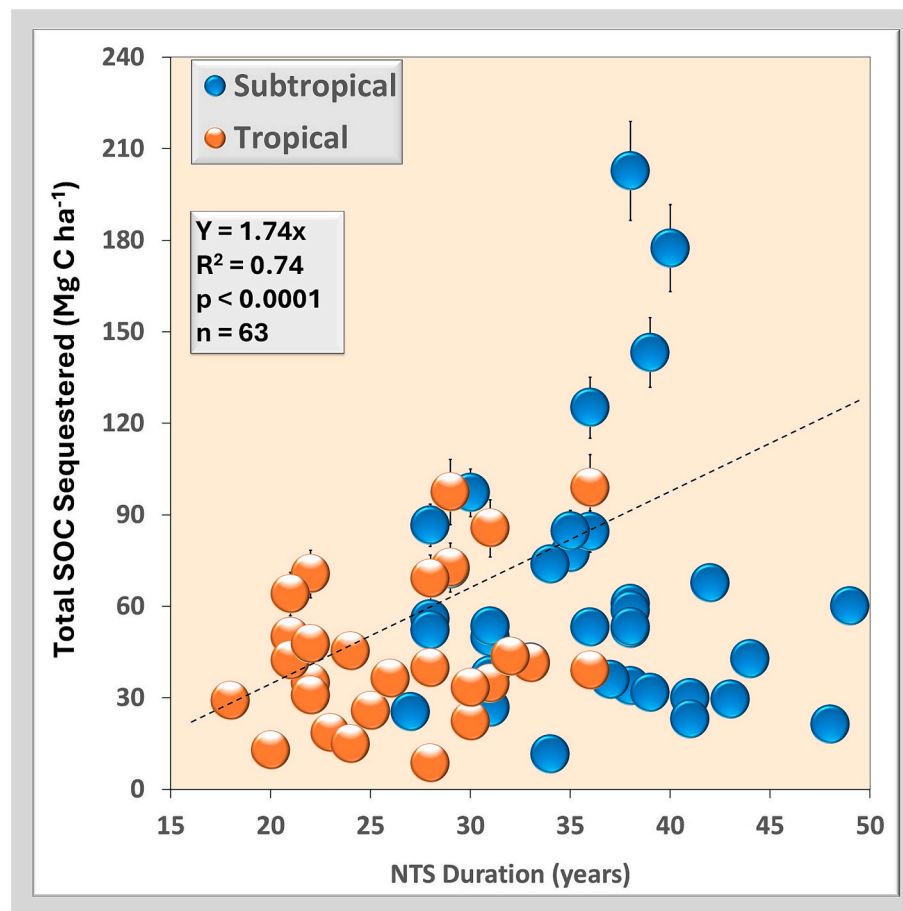
Cerrado biome (Fig. 2a). The dominant soil pattern in the Atlantic Forest biome; particularly in the southern Brazilian states of Paraná, Santa Catarina, and Rio Grande do Sul; is clay-rich soil types derived from basalt (Schaefer et al., 2023). These soil types have higher total SOC stock under NV than those for the sandy and sandy-clay soil types of the Cerrado biome. The weighted average of SOC stocks (1-m depth) under NV within the Atlantic Forest and the Cerrado biome were  $219.0 \pm 29.3$  Mg C ha $^{-1}$  and  $170.4 \pm 22.2$  Mg C ha $^{-1}$ , respectively. It was expected that the maximum SOC losses would occur within the Cerrado biome, because of the impact of high MAT and MAPs on the decomposition of SOC. The comparison of SOC stock between the Atlantic Forest and Cerrado biome indicates a 30 % reduction. This difference is due to intense precipitation during the summer and winter seasons, absence of a definite dry period in the Atlantic Forest biome, in conjunction with the elevated slope and high altitude, which resulted in a considerable adverse impact on SOC stocks, as is shown by a 45.8 % decrease under PBT compared to that of a soil under NV. Despite a greater average loss of SOC in the Atlantic Forest biome, the results varied among different clusters within the biome. The mild summer and cold winter conditions characteristic of clusters C12 and C13 (i.e., Subtropical-Temperate climate zone, Table 1) are conducive to protection of SOC stock. Conversely, a hotter summer with more intense rainfall in the subtropical humid zone (i.e., C7 to C11) resulted in greater SOC stock loss and represents the primary distinction in environmental variables among these climate zones. In contrast to the findings observed in the Cerrado biome, the losses recorded in the Atlantic Forest biome for the 0–40 and 40–100 cm layers were  $57.2$  Mg C ha $^{-1}$  and  $44.3$  Mg C ha $^{-1}$ , respectively. These values indicate a 29.1 % higher loss in the 0–40 cm layer compared to that for 1-m depth. Furthermore, the mean SOC losses in the tropical and subtropical zones were  $73.8$  and  $95.4$  Mg C ha $^{-1}$  (1-m depth), respectively (Fig. 2a). The primary factors contributing to the reduction of SOC in the subtropical humid climate zone, as ranked by elevation, clay content, MAT, MAP, and high slope (Table S3). A linear regression was used to estimate the SOC stocks loss as a function of latitude. The SOC loss of  $3.8 \pm 0.4$  Mg C ha $^{-1}$  for 1-m depth was computed for each increase of one degree in latitude (Fig. 2b) for each site. This loss indicates a strong impact of rainfall intensity on sloping land and leading to a high SOC depletion in the subtropical climate zone of the Atlantic biome.

