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RESEARCH ARTICLE

Long-Term impact of rice cultivation on soil quality indicators in Northern Amazonia Savanna

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Abstract
Changes in soil attributes following changes in management strategies in rice cultivation can alter soil quality, either positively or negatively. The aim of this study was to evaluate soil quality indicators, based on physical, chemical and microbiological soil attributes in Amazonian savanna under rice cultivation with different ages. The research was conducted in five areas under continuous rice cultivation during 1, 3, 8, 13, and 25 years, compared with local reference native vegetation. Soil samples were collected at 0-10 cm depth and evaluated for physical attributes such as texture and bulk density, chemical properties such as soil organic carbon, total nitrogen, exchangeable cations, available P, pH, CEC and C/N ratio; and microbiological attributes like microbial biomass carbon, soil microbial respiration, metabolic ratio and microbial coefficient. Acid phosphatase and urease activity were evaluated. With longer periods of time of rice cultivation, soil quality was enhanced with increasing soil fertility, increased soil organic carbon content and enzyme activity. The paddy soil indicators were sensitive to changes in rice cultivation and its duration. Acid phosphatase activity and available phosphorus increased with longer time of rice cultivation, indicating a possible conversion of inorganic into organic phosphorus forms, corroborated by increasing phosphatase activity. Microbiological (SMB-C, SBR, qMIC and qCO_2) and biochemical (urease and acid phosphatase) indicators, as well as soil organic carbon and total nitrogen were highly sensitive to land use changes. Chemical and microbiological indicators are suitable for estimating paddy soil quality in lowland of Amazonian savanna.

Keywords: enzymatic activity; soil microbiology; soil indicators; Amazon soils.

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1. Introduction
Rice is one of the most important crops globally due to its fundamental role in feeding the word's populations. Approximately 50% of the global population depends on rice as their primary source of calories and nutrients (FAO, 2021). This crop is especially crucial in regions such as Asia, Africa, and Latin America, where it is an essential part of the daily diet and significantly contributes to food security (Vitali et al., 2024). Rice cultivation is one of the few commercial crops of importance in Roraima State that generate local employment and income. Nearly 80% of the rice production is located on lowland soils (Sakazaki et al., 2008), in total 160,000 hectares of savanna. These soils are traditionally used for flood irrigated rice cultivation (Barberena et al., 2011), but the long-term consequences for soil quality (SQ) and crop yield have not yet been investigated.
Assessment of SQ is often difficult, since the complex interplay in the soil system is a rule. Consequently, any attributes used to estimate SQ should consider this complexity (Lima et al., 2013). Since individual indicators are insufficient to measure losses or gain in SQ (Li et al., 2022), it is essential to assess the combination of diverse soil attributes, through multivariate analysis (Lima et al., 2013). Lima et al. (2013) studied a small set of SQ indicators of rice

cultivation in Rio Grande do Sul (Brazil) and reported that microbiological attributes and Soil Organic Matter (SOM) were the most important SQ indicators. In China, Li et al. (2022) found the content of N, P, acid phosphatase, total number of bacteria and mycorrhizae as key variables to measure SQ for rice cultivation. Liu et al. (2015) indicated that the activities of enzymes such as urease, acid and alkaline phosphatase, and β-glucosidase, are considered the most reliable indicators of SQ due to their high sensitivity to changes resulting from soil management.

Research on SQ indicators for rice strategies cultivation has been carried out, both within Brazil (Barberena et al., 2011; Lima et al., 2013) and elsewhere (Li et al., 2022). These studies allowed the establishment of a minimum data set from which soil quality in paddy soils can be assessed. However, information concerning these soil indicators for rice cultivation paddy soils from Amazonia is currently unknown or nonexistent. Due to the increasing economic importance of commercial rice cultivation in Northern Amazonia, studies that analyze and monitor soil attributes are very important.

The hypothesis is that in flood rice cropping, soil tillage and crop residue management could be considered the prime factors in the control of the long-term soil nutrient balance, and in the enzymatic and soil microbiological activities. In addition, to keep the soil productive for long-term, the low fertility of soil must be considered under natural vegetation. In this way, a comprehensive assessment of soil quality integrating soil physical, chemical and biological properties would be greatly desired.

Considering the above statements and bearing in mind that the search for the appropriate attributes with which to measure SQ for rice cultivation and other forms of agricultural activities is still incipient for Amazonian soils under savanna. Therefore, the aim of this study was to evaluate SQ by studying physical, chemical, microbiological, and biochemical soil properties over different times under rice cultivation.

2. Methodology

2.1 Study area

The study was conducted in rice fields at Paraiso Farm, Northeastern Roraima, Amazonia, Northern Brazil (03° 19' 01.56'' N; 60° 23' 43.65 '' W: altitude, 70 m) (Figure 1).

