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Microbial enzymatic stoichiometry and the acquisition of C, N, and P in soils under different land-use types in Brazilian semiarid

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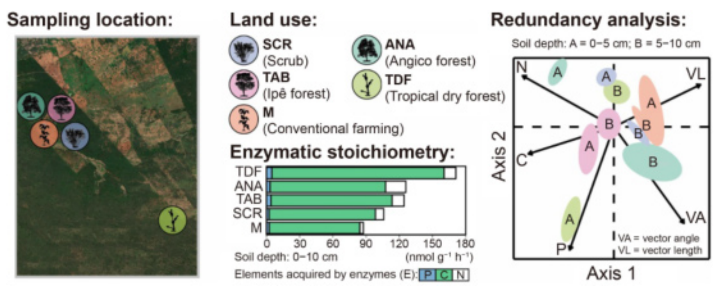
ABSTRACT

- The enzymatic stoichiometry varied between land-use in both soil depth.
- The values of C- and N-acquiring enzymes were higher at 0−5 cm depth.
- Soils under different land-use types in the Brazilian semiarid are P-limited.



This study hypothesized that different land-use affect the microbial enzymatic stoichiometry and C-, N-, and P-acquisition in Brazilian semiarid soils. Thus, the enzymes β-glucosidase (C-acquiring enzyme), urease (N-acquiring enzyme), and acid phosphatase (P-acquiring enzyme) were assessed in soil samples collected at 0−5 and 5−10 cm depth from a tropical dry forest, a protected area with Angico, a protected area with Ipê, scrub area, and an agricultural area with maize. The values of C-, N-, and P-acquiring enzymes were used to calculate the enzymatic C:N, C:P, and N:P ratios. The values of C:P and N:P ratios were higher at 0−5 cm depth, while no significant variation, between soil depth, was observed for C:N ratio. The values of C- and N-acquiring enzymes were higher at 0−5 cm in tropical dry forest areas and Angico forest, respectively. In all land use types, the values of vectors L and A were higher than 1° and 45°, respectively. This study showed that both land-use and soil depth influence the enzymatic stoichiometry, showing higher values of C- and N-acquiring enzymes in native and protected forests at soil surface.

Keywords C:N:P stoichiometry, tropical forests, soil depth, Luvisols.

1 Introduction

Brazil has an estimated area of one million km^2 under semi-arid conditions which is dominated by native vegetation known as Caatinga. This region presents soils with high fragility and vulnerability to degradation due to their natural preconditions (geology and climate) associated to different land-use, such as those applied in agriculture (Ferreira et al., 2016). Thus, these different land-use types applied in semi-arid have brought uncertainty and concerns about their potential negative effects on soil properties, mainly those associated with soil microorganisms (Lacerda-Junior et al., 2019). Indeed, previous studies have reported that different land-use types have affected the responses of soil microbial properties in Brazilian semiarid (Ferreira et al., 2016; Lima et al., 2020).

A better understanding of the potential effect of land-use type on soil microbial properties is particularly important since microorganisms play essential functions in soils and are involved in the turnover processes of soil organic matter

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(SOM) (Mendes et al., 2017; Naylor et al., 2022). These microorganisms produce and release extracellular enzymes that act mineralizing nutrients, such as C, N, and P (Liu et al., 2020), and provide both a measurement of nutrient availability and soil metabolism (Hill et al., 2012). Although the enzymatic activity is driven by microorganisms, the microbial activity is limited by the availability of C, N, and P (Araujo et al., 2022), which evidence the need to assess C, N, and P limitation in soils, which in turn can be influenced by different land-use types.

One method to estimate the nutrient demands by microbes and C, N, and P availability in soils is the stoichiometry of C-, N-, and P-acquiring enzymes (Sinsabaugh et al., 2009) and it has been applied in several previous studies (Zhang et al., 2021; Araujo et al., 2022; Pereira et al., 2023). In addition, the enzymatic stoichiometry can indicate which conditions C, N, and P are limited to soil microorganisms. For instance, Liu et al. (2020) assessed the enzymatic stoichiometry in soils from native forests in China and found P limitation to soil microbes. In Brazil, Araujo et al. (2022) reported that soils from native Cerrado are P-limited to microbes. However, these studies assessed the stoichiometry of C-, N-, and P-acquiring enzymes in native and preserved ecosystems, while it is unclear the responses of the enzymatic stoichiometry and microbial acquisition of C, N, and P in soils submitted to different land-use types.