#### 4. Discussion

##### 4.1. Total soil organic carbon stock sequestered in each cluster through no-till systems

The long-term NTS management has restored the SOC stock of the Cerrado and Atlantic Forest biomes (Fig. 3a).

In the Cerrado biome, the SOC sequestered through NTS management to 1-m depth ranged from  $17.8 \pm 2.13$  to  $45.9 \pm 4.15$  Mg C ha $^{-1}$  across clusters 1, 2, and 3 (Fig. 3a), representing a net sequestration equivalent to SOC loss from PBT of  $20.2 \pm 2.1$  to  $41.0 \pm 3.9$  Mg C ha $^{-1}$ . Furthermore, this SOC sequestration occurred consistently across all depth intervals, indicating the effectiveness of NTS management system based on input of biomass-C. The predominant cropping sequence in these clusters, over the five years prior to soil sampling, included soybean (*Glycine max*) as the main crop from October to early February, followed by an intercropping system comprising of corn (*Zea mays*) or sorghum (*Sorghum bicolor*) plus Congo grass (*Brachiaria ruziziensis*) with Congo grass from February to September alternating this sequence each year. These intercropping systems, imposed immediately after the soybean harvest, developed deep root systems (Fig. 4) especially because of the roots of Congo grass which penetrated deeper in sub-soil that is acidic and contain high Al concentrations (Jia et al., 2024). Hence, the deep root system leads to deposition of root biomass-C into the deep subsoil.



**Fig. 5.** Soil organic carbon (SOC) sequestration rates in function of no-till systems (NTS) duration (years) as independent variable and total SOC sequestered by NTS including all 63 sites of tropical and subtropical climatic zones. Blue balls refer the subtropical and orange balls the tropical climatic zones for 1-m depth.

The intercropping system added C input of  $9.5$  to  $11.0 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ , exceeding the minimum threshold  $5.1$  to  $6 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  (Sá et al., 2015) required for sustaining the SOC dynamic equilibrium. A review of cluster performance in the Cerrado revealed that nine out of twelve sites in clusters 1, 2, and 3 experienced increased SOC sequestration to 1-m depth, with SOC stocks exceeding those under NV. In contrast, clusters 4, 5, and 6, located within the tropical central climate zone, did not exhibit this trend, and SOC sequestration reached between 55 % to 85 % of the losses under PBT practices. These data indicate that the NTS has the potential of SOC recovery, regardless of the prevailing environmental conditions. In the Atlantic Forest biome, clusters C7 to C11, situated in a subtropical humid climate zone (Table 1), increase in SOC stock under NTS for 1-m depth was observed for 10 out of 24 sites exceeding NV. This trend of increasing SOC stock under NTS is similar to that observed under the Cerrado biome. The NTS sites comprising clusters 9 and 10, characterized by clayey to very clayey soils, exhibited SOC stock to 1-m depth of  $181.8 \pm 17.5$  to  $236.2 \pm 20.6 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ . Conversely, soil of a sandy-clay texture had markedly lower SOC stocks of  $66.7 \pm 7.5$  to  $98.9 \pm 10.5 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ . Furthermore, NTS in Clusters 12 and 13 were characterized by a high SOC recovery at five of the 11 sites surpassing NV SOC stocks and indicated a high capacity to sequester C in this biome.

