Journal of Arid Environments 229 (2025) 105401

Contents lists available at ScienceDirect

Journal of Arid Environments

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

Microbial biomass, carbon and nitrogen stocks across land uses and soil types in the Brazilian tropical dry forest region

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ARTICLE INFO

Keywords:
Microbial biomass
Land degradation
C-stability
Soil organic matter

ABSTRACT

The Brazilian native dry forest (Caatinga) is used as a natural pasture or converted to unfertilized and overgrazed pastures. We investigated the restoration process, measuring soil attributes after three years of cattle exclusion in four soil types under the most common land covers of the region (dense and open Caatinga and pasture). C and N stocks tended to be higher under the dense Caatingas than under the other vegetation covers, particularly in the Regosol (80 and 8 Mg ha^{-1}), but tended not to significantly differ between the open Caatingas and the pastures. Microbial biomass C had the same trend, higher under dense Caatingas and in the Regosol (553 mg kg^{-1}). Basal soil respiration and C decay constant (0.02 day^{-1}) tended to be lower (thus higher C half-life, 347 days) in the Regosol (higher rainfall sites). The Oxisol (lower fertility soil) separated from the other soils in a Principal Component Analysis, especially from the Luvisol (highest fertility soil). Therefore, studies in the region must consider soil types and rainfall. The differences between open Caatingas and pastures in the dense Caatinga indicate that the period necessary for soil recovery after grazing exclusion is longer than three years, including for the microbial population.

1. Introduction

Overexploitation of natural resources and deforestation drive increased soil degradation in most semiarid areas of the world (Kooch et al., 2024; Shao et al., 2024). They are also degrading lands in the Brazilian semiarid region, which has an average annual rainfall of less than 800 mm, intense sunlight radiation, and high temperatures, leading to high potential evapotranspiration (Vieira et al., 2023). Most of the semiarid region was originally covered by a dry tropical forest, locally known as "Caatinga", which has high species richness, endemism, and functional diversity of plants and animals (Silva et al., 2020). The Brazilian semiarid region exhibits a diverse mosaic of soil types influenced by geological formations (sedimentary or crystalline) and pedogenetic factors (Krause et al., 2020). Sedimentary soils, such as Oxisols, are usually deep and of low fertility. Crystalline soils, such as Luvisols, may be shallow, stony, and rich in bases. Planosols have dense sub-superficial layers and low permeability, and Regosols have poorly differentiated horizons (Araújo Filho et al., 2023). In all these soils, the accumulation of organic matter and N availability is low due to low plant biomass production and litter input (Moura et al., 2016), combined with high photo and biological oxidation (Parton et al., 2007; Gava et al., 2022; Tomaz et al., 2024).

Since the introduction of cattle in the sixteenth century, the Caatinga has been used as a native pasture with high animal loads, leading to changes in its botanical composition (Schulz et al., 2016; Ferreira et al., 2024; Silva et al., 2024). Large areas have also been deforested to increase the growth of the herb layer and sometimes to plant pasture species. In these areas, overgrazing leads to vegetation dominated by plants mainly in the initial ecological succession stages (Freitas et al., 2012; Araujo Filho et al., 2018; Borovyk, 2020) and continuous overgrazing reduces litter production, nutrient inputs, and soil fertility, and may result in soil degradation, damage of ecological resilience potential

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https://doi.org/10.1016/j.jaridenv.2025.105401
Received 6 November 2024; Received in revised form 16 February 2025; Accepted 4 May 2025
Available online 16 May 2025

and, eventually, in a barren landscape (Barbosa Neto et al., 2020; et al., 2022). When livestock or agricultural activity ceases, the dry forest slowly regenerates with the initial growth of shrubs, mainly species of the genera *Mimosa*, *Caesalpinia*, and *Croton* (Santos et al., 2023; Oliveira et al., 2023; Silva et al., 2024). Although less documented, total C and N stocks also slowly increase, but it may take several decades before reaching the levels in the forest (Althoff et al., 2018; Medeiros et al., 2023, 2023b; Medeiros et al., 2023a,b; Oliveira et al., 2023). Published information on restoring microbial activities in the Brazilian semiarid region is scarce (Souza et al., 2022; Oresca et al., 2024). In severely degraded Planosols and Luvisols, carbon stocks are less than 30 Mg ha^{-1}, soil C-CO_2 emissions may be more than 40 mg kg^{-1} day^{-1}, and microbial biomass carbon rarely exceeds 130 mg kg^{-1} in the superficial soil layer (Neves et al., 2021; Santos et al., 2022), less than half of the amounts found in rainforests.

Among soil quality indicators, the soil microbial biomass, constituting a small but very active part of soil organic matter, has been considered to be a sensitive soil quality indicator, particularly for land degradation, and can be used to predict short- and long-term soil quality trends (Tarrason et al., 2010; Bargali et al., 2019; Manral et al., 2022). Soil microbial biomass plays an important role in indicating soil fertility and is easily altered by changes in plant litter's quantity and biochemical composition (Singh et al., 2021; Padalia et al., 2022; Manral et al., 2023). Due to the presence of litter and organic matter, most of the microbial communities and their activities are confined in the upper-most soil layer (Srivastava and Singh, 1989; Adeboye et al., 2011; Bargali et al., 2018; Padalia et al., 2018; Manral et al., 2022; Pandey et al., 2024). Few reports correlate soil chemical and microbiological attributes with environmental factors in the Brazilian tropical drylands. Losses in soil fertility, reductions in the microbial community diversity, and increased respiration activity of Planosols, Luvisols, and Neosols triggered by the decline of soil nutrient stocks after the conversion of Caatinga to crops and pastures have already been documented by Araújo Araujo Filho et al. (2018), Neves et al. (2021), and Santana et al. (2019). Over time, some of the converted areas became less fertile and degraded, which led to a reduction in the diversity of microorganisms and a boost in their respiratory activity so that C stability decreased by more than half compared to the preserved Caatinga (Wardle and Ghani, 2018; Gava et al., 2022).

Investigating C and N stocks and soil microbial functionality in this region is extremely necessary to understand the influence of grazing on pastures, the potential of forage crops, and soil nutrient cycling. Information on the restoration of microbial biomass and activity may guide the development of pasture management strategies, in order to decrease overgrazing occurrence, create grazing exclusion intervals, and calibrate the length of restoration periods.

Considering the scarcity of information on soil microbial activity related to C and N stocks in areas under pasture and native dry forest submitted to different environmental conditions in the Brazilian semiarid region, we established a long-term study to monitor changes in soil C and N stocks, respiration, and microbial variables, after exclusion of grazing, n four soil classes and in areas which were under pasture and open and dense Caatinga). We hypothesize that the Caatingas would still have better attributes than the pastures after these first three years of grazing exclusion and that the attributes would be influenced by the four areas' different soil classes and climate conditions.

