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Impact of forest conversion to pasture on soil enzymatic activity in the northern Amazon

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ABSTRACT

The conversion of forests to pastures in the Amazon results in deforestation and the loss of environmental services. This practice affects biogeochemical cycles and impairs soil enzyme activity, which is essential for maintaining soil quality. This study aimed to investigate the impact of converting part of the Amazon forest into pastures, focusing on soil enzyme activity. The study was conducted at Fazenda Canto Verde, Roraima, comparing native forest areas and pastures of *Brachiaria brizantha* and *Brachiaria humidicola*, established on Haplic acrisol, without fertilization or tillage, under a management regime of 30 days of grazing and 60 days of rest. Sampling involved 12 mini trenches per hectare at two depths, with analysis of enzyme activity post-incubation. Higher activity of carbon cycle enzymes (Cellulase, Invertase, β-Glucosidase) was observed in the forest compared to pastures, especially with *B. humidicola*. Nitrogen cycle enzymes (Urease, BAA-Protease) were more active in the forest, while *B. humidicola* showed the highest Casein-Protease activity. In the phosphorus and sulfur cycles, the forest led in Phosphomonoesterase and Phosphodiesterase, while *B. humidicola* excelled in Arylsulfatase. This study demonstrates that replacing forest with pastures significantly alters soil functionality, impacting biogeochemical cycles and their ecological functions.

Keywords: *Brachiaria*, Biogeochemical Cycles, Livestock Farming in the Amazon, Environmental Services.

Impacto da conversão de floresta em pastagem na atividade enzimática do solo no norte da Amazônia

RESUMO

A conversão de florestas em pastagens na Amazônia resulta em desmatamento e perda de serviços ambientais. Essa prática afeta os ciclos biogeoquímicos e prejudica a atividade enzimática do solo, essencial para a manutenção da qualidade desse recurso. Objetivou-se, através do presente trabalho, investigar o impacto da conversão de parte da floresta amazônica em pastagens, com foco na atividade enzimática do solo. O estudo, realizado na Fazenda Canto Verde, Roraima, comparou áreas de floresta nativa e pastagens de *Brachiaria brizantha* e *Brachiaria humidicola*, estabelecidas em Argissolo Vermelho-Amarelo, sem adubação ou revolvimento, sob manejo de 30 dias de pastejo e 60 de pousio. A amostragem envolveu 12 minitrincheiras por hectare, em duas profundidades, com análise de atividade enzimática pós-incubação. Observou-se maior atividade de enzimas do ciclo do carbono (Celulase, Invertase, β-Glucosidase) na floresta, em comparação com as pastagens, especialmente com *B. humidicola*. Enzimas do ciclo do nitrogênio (Urease, BAA-Protease) foram mais ativas na floresta, enquanto *B. humidicola* destacou-se com a maior atividade de Caseína-Protease. No ciclo do fósforo e do enxofre, a floresta liderou em Phosphomonoesterase e Phosphodiesterase, e a *B. humidicola* em Arilsulfatase. Este estudo evidencia que a substituição de floresta por pastagens altera significativamente a funcionalidade do solo, impactando os ciclos biogeoquímicos e suas funções ecológicas.

Palavras-chave: *Brachiaria*, Ciclos Biogeoquímicos, Pecuária na Amazônia, Serviços Ambientais.

1. Introduction

The extensive, conventional livestock farming system, conducted rudimentarily with low technology, without selecting suitable cultivars or applying the recommended soil correction and fertilization, has led to low farm yields. This management has generated pressure to clear new areas, mainly due to soil depletion and loss of vegetation cover, which has irreversible long-term impacts (Batista et al., 2014; Assis et al., 2015).

Only in the Amazon biome, 481,000 km^2 were deforested between 1988 and 2022 (INPE, 2023). This deforestation leads to the loss of environmental services, which include the maintenance of biodiversity, water cycling, and carbon stocks, exacerbating the impacts of the greenhouse effect (Barni et al., 2016). In addition, Lange et al. (2019) state that converting native forests into pasture reduces phosphorus, potassium, calcium, and magnesium levels by more than 50%. This reduction is due to the loss of organic matter, soil erosion, compaction, changes in the soil microbiota, differing nutritional requirements of pastures, and the disruption of natural nutrient cycles.

The soils of the Amazon are low in fertility and acidic, requiring correction and fertilization to become productive (Benedetti et al., 2011; Zaninetti et al., 2016; Henrique et al., 2018; Matos et al., 2023). However, agricultural systems established in this biome are generally not managed correctly, undermining their stability and accelerating soil degradation, causing a vicious cycle of forest clearing and land abandonment.

