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RESEARCH PAPER





Land-use change alters the stocks of carbon, nitrogen, and phosphorus in a Haplic Cambisol in the Brazilian semi-arid region

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Abstract

Land-use change (LUC) can impact soil quality. In semi-arid areas of Brazil, impacts of LUC need to be understood for better soil management. This study evaluated the impact of LUC on soil organic carbon (SOC), nitrogen (N), and phosphorus (P) distributions through the soil profile and stocks of a Haplic-Cambisol in the Brazilian Semi-arid region. Three land-use systems (LUS) were investigated: agricultural management (30 years), regeneration under controlled grazing (25 years) after 5 years arable management, and native dry forest. Soil contents of P, total C, total N, and N fractions were used to calculate stocks and their stoichiometric ratios for layers 0-5, 5-10, 10-20, and 20-40 cm. Data from these LUS were compared using Kruskal-Wallis non-parametric tests. Changes to soil microbial biomass reflected the substantially reduced SOC concentration and stock in managed soils compared with that of the natural dry forest area. Total N stock was not affected by LUC, although increases in nitrate and ammonium offset a significantly reduced organic N fraction in the agricultural area. The largest P stock was found in agricultural land, followed by the grazed fallow regeneration site. LUC significantly influenced the stoichiometric ratio of C, N, and P, with the change from Caatinga to agriculture affecting the equilibrium between organic residues' input and mineralization. LUC resulted in significant changes to C, N, and P stocks, which did not recover to the original values, even after 25 years of regeneration under controlled grazing.

KEYWORDS

drylands, soil carbon, soil nitrogen, soil phosphorus

Highlights

- Agriculture and grazing reduces the stocks of C and N in a Brazilian Cambisol;
- 25 years of regeneration under controlled grazing do not recover the original C stocks
- Fertilizations, even after 25 years, increase the stock of P in former agricultural soil.

1 | INTRODUCTION

Carbon (C) and Nitrogen (N) stocks in the soil are controlled by the addition and removal rates. Soil organic matter (SOM) is the primary reserve of these elements, and, under natural conditions, it remains in a dynamic equilibrium, slightly fluctuating over time. Under these conditions, C and N stocks in the soil are mainly determined by moisture availability, temperature, and intrinsic soil characteristics. The replacement of native vegetation by agricultural systems dramatically reduces the stocks of C and nutrients in the soil, accelerating the mineralization of SOM (Corsi et al., 2012; Don et al., 2011; Giongo et al., 2020; Menezes et al., 2021).

The main mechanisms that limit SOM mineralization in weathered tropical soils are intra-aggregate protection and formation of organo-mineral complexes (Kravchenko et al., 2015). However, these mechanisms are relatively uncommon in the Brazilian semi-arid region because most of the soil has a sandy texture, with little charge or cations (Giongo et al., 2011; Menezes et al., 2021; Santana et al., 2019). Soils that originate from metamorphic rocks, rich in calcium carbonate, are, perhaps, the few exceptions (Fernández-Ugalde et al., 2014). As highlighted by Don et al., (2011) and Ademe (2015), the lack of adequate protective mechanisms implies that SOM is greatly affected by continuous soil disturbance and erosion in tropical ecosystems. Thus, SOM distribution and stocks in the soils of the Brazilian semi-arid region is dependent on a delicate balance between the supply and mineralization of organic residues (Giongo et al., 2011).

In highly weathered soils, P bioavailability is mostly controlled by the mineralization processes of SOM. At the same time, biological nitrogen fixation is the primary source of N. The most labile forms of P tend to decrease during the pedogenetic development of tropical soils, with a fraction of P immobilized into sesquioxides and soil erosion becoming predominant in the final stages of its evolution (Vitousek et al., 2010; Weil et al., 2016). The release of non-labile forms of P to soil solution can occur through microbial consumption of SOM, specifically by P-mining activity mediated by phosphatases (Vitousek et al., 2010).

Land-use change (LUC) affects content, distribution, and stoichiometric relationships between nutrients over time, thereby disturbing microbial activity and changing biogeochemical cycles, thereby resulting in a loss of soil fertility and productive potential (Erb et al., 2016). However, there is still a lack of information about the importance of long-term alterations caused by agriculture in the processes driven by the stoichiometric ratio in tropical environments. Such knowledge is even rarer for Caatinga, a predominant biome in the Brazilian semi-arid region.