2. Materials and methods

2.1. Area description and sampling strategy

The study was conducted in four experimental stations, following an East-West transect in the semiarid area of Pernambuco state, Brazil (Table 1). The three eastern sites have the typical Caatinga vegetation, and the western one a mixture of Caatinga and Savanna vegetation (Freitas et al., 2015). The species present in each site, together with their areal density, are listed in Table 2. Other studies report that the number of species and the height and diameter of trees and large shrubs are higher in the eastern than in the western sites (Freitas et al., 2010). The three eastern sites have shallow soils (Luvisol, Regosol, and Planosol, covering a crystalline substrate, while the western area has a deep soil (Oxisol) of sedimentary origin (Table 1). The sites have mean annual rainfalls from 549.7 to 798.7 mm (Table 1).

In each of the four sites, two stands of pasture and open and dense Caatinga were selected, considering that these three vegetation covers are the most common in the semiarid region:

1) Dense dry forest (hereafter also mentioned as "DDF") - Caatinga in an advanced stage of growth and regeneration based on ecological succession (Fig. 1), with a canopy that intercepts more than 80 % of radiant sunlight, a high density of trees and few points of bare soil in the rainy season. They are all native forests without major disturbances (mainly tree cutting) over the last 50 years, according to the experimental station records.
2) Open dry forest (hereafter also mentioned as "ODF") - Caatinga with a lower density of trees and tall shrubs and greater coverage of herbaceous stratum, in an intermediate stage of regeneration and ecological succession. The current open forests underwent land disturbance events, firstly triggered by wood cutting and burning over 30 years ago, followed by occasional fire-wood harvest and infrequent fire to increase herbage growth. After the initial fires, the areas were recolonized by native species in a natural regeneration process, starting with herbaceous and followed by shrub and tree species. Most of the trees and large shrubs survived the subsequent fires, as usual in Caatinga (Sampaio et al., 1993)
3) Pasture (hereafter also mentioned as "P") - vegetation formed mainly by herbaceous plants, with few tall shrubs and regenerating trees with stem diameters greater than 6 cm. Anthropic activities (mowing and burning) and excessive grazing generally prevent these areas from advancing in ecological succession. The pastures were established over 30 years ago after the native vegetation had been cut, and the land was cultivated to short-cycle crops for some years (mainly maize and cowpeas). The pastures comprised introduced grasses (commonly buffelgrass, *Cenchrus ciliaris* L.) or spontaneous native grasses and forbs. They were usually overgrazed by cattle or goats, leading to a continued degraded condition. Intentional burning

Table 1
Coordinates and main characteristics of the studied areas in the Brazilian semiarid region.

Municipality				
	São Bento do Una	Arcoverde	Sertânia	Araripina
Coordinates	8°31′37.8"S, 36°27′35.9"W	8° 25′ 15″ S 37° 03′ 41″ W	8°03′51.5"S, 37°13′28.3"W	7°27′32.7"S, 40°24′58.6"W
Altitude (m.a.s.l.)	614	663	570	830
Soil type	Planosol	Regosol	Luvisol	Oxisol
Soil depth (cm)	50 to 70	60 to 100	60 to 100	>100
Mean annual rainfall (mm)	595.9	798.7	549.7	711.2
Mean annual temperature (°C)	23.8 °C	23.5 °C	25.0 °C	24.6 °C
Region	Agreste	Sertão	Sertão	Sertão
Vegetation type	Subhumid Caatinga	Subhumid Caatinga	Semiarid Caatinga	Transitional between Caatinga and Savanna formations

Table 2
– Tree and large shrub species and their densities in open and dense Caatinga plots in four municipalities in the Brazilian semiarid region, after three years without grazing.

Municipality/plant species	Famíly	Density (plant ha^{-1})	
		Open Caatinga	Dense Caatinga
São Bento do Una			
Thiloa glaucocarpa Mart.	Combretaceae	25	51
Croton sonderianus Müll. Arg.	Euphorbiaceae	59	26
Piptadenia stipulacea (Benth.) Ducke	Fabaceae (Mimosoideae)	2	3
Capparis flexuosa (L.) L.	Capparaceae	1	0
Poincianella pyramidalis [Tul.] L.P.Queiroz	Fabaceae (Caesalpinioideae)	3	53
Manihot glaziovii Müll.Arg.	Euphorbiaceae	2	0
Aspidosperma pyrifolium Mart	Apocynaceae	1	5
Jatropha molíssima (Pohl) Baill	Euphorbiaceae	0	9
Ziziphus joazeiro Mart.	Rhamnaceae	0	1
Mimosa tenuiflora (Willd.) Poir.	Fabaceae (Mimosoideae)	0	1
Capparis jacobinae Moric.	Capparaceae	0	1
Arcoverde			
Lantana camara L.	Verbenaceae	57	0
Lachesiodendron viridiflorum (Kunth) P.G. Ribeiro, L.P. Queiroz & Luckow	Fabaceae (Mimosoideae)	6	41
Piptadenia stipulacea (Benth.) Ducke	Fabaceae (Mimosoideae)	30	0
Croton heliotropiifolius Kunth	Euphorbiaceae	1	0
ordia leucocephala Moric.	Boraginaceae	8	0
Solanum paludosum Moric.	Solonaceae	9	0
Capparis flexuosa (L.) L.	Capparaceae	1	0
Aegiphila verticillata Vell.	Lamiaceae	7	0
Calotropis procera (Aiton) W.T. Aiton	Apocynaceae	1	0
Cochlospermum vitifolium (Willd.) Spreng.	Bixaceae	1	0
Cordia goeldiana (Huber) M. Kuhlm. & Mattos	Boraginaceae	1	0
Senna spectabilis (DC.) H.S. Irwin & Barneby.	Fabaceae (Caesalpinoideae)	3	0
Mimosa oftalmocentra Mart. ex Benth.	Fabaceae (Mimosoideae)	1	0
Mimosa tenuiflora (Willd.) Poir.	Fabaceae (Mimosoideae)	1	0
Ziziphus joazeiro Mart.	Rhamnaceae	0	2
Zanthoxylum syncarpum Tul.	Rutaceae	0	12
Euphorbia phosphorea Mart.	Euphorbiaceae	0	17
Combretum leprosum Mart.	Combretaceae	0	17
Bauhinia cheilantha (Bong.) Steud.	Fabaceae (Caesalpinioideae)	0	1
Mimosa arenosa (Willd.) Poir.	Fabaceae (Mimosoideae)	0	2
Capparis jacobinae Moric.	Capparaceae	0	3
Melloa sp.	Bignoniaceae	0	5
Vitex polygama Cham.	Lamiaceae	0	2
Caesalpinia ferrea Mart. ex Tul.	Fabaceae (Caesalpinioideae)	0	1
Sertânia			
Croton heliotropiifolius Kunth	Euphorbiaceae	5	1
Not identified 1		2	6
Poincianella pyramidalis [Tul.] L.P.Queiroz	Fabaceae (Caesalpinioideae)	1	24
Jatropha molíssima (Pohl) Baill	Euphorbiaceae	3	0
Croton sonderianus Müll. Arg.	Euphorbiaceae	12	37
Piptadenia stipulacea (Benth.) Ducke	Fabaceae (Mimosoideae)	0	5
Guapira sp	Nyctaginaceae	0	3
Mimosa ophthalmocentra Mart. ex Benth	Fabaceae (Mimosoideae)	0	9
Mimosa tenuiflora (Willd.) Poir.	Fabaceae (Mimosoideae)	0	2
Not identified 2		0	4
Caesalpinia ferrea Mart. ex Tul.	Fabaceae (Caesalpinioideae).	0	1