#### 4.2. Soil organic carbon sequestration rates

Long-term use of NT has a high potential for SOC sequestration that exceeds the previously documented inventories from 2007 (Cerri et al., 2007) and 2020, which indicated the sequestration rate of  $0.5$  (for 0 to 20 cm depth) and  $0.8 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  (0 to 40 and 0 to 100 cm depth),

respectively (Sá et al., 2024). A linear regression, plotted between the duration of NTS and the total SOC sequestered for the 63 sites (Fig. 5) indicate the observed sequestration rate of  $1.74 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  ( $R^2 = 0.74$ ,  $p < 0.0001$ ). Furthermore, the correlation between NTS duration and SOC sequestration in tropical and subtropical climate zones (Fig. S5) show rates of  $1.71 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  ( $R^2 = 0.77$ ,  $p < 0.0001$ ) to  $1.73 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  ( $R^2 = 0.68$ ,  $p < 0.001$ ). Thus, the duration of the NTS has a significant impact on the sequestration rate with the mean SOC sequestration rate for both biomes of  $1.74 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ . The data also show that increase in duration results in a corresponding increase in system stability (Briedis et al., 2016). Regardless of other environmental variables affecting SOC stocks, clay content plays a crucial role (Table S3, Fig. S7) in influencing the rate of SOC sequestration. Furthermore, the correlation between exchangeable calcium and C content demonstrated a substantial contribution to C sequestration, suggesting that as C content rises, C content concomitantly increases (Fig. S8).

#### 4.3. Restoring soil organic carbon stocks in the Cerrado and Atlantic Forest biomes

The potential for SOC restoration in the Cerrado and Atlantic Forest biomes via NTS is observed in both sandy-clayey and clayey soils, at low and high altitudes, across a latitudinal range from  $-7$  to  $-28^\circ \text{ S}$  and climatic zones including tropical equatorial and central, subtropical humid, and temperate. The data presented show that seven of the 13 clusters had high SOC stocks under NTS to 1-m depth at a level comparable to those observed in soil under NV (Fig. 6). The critical determinant of achieving C sequestration potential is the adoption of NTS