The Caatinga biome encompasses about 850,000 km² and represents more than 11% of the Brazilian territory, however, it is still inadequately studied. The origin of the Caatinga is still debated, but the most accepted hypothesis proposes its evolution from a seasonally dry tropical forest developed during glacial periods in South America. The Caatinga is characterized by recurrent droughts leading to characteristic vegetation mainly formed by shrubs, cactus, deciduous trees, and some evergreen species (de Albuquerque et al., 2012).

Relief and orientation determine the pedogenesis from the calcareous metamorphic rock formation in the Chapada Diamantina region, the geomorphological subdivision of the landscape and, with land cover, lead to the formation of various soil types (Lima & Nolasco, 2015). Among these soils, Cambisols contain clays with great activity, rich in Ca⁺⁺ and carbonates, having well-developed structures (Cunha & Ribeiro, 1998). The intrinsic characteristics of these soils have led to the development of a SOM with particular features but little studied in terms of the impact of LUC on its stocks. The objective of this study was to evaluate changes in the stocks and distributions with the depth of SOC, N, and P, organic N, and C fractions induced by the LUC in a loamy eutrophic Haplic Cambisol from the Chapada Diamantina region. We compared an area under cultivated crops with a reference area of native vegetation and an area being regenerated under controlled grazing.

2 | MATERIALS AND METHODS

2.1 | Site location and description

The study area was located in the municipality of Irecê, in the north-central region of the State of Bahia (11°18'56 "S: 41°49'47", 760 m). The climate of the region was semi-arid, Bsh, according to the Köppen-Geiger classification, with an average daily temperature ranging from 18 to 36 °C annually. Annual rainfall averaged 650 mm, which was irregularly distributed in time and space, concentrated in summer and contrasted with the dry winters. Potential evapotranspiration was 1300 mm year⁻¹. The experimental area was set in a loamy eutrophic Haplic Cambisol [Loamy Calcaric Cambisol, according to FAO (2014) classification system], which originated from dark-grey calcitic limestones from the Bambuí Group (Schobbenhaus & Neves, 2003). The area had a flat geographical conformation with hypoxerophytic vegetation (dry forest), known as Caatinga (Brazilian Stepic Savannah), formed by shrubs and deciduous trees with a xerophytic character, a large number of cacti and bromeliads, deciduous trees and some evergreen species (Pennington et al., 2009). Three profile descriptions were made in trenches excavated in the studied area for soil classification. The areas of the study had differing use histories:

2.1.1 | Native dry forest (Nat)

A preserved area of stepic savannah (Caatinga) that received little human interference since 1980's, considered as the reference treatment. The predominant species in the natural vegetation were: *Aspideoperma* sp., *Combretum* sp., *Bauhinia* sp., *Commiphora leptoploeos*, *Anadenanthera* spp., *Myracrodruon urundeuva*, *Cereus jamacaru*, and *Jatropha molissima*.

2.1.2 | Regeneration area under controlled grazing (R)

The Caatinga area was deforested in 1987. Rainfed maize (Zea mays) was then grown as the main crop and intercropped with cassava (Manihot sculenta). From 1987 to 1991, maize was sown after conventional tillage, using mechanical ploughing and harrowing. The average annual fertilizer application for maize was 80 kg N ha⁻¹; 45-60 kg P ha⁻¹ and 25–35 kg K ha⁻¹, preferentially applied in the plant rows. Cassava received was 0-30 kg N ha⁻¹ and 0-20 kg P ha⁻¹. The rainfall regimen greatly influenced maize and cassava productivity, varying from 1.4 to 3.7 Mg ha⁻¹ year⁻¹ for maize and 10.8 to 14.4 Mg ha⁻¹ year⁻¹ for cassava. For the last 26 years, the area has been under a natural regeneration process, with the predominant species being Croton sp., Mimosa hostilis, Mimosa tenuiflora, Caesalpinia sp., Croton sp., Piptadeina sp., Manihot glaziovii, Neoglaziovia variegata, Myracrodruon urundeuva, and Combretum sp. Dairy cows grazed the area with access restricted to the beginning of the dry season. The current herd grazing in the area was made up of 12 lactating cows and their calves.