Replacing natural forests with pasture is the most common practice in the Brazilian Amazon, which is precariously sustained through the cycling of carbon and the use of forage plants, with *Brachiaria brizantha* and *Brachiaria humidicola* species being the most adapted to the soil and climate conditions (Moreira and Malavolta, 2004; Rodrigues et al., 2017). *Brachiaria brizantha* can grow satisfactorily in the acidic, low-fertility soils characteristic of the Amazon region (Rodrigues et al., 2017). *Brachiaria humidicola*, known as "quicuio da Amazônia", is undemanding in soil fertility, tolerant to aluminum, and does not require high levels of phosphorus for its development (Moreira and Malavolta, 2004). *Brachiaria brizantha* generally outperforms *Brachiaria humidicola* in biomass production, crude protein content, palatability, and growth rate (Laiton Medina et al., 2021).

Biochemical properties, including soil enzyme activity, can be determined to diagnose soil degradation caused by pasture implementation, including soil enzyme activity (Soltangheisi et al., 2019; Durrer et al.,

2021). Enzyme activity provides information on the capacity of soils to conduct biogeochemical reactions and can be used as an index to detect the impacts of anthropogenic management or pollution on soils. The advantages of using this property lie in its simplicity, speed, precision, and cost-effectiveness (Nannipieri et al., 2017).

In addition, enzyme activity indicates soil quality, as it is linked to forming complexes with organic matter and the clay fraction. This activity reflects the effectiveness of soil management in stabilizing organic molecules and the structural properties of the soil, such as aggregation and porosity (Assis et al., 2015). Thus, enzymes act as markers of the management practices adopted and are strongly influenced by the availability and form of specific nutrients, such as C, N, P, and S (Soltangheisi et al., 2019; Durrer et al., 2021; Silva Olaya et al., 2021).

There are few studies on soil enzymes in the northern states of Brazil, where the Amazon Rainforest is located. Among them, Silva Olaya et al. (2021) state that the enzymes that make up the forest litter play a fundamental role in the dynamics of the Amazon forest, mainly in the regulation and decomposition of organic matter (OM), as well as helping with C sequestration and the biogeochemical cycling of elements. Schaap et al. (2023) conclude that microbial activity is related to enzymes in the soil, strongly influenced by the OM content in the surface layers. According to Sobucki et al. (2021), most studies have focused on just a few soil enzymes. This highlights the need to calibrate enzyme activity interpretation tables, considering different soil types and crops.

This study aimed to evaluate the activity of soil enzymes related to the biogeochemical cycles of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) in Amazonian soil, comparing native forest systems and two pastures.

2. Material and Methods

The areas used for this study are located at the Canto Verde farm in Iracema, Roraima, Brazil. The 400-hectare property is located on the BR-174 highway, 110 km from the capital, Boa Vista, with the following geographical coordinates: 2°10′55″ N, 61°2′27″ W (Figure 1). According to the Köppen classification, the region's climate is Aw-type, tropical rainy with a defined dry period (spring and summer). The average annual temperature is 27 °C, and the average annual rainfall is 2,000 mm, with April to July being the wettest months (Barbosa et al., 1997).
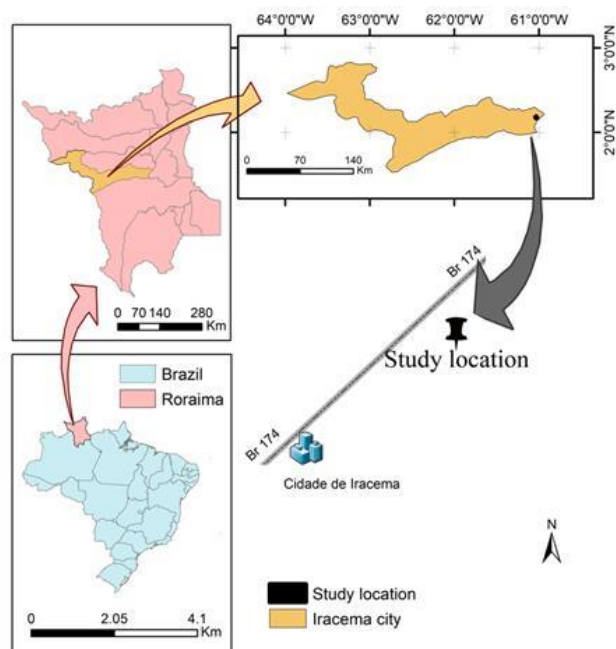
Figure 1. Location Map of Canto Verde Farm in the Municipality of Iracema-RR

The soil in the study areas is classified as Argisolo Vermelho-Amarelo (EMBRAPA, 2018) or Haplic acrisol (FAO, 2015), and its characterization is shown in Table 1. The reference forest formation is called a Dense Ombrophilous Forest, characterized by less than 60 consecutive days without rainfall. In this region, the forest size tends to be larger, and the species found include: Angelim-Pedra (*Hymenolobium petraeum Ducke*), Copaíba (*Copaifera landesdorffii*), Roxinho (*Peltogyne spp.*), Atamenju, Caferana (*Picrolemma pseudocoffea*), Cupiúba (*Goupia glabra*), Balsamo, Itauba (*Mezilaurus itauba*), Canelas (*Lauraceae*), and *Lecythidaceae*, the latter two being more tolerant of wetter environments. The canopy is always closed (dense).