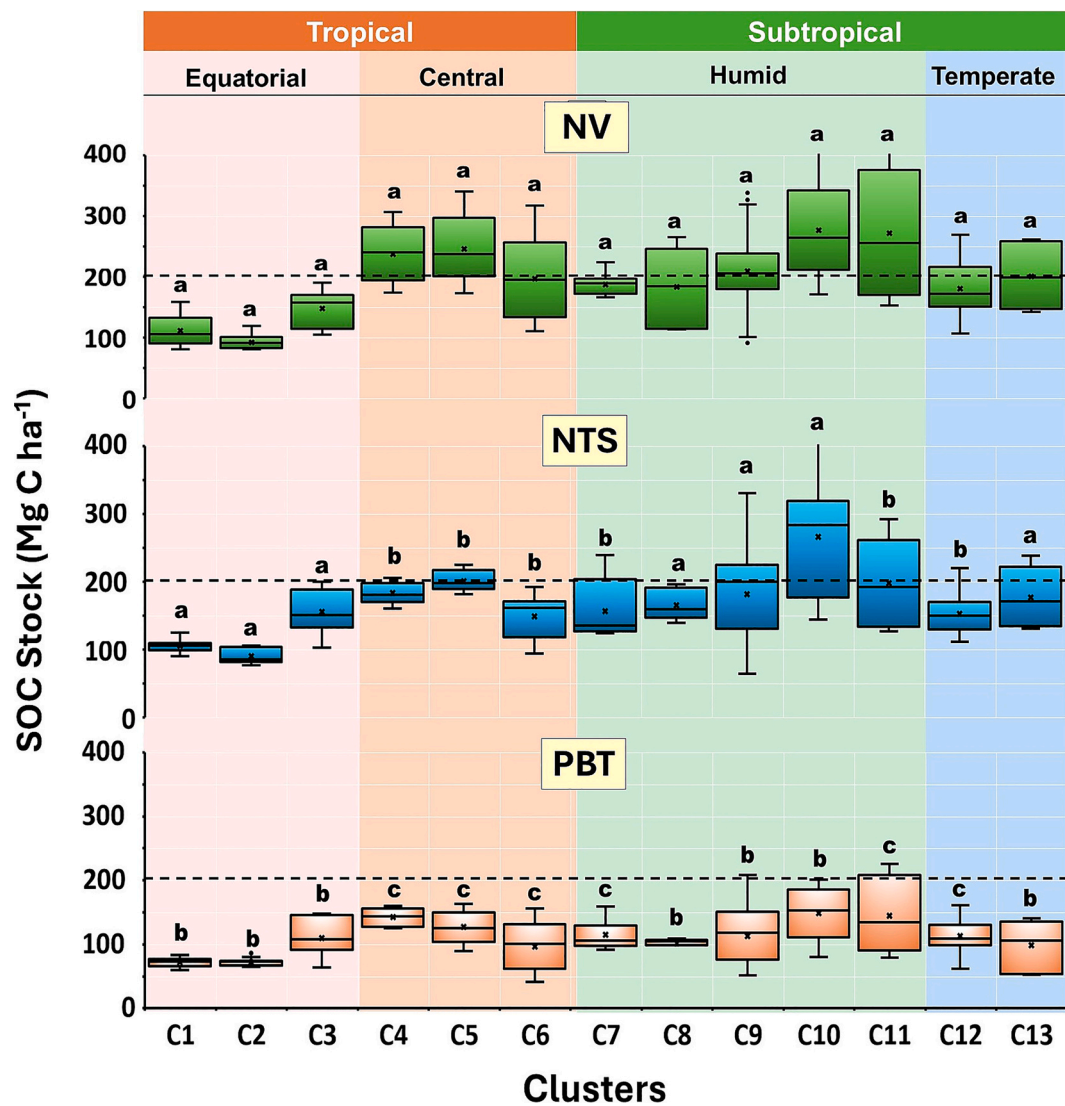


Fig. 6. Boxplots illustrating soil organic carbon (SOC) stock data distribution for 1-m depth for three land use types (LUT): native vegetation, (NV), no-till systems (NTS) and plow-based tillage (PBT). Different letter within a cluster shows the statistical differences among the LUT.

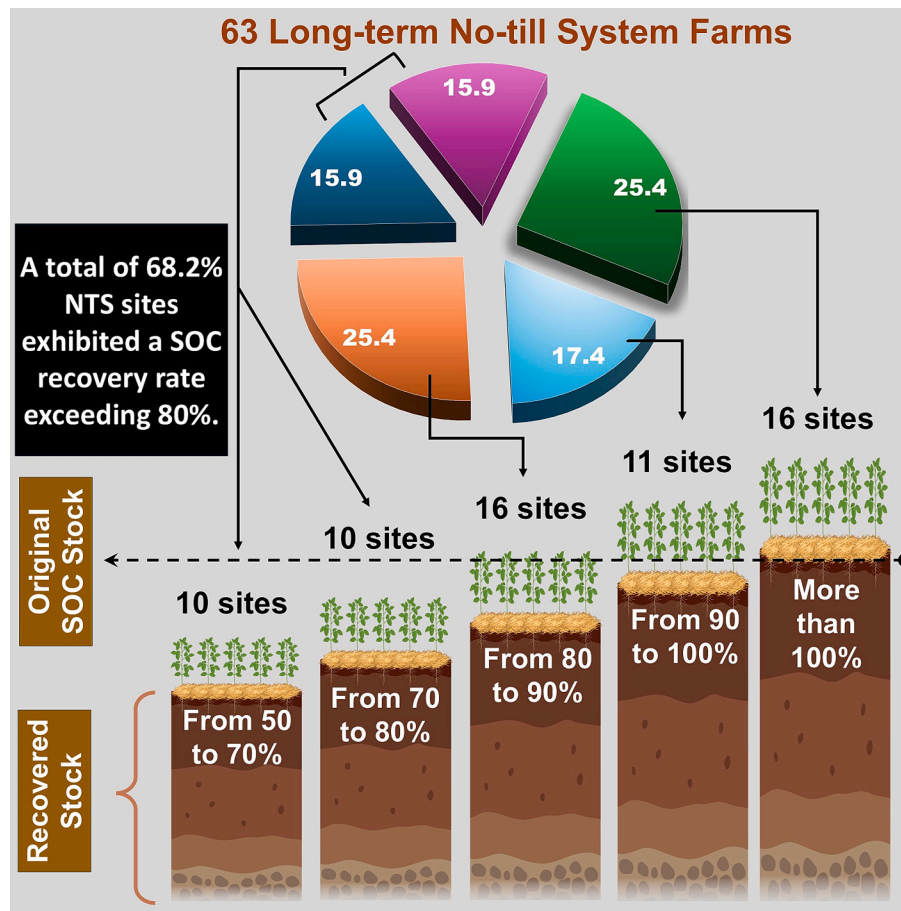
based on its key principles. In the Cerrado biome of the tropical-equatorial zone, the high SOC sequestration potential of clusters 1, 2, and 3 exhibited SOC stock restorations in 1-m depth. It may be primarily attributable to the root system of Congo grass combined with that of corn or sorghum resulting in higher biomass-C input ( $> 9 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) than the expected amount needed to achieve dynamic SOC equilibrium (Fig. 4).