2.1.3 | Cultivated area (Agr)

Area under continuous rainfed cultivation alternately with different crops [maize, common beans (*Phaseolus vulgaris*), and sorghum (*Sorghum bicolor*)] since 1992. It had been used to cultivate castor bean (*Ricinus communis*) from 1987 to 1992. Soil preparation was by conventional tillage using mechanical ploughing and harrowing. The regular fertilizer application to the cultivated area was: 60 kg ha⁻¹ of N (mainly as urea), 61 kg ha⁻¹ P (single superphosphate); and 33.2 kg ha⁻¹ K (exclusively KCl) for maize and sorghum crops. No lime was needed, given the low aluminium content, neutral to alkaline pH, and large content of Ca²⁺ in the soil. Fertilizer P was applied at sowing, whereas N and K applications were

split, applying 25% of N and 50% of K at sowing. A sidedress fertilizer application (20–30 days after emergence) used 50% of N, and all remaining nutrients were applied at the flowering stage (vegetative stage V10). *Phaseolus* bean received 60 kg N ha⁻¹ (20 kg at sowing and 40 kg 30–40 days after emergence); 52 kg P ha⁻¹ were applied in the plant rows at sowing, 42 kg K ha⁻¹ were applied, with 50% applied at sowing and the remainder as side-dressing at flowering. In the first crop season, castor beans received 150 kg N ha⁻¹, 10.8 kg P ha⁻¹ and 50 kg K ha⁻¹ of K in the plant row. Subsequently, 80 kg N ha⁻¹, 7.8 kg P ha⁻¹, and 26.6 kg K ha⁻¹ were applied in side-dressing.

Crop productivity was very dependent on rainfall, with maize grain production for 2001–2011 being similar to previous values, roughly varying from 1.8 to 4.0 Mg ha⁻¹ per year; sorghum grain production varied from 2.5 to 3.4 Mg ha⁻¹ year⁻¹. *Phaseolus* bean productivity in the same period varied between 0.31 and 0.67 Mg ha⁻¹ year⁻¹. As part of the production system, the dairy cow herd gained access to the crop areas after harvest. Castor bean cultivation started in 2012 at the start of a prolonged regional drought period; therefore, productivity was low, ranging from 0.3 to 0.75 Mg ha⁻¹ year⁻¹.

2.2 | Soil sampling and chemical, biological and physical analysis

Soil sampling was in August 2017 using six trenches (1.0 x 0.5 m) in each sampling area. Samples were collected at depths of 0–0.05; 0.05–0.10; 0.10–0.20, and 0.20–0.40 m for chemical and physical analyses. Subsamples of 4 kg for each soil layer were collected from the side of the trench walls, stored in plastic bags, and kept in a refrigerated box for transportation. Analysis of the soil bulk density (BD) was determined using a volumetric ring (d = 0.05; h = 0.06 m) collected in triplicate from each layer and all trenches. Soil texture was determined using the sedimentation and pippet method (Gee & Or, 2002).

Subsamples of soil from each layer were separately processed for chemical analysis. After air-drying and sieving (<2 mm), the subsamples were ground in a Wiley mill to pass through a 100 mesh (0.149 mm) sieve and used for the determination of total contents of C and N using dry combustion in an Elemental Analyzer TruSpec model CN (Leco Inc.). Dissolved organic carbon (DOC) was measured, according to Bolan et al., (2011). Briefly, aqueous extracts obtained in 0.5 mol L $^{-1}$ K $_2$ SO $_4$ were filtered through a 0.45-µm filter membrane and analysed by wet oxidation in K $_2$ Cr $_2$ O $_7$ 0.0667 mol L $^{-1}$ + H $_2$ SO $_4$ /H $_3$ PO $_4$ 2: 1 and titrated with ferrous ammonium sulphate 0.0333 mol L $^{-1}$. The analyses of available P was performed according to Klute and Page (1982). All the analyses were made in triplicate for each subsample.

Nitrate contents were analysed using the colorimetric determination of soil NO_3 -N by transnitration of salicylic acid, as described by Yang et al., (1998). The ammonium contents were determined by applying the colorimetric determination of soil N-NH₄ titration of Kjeldahl digestion products. Organic N was determined by the difference between the total N (determined by dry combustion method) and inorganic N ($NH_4^+ + NO_3^-$).