Three land use systems were considered for the study: native forest at the interface with the cultivated areas (200 ha; NF); pasture with *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf, cultivar Marandu (10 ha; BB) and Pasture with *Brachiaria humidicola* (Rendle) Schweick (10 ha; BH). The pasture areas were established over 10 years, from 2002 to 2012, by clearing the native (primary) forest. The pasture areas evaluated in the study have not undergone any tilling, fertilizing, or liming since

they were opened. Cattle were managed for 30 days of grazing and 60 days of fallow, with an average stocking rate of one animal unit per hectare.

The areas were sampled during the dry season 2012, and four representative areas of 1 ha were delineated for each system. In each area, 12 mini trenches were dug to a depth of 0.20 m, subdivided into two layers of 0.0-0.10 m and 0.10-0.20 m. This division constituted an experiment in a 3x2 factorial design (land use systems x soil layers) with a randomized block design. The mini trenches were distributed using the free-walk method, choosing a representative diagonal in the area. Each sampled point corresponded to a single sample of 1 dm^3, which was then combined according to depth to form a composite sample. The samples were packed in airtight plastic bags and transported to the drying facility.

There, the samples were air-dried, crushed, and passed through a sieve (4 mm), and 500 g of each was separated for biochemical analysis and determination of enzymatic activity. For the biochemical analysis and determination of enzyme activity, the dried soils passed through a 4 mm mesh sieve were moistened to 60% of field capacity, kept for eight days, incubated at room temperature in the dark, then stored at 4 °C in a refrigerator (Tabatabai, 1994). Before analysis, the sample moisture content was measured gravimetrically in an aliquot of soil dried in an oven at 105 °C for 24 hours.

The activity of the hydrolytic enzymes Phosphomonoesterase, Phosphodiesterase, Casein-Protease, BAA-Protease, Urease, Cellulase, Invertase, β-D-glucosidase, and Arylsulfatase were analyzed using methods described by Trasar-Cepeda et al. (2000). The specific activity of these enzymes, referring to the rate of the reaction catalyzed by each enzyme per unit of protein present, was also calculated. The soil samples were moistened to approximately 80% of field capacity for these analyses and kept refrigerated. The data were submitted to analysis of variance (ANOVA) at a 5% probability. The means were compared by the Tukey test at a 5% significance level. The analyses were conducted using the SISVAR statistical package, version 5.3 (Ferreira, 2011).

Table 1. Chemical and physical characterization of the soils in the study area (Iracema, Roraima, Brazil).

System	Layers	pH	P	K	Ca	Mg	Al	H+Al	SB	ECEC	CEC	V	m	OM	Sand	Silt	Clay
		H$_2$O	mg/dm^3				cmol$_c$/dm^3					%		dag/kg	%		
NF	0.00 – 0.10 m	5.4	2.6	14.0	0.0	0.0	1.3	9.8	0.0	1.3	9.8	0.4	96.9	5.2	73.1	10.1	16.8
	0.10 – 0.20 m	5.6	1.4	5.0	0.0	0.0	0.8	6.4	0.0	0.8	6.4	0.2	98.7	3.3	75.7	4.9	19.5
BB	0.00 – 0.10 m	5.2	1.9	16.0	0.1	0.1	0.4	4.2	0.3	0.7	4.5	6.3	58.2	1.8	79.0	4.4	16.6
	0.10 – 0.20 m	5.2	1.4	7.0	0.0	0.0	0.5	4.0	0.0	0.5	4.0	0.7	94.2	1.0	78.3	2.6	19.1
BH	0.00 – 0.10 m	5.6	4.0	40.0	0.5	0.3	0.2	3.7	0.8	1.0	4.5	18.1	19.6	2.5	80.2	4.0	15.8
	0.10 – 0.20 m	5.5	2.0	26.0	0.2	0.1	0.2	2.7	0.4	0.6	3.1	12.6	33.9	0.5	76.7	5.5	17.8

SB – Sum of bases; ECEC - Effective cation exchange capacity; CEC - Cation exchange capacity; V – Base saturation; m - Aluminum saturation; OM – Organic matter.

3. Results and Discussion

The activity of hydrolytic enzymes in the carbon (C) cycle was significantly affected by the systems and layers studied (Table 2, Figure 2). In general, enzyme activity decreased with depth, and the change in land use altered the availability of substrates. This was most critical in the BH system, which saw a 51% reduction in cellulase activity and an 83% reduction in Invertase activity compared to NF in the 0.00-0.10m layer. The β-Glucosidase activity was higher in NF and did not differ between the BB and BH systems, which had losses of up to 72% (Table 2). These changes reflect the lower availability of specific compounds such as cellulose and sucrose in pastures compared to native forests. In addition, other factors, such as the microbial community composition and the soil's physical and chemical conditions, also influence enzyme activity.