In contrast, the diversification of crop rotation in the Atlantic Forest biome had a particularly pronounced impact, due to the introduction of a mixed species (Zhang et al., 2021) during the winter or after the soybean harvest. The blend of species used combines 6 to 9 species with different dry matter composition and type of root system. The mean percentage of SOC restored by NTS vis-a-vis NV soil in the 13 clusters ranged from 72.5 to 105.8 %. However, when considering the 63 sites, the range was much wider 54.8 to 138.6 %, indicating that in the highest case, there was a 38.6 % greater SOC stock under NTS than that observed in soil under NV. The restoration of SOC is contingent upon the input of biomass-C, which depends on three variables: 1) The quantity of C introduced by the above- and below-ground sources; 2) The quality of the biomass input, including the combination of species (i.e., grasses and legumes) that can simultaneously promote equilibrium in C and N, thereby stimulating C accumulation in the soil, and 3) the frequency of

the biomass input based on the number of times per year that additions are made (Sá et al., 2022).

Conversely, the SOC stock restoration turnover time at the same level as that of the NV soil was observed to range from 36.4 to 55.0 years, for the Cerrado and Atlantic Forest biomes, respectively. The environmental variables, with substantial impact on SOC sequestration in the Cerrado biome (i.e., solar irradiation, MAP and MAT) stimulated the growth of species with high C potential to produce biomass-C that triggered a high C production in grasses like Congo grass. The turnover time for all sites in clusters 1, 2, and 3 to restore SOC at the NV level is 18.4 years. In contrast, the turnover time for clusters 4, 5, and 6 is approximately 54.4 years. The primary reason for this discrepancy is the prolonged dry period in these clusters (6 to 7 months) and the prevalence of clayey to very clayey soils. However, in the Atlantic Forest biome, there is a considerable range in the SOC turnover time from 36.4 to 71.5 years. This variation is closely linked to the diverse scenarios observed in the clusters, particularly the influence of clay content. For instance, in the Ponta Grossa-PR, Southern Brazil region, sandy-clay soils are developed from Devonian shale, comprising primarily of sandstone material. In contrast, clay to very clayey soil from Paraná to Rio Grande do Sul are derived from basalt (Schaefer et al., 2023).





**Fig. 7.** The percentage of soil organic carbon (SOC) stock restoration by no-till system (NTS) for 1-m depth by sites compared to the SOC stock of native vegetation (NV) soil level.

#### 4.4. Agriculture as a part of the solution to climate change

The results from diverse scenarios presented herein raise questions about evidence to substantiate the assumption that agriculture-based NTS is capable of contributing to the mitigation of climate change through SOC sequestration? In response to this question, the analysis of results was based on four scenarios: 1) Data from the 63 sampled sites in the Cerrado and Atlantic Forest biomes revealed that 16 of them (i.e., 25.4 %) exhibited SOC stocks in the 1-m depth in the NTS that were equal to or greater than those observed in the soil under NV (Fig. 7). In addition, 27 NTS sites (i.e., 42.8 %) recovered between 80 and 100 % of NV SOC stock. This trend indicates that management of biomass-C inputs associated with agricultural practices (i.e., liming for alleviating soil acidity, judicious fertilization, and the maintenance of the NTS over an extended period) can sequester greater quantities of SOC than that under NV soil; 2) The turnover time for SOC recovery in both the Cerrado and Atlantic Forest biomes ranging from 36.4 to 55.0 years implies that it is possible to recover the lost SOC in about one human generation; 3) The sequestration rate obtained in the present study ( $1.74 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) was up-scaled (10 % of the current NTS in Brazil is 3.6 M ha in accordance with the principles of NTS) to provide an estimate of potential mitigation of  $6.3 \text{ Tg C}$  (i.e.,  $21.3 \text{ Tg CO}_2\text{e}$ ). Upscaling of this scenario increasing the land area under NTS to 30 % (equivalent to 10.8 M ha) and maintaining the aforementioned sequestration rate, the  $\text{CO}_2$  mitigation can increase to  $68.9 \text{ Tg CO}_2$ . Thus, the data presented herein suggest that the emissions level can be offset through the expansion of the land area under NTS in agroecosystems of Brazil.