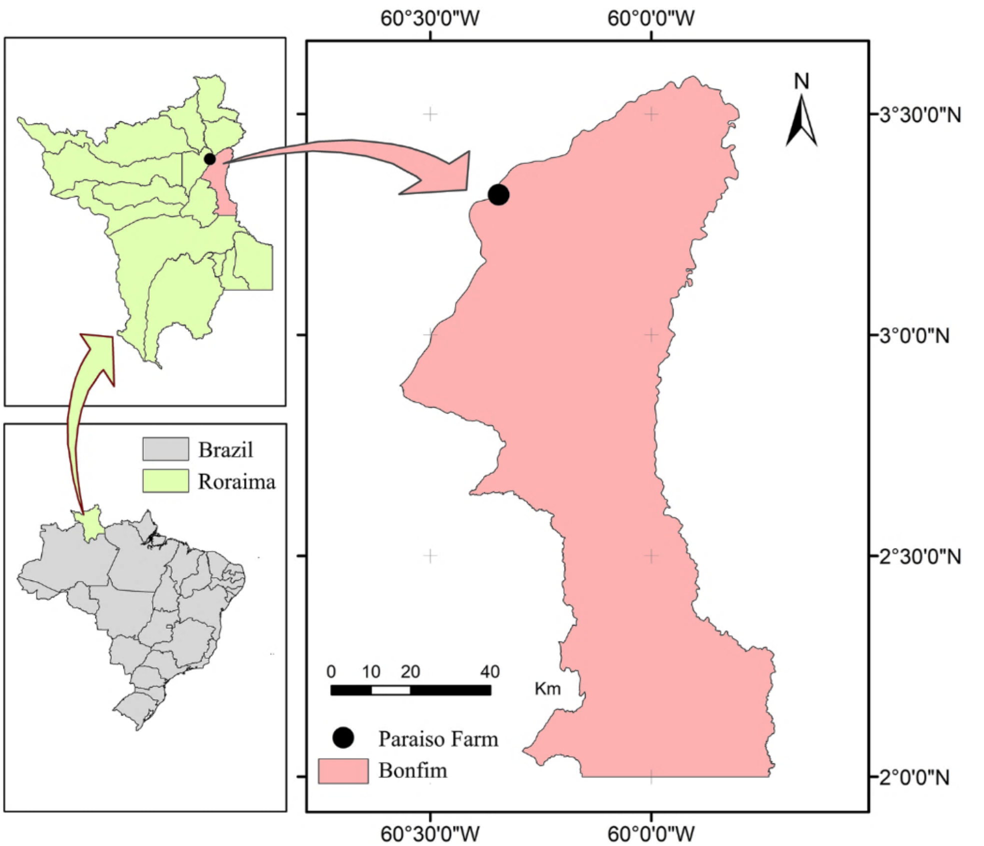

Figure 1. Location of the study area.

The climate is classified as rainy tropical (AW) by the Köppen system, mean annual rainfall of 1,500 mm, and average temperature of 27.5 ºC (Araújo et al., 2024). The soil under investigation is classified as Typic Fluvaquent according to the Soil Taxonomy System (Soil Survey Staff, 2022) and Hyperdystric Ferralic Gleysol in WRB (Anjos & Schad, 2018). The native vegetation of the area is hygrophilous lowland field, composed of arboreal, shrub and herbaceous strata. Before the cultivation of rice crops, all areas were with native vegetation.

2.2 Experimental design and management history of the area

The experimental design consisted of complete randomized blocks with six treatments and four replicates. The treatments were: native vegetation (ArN) as control, one year of rice cultivation (Ar1), three years of rice cultivation (Ar3), eight years of rice cultivation (Ar8), thirteen years of rice cultivation (Ar13), and twenty-five years of rice cultivation (Ar25). In the one-year treatment, conventional tillage was used with two harrowing, leveling and a flattening of the soil, followed by the construction of bunds and paddies (Table 1).

Fertilization at planting consisted of 500 kg ha^{-1} of NPK (formula 5:25:25) in furrow and 2 Mg ha^{-1} of dolomitic lime applied in the first year of cultivation, with a follow-up application every four years in older cropping areas. A 225 kg ha^{-1} top dressing of N via urea was split into three applications of 75 kg ha^{-1}, with the first application 15 days after planting, the second 40 days after planting, and the third 60 days after planting. In all fields, following harvest, the area was used for cattle grazing for two months and left free of animals until the next planting.

2.3 Soil sampling and analysis

Composite soil samples were collected in March 2015, one week after the harvest, in 100 x 100 m squares (10,000 m^2) in each study area at the 0 – 10 cm depth. To provide replicates of each treatment, squares were sub-divided into four plots of 50 x 50 m each, from which the composite samples were collected, as four replicates. Samples were divided into two parts, one for physical and chemical analysis, and another for microbiological and biochemical analysis. Soil for microbiological analysis was packed in plastic bags and stored in a cooler. In the laboratory, samples were stored under refrigeration with temperature between 4 °C and 7 °C prior to the analysis.

The chemical analysis was carried out according to Embrapa (2009). Samples were determined for Soil organic carbon (SOC) using Walkley-Black method; Ca^{+2} and Mg^{+2} extracted with 1mol L^{-1} KCl determined by atomic absorption spectrophotometry; P and K extracted by double acid solution (0.05 M HCl + 0.0125 M H_2SO_4), and determined by UV-Visible spectrophotometry and flame photometry, respectively; total nitrogen using Kjeldahl method; Al^{+3} + H^+ (exchangeable) extracted with calcium acetate; exchangeable Al^{+3} by 1 mol L^{-1} KCl extraction; pH (H_2O) (1:2.5); SOC stock was calculated from the carbon content multiplied by the soil bulk density at the 10 cm depth; N stock was calculated from the N content multiplied by the soil bulk density at the 10 cm depth.