Regarding the specific activity of the enzymes in this cycle (Figure 2), cellulase activity was higher in the BB system in the 0.00-0.10 m layer and significantly similar to the NF system in the 0.10-0.20 m layer. The specific activity of Invertase and β-glucosidase was higher in the NF system at both layers, but the activity of Invertase in the 0.00-0.10 m layer was significantly similar in the NF and BB systems. The specific activity of the carbon cycle enzymes was similar or close between the NF and BB systems.

Cellulase is an enzyme that catalyzes the hydrolysis of cellulose, Invertase hydrolyzes sucrose, and β-Glucosidase- catalyzes cellobiose. These carbon-degrading enzymes have their activity stimulated in environments with more litter, which is the case in the NF system, which is rich in these three carbohydrates (Silva Olaya et al., 2021). The differences observed for Cellulase and Invertase in the systems involving pasture may be related to the greater root development of BB and the excretion of organic compounds in greater quantities by this species, which are part of the reactions catalyzed by these enzymes (Hout et al., 2020).

Batista et al. (2014), in their evaluation of soil enzymes related to the crop-livestock integration system with soybeans and *Brachiaria* spp., observed an increase in Cellulase activity at different grazing intensities, which agrees with the results of this study that showed higher Cellulase activity in the BB system. Coura et al. (2020) found maximum Invertase activity in shaded environments cultivated with *Brachiaria brizantha* compared to full sun pasture; however, the present research found no differences in similar systems. Durrer et al. (2021) concluded that the conversion of primary forest to pasture significantly increased β-glucosidase activity in older pastures (>24 years) and remained stable in more recent pastures (<7 years).

The activity of the nitrogen (N) cycle enzymes was significantly influenced by the systems and layers studied (Table 3, Figure 3 A, B and C). The activity of the Urease enzyme was higher in NF and lower in BH, with its activity decreasing by 78% in the 0.00-0.10 m layer and 49% in the 0.00-0.20 m layer compared to the forest. The same occurred with the BAA-Protease enzyme, with 55% and 61% reductions between NF and BH at the respective layers. On the other hand, Casein-Protease activity was higher in BH at both layers studied. NF and BB had equal enzymatic activity for this enzyme at the 0.00-0.10 m layer, and NF had lower activity at the 0.00-0.20 m soil layer (Table 3).

The specific activity of the enzymes involved in the (N) cycle was influenced by depth, especially for BAA-Protease (Figure 3A, B and C). The NF and BB systems had a higher specific activity of Urease and BAA-Protease, while in NF, the specific activity of Casein was lower than in the other systems. Proteolytic enzymes rapidly degrade Urease in the soil. However, it is relatively persistent in the soil when associated with the humic complex or clays, remaining stable for a long period (Borghetti et al., 2003), which may have occurred in the NF system.

Table 2. Mean values (±s.d.) of the activities of the hydrolytic enzymes of the C cycle in the land use systems (NF, BB, and BH) and soil layers studied in Iracema, Roraima, Brazil.

System	Layer	Cellulase[a]	Invertase[a]	β Glucosity[b]
NF	0.00–0.10 m	0.089±0.008*aA*	1.171±0.109*aA*	0.281±0.024*aA*
	0.10–0.20 m	0.060±0.007*bA*	1.074±0.133*bA*	0.184±0.038*bA*
BB	0.00–0.10 m	0.072±0.006*aB*	0.528±0.031*aB*	0.110±0.010*aB*
	0.10–0.20 m	0.042±0.007*bB*	0.409±0.033*bB*	0.068±0.009*bB*
BH	0.00–0.10 m	0.044±0.008*aC*	0.298±0.035*aC*	0.102±0.026*aB*
	0.10–0.20 m	0.019±0.004*bC*	0.180±0.014*bC*	0.052±0.018*bB*