Similarly, soil microbial biomass (SMB) was determined for the subsamples of each layer. Processing of soil began immediately after arrival at the laboratory by passing it through a 10 mm mesh sieve and was followed by applying the fumigation-extraction method (Vance et al., 1987). Soil microbial carbon (or soil microbial biomass) was estimated by the difference between the carbon content in fumigated and non-fumigated samples and corrected by the multiplication by the correction factor (K) of 2.64 (Anderson & Ingram, 1993).

2.3 | Estimating the soil stocks of phosphorus, carbon, and nitrogen

Stocks of P, SOC, and N and their fractions in the bulk soil were calculated for each layer as described by Bayer et al., (2006) using the equation $D_{X_{ij}} = -X_{ij} \cdot h \cdot \gamma$ where D represents the stock of the variable (Mg ha⁻¹); $-X_{ij}$ is the average concentration of the variable (kg Mg⁻¹); γ is the soil bulk density (Mg m⁻³); and h is the sampled layer (m). Stocks were corrected for the mass of the equivalent layer using the bulk density of the reference area according to Ellert and Bettany (1995): $SD_c = \gamma_{ref}/\gamma_{cor} \times SD_{ref}$, where, SD_c =soil layer depth of the reference area; γ_{ref} =soil density in the reference area (kg dm³); γ_{cor} = soil density in the corrected area; SD_c =soil layer depth of the corrected area. Gross and corrected stock were calculated for the whole depth by the summation of the stock of each layer.

Sensitivity indexes (SIs), defined as the reduction caused by LUC in the P, SOC and N pool component stocks using area Nat as the reference, were calculated as Liang et al., (2012) using the equation $SI = \frac{-X_{ij} - -X_{nv}}{-X_{nv}} x 100$ for each layer, where $-X_{ij}$ is the average stock of the variable of a given land use in a layer and $-X_{nv}$ is the average stock in the same layer of the reference area. The SIs were estimated for each depth using the equivalent layer of corrected stocks.

2.4 | Estimating stoichiometric C, N, and P ratio

C, N and P ratio, the soil total C, N and P concentrations (mg kg⁻¹) were transformed to units of mmol kg⁻¹, and C: N, C: P and N: P ratios for each layer and sampling

site were calculated as molar ratios (atomic ratio), rather than mass ratios as in Redfield (1958). This procedure was adopted to correct limitations reported in the methods applied to estimate soil and microbial organic C, N, and P contents (Cleveland & Liptzin, 2007). Therefore, estimates of microbial biomass were adjusted using the conversion factors 0.45, 0.45, and 0.40 for C, N, and P, respectively (Vance et al., 1987).

2.5 | Statistical analysis

The data for the P, SOC, DOC, and N contents and stocks were analysed using the non-parametric Kruskal–Wallis (K-W) test for k samples (p < .05) using Statistica 12.0 (Statsoft Inc., USA).