In this study, soils from each land-use type present different availability of nutrients (C, N, and P) and it is known that microorganisms invest metabolic resources to acquire nutrients that could limit their activity and growth (Chen et al., 2020; Hu et al., 2021). Therefore, we hypothesized that these differences could influence the enzymatic stoichiometry and the acquisition of C, N, and P in the soil. To address this hypothesis, this study evaluated the stoichiometry of C-, N-, and P-acquiring enzymes in soils under different land-use types in the Brazilian semiarid.

2 Materials and methods

This study assessed sites under different land-use types located in a semiarid region from Pernambuco State, Brazil (7°59′31″ S and 38°17′59″ W, 430 m). This region presents an average temperature and precipitation of 28°C and 600 mm, respectively (mostly concentrated from January to April). According to the Brazilian classification system, the soil is classified as chromic Luvisols. In this study, five different land-use types (Fig. 1) were assessed, being a tropical dry forest (TDF), a protected area with Angico (ANA), a protected area with Ipê (TAB), a scrub area (SCR), and an agricultural area with maize (M) (Table 1). Each site was divided into five transects (~4000 m^2, each one) where soil samples (three points per transect) were collected at 0−5 and 5−10 cm depth, during the wet season. In all soil samples, available P (Mehlich-1), total N (combustion method), and SOC content (wet oxidation method) were evaluated, and the values are in Table 2.

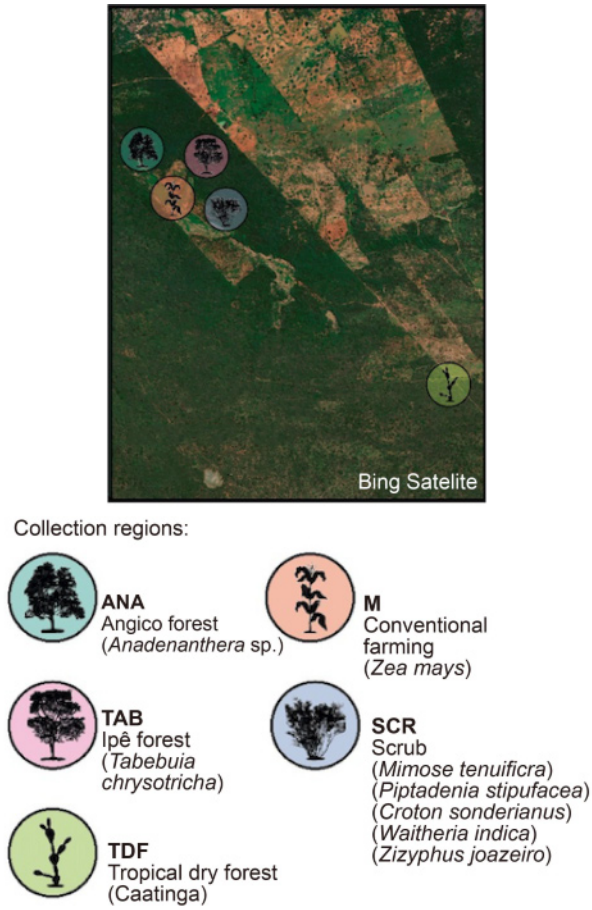

Fig. 1 Map showing the localization of each land-use types.

The estimations of C-, N-, and P-acquiring enzymes were obtained through the activities of β-glucosidase (BG), urease (U), and acid phosphatase (AP), respectively. Briefly, the β-glucosidase (EC 3.2.1.21; Eivazi and Tabatabai, 1988) was measured using ρ-nitrophenyl β-glucopyranoside as substrate and the ρ-nitrophenol formed was measured spectrophotometrically at 410 nm. The acid phosphatase (EC 3.1.3.2; Tabatabai and Bremner, 1969) was measured using disodium ρ-nitrophenyl phosphate as substrate and the amount of ρ-nitrophenol formed was measured spectrophotometrically at 420 nm. The urease (EC 3.5.1.5; Kandeler and Gerber, 1988) was measured using urea as substrate and the amount of ammonium produced was determined using the "Kjeldahl" method. C-, N-, and P-acquiring enzymes were expressed by BG, U, and AP in nmol g^{-1} h^{-1}. The values of C-, N-, and P-acquiring enzymes were used for calculating the enzymatic C:N, C:P, and N:P ratios, respectively. The microbial resource acquisition was estimated by the vector method (Moorhead et al., 2016) as follows:

Table 1 Localization and history of each land-use.