[a] μmol glucose g^{-1}h^{-1}; [b] μmol p-nitrophenol g^{-1}h^{-1}. Means followed by different lowercase letters indicate a statistical difference between the soil layers for each system. Means followed by different uppercase letters indicate a statistical difference between the systems for each soil layer. Tukey test at 5% probability.
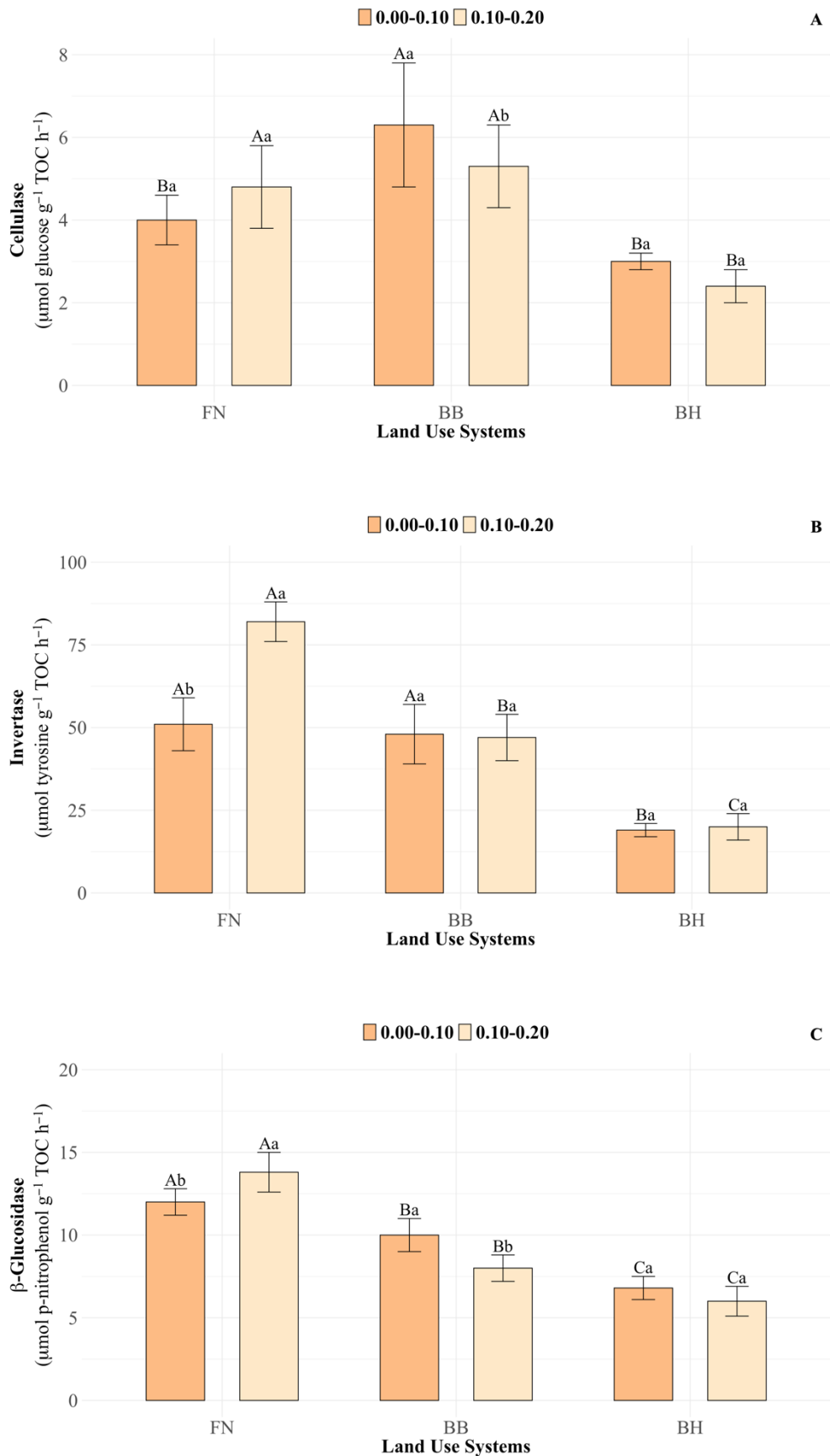
Figure 2. Specific activities of the hydrolytic enzymes of the Carbon cycle in the land use systems (NF, BB, and BH) and soil layers studied. A: Cellulase (µmol glucose g^{-1} TOC h^{-1}); B: Invertase (µmol tyrosine g^{-1} TOC h^{-1}); C: β Glycosidase (µmol p-nitrophenol g^{-1} TOC h^{-1}).

Proteases make up a group of different enzymes that catalyze reactions in proteins with different molecular structures, the type of reaction catalyzed, and the affinity of the active site for the substrates (Wang et al., 2020). In this sense, BAA-Protease and Casein-Protease differ in that the former is associated with the degradation of humic compounds, while the latter remains in the soil as a compound that is more resistant to degradation.

Vasconcellos et al. (2013), in a study on factors that limit the recovery of riparian forests, found a positive correlation between the activity of Urease and the total (C) and (N) content of the soil, agreeing with the observations of this study on the higher activity of Urease in NF. Nannipieri et al. (2012), in a pyrolysis gas chromatography mass spectrometry analysis, showed that the activity of BAA-Protease was higher in environments with higher levels of organic matter, while the action of Casein-Protease was maintained in environments with greater adsorption of soil particles and a favorable microenvironment, stable pH and humidity.