Table 2 (*continued*)

Municipality/plant species	Famíly	Density (plant ha^{-1})	
		Open Caatinga	Dense Caatinga
Myracrodruon urundeuva Allemão	Anacardiaceae	0	1
Schinopsis brasiliensis Engl.	Anacardiaceae	0	2
Anadenanthera colubrina (Vell.) Brenan	Fabaceae (Mimosoideae)	0	12
Capparis flexuosa (L.) L.	Capparaceae	0	1
Araripina			
Eugenia speciosa Cambess.	Myrtaceae	1	0
Annona leptopetala (R.E.Fr. H. Rainer	Annonaceae	10	0
Scoparia dulcis L.	Plantaginaceae	1	0
Solanun capsicoides All.	Solanaceae	6	1
Not identified 3		3	0
Not identified 4		0	1
Croton sonderianus Müll. Arg.	Euphorbiaceae	1	0
Coccoloba alnifolia Casar.	Polygonaceae	2	1
Brosimum rubescens Taub.	Moraceae	0	44
Campomanesia xanthocarpa O. Berg	Myertaceae	0	3
Nothoscordon fragrans Kunth.	Liliaceae	0	3
Mimosa arenosa Willd.	Fabaceae (Mimosoideae)	0	13
Erythroxylum sp	Erythroxylaceae	0	4

occurred every few years. No fertilization or liming has been done in this area.

In 2018, two 25 × 25 m plots were delimited in each vegetation cover type and each study area. One of the two plots was fenced to prevent animals from entering and grazing (exclusion plots), and the other plot was left open to grazing (control plots). The control plots are still maintained for future measurements but in two of the areas the experimental stations remained without herds for long periods, after the animals were sold or transferred, and in another area, with fewer animals, grazing was restricted to the pasture stand, also for long periods. Therefore, they were not considered appropriate controls, and they were not sampled. Soil sampling was carried out in 2021, three years after exclusion, but exclusively in the fenced exclusion plots. Six simple disturbed samples were collected in the superficial layer (0–20 cm), at random points in each plot to perform chemical, physical and microbiological analyses. The samples were packaged in plastic bags and transported to the laboratory under refrigeration. Subsamples were air-dried, homogenized, and sieved at a 2 mm opening. One undisturbed sample was collected adjacent to each of the six disturbed samples of each plot, in two layers, 0–10 cm, and 10–20 cm. These samples were used to determine the soil bulk densities, and the density in the 0–20 cm layer was calculated as the average of the densities in the two layers. This collection procedure was adopted due to the difficulty of obtaining proper undisturbed samples for the 0–20 cm layer.

2.2. Soil characterization

The chemical attributes determined in the disturbed subsamples were (Table 3): pH in water (soil: water ratio of 1:2.5); sodium (Na^{+}), potassium (K^{+}), and phosphorus (P), extracted with Mehlich1 and measured by flame emission photometry (Na^{+} and K^{+}) and colorimetry (P); calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminum (Al^{3+}), extracted with KCl 1 mol L^{-1} and measured by titration. The physical attributes were (Table 3): granulometry (proportions of sand, silt, and clay), determined by the pipette method, natural clay; degree of flocculation; and residual moisture (Teixeira et al., 2017). In the undisturbed samples of both 0–10 cm and 10–20 cm, bulk density was determined by the clod method.