3 | RESULTS

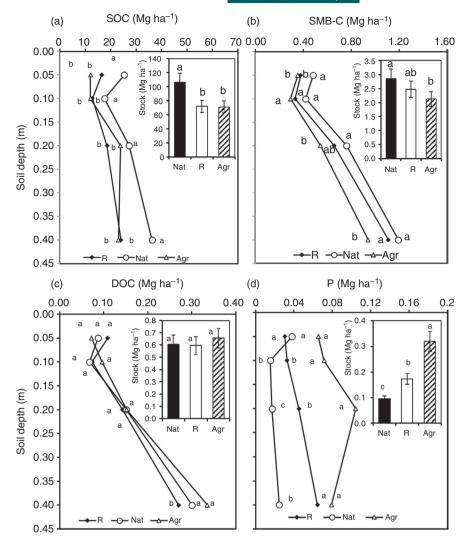
3.1 | Effect of LUC on SOC and C stocks

LUC resulted in an intense alteration in the SOC of the different layers of a loamy eutrophic Haplic Cambisol in the Chapada Diamantina. There was a significant reduction in SOC concentration and stock than in the native area (Nat) (Figure 1a). In general, LUC significantly (p < .05) reduced the distribution of SMB in the layers and stock (Figure 1b), but not for DOC (Figure 1c). The SOC distribution was significantly smaller in the different soil layers surface layers, for both the cultivated (Agr) and regeneration under controlled grazing (R) areas, showing negative sensitivity index (SI) values in all layers (Figure 2b)., resulting in a negative SI in all layers for SMB (Figure 2d). SMB in the Agr treatment was less than that under natural vegetation (Nat) for all evaluated layers, with SI values varying from -26.4 in the soil surface to -30% in the 20–40 cm layer. The stock of C as SMB in Agr was significantly smaller than that in Nat for the 0-20-cm layer, whereas results for the soil under R were statistically similar to Agr. The amount of DOC in Nat and AGR was significantly greater than in R at 0.45 m, but there was no significant effect of LUC on DOC stock.

3.2 | Effect of LUC on P distribution and stock

Results for phosphorus (P) distribution and stocks relate to the available form of P. The content and stock of P in Agr were significantly greater than in other areas (approximately 2.5 times). Under Nat, P distribution showed the greatest amount in the top layer, but the concentration of this element decreased to smaller values than for other treatments, showing little further

FIGURE 1 Stocks (Mg ha⁻¹) of total soil organic carbon (a), soil microbial biomass (b), dissolved organic carbon (c), and phosphorus (d) influenced by land-use change at different depths of a loamy eutrophic Haplic Cambisol in the Brazilian semi-arid region. The inner graph shows the total stock of each variable (0–40 cm), and letters above the columns represent significant differences between land uses according to the Kruskal–Wallis test (p < .05). R: Regeneration area under controlled grazing; Nat.: Native dry forest; Agr: Cultivated area



variation with depth; consequently, Nat had the smallest stock of P (Figure 1). The P content in R was relatively homogeneous in all layers, statistically similar to Nat in the surface layer and with a slight increase at 0.4 m depth. SI for P was huge and positive in Agr and R, mainly in the subsurface layers.

3.3 | Effect of LUC on N pool content and stock

Total nitrogen pool contents (N) and stocks were not significantly affected by LUC (Figure 3). However, the concentration and stocks of mineral forms of N (N-Min =NO3 + NH4) were remarkably larger under Agr at all depth, with N-Min stock in Agr being 78.9% greater than in Nat. Most of the effect in the SI for N-Min was caused by the content of NO_3 (Figure 3).

The organic N (N-Org) content was smaller in the soil surface layer under Agr, whereas in R it was similar to Nat. The soil nitrate stock SI was markedly large in Agr, increasing in the subsurface layers. R area results showed SI near zero for

 $N-NO_3$ in every layer, indicating that the $N-NO_3$ content was very close to Nat.

In contrast to these findings for C, N and, P contents, K, Mg, Ca, and micronutrients did not change with LUS (Table S1). The values of density (particle and soil) and total porosity also were not influenced by the LUS (Table S2).

3.4 | Effect of LUC on C, N, and P stoichiometry

LUC produced a remarkable change in the stoichiometry of C, N, and P (mol mol⁻¹) throughout soil depths. The C:N ratio of the disturbed areas were close to each other and smaller than Nat at all depths, but mainly in the surface layers (Figure 4). The LUC's effects on C, N, and P stoichiometry were most striking in the C:P and N:P ratios, which were lower in Agr and R than in the Nat area, except for the surface layer (0–0.05 cm). The estimated values for C:N:P ratio between the stocks were: Agr=275.1, 18.95, 1.0; R = 509.54, 34.2, 1.0; and Nat=1,315.8, 65.8, 1.0.

20 -40

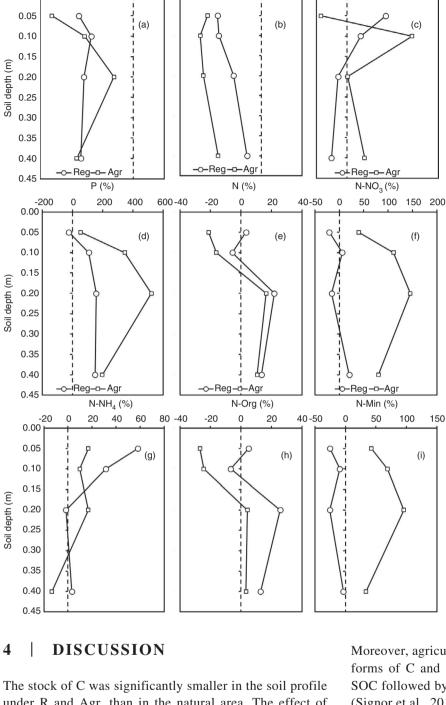
SOC (%)