These results corroborate the data from this research and indicate the influence of the soil physical, chemical, and biological conditions on enzyme activity. In a study of winter cover crops, Wang et al. (2020) stated that Urease and Protease activity are closely linked to the diversity and richness of microorganisms, as well as to (C) and (N) content. The specific activity of Phosphomonoesterase was not affected by the systems in the 0.10-0.20 m layer. The BB system showed the highest Phosphomonoesterase specific activity, and the NF and BH systems were significantly similar in the 0.00-0.10 m layer (Figure 4). The specific activity of Phosphodiesterase was higher in the BB system and significantly similar between the NF and BH systems in the 0.00-0.10 m layer (Figure 4).

In the 0.10-0.20 m layer, the specific activity of Phosphodiesterase was much higher in the NF system and significantly similar in the BB and BH systems. The specific activity of Phosphomonoesterase was not affected by the systems in the 0.10-0.20 m layer. The BB system showed the highest Phosphomonoesterase specific activity, and the NF and BH systems were significantly similar in the 0.00-0.10 m layer (Figure 4). The specific activity of Phosphodiesterase was higher in the BB system and significantly similar between the NF and BH systems in the 0.00-0.10 m layer (Figure 4).

In the 0.10-0.20 m layer, the specific activity of Phosphodiesterase was much higher in the NF system and significantly similar in the BB and BH systems. In the 0.00-0.10 m layer, the BB and BH systems showed significantly similar and higher specific Arylsulphatase activity than the NF system. In contrast, in the 0.10-0.20 m layer, only BH showed higher specific Arylsulphatase activity, and the NF and BB systems did not differ (Figure 4). Soltangheisi et al. (2019) observed higher activity of the enzymes Phosphomonoesterase and Phosphodiesterase in pastures compared to adjacent forests. The high activity of these enzymes in pasture systems represents high organic P mineralization and low organic P content.

When sufficient P is available in the soil, Phosphatase activity is suppressed because mineralization of organic P is not necessary, and therefore, organic P accumulates in the soil (Soltangheisi et al., 2019). These results differ from ours, which showed greater Phosphomonoesterase activity in NF. However, they partially agree in the case of Phosphodiesterase, where we found similar activity between NF and BB on the surface.

Table 3. Mean values (±s.d.) of hydrolytic enzymes in the nitrogen (N) cycle in the land use systems (NF, BB, and BH) and soil layers studied in Iracema, Roraima, Brazil.

System	Layer	Urease[a]	BAA-Protease[a]	Casein[b]
NF	0.00–0.10 m	6.203±0.438aA	3.045±0.805aA	0.242±0.054aB
	0.10–0.20 m	2.185±0.291bA	2.692±0.285aA	0.186±0.033bC
BB	0.00–0.10 m	2.498±0.317aB	2.083±0.236aB	0.255±0.046aB
	0.10–0.20 m	1.703±0.126bB	1.283±0.179bB	0.232±0.043aB
BH	0.00–0.10 m	1.356±0.183aC	1.380±0.232aC	0.468±0.016aA
	0.10–0.20 m	1.125±0.099aC	1.053±0.247bB	0.274±0.047bA