Table 1
Soil management and rice production (t ha^{-1}) of the last five years of cultivation of the studied areas

Treatments	Ar1	Ar3	Ar8	Ar13	Ar25
Harvest	Soil management				
2014/2015	CT	NT	NT	NT	NT
2013/2014	-	NT	NT	NT	NT
2012/2013	-	CT	CT	NT	NT
2011/2012	-	-	CT	CT	CT
2010/2011	-	-	CT	CT	CT
Harvest	Soil liming (dolomitic limestone 85% of ECCE)				
2014/2015	soil liming	-	soil liming	soil liming	soil liming
2013/2014	-	-	-	-	-
2012/2013	-	soil liming	-	-	-
2011/2012	-	-	-	-	-
2010/2011	-	-	soil liming	soil liming	soil liming
Harvest	Productivity (t ha^{-1})				
2014/2015	7.90	7.05	7.10	7.00	7.00
2013/2014	-	6.95	7.00	6.90	6.70
2012/2013	-	7.05	7.10	6.90	6.75
2011/2012	-	-	6.90	6.80	6.60
2010/2011	-	-	6.75	6.50	6.40

CT - conventional tillage. NT – no tillage.

Microbiological attributes assessed were: carbon from microbial biomass (SMB-C) with fumigation and extraction method (Tate et al., 1988); soil microbial respiration (SBR) via evolved CO_2 extracted with NaOH (Jenkinson & Powlson, 1976); the metabolic quotient (qCO_2) calculated as the ratio between SBR and BMS-C; microbial quotient (qMIC) calculated as the ratio between SMB-C and SOC (Anderson & Domsch, 1993). Acid phosphatase was determined by p-nitrophenol phosphate release (Tabatabai, 1994); Urease was determined by the salicylate method (Kandeler & Berber, 1988). For physical analysis, soil texture and bulk density were determined.

2.4 Statistical analysis

Data were subjected to variance analysis F test ($p < 0.05$) using the software R version 4.3.2 and, in case of significant differences, means were compared by the post-hoc Tukey test ($p < 0.05$). All treatments under rice were compared with the ArN area by applying Dunnett's test ($p < 0.05$). Multivariate analysis was performed by canonical analysis. To perform these analyses, the data were reduced to a set of linear combinations, generating the scores for the first two canonical variables, which explained over 80% of the total variation, as recommended by Cruz & Regazzi (2001). All analyses were performed using FactoMineR, factoextra, ggplot2, tidyverse and boot packages (R CORE TEAM, 2023).

3. Results and discussion

3.1 Indicators of soil chemical quality

Soil chemical properties showed significant variation ($p < 0.05$) between the different times of cultivation when compared with native vegetation as reference (Table 2).

The soil pH at cultivated sites differed from ArN (4.15) only for AR25 (4.53), with higher pH. Although there were no significant differences between other areas and ArN, all showed pH values higher than those at ArN. This range of low pH values of these Gleysols from Northern Amazonia does not negatively influence rice crop yield. The effects of flooding stabilize the soil pH at close to 6.0 and remain stable for up to 30 days after flooding. Soil pH increases due to consumption of protons in acidic soils, whereas pH decreases in alkaline soils due to the accumulation of carbon dioxide after flooding, which neutralizes alkalinity (Kaur et al., 2020). The management practices associated with rice cultivation increased soil pH over time, and it is evident that low pH values found in this study did not negatively influence the rice crop yield. This stability in pH can be attributed to the flooding regime, which maintains soil pH values close to 6.0 for up to 30 days after flooding. Additionally, flooding can promote the concentration of Fe^{2+} and Mg^{2+} in solution. Some phosphorus adsorbed by Fe-oxides can be partially released during flooding and reduction, increasing its bioavailable concentration (Eshun et al., 2024).

Long-term cultivation of these Gleysols increased the contents of available phosphorus (P), except in the most recent cultivation (Ar1), where values did not differ from ArN. The older cultivated area (Ar25) showed an increase in phosphorus content of approximately 121.61 mg kg^{-1} in comparison to Ar1. Continuous cultivation promoted the contents of available P, with Ar25 having 120 mg kg^{-1} more than ArN and 83.51 mg kg^{-1} more than Ar1. This marked difference can be attributed to P fertilization during cultivation. Additionally, the release of adsorbed P by Fe-oxides during flooding and reduction increased its bioavailable concentration (Eshun et al., 2024). This stimulates P solubility for rice plants and may occur because of the formation of citrate-soluble P-metal complexes or metal ion chelates that immobilize P (Li et al., 2023). Low rice productivity in paddy soils may be related to the mechanisms involved in the mobilization and solubilization of P in the rice rhizosphere, including decreasing the pH within the rhizosphere (Martinengo et al., 2023). A study of rice cultivation in China (Li et al., 2023) emphasized that although P has not been included as one of the main soil quality indicators, this nutrient is a key factor limiting rice yield (Liu et al., 2013).

The exchangeable potassium (K^+) contents of the ArN differed statistically from all treatments (Table 2). However, all treatments had lower K content than those of ArN, close to the critical limit for irrigated rice. Only the treatment Ar3 showed a good level of potassium. The available K^+ contents decreased with cultivation, and ArN values were close to the critical limit for irrigated rice (60 mg kg^{-1}) (CQFS-RS/SC, 2004). Soil K leaching has been detected in irrigated rice cultivation, especially under conditions of continuous water management (Santos et al., 2016), reaching depths of 80 cm.

For Ca^{2+} contents, all samples except Ar1 differed statistically from the ArN values. There was an increase in Ca^{2+} over time, compared to the control area, with Ar8 having the highest Ca^{2+} content. Incidentally, the liming of Ar8 occurred just before the study and may have contributed to these results (Table 2). The increase in Ca^{2+} over time in cultivated areas reached the highest content in Ar8, where liming had just occurred, coupled with the greatest SOC values (Table 2).