-40

-60

0.00

-20



SMB-C (%)

-20

DOC (%)

20

40

(c)

(f)

100

(i)

150

60

0

20 -20

FIGURE 2 Variation in the sensitivity index (SI) of soil P, C, and N and its fractions influenced by land-use change in a loamy eutrophic Haplic Cambisol in the Brazilian semi-arid region. a - soil organic carbon; b - soil microbial biomass C; c - dissolved organic C; d - phosphorus; e total N stocks; f - nitric N; g - ammoniacal N; h – organic N; i – mineral N. R: Regeneration area under controlled grazing; Nat.: Native dry forest; Agr: Cultivated area

under R and Agr, than in the natural area. The effect of LUC on SOC reductions in the soil profile was also shown by Sheng et al., (2015), even at depths greater than 20 cm - previously considered to be a more stable environment. Under Agr, crop debris became the only source of organic residues, differing qualitatively, and quantitatively from the residues of Caatinga in R and Nat areas. In such conditions, fresh carbon and nutrient availability produced by agriculture may result in a priming effect, which in the long term reduced the SOC and SMB content, interfering with microbial-mediated processes in soil (Kuzyakov, 2010).

Moreover, agricultural operations produce a burst of stable forms of C and microbial activity, resulting in losses of SOC followed by a rapid reduction in microbial processes (Signor et al., 2018).

SOC, C stocks, and SI for SOC observed in R and Agr areas show that, in this semi-arid environment, even under fallow for 25 years, it is not easy to accumulate stable forms of C in the soil. This behaviour reflects semi-arid precipitation and temperature conditions that limit plant biomass production in the R area (and also in the Nat) to the short rainy season. The leaves fall due to the lack of water throughout the dry season, and this material accumulates under the soil surface and will be quickly decomposed when water becomes available in future rainy seasons. In this case, as SMB also

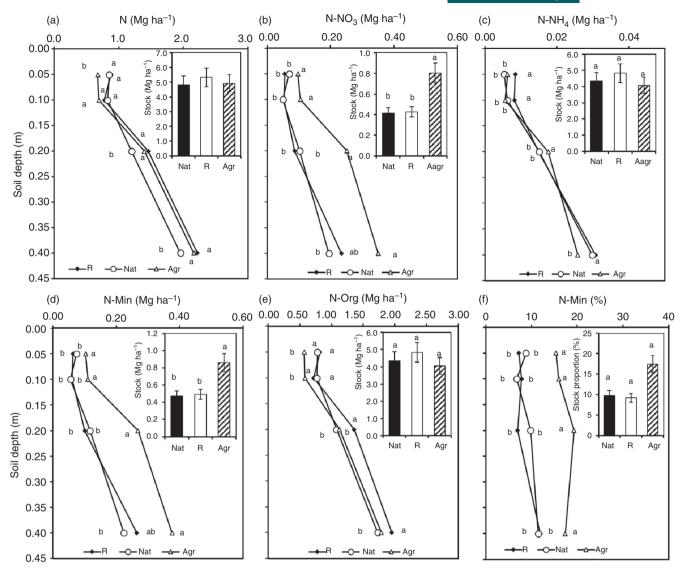


FIGURE 3 Stocks (Mg ha⁻¹) of total nitrogen (a), nitric nitrogen (b), ammoniacal nitrogen (c), mineral nitrogen (d), organic nitrogen (e), and proportion (%) of mineral nitrogen (f) influenced by land-use change in different depths of a loamy eutrophic Haplic Cambisol in the Brazilian semi-arid region. The inner graph shows the total stock of each variable, and the letters above the columns represent significant differences between land uses according to the Kruskal–Wallis test (p < .05). R: Regeneration area under controlled grazing; Nat.: Native dry forest; Agr: Cultivated area

showed similar behaviour, we can affirm that much of the C in the decomposed materials in the litter of the R will be converted to CO_2 that will be emitted to the atmosphere, explaining why 25 years of fallow did not increase soil C stocks.