[a] μmol NH3 g^{-1} h^{-1}; [b] μmol tyrosine g^{-1} h^{-1}

Equal uppercase letters indicate that the values for the same soil layer in the different systems are not significantly different; equal lowercase letters indicate that in each study system, the values in the two soil layers are not significantly different (p < 0.05).
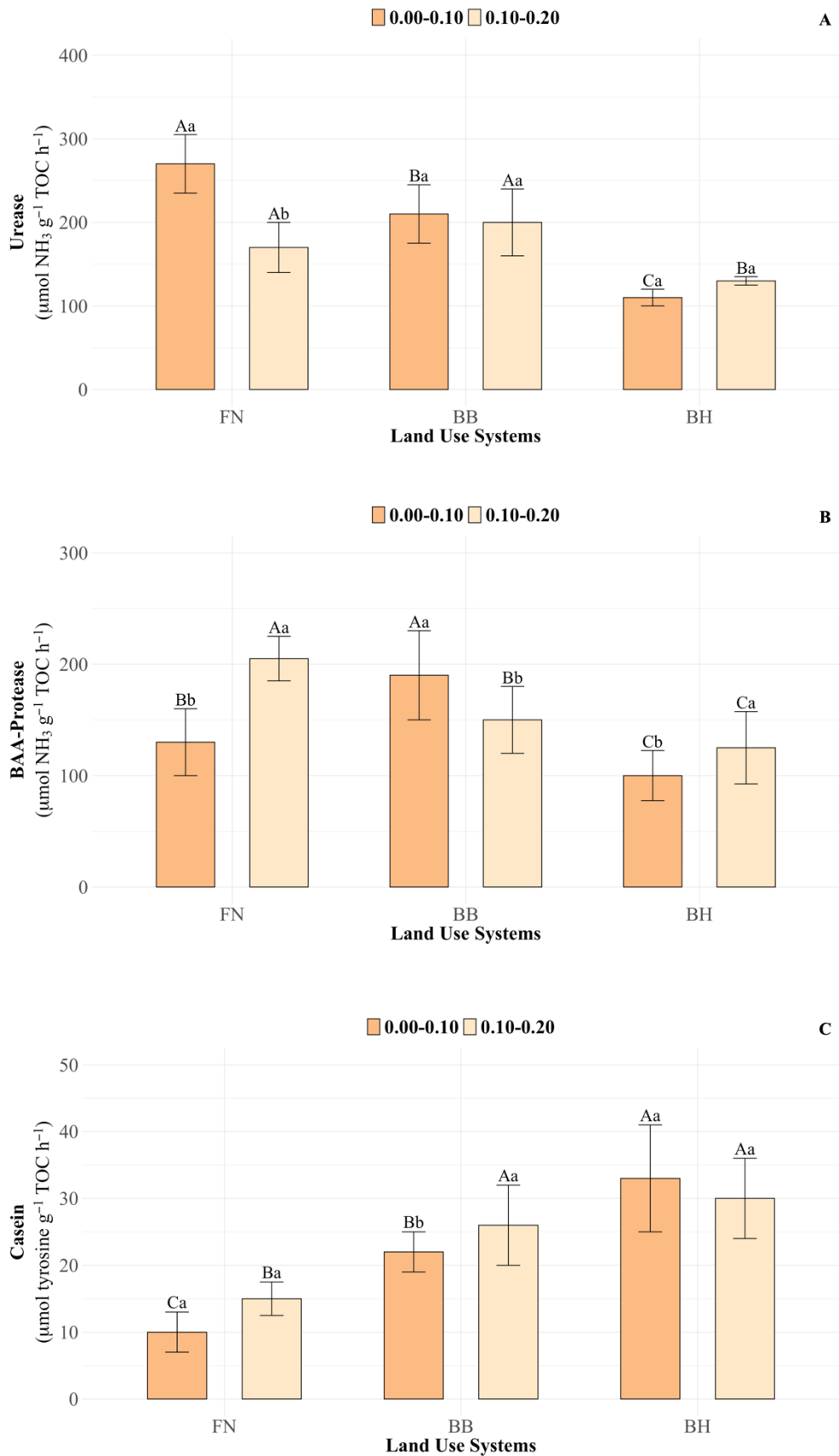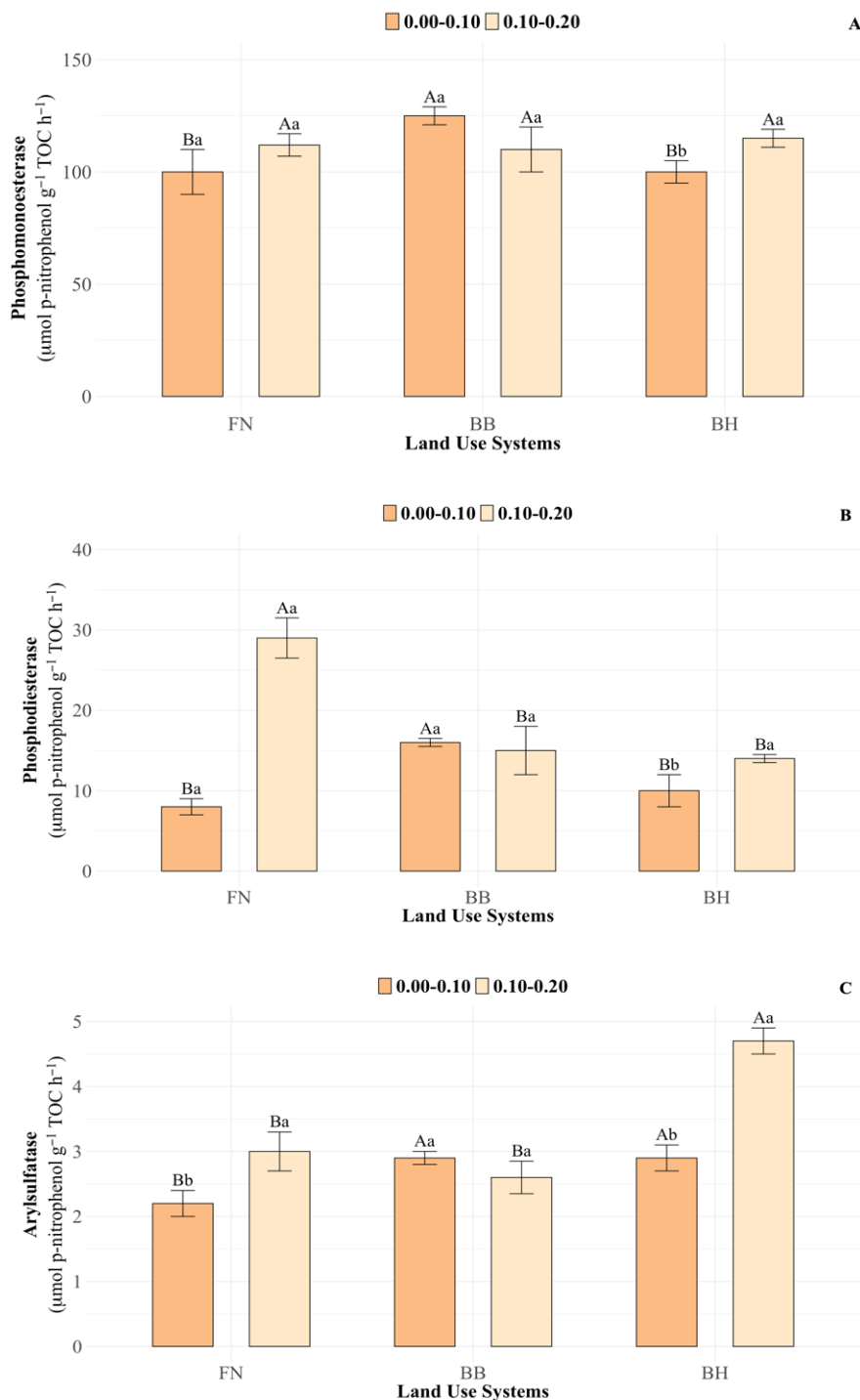
Figure 3. Specific activities of the hydrolytic enzymes of the N cycle in the land use systems (NF, BB, and BH) and soil layers studied. A: Urease (µmol NH3 g^{-1} TOC h^{-1}); B: BAA-Protease (µmol NH_3 g^{-1} TOC h^{-1}); C: Casein (µmol tyrosine g^{-1} TOC h^{-1})