Total soil organic carbon and nitrogen concentrations (Table 4) were

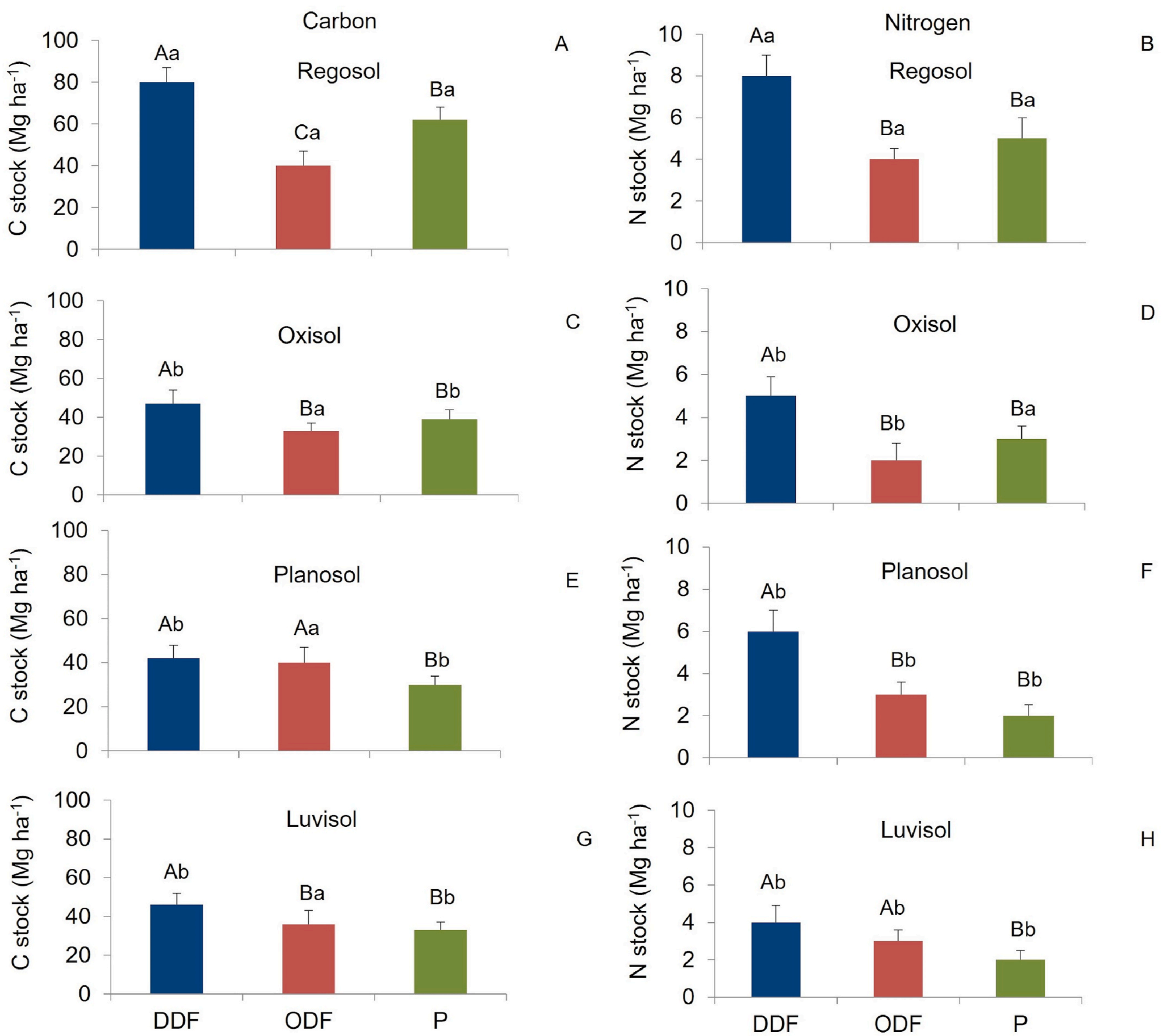

Fig. 1. C and N stocks in the 0–20 cm superficial layer of soils of four different classes under pasture (P) and open (ODF) and dense, dry forest (DDF, Caatinga) in the Brazilian semiarid region after three years without grazing. Uppercase and lowercase letters indicate significant differences between covers and soil types, respectively (Tukey $p < 0.05$).

also determined in the disturbed samples by dry combustion using LECO-CN 2000 aparatus. The C and N stocks (Fig. 1) were determined using the equivalent soil mass method, according to Ellert and Bettany (1995):

$$\text{Soil element stock}\left(\text{Mg ha}^{-1}\right)=\text{conc}\left(\text{kg Mg}^{-1}\right)*\text{Ds}\left(\text{Mg m}^{-3}\right)*\text{T}\left(\text{m}\right)*10^{4}$$

Where: soil element stock = C or N mass per unit area (Mg ha^{-1}); conc = C or N concentration (kg Mg^{-1}); Ds = average soil bulk density (Mg m-^{3}); T = thickness of soil layer (0.2 m); and 10^4 = conversion from m^2 to ha.

2.3. Soil respiration and C stability

Soil basal respiration (SBR) was determined by incubating soil subsamples with sodium hydroxide for seven days and titrating the absorbed bicarbonate (Mendonça and Matos, 2005). The mineralization quotient (qMin) was estimated by the relationship between the soil basal respiration during the seven days and the soil C concentration (Shao et al., 2024). The C decay constant (k_{dec}) was estimated by the relationship between soil basal respiration and carbon stocks, according to the model determined by La Scala Jr et al. (2009). The soil C half-life ($T_{1/2}$), which represents the time needed for 50 % of the soil C to be decomposed (Silva et al., 2020). was obtained according to Bayer et al. (2006).

Soil microbial biomass C (Cmic) was determined by the irradiation-extraction method (Anderson and Domsch, 1985), which is similar to the chloroform fumigation-extraction method (Powlson and Jenkinson, 1975), fumigation being substituted by irradiation. The metabolic quotient (qCO_2) was determined from the ratio between soil basal respiration and soil microbial biomass C. The microbial quotient (qMic) was calculated from the ratio between Cmic and soil C concentration (Anderson and Domsch, 1985).

Table 3
Chemical and physical attributes (0–20 cm depth) of soils of four soil classes under pasture (P) and open (ODF) and dense dry forest (DDF, Caatinga) in the Brazilian semiarid region, after three years without grazing.

Soil atribute	Soil type/vegetation cover											
	Regosol			Oxisol			Planosol			Luvisol		
	DDF	ODF	P	DDF	ODF	P	DDF	ODF	P	DDF	ODF	P
pH (water 1:2.5)	4.1	5.1	5.1	4.1	4.1	4.1	5.1	5.1	4.1	6.1	6.1	5.1
P (mg dm^{-3})	9.3	18.0	15.6	3.8	2.6	3.0	9.0	8.5	32.0	90.5	67.8	41.8
Ca^{2+} (cmol dm^{-3})	4.5	2.6	3.4	0.7	0.3	0.5	4.2	2.8	3.8	13	6.8	7.5
Mg^{2+} (cmol dm^{-3})	2.0	1.4	1.4	0.8	0.7	0.5	1.7	1.9	2.1	2.2	2.2	1.7
Na^{+} (cmol dm^{-3})	0.03	0.01	0.02	0.01	0.01	0.01	0.04	0.03	0.1	0.04	0.04	0.05
K^{+} (cmol dm^{-3})	0.9	0.4	0.4	0.1	0.05	0.07	0.3	0.2	0.5	0.6	0.6	0.5
Al^{2+} (cmol dm^{-3})	0.1	0.1	0.1	1.0	0.7	0.6	0.1	0.2	0.3	0	0	0
CEC (cmol dm^{-3})	12.4	7.6	8.5	7.2	5.9	5.4	8.9	8.5	11.4	18.8	11.3	11.4
BD (g cm^{3})	1.30	1.35	1.41	1.41	1.42	1.40	1.49	1.39	1.48	1.40	1.39	1.38
CS %	41.8	43.1	46.5	54.3	56.0	58.1	38.0	40.6	23.3	44.0	46.8	46.3
FS %	18.7	24.0	21.0	20.8	21.0	20.1	22.0	23.3	14.8	18.3	22.6	17.8
Silt %	24.0	19.8	19.5	4.5	3.3	4.0	28.0	26.0	44.0	19.1	19.5	22.5
Clay %	16.0	14.6	13.0	20.3	16.6	17.6	11.6	8.6	17.3	18.5	11.0	13.3
NC %	3.3	1.3	1.3	5.3	4.0	2.0	0.3	1.0	4.6	3.6	0.0	3.3
DF %	80.1	90.1	91.1	75.1	80.0	88.8	98.1	92.8	81.1	80.1	100.0	75.8

CEC: Cation exchange capacity; BD: bulk density; CS: coarse sand; FS: fine sand; NC: natural clay; DF: degree of flocculation.