It is thus pertinent to re-evaluate the current agricultural practices with the objective of identifying efficacious strategies for the large-scale

implementation of production systems that effectively sequester SOC. These findings suggest that one hectare of land under NTS has the potential to avert deforestation of  $1.01 \pm 0.15 \text{ ha}$  of NV in the Tropical Equatorial zone and  $0.81 \pm 0.18 \text{ ha}$  in the Tropical Central zone within the Cerrado biome. The implementation of NTS on one ha has the potential to avert deforestation for food production of  $0.88 \pm 0.19 \text{ ha}$  for the Atlantic Forest biome within the subtropical humid zone, compared with  $0.87 \pm 0.17 \text{ ha}$  in the subtropical temperate climate zone. This trend suggests that NTS land use has the potential to play a significant role in SOC restoration, thus becoming a contributing factor to global climate change solutions and, consequently, protecting the undisturbed forest from direct, human-induced disturbances. Furthermore, it is estimated that 10 % of the no-till area in Brazil, practiced according to the NTS principles, could result in the avoidance of deforestation on 3.1 M ha of forest. Furthermore, a 5 % expansion of land area under NTS each year for a 10-year period would result in the avoidance of deforestation of 22.6 M ha, which can contribute to land sparing in which high-yield farming is combined with protecting natural habitats from conversion to agriculture. Indeed, the integration of long-term no-till farming with the addition of biomass-C has the potential to achieve multiple objectives, including the concurrent production of food, the prevention of deforestation, and the effective contribution of agriculture to climate change mitigation.

#### 5. Conclusion

In conclusion, this comparative analysis of land use types across the 63 sites evaluated can be summarized as follows: Firstly, the conversion of native vegetation to agricultural areas and the frequent use of soil

disturbances resulted in losses of 38.1 % and 45.8 % of the original SOC stock (1-m depth) under NV in the Cerrado and Atlantic Forest biomes, respectively. Secondly, the SOC stocks (1-m depth) in the NTS were equal to those found in the soil under NV in seven of the thirteen clusters assessed. Thirdly, the SOC stocks for 1-m depth in 16 NTS sites (i.e., 25.4 %) of the 63 sites evaluated, were found to exceed the SOC stock in the soil under NV and in another 27 NTS sites, the SOC stock restored in relation to NV was between 80 and 100 %. Fourthly, the recovery time of SOC stocks to the NV level by NTS was found to be between 36 and 54 years. Fifthly, the findings suggest that one hectare of land under NTS has the potential to avert deforestation for food production of  $1.01 \pm 0.15$  and  $0.87 \pm 0.17$  ha of NV in the Cerrado and Atlantic Forest biome, respectively.

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### CRediT authorship contribution statement

**João Carlos de Moraes Sá:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rattan Lal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Klaus Lorenz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Yadunath Bajgai:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Carla Gavilan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation. **Manan Kapoor:** Writing – review & editing, Validation, Data curation. **Ademir De Oliveira Ferreira:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Clever Briedis:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Thiago Massao Inagaki:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Lutecia Beatriz Canali:** Writing – review & editing, Writing – original draft. **Daniel Ruiz Potma Gonçalves:** Writing – review & editing, Writing – original draft, Visualization. **Jeankleber Bortoluzzi:** Writing – review & editing, Project administration, Methodology, Funding acquisition.

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### Declaration of competing interest

The authors have no competing interests to declare.

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### Data availability

All data are in the main text and supplementary material of this article.

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