Table 2
Chemical attributes of a Typic Fluvaquent in area of native vegetation and areas with different durations of flooded rice cultivation and management at 0 - 10 cm depth

Treat.	Variables										
	pH	P	K^+	Ca^{+2}	Mg^{+2}	Al^{+3}	H^+	CEC	SOC	Total N	C:N
	H_2O	--- mg kg^{-1} ---		--------------------- $cmol_c\ kg^{-1}$ ---------------------					----- g kg^{-1} -----		
ArN	4.15	7.03	31.19	0.19	0.11	3.22	6.63	3.75	10,73	1,86	5,77
Ar1	4.23ab	5.48c	68.17b*	0.23c	0.03b*	1.37bc*	2.97b*	1.80c*	4.64c*	0,63b*	7.37a
Ar3	4.02b	43.58bc*	89.56a*	1.19b*	0.22a*	1.85ab*	4.50a*	3.49b	6.22bc*	0.91ab*	6.83a
Ar8	4.48a	77.27b*	68.00b*	1.99a*	0.28a*	2.36a*	4.68a*	4.81a*	8.99a*	1.08a*	8.32a
Ar13	4.40ab	82.98b*	52.81b*	1.08b*	0.27a*	1.34bc*	4.02ab*	2.82bc	8.78b*	1.09ab*	8.81ab
Ar25	4.53a*	127.09a*	65.79b*	0.69bc*	0.28a*	0.94c*	4.05ab*	2.07c*	8.59ab*	1.07a*	8.03b
VC (%)	4.48	27.86	13.77	25.95	18.07	23.28	15.78	17.72	14.85	19.66	13.45

Treat = Treatments. Means followed by the same letter in the column do not differ significantly (Tukey test: $p < 0.05$); those followed by an asterisk (*) are significantly different from the native vegetation (Dunnett's test: $p < 0.05$).
CEC = Cation exchange capacity; SOC = Soil organic carbon; Total N = Nitrogen total.

The same trend of increasing Mg^{2+} with time of cultivation resulted from liming. Mg^{2+} values at Ar1 were far below those from ArN. All other sites had Mg^{2+} values higher than those from ArN, indicative of the contribution of limestone and organic matter. In southern Brazil floodplain soils, Lima et al. (2008) reported adequate Ca^{2+} and Mg^{2+} levels as key for successful rice crop management since most floodplain soils are acidic with low levels of Ca^{2+} and Mg^{2+} (Provam, 1996), so limestone application is required, also helping to reduce the Al^{3+} contents. Despite the above scenario, this situation can be reversed at the time of soil flooding, where changes in the redox system occur, with an increase in pH close to neutrality in acid soils due to reduction. As a result, nutrients such as K^+, Ca^{2+}, and Mg^{2+} increase their availabilities (Kögel-Knabner et al., 2010; Lee et al., 2011).

All areas under rice cultivation had Al^{3+} values significantly greater than those from ArN (Table 2). The practice of liming in rice cultivation management is mainly conducted to provide Ca^{2+} and Mg^{2+} and is not effective in neutralizing the high $H+Al^{3+}$ contents in soil with greater age under cultivation. However, the management system used promoted decreasing Al^{3+} contents in soil with Ar25 showing the lowest aluminum content (0.94 cmolc kg^{-1}) of all sample areas (Table 2). Over the years, rice cultivation and the crop management system used promoted decreasing Al^{3+} contents, with Ar25 showing the lowest content (Table 2).

The CEC values for Ar1, Ar8, and Ar25 differed from those at ArN (Table 2). However, only those from Ar8 were higher (4.81 $cmol_c\ kg^{-1}$) compared to the ArN control area (3.75 $cmol_c\ kg^{-1}$). All other sites had lower CEC in comparison to the control area, despite the overall reduction in CEC with cultivation time, except for Ar8, where liming had just been added. In China, Liu et al. (2023) observed higher CEC values in Chinese floodplain soils with higher levels of rice productivity, positively correlated with the SOC contents. Ar8 had the highest Ca^{2+} values, as well as a higher SOC content. Longer rice cultivation promoted high negative charges in the soil, thereby increasing cation adsorption.

The SOC and N levels decreased with the conversion of native vegetation to rice cultivation. However, from the third year onwards, they started to increase, reaching values even close to natural vegetation. The C:N ratio did not differ between cultivated areas and the ArN (Table 2), although Ar25 had the smallest C:N (4.95). The low C:N ratio indicated a higher rate of SOM mineralization, attributed to nitrogen fertilizers, enhancing organic-C stabilization and helping to mitigate CO_2 emissions (Kirkegaard et al., 2023).

3.2 Soil carbon and nitrogen stocks

Figure 2A and 2B show the soil C and N stocks of the six areas studied. The native vegetation (ArN) showed the highest C stock in comparison to cultivated areas. Ar8 had the highest SOC stock, whereas Ar13 and Ar25 showed decreasing SOC stocks compared to Ar8.

The carbon stock loss of the ArN area converted to rice cultivation, indicated by the Ar1 was 6.76 Mg ha^{-1} of C. The vegetation clearing of soil and tillage, exposure to high temperatures, and accelerated decomposition of organic matter causes this abrupt change in soil C stock levels. However, the difference between ArN to the AR8 (area with higher C stock) was 2.4 t ha^{-1} C, which indicates some SOC recover over time. This study indicated that SOC is a useful indicator of soil quality, as pointed out by Laurentiis et al. (2024), with age of cultivation. This is illustrated by the 57% reduction of SOC content after one year (ArN to Ar1), followed by a recovery of 72% after 8 years (Ar8) when compared to Ar1. A similar trend was observed by Omer et al. (2024) during a long-term experiment on the effects of crop plantation in an irrigated semi-arid agroecosystem. The abrupt losses of SOC within one year were caused by severe disturbance of native soil, exposure to high temperatures, and accelerated decomposition of organic matter, consistent with observations by Laurentiis et al. (2024). However, the recovery of C stocks (2.4 t ha^{-1} C) after 8 years of cultivation is explained by the no-tillage management adopted in the last three years, resulting in organic matter concentration. Since rice has a well-developed fasciculate root system, with very fine roots, carbon recovery can be facilitated, as suggested by Ghestem et al. (2011), who reported that soil structure development by root decomposition enhances SOM protection from decomposing agents.