Table 4
Soil C and N concentrations (C conc and N conc), soil basal respiration (SBR), mineralization quotient (qMin), C decay constant (k_{dec}) and soil C half-life (T 1/2), in the 0–20 cm superficial layer of soils of four different classes under pasture (P) and open (ODF) and dense dry forest (DDF) in the Brazilian semiarid region, after three years without grazing.

Soil/ Cover	C conc (g kg^{-1})	N conc (g kg^{-1})	SBR (mg kg^{-1})	qMin (day^{-1})	kdec (%)	T ½ (day)
Regosol						
DDF	39 Aa	4 Aa	60.9 Ab	0.1 Cb	0.002 Bb	347 Aa
ODF	15 Ca	2 Ba	64.2 Bc	0.4 Aa	0.004 Ab	173 Ba
P	27 Ba	2 Ba	43.2 Cd	0.2 Bc	0.002 Bc	347 Aa
Oxisol						
DDF	20 Abc	2 Ab	65.3 Bb	0.3 Aa	0.004 Ba	173 Ab
ODF	13 Ba	2 Aa	76.9 Ab	0.4 Aa	0.007 Aa	99 Bb
P	15 ABb	1 Aa	55.7 Cc	0.4 Ab	0.004 Bb	173 Ab
Planosol						
DDF	16 Ac	2 Ab	57.9 Cb	0.3 Ba	0.004 Ba	173 Ab
ODF	14 Aa	2 Aa	71.4 Bb	0.4 Ba	0.005 Bb	139 Bb
P	15 Ab	1 Aa	85.4 Ab	0.8 Aa	0.007 Aa	99 Cc
Luvisol						
DDF	25 Ab	1 Ab	83.4 Ba	0.4 Ba	0.004 Ba	173 Ab
ODF	17 Ba	1 Aa	93.3 Aa	0.3 Ba	0.007 Aa	100 Bb
P	13 Bb	1 Aa	95.9 Aa	0.8 Aa	0.007 Aa	100 Bc

Uppercase and lowercase letters indicate significant differences between different covers and soil types, respectively (Tukey test at 5 % probability).

2.4. Data analysis

Data of the estimated variables were tested in relation to their normal distribution and homoscedasticity and, when necessary, transformed to meet the assumptions of the analysis of variance. The data were subjected to analysis of variance (ANOVA) in a completely randomized design with six replications (samples in each plot) to test the effects of soil cover on the chemical, the physical, and the microbiological attributes. The means were compared by the Tukey test ($p < 0.05$).

Soil attribute data was subjected to principal component analysis (PCA - using the correlation matrix among all soil variables) to observe edaphic differences between the four studied areas. Spearman's rank correlation analysis was performed to identify interrelationships between the variables using the data of all experimental plots on the field (6 replications x 3 vegetation covers x 4 areas = 72). PCA and Spearman correlation were conducted using the "factoextra" R package (Kassambara, 2017).

3. Results

3.1. Soil C and N concentrations and stocks

C and N concentrations (Table 3) and stocks (Fig. 1) tended to be higher under the dense Caatingas (DDF) than under the other vegetation covers in all four soil types, especially in the Regosol area, which had the highest values (39 g kg^{-1} and 80 Mg ha^{-1} of C and 4 g kg^{-1} and 8 Mg ha^{-1} of N). In general, C and N concentrations and stocks did not significantly differ between the open Caatingas and the pastures despite absolute values being slightly lower under the pastures.

Unlike the C concentrations and stocks, basal soil respiration (Table 4) tended to be lower in the Regosol and higher in the Luvisol area, especially under the pasture cover (43.2 and 95.9 mg kg^{-1} day^{-1}, respectively). Comparing the pastures to the Caatingas, the soils were divided into two groups: the pastures were higher in the Luvisol and also in the Planosol and lower in the Regosol and Oxisol. The pattern for the carbon decay constant (k_{dec}) was similar to that of the SBR, being lower in the Regosol and higher in the Luvisol, particularly under the pastures (0.02 and 0.07 day^{-1}, respectively). Within each soil type, the comparisons of open Caatingas and pastures had a complex pattern, k_{dec} being higher in the open Caatingas than in the pastures in the Regosol and Oxisol, lower in the Planosol and similar in the Luvisol. As expected, the pattern for the C half-lives ($T_{½}$) was almost the opposite, with higher half-lives in the Regosol (173–347 days) and lower ones in the Luvisol (100–173 days), and higher half-lives in the dense than in the open caatinga in all soil types. Open Caatinga also had lower half-lives than the pastures in the Regosol and Oxisol, similar ones in the Luvisol, and higher in the Planosol.

Contrary to the C stock, the C mineralization quotient (Table 4) tended to be higher under the pastures, particularly in the Planosol and Luvisol areas (0.8 %) and except in the Regosol area. In this area, this quotient under the pasture (0.2 %) was similar to that under the dense Caatinga, which was the lowest absolute value (0.1 %). All other values oscillated from 0.3 to 0.4 %.

3.2. Microbiological analyses

Microbial C (Table 4) had a similar trend as the total organic C stocks (Fig. 1), tending to be higher under the dense Caatingas than under the other covers in all areas, except in the Regosol, where it was statistically similar to and slightly lower in absolute value than that of the pasture. The highest values also occurred in this soil type (553 mg kg^{-1} under the dense Caatinga and 580 mg kg^{-1} under the pasture), as occurred with the total soil C stocks. Except in the Regosol, microbial C under the open Caatingas and the pastures did not significantly differ.

The microbial quotients (qMic; Table 5) tended to be higher under the pastures in all soils (2.1–2.8 %) except in the Oxisol (1.2 %), with similar values for the dense and open Caatingas (1.2–1.9 %) in three of the soils but not in the Planosol. In this soil, the microbial quotient was higher under the dense Caatinga than the open Caatinga (2.2 versus 1.5 %). The metabolic quotient (qCO_2) also tended to be higher under the pastures (Table 5), particularly in the Planosol and Luvisol (0.4 %) and except in the Regosol area. In this area, this quotient under the pasture was similar to that under the dense Caatinga, which was the lowest absolute value of all (0.1 %). All other values oscillated from 0.3 to 0.4 %.

3.3. PCA and spearman correlation

The biplot Principal Component Analysis (Fig. 2) explained 51.5 % of the observed variance, 35.1 % in the first axis (PC1), and 16.4 % in the second axis (PC2). The samples of the Oxisol clearly separated from the other soils, clustering at the left in the PC1, while the Luvisol samples tended to cluster at the right in the PC1. The Regosol and Planosol samples were not separated in the PC1, but in the PC2 axis, the Regosol ones tended to cluster above those of the Planosol. The three covers within each soil class did not separate in PC1, but, in PC2, the samples of the dense Caatinga tended to cluster above the samples of the open Caatinga and the pastures, which had no clear separation.