The distribution of DOC in the soil profile was homogenous and did not match the theoretically expected higher concentration in the surface layers, indicating an undercurrent SOC translocating process. For methodological purposes, DOC is defined as the organic matter in a solution that passes a 0.45-µm filter, representing only a small proportion of the SOC (Bolan et al., 2011). The sources and degradation processes of DOC have long been discussed, and it is known to influence different biological systems and processes (Kiikkila et al., 2014). Its accumulation at depth is caused by

leaching of soluble organic compounds, such as fulvic acid, or illuviation of organic matter (Bot & Benites, 2005). The first hypothesis is supported by a study of Cunha and Ribeiro (1998), who found high levels of soluble fulvic acids in this same soil, which can be transported by rainwater to the lower layers.

Organic N was the main form of N in the three evaluated land uses. Despite this, the Agr site showed the larger mineral N contents, mainly represented by nitrate, due to the frequent N fertilizer application. The SI for the nitrate stock was markedly large in the Agr area, increasing in the subsurface layers, which also reflects the nitrogen fertilizer application to successive cropping cycles. The SI for N-min showed the same behaviour as the SI of N-NO₃. This results from nitrate

Stoichiometric relationships between the C, N, and P contents as a function of land-use change through different layers of a loamy eutrophic Haplic Cambisol in the Brazilian semi-arid region. R: Regeneration area under controlled grazing; Nat.: Native dry forest; Agr: Cultivated

—R -0-Nat. -△-Agr

being the primary form of mineral N in both Agr and R land uses. The R area showed an SI around zero for N-NO3 in every layer, meaning that nitrate was consumed throughout the years of natural vegetation recovery and partially converted to organic forms, explaining why N-Org was higher in the R area.

-o-Nat. -△-Agr

0.40

0.45

Although P is a limiting nutrient in tropical soils, relatively few studies have considered the impact of LUC on stocks of this element in the soil (Franco et al., 2015). We found that P soil stock in Agr was approximately 2.5 times larger than under Nat, with the greatest stock in the 0-20 cm layer. The P stock in R was significantly smaller than in Agr but larger than in Nat. Phosphate fertilizer application in Agr resulted in the largest P stock, with a homogeneous distribution within the soil layers (0–40 cm depth). Despite its known restricted mobility, P stocks both in Agr and R areas had a homogenous distribution among soil layers that was caused by ploughing and harrowing operations of successive cropping years (Deubel et al., 2011). Phosphorus fertilizer application and redistribution had a long-term impact on P stocks resulting in P stock under R being twice that of the reference area and homogeneously distributed down to 40 cm depth even after a long period of fallow.

The area under regeneration with controlled grazing (R) has been in fallow for 25 years and had received P fertilizer only during the cropping before the fallow. As this area was cultivated for a shorter period than was Agr, the amount of P added was less. Revegetation in R occurred by the natural growth of shrub and arboreous species highly adapted to the climate conditions. The predominant species among

shrub and shrub-tree in R were Mimosa spp., Piptadenia sp., Caesalpinia sp., and Croton spp., interspaced with young tree species (Anadenanthera sp., Cammiphora sp., and Myracrodruon urundeuva). As common for species highly adapted to arid and semi-arid environments, these species have deep root systems able to mobilize large amounts of nutrients from deep soil layers, accumulating them in soil surface through leaf and thin branch deposition over the years (Dalla Lana et al., 2019; Menezes et al., 2021). This characteristic helps to explain P accumulation in Nat areas. However, after 25 years in fallow and with natural reforestation under controlled grazing, P content in the surface layer was less than in Nat as a result of the nutrient export by foraging (Raiesi & Asadi, 2006). Although the large calcium content of this soil could promote P fixation by forming calcium phosphates, it would have been dissolved by the acidic Mehlich I extractant used in the soil analysis. The smallest P stock was observed in the native area, but the upper soil layer (0-0.05 m depth) showed larger P concentration than did deeper layers. Likely, P is captured in deeper layers by tree roots and deposited on the surface as organic residues when leaves and branches fall on the soil surface (Kertesz & Frossard, 2015; Tian et al., 2010).