The increased soil nitrogen stocks over time of cultivation may be due to the annual application of N (300 kg ha^{-1}). The high SOC and TN at the topsoil are directly related to soil management systems adopted (Omer et al., 2023), and high N levels promote faster decomposition of organic matter. The C and N stocks of soil showed that the native vegetation (ArN) had the highest C stock compared to cultivated areas. However, the rice cultivation areas showed recovery of the C stock over time (Figures 2A and 2B), with Ar8 having the highest SOC stock compared to other rice-cultivated areas. The decrease of C in Ar25 can be explained by changes in soil management, as it was necessary to systematize the area in the last three years.

3.3 Soil physical indicators

Soil bulk density (SBD) in cultivated areas increased over time in relation to the native vegetation (Table 3), with significant differences between ArN and Ar13 and Ar25.

The soil bulk density increased with time of cultivation, probably due to machinery traffic, resulting in soil structure disruption and compaction. According to Liu et al. (2023), rice cultivation may result in soil compaction due to heavy traffic. Rice cultivation can be reduced when soil bulk density reaches near 1.63 Mg m^{-3} (Evald et al., 2021), although critical SBD for inundated rice cultivation is not reported in the literature. Compacted soils have higher penetration resistance and lower aeration, which hinder the development of plant roots and reduce crop production (Hamza & Anderson, 2005).

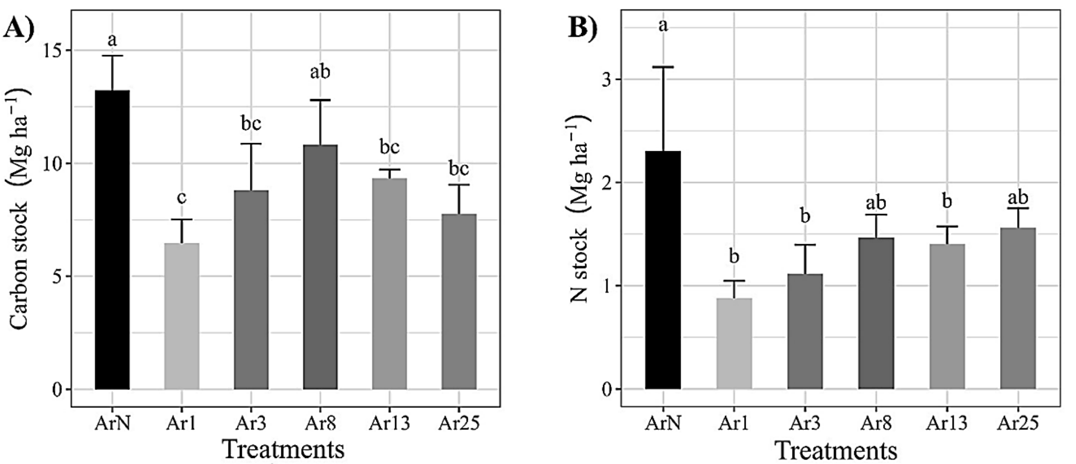

Figure 2. Carbon (A) and nitrogen (B) stock typical Hyperdystric Ferralic Gleysol under native vegetation area and flooded rice cultivation with different times of continued cultivation and management.

Table 3
Soil bulk density (SBD) and granulometry of a Typic Fluvaquent in area of native vegetation and different durations of flooded rice cultivation and management types at 0 - 10 cm depth

Treatments	Variables			
	SBD	Clay	Silt	Sand
	--- Mg m^{-3} ---	----------------- g kg^{-1} -----------------		
ArN	1.24	394.20	513.90	91.90
Ar1	1.40ab*	395.30c	509.50ba	95.50c
Ar3	1.25b	479.20bc*	347.60c*	131.60ab*
Ar8	1.35ab	524.70ab*	355.80cb*	119.50cb*
Ar13	1.49a*	452.20bc	503.50ab	144.30ab*
Ar25	1.47a*	418.90bc*	479.30cb*	101.80c
VC (%)	5.88	19.15	19.16	65.92

Means followed by the same letter in the column do not differ significantly (Tukey test: $p < 0.05$); and followed by an asterisk (*) are significantly different from the native vegetation by (Dunnett's test: $p < 0.05$).

However, soil compaction alone should not be considered as limiting factors for floodplain rice cultivation, since soil remains saturated for most of the crop cycle (Medeiros et al., 2005). As flooding advances, rice plants grow roots into deeper soil, and surface soil compaction has no negative influence on rice production. Such moistened soil has a semifluid consistency, with low cohesion between particles and so less resistance to deformation and root penetration (Tomobe et al., 2023). According to Medeiros et al. (2005), some compaction of paddy soil promoted an increase in plant dry mass, and P and K accumulation in shoots, enhancing rice production. For soil texture, the clay contents differ significantly between areas with different ages of cultivation. The Ar8 had the highest clay content (524.70 g kg^{-1}), and ArN the lowest (394.20 g kg^{-1}). All areas investigated had high levels of clay and silt, with clay ranging from 418.90 g kg^{-1} to 595.30 g kg^{-1} and silt from 292.20 g kg^{-1} to 503.50 g kg^{-1}. All areas investigated showed high levels of clay and silt, with clay Ar13 showed highest clay and silt content. In general, all areas investigated showed high levels of clay and silt, with only Ar3 showing approximate values in the three soil fractions, varying around 350 g kg^{-1}. Such variation in texture can be attributed to the addition of sediment transported from the river, both by the irrigation system and by the uncontrolled flooding of areas during the rainy season. Such changes in soil texture do not occur via management practices, but by additions of materials to the system (Brady & Weil, 2016). This is evident in the present study mainly because the layer studied lies at 0-10 cm, a stratum highly subject to change by sediment addition.