C and N concentrations and stocks were strongly correlated (0.81; p, 0.001) to each other (Fig. 3). C stocks were also positively correlated with concentrations of Ca^{2+} (0.37; p, 0.01) and Mg^{2+} (0.37) and negatively correlated with sand proportions (−0.37), while N stocks were also correlated with Ca^{2+} and Mg^{2+} concentrations (0.47 and 0.38, respectively), plus K^{+} and available P concentrations (0.39 and 0.39), and also negatively correlated with sand proportions (−0.37). Soil basal respiration was positively correlated with Na^{+} concentrations.

Soil basal respiration also correlated with the concentrations of these

Table 5
Microbial biomass C (C mic), metabolic quotient of microbial C (qCO_2) and microbial C quotient (qMic) in the 0–20 cm superficial layer of soils of four different classes under pasture (P) and open (ODF) and dense dry forest (DDF, Caatinga), in the Brazilian semiarid region, after three years without grazing.

Soil	Land cover	C mic (mg kg^{-1})	qCO_2 (%)	qMic (%)_
Regosol	DDF	553 Aa	0.1 Bb	1.2 Bb
	ODF	209 Ba	0.3 Aa	1.4 Ba
	P	580 Aa	0.1 Bb	2.6 Aa
Oxisol	DDF	347 Ab	0.2 Aa	1.6 Ab
	ODF	197 Ba	0.3 Aa	1.7 Aa
	P	170 Bc	0.3 Aa	1.2 Ab
Planosol	DDF	383 Ab	0.2 Ba	2.2 Aa
	ODF	243 Ba	0.3 Aa	1.5 Ba
	P	282 Bb	0.4 Aa	2.8 Aa
Luvisol	DDF	353 Ab	0.2 Ba	1.9 Aa
	ODF	270 Ba	0.3 Ba	1.5 Ba
	P	245 Bb	0.4 Aa	2.1 Aa

Uppercase and lowercase letters indicate significant differences between covers and soil types, respectively (Tukey $p < 0.05$).

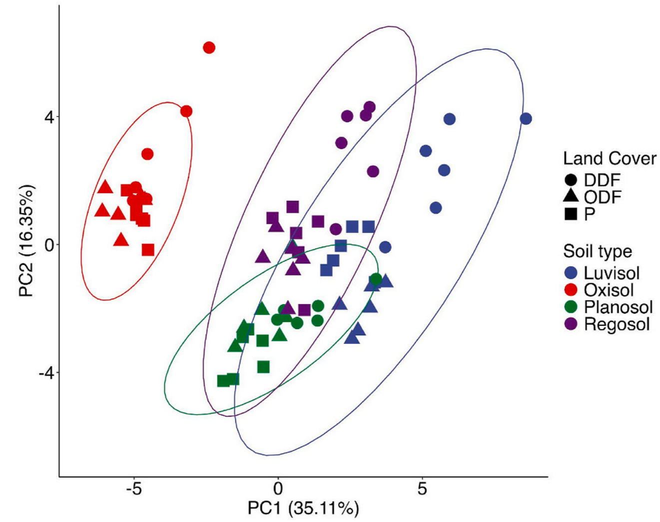

Fig. 2. Principal component analysis (PCA) biplot constructed with the correlation matrix of all soil characteristics in the 0–20 cm superficial layer of soils of all experimental plots (n = 72), in four different classes under pasture (P) and open (ODF) and dense dry forest (DDF, Caatinga), in the Brazilian semiarid region, after three years without grazing. PC1 and PC2 are the principal components 1 and 2, respectively. Both components explain 51.46 % of the observed variance.

elements (Ca^{2+}, 0.34; Mg^{2+}, 0.38; P, 0.39), plus Na^{+} concentrations (0.49) and pH (0.38) and negatively correlated with Al^{3+} concentrations (−0.34). On the other hand, it was not correlated with C and N concentrations and stocks. In contrast, the microbial biomass C was correlated with the soil C and N concentrations (0.31 and 0.33) and stocks (0.24 and 0.31), but not with soil basal respiration.

As expected, the derived quotients were correlated with the variables that were part of their calculations, but these correlations cannot be considered valid ones. As usual, some chemical (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, CEC and P) and one physical variable (silt) were positively correlated to some degree among themselves, while some of them were negatively correlated with Al^{3+} and sand).

4. Discussion

Carbon stocks in soils are determined by the balance between plant biomass production and plant necromass decomposition in the soil (Das et al., 2023). Commonly, soils under dense vegetation in the Brazilian drylands have higher carbon stocks than soils under open vegetation, which, in turn, have carbon stocks similar to those of pasture or agricultural soils (Menezes et al., 2021; Gava et al., 2022; Santana et al., 2019). In the extensive survey of Menezes et al. (2021), sandy soils had the highest average C stocks, while Leptosols and Planosols had the lowest stocks, but these differences were mostly derived from the stocks in areas of dense Caatingas since there were no differences among soil classes within the other land-use systems (Menezes et al., 2021). After three years of excluding ruminants, the present study showed that the C and N stocks in the top 0.2 m layer of the soil under dense vegetation were higher than those under open vegetation and pasture, except for the open Caatinga in the Planosol area. The higher stocks in the Regosol area can be attributed to a combination of higher rainfall and adequate soil chemical attributes. The stocks in the Luvisol area are limited by low rainfall despite the higher soil P and base contents. In the Oxisol area, they are limited by low fertility despite its higher rainfall.

Results in the present study indicated that the soils under the forests and pastures differ markedly in their physical and chemical properties and microbial biomass carbon. Vegetation and their trait properties vary in space and time because of variations in soil characteristics,