Table 4. Mean values (±s.d.) of hydrolytic enzymes of the P and S cycle in the land use systems (NF, BB, and BH) and soil layers studied in Iracema, Roraima, Brazil.

System	Layer	Phosphomonoesterase[a]	Phosphodiesterase[a]	Arylsulfatase[a]
NF	0.00–0.10 m	2.623±0.248*aA*	0.184±0.023*aA*	0.242±0.054*aB*
	0.10–0.20 m	1.316±0.207*bA*	0.377±0.029*bA*	0.186±0.033*bC*
BB	0.00–0.10 m	1.394±0.182*aB*	0.173±0.013*aA*	0.255±0.046*aB*
	0.10–0.20 m	0.928±0.329*bB*	0.120±0.012*bB*	0.232±0.043*aB*
BH	0.00–0.10 m	1.426±0.218*aB*	0.152±0.010*aB*	0.468±0.016*aA*
	0.10–0.20 m	1.071±0.152*bAB*	0.121±0.010*bB*	0.274±0.047*bA*

[a] μmol *p*-nitrophenol g^{-1} h^{-1}. Equal uppercase letters indicate that the values for the same soil layer in the different systems are not significantly different; equal lowercase letters indicate that in each study system, the values in the two soil layers are not significantly different ($p < 0.05$).

Figure 4. Specific activities of the hydrolytic enzymes of the P and S cycle in the land use systems (NF, BB, and BH) and soil layers studied. A: Phosphomonoesterase (μmol *p*-nitrophenol g^{-1} TOC h^{-1}); B: Phosphodiesterase (μmol *p*-nitrophenol g^{-1} TOC h^{-1}); C: Arylsulfatase (μmol *p*-nitrophenol g^{-1} TOC h^{-1}).

Zaninetti et al. (2016) concluded that removing primary forest increases the metabolic quotient and reduces organic C contents, reflecting a decrease in the activity of several soil enzymes, which aligns with the lower enzymatic activity observed in NF for some enzymes in our study. Arylsulfatase, which is present in fungi in the form of sulfate esters, also participates as a component in the formation of soil microbial biomass and is directly affected by the replacement of forests with pastures (Bandick and Dick, 1999).

4. Conclusions

The soil in parts of the Amazon region, under native forests, shows higher activity of carbon cycle enzymes than soil under pasture. In the nitrogen cycle, the activity of Urease and BAA-Protease is predominantly higher in native forests, while Casein-Protease stands out in pastures. In the phosphorus and sulfur cycles, Phosphomonoesterase and Phosphodiesterase activity are higher in native forests, while Arylsulphatase is more active in pastures.

Authors' Contribution

Sandra Cátia Pereira Uchôa: Conceptualization, data collection, laboratory data analysis, data interpretation, and manuscript writing. Lucas Feitosa Pereira: Data interpretation and manuscript writing. Carlos Henrique Lima de Matos: Statistical data analysis, preparation of graphs and tables, data interpretation, and manuscript revision. Ingridy do Nascimento Tavares: Preparation of graphs and tables, data interpretation, and manuscript revision. José Maria Arcanjo Alves: Data collection, data interpretation, and manuscript revision. José Frutuoso do Vale Júnior: Data interpretation and manuscript revision

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