3.4 Soil microbiological indicators

All the microbiological indicators, except for qCO_2, showed significant variation ($p < 0.05$) between cultivated areas (Table 4). Ar3 and Ar8 showed the highest values. However, the time of tillage does not contribute to increasing the values of SMB-C.

Soil microbial biomass carbon (SMB-C) decreased in the first year of rice cultivation, with all areas differing statistically from ArN (Table 4). Site Ar3 had the highest SMB-C (193.80 mg of C kg^{-1}), and Ar1 the lowest (73.06 mg of C kg^{-1}). A reduction of approximately 54% in SMB-C between Ar3 and ArN was recorded. Soil microbial biomass was higher in the ArN area, resulting from the continuous supply of diverse organic materials of varying compositions. This greater diversity of organic compounds deposited in the rhizosphere contributes favorably to the survival and growth of different groups of soil microorganisms (Li et al., 2023). Therefore, soils under native vegetation tend to have higher SMB-C, indicating greater balance of soil microbiota. The low SMB-C values in soils of cultivated systems may also be explained by the flooding regime management. According to Carvalho et al. (2012), the abundance of microorganisms is lower in anoxic conditions, although an increase in SMB-C in the third year of cultivation was observed. This may be due to a new microbiota equilibrium following cultivation (Tang et al., 2021), and SOC recovery.

Soil basal respiration (SBR) at the Ar1 site was approximately 29% less than at the ArN site (Table 4), but no differences in SBR were recorded for any other age of cultivation. When time of cultivation was compared, Ar8 had the highest (18.14 mg kg^{-1} h^{-1} CO_2-C) and Ar1 the lowest SBR values (4.43 mg kg^{-1} h^{-1} of CO_2-C). This result may be related both to the SOC levels (8.99 and 4.64 g kg^{-1}, respectively), and the SMB-C (169.04 and 73.06 mg C kg^{-1} of soil, respectively). A positive and significant correlation between SBR and SOC was observed by Zhou et al. (2023), with a close relationship between microbial activity and the availability of organic material (SOC). However, high SBR may not only mean high microbial activity due to high organic matter, but also to inundation (Li et al., 2023), highlighting the importance of using an area of native vegetation as control.

Table 4
Microbiological indicators of a Typic Fluvaquent in area of native vegetation and different durations of flooded rice cultivation and management at 0 - 10 cm depth

Treatments	Variables			
	SMB-C (mg of C kg^{-1} soil)	SBR (mg kg^{-1} h^{-1} of CO_2-C)	qCO_2 (mg kg^{-1} h^{-1} of CO_2-C of MB-C)	*q*Mic %
ArN	424.10	14.99	0.04	3.94
Ar1	73.06c*	4.43b*	0.07ª	1.58a*
Ar3	193.80a *	16.15ab	0.09ª	2.59ab*
Ar8	169.04ab*	18.14ª	0.11ª	2.16ab*
Ar13	154.90ab*	12.34ab	0.08ª	2.59ab*
Ar25	159.62ab*	14.63ab	0.09ª	3.15a
VC (%)	32.41	39.75	41.37	29.26

Means followed by the same letter in the column do not differ significantly (Tukey test: $p < 0.05$); and followed by an asterisk (*) are significantly different from the native vegetation (Dunnett's test: $p < 0.05$).
[SMB-C = carbon from microbial biomass; SBR = soil microbial respiration; qMIC = microbial quotient; qCO_2 = metabolic quotient].

The levels of qCO_2 did not differ statistically between the tillage areas and the ArN (Table 4). The qCO_2 was not a good attribute for assessing soil quality in rice cultivation, consistent with Lima et al. (2013), who reported similar qCO_2 values between cultivated areas and native vegetation. The qMIC index was highest at the ArN site, differing significantly from all areas under tillage (Table 4), except Ar25. For the sites under different ages of cultivation, Ar25 had the highest qMIC (3.15%). The recorded qMIC values are considered normal, according to Sarto et al. (2020). The qMIC is apparently more efficient than the SMB-C and SBR in detecting changes in agroecosystems and shows considerable variation, allowing its use as an indicator of soil quality (Rao et al., 2021).

3.5 Soil biochemical indicators

Acid phosphatase activity differed significantly between ArN and the cultivated areas, except at Ar3 (Table 5). The Ar8, Ar13 and Ar25 areas showed greater phosphatase activity (407.38; 430.33 and 442.75 mg p-nitrophenol kg^{-1} of soil h^{-1}, respectively) than Ar1 (322.85 mg p-nitrophenol kg^{-1} of soil h^{-1}). However, activity at Ar1 was less than at ArN.

The highest acid phosphatase activity levels were recorded at the Ar13 and Ar25 areas, and the lowest at Ar1. The amount of SOC, SMB-C, and Po (organic phosphorus) may explain this variation. The higher phosphorus concentrations at Ar8, Ar13, and Ar25 may represent residual organic P forms, resulting from the incorporation of crop residues. Thus, areas with greater Pi (inorganic phosphorus) had greater Po in organic matter, favoring acid phosphatase activity.