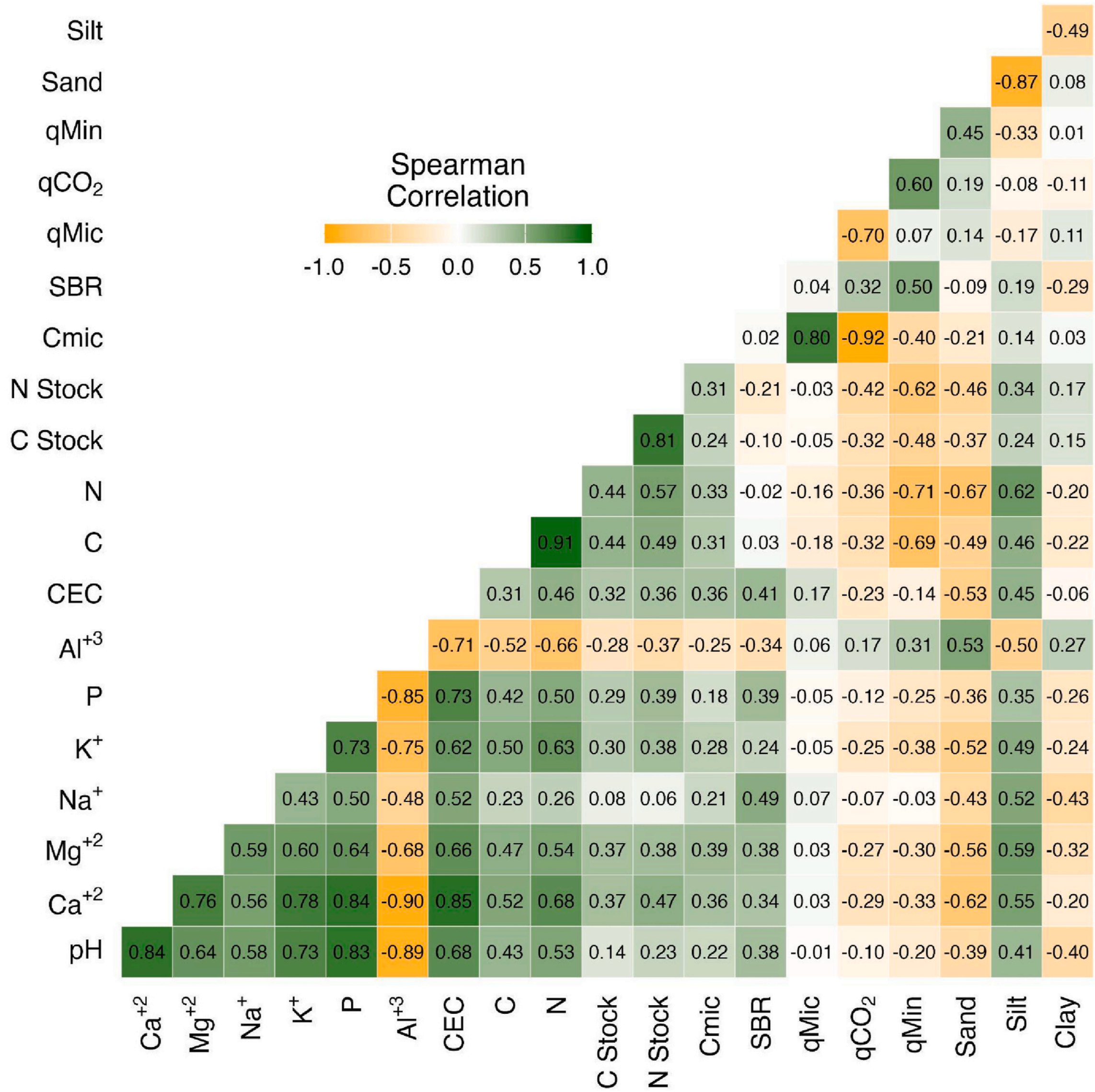

Fig. 3. Spearman rank correlation (r) among all variables evaluated in the 0–20 cm superficial layer of soils of all plots (n = 72), in four different classes under pasture (P) and open (ODF) and dens, dry forest (DDF, Caatinga), in the Brazilian semiarid region, after three years without grazing. Positive and negative r values represent positive and negative correlations, respectively. Significative thresholds for Spearman correlations (n = 72) are $r > 0.38$ or $r < -0.38 = p < 0.001$; $r > 0.30$ or $r < -0.30 = p < 0.01$, $r > 0.23$ or $r < -0.23 = p < 0.05$, $r < 0.23$ or $r > -0.23$ = non-significant.

topography, climate, weathering processes, and microbial activities (Paudel and Sah, 2003; Manral et al., 2023) and several other biotic and abiotic factors (Pandey et al., 2024). Soil properties, therefore, vary within short distances according to parent rocks, vegetation cover, and land use. In the highly dissected landscapes of any region, bioclimatic conditions change rapidly with altitude and may vary within short distances, resulting in a pronounced heterogeneity of soil types (Bäumler, 2015; Awasthi et al., 2022), hence influencing the distribution of vegetation, their traits, microbial activities and production (Pant et al., 2017; Bargali et al., 2019; Manral et al., 2022).

Notably, the soils we studied have not significantly improved carbon stocks in the open Caatingas compared to the dense Caatingas, probably due to the short grazing exclusion period. The water deficit in the Brazilian semiarid region imposes water limitations on vegetation growth even in fertile soils. Thus, after intense land use, plant biomass and soil C recoveries to match the values in the dense Caatinga can take over 60 years (Althoff et al., 2018). Nevertheless, despite the lack of differences comparing the grazing exclusion times within the open Caatinga and pasture samples in the same soil, our results highlight the influence of different soil types on the potential storage and stability of C, influenced by the intrinsic soil characteristics, and those that are the results of the interaction of soil pedogenesis and management, for example, the microbial community activity and functionality (Fracetto et al., 2012; Sokol et al., 2022).

The C storage in Regosol achieved the levels predicted for preserved dry forests independently of the cover management applied, being higher than 2 Mg ha^{-1} y^{-1} (IPCC, 2023-AR6), meeting the SDG 12, 13, and 14 proposed by the United Nations (Hilmi et al., 2022; Rodrigues do Nascimento, 2023). Moreover, even after three years of complete animal exclusion, the dense Caatinga stored twice as much carbon and nitrogen as the open Caatinga in this soil type. In general, the high environmental variability of the Caatinga biome, mainly of climate and soil type, considerably determines the total C and N stocks (Menezes et al., 2021; Santana et al., 2019).

Remarkably, the Regosol under the dense Caatinga has a reasonable availability of Ca, and Mg, which favors cation exchange in these microenvironments. Furthermore, the high total K content in the dense Caatinga increases the degree of soil dispersion, retaining the hydration and stabilizing a diffuse double layer, increasing the volume of macropores, and reducing soil compaction. These findings trigger the sealing of aggregate pores, increasing the density and cohesion between the particles and allowing greater accumulations of C in the organic matter (Moraes et al., 2022).