→R -○-Nat. -△-Agr

Plant-mediated P remobilization is essential in soils with a high potential for P immobilization by Ca⁺², as in the studied area, making it available by microbial mineralization of the organic residues deposited. P fertilizer increased P stock drastically and positively affected the SI, even in the regeneration area, with 25 years to natural restoration. It provides solid evidence for the permanent alteration produced by

agriculture in the soil and environment. In a previous study in the same region in another soil type (Oxisol) covered with castor bean, Fracetto et al., (2012) observed a reduction of 50% in the C and N stocks. We also found C stocks decrease of 33.2% and 32.4% for Agr and R areas, respectively, confirming C stock behaviour and the slow progress of C content recovery in a tropical semi-arid region. However, total N stocks increased 12.1% and 17.2% in the same areas, driven by a increase of 69.6% in mineral N stock in cultivated area and 20.0% in organic N stock in the area under regeneration and controlled grazing. Similarly, we also found that LUC significantly increased P stock even at the deeper soil layers studied, resulting in an increase of 215.4% in the stock estimated for the continuously cultivated land, and 73.0% of the stock in the area under natural revegetation. The increase of P stock caused by agriculture in tropical areas has been reported previously. For example, Franco et al., (2015) studied three chronosequences of land-use change in the Atlantic rain-forest biome, an environment different from Caatinga. However, they also showed that sugarcane cultivation reduces C and N stocks, but that changes in the P stock were dependent on fertilizer application, as in the present study.

In the C-richer surface layers (0–5 cm), the values of C:N ratios were between 18 and 30% greater than those predicted by Cleveland and Liptzin (2007). C:N ratios larger than 20 were also observed in deeper layers for Nat, while the values for Agr and R were smaller than that in deeper layers. The reduction in the variability of the C:N ratio in the subsurface is consistent with reports of Tian et al., (2010) and Groppo et al., (2015). C:P and N:P ratios were more affected by LUC than C:N ratio. While C:P in Nat varied from 74.9 to 200 in the evaluated layers, in Agr and R areas, it varied between 21.3 and 63.34, values that are smaller than those found by Cleveland and Liptzin (2007) as a result of P fertilizer application in these both areas.

It is hard to set a precise limit but it is believed that the C:N ratio threshold in SOM that allows its accumulation is approximately 20:1 (Mooshammer et al., 2014). In this study, only the native vegetation showed values greater than this threshold, showing potential for accumulation in the soil. In N and P-depleted soils, the microbial population will allocate more resources to nutrient mining than growth, especially when multiple nutrients are lacking. This resource allocation reduces the growth rate of microbial population and allows for a balance between mineralization and transformation of SOC that could increase C stocks over time (Sinsabaugh et al., 2013). Thus, excessive nutrient disposal, as occurred under Agr, accelerates SOM decomposition, negatively affecting soil physical—chemical attributes, notably CEC, aggregation, and water retention (Manzoni et al., 2012).

LUC significantly influenced the balance between the addition and loss of C in the soil mainly due to fertilizer application events, and these changes may extend to layers

below 20 cm that were previously considered to be protected. Comparing our results to those in the literature, we verified that the intensity of change in SOC is influenced by the intrinsic characteristics of the soil and climate. In the studied Haplic Cambisol, the large content of calcium carbonate and clay protected SOM from the impact of LUC, keeping large contents compared to the most common soils in the tropical semi-arid. LUC did not change the N stock significantly, mainly due to the increase in the mineral N stock in Agr, most notably in nitrate form. The natural regeneration allowed the recovery of the total and organic N stocks, perhaps accumulating part of N added by agriculture fertilizer use in residues and later in SOM. The stoichiometric ratio of C, N, and P was profoundly altered by LUC, even at depth, and the reduction of C:N and C:P ratios, may cause a significant reduction in the C stock. However, detailed studies with more in-depth sampling are necessary to confirm this information.

Regeneration of the Caatinga under controlled grazing, an economic strategy for degraded land recovery, has not allowed the C stock's recovery after 25 years. However, it was evident that the stocks of P and mineral N decreased, while the stock of SMB-C increased. These characteristics started to distinguish the area under regeneration and controlled grazing from the cultivated area. The stoichiometric ratios of nutrients in the regeneration area are still unbalanced at the expense of its evolution and recovery to the reference values.

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