Urease activity only differed statistically between ArN and Ar1 (Table 5). However, increased activity of urease was observed in Ar8 and Ar13, areas with higher SOC and TN content (8.99 and 8.78 g kg^{-1} of SOC and 1.08 and 1.09 g kg^{-1} of TN, respectively), compared to ArN (23.08 mg of NH_4^+ kg^{-1} h^{-1} soil) and Ar8 (22.77 mg of NH_4^+ kg^{-1} h^{-1} soil). Highest acid phosphatase activity levels were recorded at the Ar25 cultivated area and may be explained by the SOC levels, SMB-C, and Po (organic phosphorus). According to Borase et al. (2020), there is a positive correlation between SOC, acid phosphatase levels, and SMB-C. The higher P concentrations at Ar8, Ar13, and Ar25 may represent residual P in organic forms, resulting from the incorporation of plant residue after several years of P fertilization. Thus, areas with greater P content will have greater organic P forms, increasing phosphatase activity. Increased activity of urease in areas with higher SOC and TN contents are expected, since greater activity of this enzyme is expected to occur in areas with the highest SOC stock (Carvalho et al., 2012), and urease activity is related to the presence of dense population of ureolytic microorganisms (Konstantinou et al., 2021). This activity favors high ammonia loss by volatilization, indicating the capacity of the soil to transform organic N into inorganic N. Liu et al. (2023) observed significant difference in urease activity and acid phosphatase in Chinese soil with different rice production (high, medium and low productivity). The area of high productivity had elevated activity levels for both enzymes (20.8 mg of NH_4^+ kg^{-1} of soil h^{-1} and 521 mg p-nitrophenol kg of $soil^{-1}$ h^{-1}), whereas the lowest productivity had lowest (12.1 mg NH_4^+ kg^{-1} soil h^{-1} and 382 mg p-nitrophenol kg^{-1} soil h^{-1}, respectively).

3.6 Principal component analysis (PCA)

To better elucidate the relation between soil properties and age of rice cultivation, a principal component analysis (PCA) was conducted, obtaining two principal components (CP1 and CP2) with accumulated variance of 71% (Figure 3). PC1 explained 43.3% of the variance and PC2 explained 27.7%.

Table 5

Biochemical indicators of a Typic Fluvaquent in area of native vegetation and different durations of flooded rice cultivation and cropping at 0 - 10 cm depth

Treat-ments	Variable	
	Acid phosphatase (mg *p*-nitrophenol kg^{-1} soil h^{-1})	Urease (mg NH_4^+ kg^{-1} soil h^{-1})
ArN	365.53	23.08
Ar1	322.85c*	10.34a*
Ar3	358.12bc	10.61a*
Ar8	407.38ab*	22.77a
Ar13	430.33a*	13.59a*
Ar25	442.75a*	12.02a*
CV (%)	4.51	49.99

Means followed by the same letter in the column do not differ significantly (Tukey test: $p < 0.05$); and followed by an asterisk (*) are significantly different from the native vegetation (Dunnett's test: $p < 0.05$).

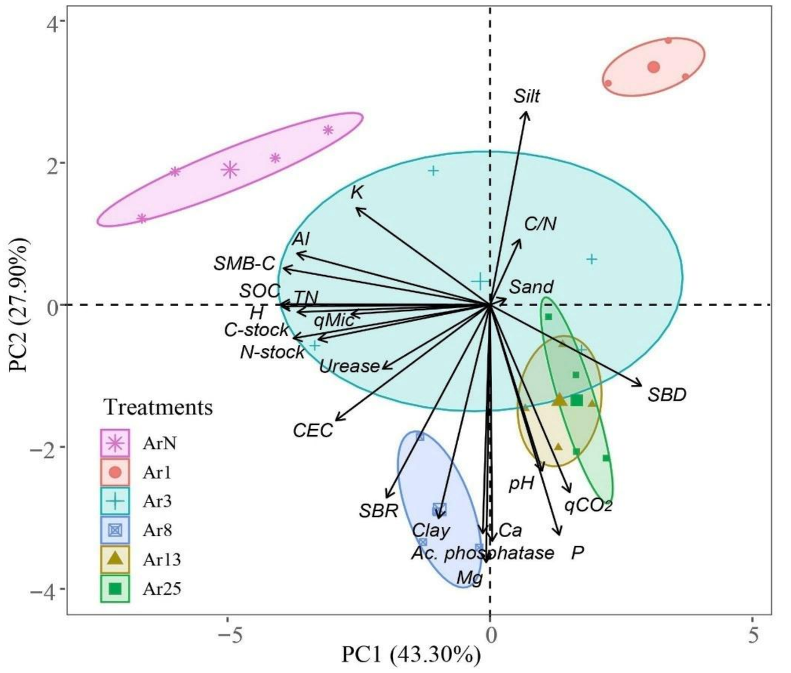

Figure 3. Principal component analysis of soil physical, chemical, microbiological, and biochemical attributes in a typical hyperdystric Ferralic Gleysol under native vegetation and flooded rice cultivation with different durations of continuous cropping and management practices. [SMB-C = carbon from microbial biomass; CEC = Cation exchange capacity; SOC = Soil organic carbon; SBR = soil microbial respiration; TN = Total nitrogen; qMIC = microbial quotient; qCO2 = metabolic quotient; SBD = Soil bulk density; C- stock = carbon stock; N-stock = nitrogen stock].