The carbon decay constant (k_{dec}) in the Regosol is lower under the dense Caatinga and the pasture than under the open Caatinga, indicating a slower organic matter decomposition, leading to an increased retention time of soil C, more than double compared to soils that released more C-CO_2, such as the Luvisol (all soil covers), Planosol (open Caatinga and pasture), and Oxisol (open Caatinga). The soil classes that accumulate humified organic matter in the top layers usually have higher microbial biomass C (Cmic) estimates and store more C and N, as observed in the Regosols of a semi-arid tropical pasture (Schaefer et al., 2022). After excluding animals and human activities for three years, the microbial communities in this soil have a greater potential for resilience due to the absence of disturbances in their oxidative metabolism, resulting thus in a higher efficiency in decomposing organic compounds (Ashraf et al., 2022; Neves et al., 2021). In contrast, the Planosol and the Luvisol under pasture and dense Caatinga showed higher fluxes of respiratory C-CO_2 and stored a smaller amount of C and N in the top layer than the Oxisol and the Regosol. The lower storage and stability of C in these two soil classes is probably due to two conditions: 1) the Luvisol area receives an annual rainfall average (550 mm) lower than those (711.2 and 798.7 mm) in the Oxisol and Regosol areas (Table 1), which results in lower vegetation contribution to litter formation and organic matter incorporation. The low total annual average but intense and short rain period disrupts aggregates, destabilizes the organic matter, and exposes its contents to decomposition by the microbial community, boosting carbon dioxide emissions (Oliveira Ferreira et al., 2018). 2) Planosols are typically shallow and of low fertility and poor drainage (Costa et al., 2021). They accumulate more water and clay only in their subsurface pedogenic horizon, diagnosed as Bt, making the surface-A horizon vulnerable to degradation and nutrient loss from plants through leaching (Krause et al., 2020). Consequently, surface microbial biomass and C and N inputs tend to be reduced.

The organic matter quality in Luvisols and Planosols is severely impacted by deforestation and wrong management, lasting over decades (Souza et al., 2022). According to Neves et al. (2021) and Santos et al. (2022), the degradation potential of the upper pedogenetic stratum of these soils after deforestation and overgrazing reduces carbon stocks by roughly 50 % compared to the dense Caatinga. Although the Luvisols and Planosols under pastures recovered the herbaceous cover during the last three years, the storage of C and N is still around 30 % and 66 % lower than those under the dense Caatingas, respectively. Furthermore, the microbial C biomass under the pasture is also 30 % lower than under the dense Caatingas. The microbial community is also susceptible to oxidative stress, as indicated by twice the metabolic and carbon mineralization quotients, suggesting that microorganisms have intense metabolic activities to break down the C and N organic compounds. Yet, this process could be inefficient due to the low microbial diversity, which exists outside of aggregates in a horizon that is susceptible to the effects of natural degradation (Schulz et al., 2016; Wardle and Ghani, 2018).

The low microbial biomass and metabolic quotients of the Oxisol under pasture (170 mg kg^{-1} and 1.2 %, respectively) are similar to those reported in soils undergoing desertification (Neves et al., 2021; Santos et al., 2022). The Oxisol C and N stocks and C stability did not improve compared to the Luvisols and Planosols. Oxisols are generally known for their good drainage and erosion resistance, but their surface horizons are characteristically poor in organic matter and nutrients and high in aluminum (Silva Cerqueira et al., 2023; Valladares et al., 2020). These conditions may inhibit microbial development.

The comparative multivariate analysis (Fig. 2), based on the overall soil chemical, physical, and biological properties, corroborated the differences among the soil classes, mainly between the Oxisol and the Luvisol, and the tendency of the vegetation covers to cluster within each soil, only the dense Caatingas separating from the open Caatingas and the pastures. The high proportions of variation explained by soil types agree with other reports on Brazilian drylands and derived mainly from their distinct chemical and physical variables, reflecting their different geological formation (Lourenço et al., 2020). The Oxisol and the Luvisol highlight these differences, the first being a deep, more sandy, previously highly weathered soil of low fertility on top of the old sedimentary plateau which formed more than a hundred million years ago, and the second a shallow, clayey and rocky soil, with high P and base contents, formed from the crystalline shield uncovered by continuous erosion of the surface layers. This separation will likely continue, despite the change in vegetation cover, if calculated with the inclusion of the chemical and especially the physic attributes, which tend to vary little for long periods, independent of management. Considering only the biological attributes, we hypothesize that in the future, after several more years of animal exclusion, the values in the pastures and open Caatingas will tend to become similar to those of the dense Caatingas. The short grazing exclusion period of the study certainly limited the tendency to similarity among the covers. Monitoring soil characteristics for more extended exclusion periods will be necessary to follow the recovery of these impacted Caatinga areas in the heart of the Brazilian drylands. Indeed, monitoring for several decades is the main objective of our long-term project, and the present results can be considered initial parameters. The inexistence of comparisons with the results of control grazed plots is a limitation of the data. Within the short period of the study, the absence of grazing for extended periods disqualified the plots as grazed controls. In the long run, with resumed grazing, the situation will probably follow the typical pattern in the semiarid region, with variable periods of grazing and animal stocks over the years. The control plots will not be continuously grazed since this pattern is not typical, but the effects of the ungrazed periods will be diluted, contrary to the initial three years, when they prevailed.

5. Conclusion

The four soil areas differed in the results of their biological attributes, certainly due to their different rainfall and chemical and physical characteristics. Under the dense, dry forest, the Regosol had the highest C and N stocks, microbial biomass C, and the lowest soil basal respiration, thus the longest soil C half-lives. This can be attributed to a combination of higher rainfall in the area than in the Planosol and Luvisol areas and better fertility than the Oxisol, despite the similar rainfall. Therefore, studies in the region must consider rainfall and soil typeswhen comparing results for different sites within the semiarid region.

Within each soil type, carbon and nitrogen stocks and the microbial biomass C in the open dry forest and the pastures tended to be still below those of the dense forest. In contrast, basal respiration tended to be higher. Therefore, the period necessary for soil recovery after grazing exclusion is longer than three years, including for the microbial population. Monitoring restoration for more extended periods will be necessary to guide the development of pasture management strategies, in order to decrease overgrazing occurrence, create grazing exclusion intervals, and calibrate the length of restoration periods.

CRediT authorship contribution statement

Pablo Acácio dos Santos Souza: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Felipe Jose Cury Fracetto:** Writing – review & editing. **Andressa Silva de Oliveira:** Formal analysis. **Juscélia da Silva Ferreira:** Methodology, Formal analysis. **Natache Gonçalves de Moura Ferrão:** Methodology. **Rômulo Simões Cezar Menezes:** Resources, Methodology. **Everardo Valadares de Sá Barretto Sampaio:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Paulo Ivan Fernandes Júnior:** Writing – review & editing, Formal analysis. **Ana Dolores Santiago de**

Freitas: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Brazilian Council for Scientific and Technological Development (CNPq; Grant Numbers: 406073/2022-8 and 316773/2021-2). This work is part of the National Observatory of Water and Carbon Dynamics in the Caatinga Biome - NOWCDCB, supported by Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE; APQ-0498-3.07/17 ONDACBC), CNPq (grants: 441305/2017-2; 465764/2014-2), and CAPES (grants: 88887.136369/2017–00). The second, third, fourth, and last two authors are research fellows of CNPq. The authors also thank INCT—Plant Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility (CNPq/Fundação Araucária STI/CAPES INCT-MPCPAgro 465133/2014-4).

Data availability

Data will be made available on request.

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