The variables SOC (0.99), C stock (0.96), SMB-C (0.96), TN (0.94), Al^{+3} (0.94), N stock (0.86), urease (0.82), qMIC (0.75), CEC (0.70) and K^{+} (0.68) comprised CP1, which are most representative of the ArN and Ar3 sites. SOC variables, SMB-C, TN, C stock, Al^{+3} and H^{+} were highly correlated with each other (above 94%). The interaction between C stock, SMB-C, TN, and C stock highlighted the importance of SOC in the responses and interactions with most soil properties, especially the microbiological attributes.

The Ar8 site had an intermediate ratio for the CP2 variables, but with a variation close to CP1. This indicates a convergence between areas of cultivation. The elevated levels of correlation between clay and other attributes at site Ar8 is explained by the higher levels of clay in this study site (Table 3). PC2 was composed of the following variables: Mg^{2+} (0.94), Ca^{2+} (0.86), acid phosphatase (0.83), P (0.81), clay (0.81), qCO_2 (0.78), basal respiration (0.71), and pH (0.68). In this group, there was a close relation between phosphatase activity and phosphorus, particularly for the two oldest cultivations (AR13 and Ar25).

The cluster analysis dendrogram (Figure 4) gave four similarity groups: Group 1 contained ArN, Group 2 contained Ar3 and Ar8, Group 3 contained Ar13 and Ar25, and Group 4 contained Ar1. From both the results of the PCA (Figure 3) and the dendrogram (Figure 4), areas Ar1 and ArN had low similarity. Ar13 and Ar25 were the most similar, but Ar3 and Ar8 also formed a distinct group. These results showed an abrupt change in soil characteristics following the conversion of a natural area to rice cultivation.

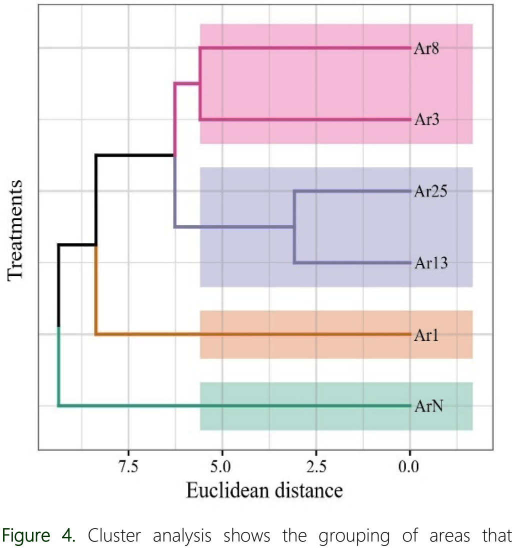

Figure 4. Cluster analysis shows the grouping of areas that exhibited greater affinity in the studied variables.

While gradual changes in soil quality occur over time (since the soils of the cultivated areas resemble each other), soil quality remains very different from that found in natural areas. Over the years, the accumulation of organic matter and the increase of nutrient levels arising from fertilization have increased the sources of energy for microorganisms. This, combined with the minimum soil disturbance associated with a no-till policy, has caused the microbial population to grow and has improved soil quality.
Using PCA analysis, Li et al. (2022) found that the variables TN, P, acid phosphatase, and total number of bacteria are the minimal variables required to infer SQ in rice paddy soils in China. Low levels of TN and P, along with soil microbial activity, were identified as limiting factors for rice productivity. Lima et al. (2013) found that microbiological (SMB-C, acid and alkaline phosphatase, β-glucosidase, and SBR) and chemical indicators (TOC, CEC, TN, Ca^{2+}, Mg^{2+}, and Al^{3+}) were major soil quality indicators in rice cultivation in Rio Grande do Sul, Brazil. Lima et al. (2013) highlighted the importance of using biological indicators and SOM in SQ assessment in rice cultivation, as these attributes had greater sensitivity. Our study corroborates Lima et al. (2013), showing that biological attributes such as SMB-C, qMIC, urease activity, acid and alkaline phosphatase, as well as SOC and TN, are sensitive to land use changes and are valuable in soil quality assessment. According to Hoffland et al. (2020), the dynamic self-organization of the soil system is dependent on high inputs of organic matter and promotes soil aggregation through adequate soil management, helping to sustain rice productivity in the long-term.

4. Conclusions

Carbon stock decreased with conversion of natural system to paddy soil in the first years but increased over the years. Long-term rice cultivation in flood systems resulted in gains of soil quality, improved soil fertility, increased soil organic carbon and enzymatic activity compared to short-term systems. Areas with shorter periods under cultivation were similar, so that longer cultivation times are necessary to detect changes in most SQ indicators. The soil indicators selected were sensitive to reveal changes in paddy soils under rice cropping, particularly the activity of acid phosphatase, and available phosphorus were positively enhanced with longer periods of rice cultivation. Microbiological (SBM-C) and biochemical (acid and urease phosphatase) indicators, as well as soil organic carbon and total nitrogen contents, showed the highest sensitivity, and are considered useful for soil quality studies of paddy soil in the lowland of Amazonian savanna. The indicators evaluated are promising for advances in soil quality studies in irrigated rice cultivation systems in tropical areas under tillage and no-till.

Author contribution

V. F. Melo, A. Evald, S. C. P. Uchôa, P. R. R. Rocha, Z. N. Senwo: Conceptualization, Development or design of methodology and Data collection. **V. F. Melo, A. Evald, S. C. P. Uchôa, R. M. Bardales-Lozano, Z. N. Senwo**: Data analysis & interpretation. **V. F. Melo, A. Evald, S. C. P. Uchôa, P. R. R. Rocha, R. M. Bardales-Lozano, A. Adandonon, Z. N. Senwo**: Writing, editing & text reviewer.

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