Area	Geographic coordinates	Area history
Tropical dry forest (TDF)	7°57′47.0″ S, 38°23′1.5″ W	Reasonably preserved, but used for uncontrolled grazing.
Angico forest (ANA)	7°57′7.5″ S, 38°23′56.1″ W	It has been covered with Angico (*Anadenanthera* sp.) since 1978. Before 1978 it was cultivated with cotton (*Gossypium hirsutum*) and palm (*Opuntia ficus-indica*).
Ipê forest (TAB)	7°57′10.1″ S, 38°23′45.5″ W	Cultivated with buffel grass (*Cenchrus ciliaris*) and cotton (*Gossypium hirsutum*). It underwent natural regeneration from 1998 by Ipê (*Tabebuia chrysotricha*).
Scrub (SCR)	7°57′16.2″ S, 38°23′45.4″ W	Covered with Scrub for > 20 years. Predominance of black jurema (*Mimosa tenuiflora*), white jurema (*Piptadenia stipulacea*), quince (*Croton sonderianus*), mallow (*Waltheria indica*), arboreal, Juá (*Zizyphus joazeiro*) and herbaceous plants.
Conventional farming (M)	7°57′15.4″ S, 38°23′49.1″ W	Cultivated with maize (*Zea mays*) conventionally from 2005 to 2015, but under fallow due to the severe drought from 2011 to 2013. The farmer has applied an unknown amount of sheep dung over the cultivated time.

Table 2 Soil organic C (SOC; g kg^{-1}), total N (TN; g kg^{-1}) and available P (mg kg^{-1}) in different land-use.

Land-use	0−5 cm			5−10 cm		
	SOC	TN	P	SOC	TN	P
TDF	19.9	3.1	7.3	13.6	1.1	14.3
ANA	18.3	1.0	34.5	14.7	1.6	11.5
SCR	15.0	2.2	14.2	10.6	1.9	22.0
TAB	13.9	1.4	30.3	13.4	1.4	10.7
M	11.9	2.0	21.8	12.2	2.3	24.3

TDF−tropical dry forest, ANA−Angico, TAB−Ipê, SCR−scrub area (SCR), M−agricultural area with maize.

$$\text{Vector L (unitless)} = \sqrt{X^2 + Y^2}, \quad (1)$$

$$\text{Vector A (degree)} = \text{Degrees (Atan2 (X, Y))}, \quad (2)$$

where:

$$X = BG/(BG + AP); Y = BG/(BG + U).$$

The multivariate statistical analyses were done in R version 4.1.1 software (R Core Team 2021) with the aid of the RStudio interface 2021.09.0-Build 351 (RStudio Team 2021). For analysis of variance (ANOVA), this study considered a double factorial design with five replicates. The first factor consisted of five land-use types (TDF, ANA, TAB, SCR, and M) and the second factor consisted of soil depth (0−5 and 5−10 cm). Before proceeding with ANOVA, assumptions of normality and heteroscedasticity of all the variables were evaluated by the Shapiro-Wilk's test and Bartlett's test, respectively, by R library 'RVAideMemoire' (v. 0.9-81-2). Analysis of variance and Fisher's LSD (Least Significant Difference) post-hoc test was performed with 'agricolae' R library (v. 1.3.5) at a 5% significance level (p = 0.05). Bonferroni's correction was applied to minimize the type I error rate of the test family as a function of the increased number of pairwise comparisons. Bar charts were constructed using the features of the 'ggplot2' R package (v. 3.3.5). The redundancy analysis (RDA) ordination, variables share calculations (Venn diagram), and multivariate analysis of variance (MANOVA) were done with functions from the 'vegan' R package (v. 2.5.7) and graphs constructed by the 'graphics' R package (v. 4.1.1). Influences of soil depth and land-use types on C-, N-, and P-acquiring enzyme were checked by multivariate regression using structural equation modeling (SEM). The SEM model was adjusted with 'lavaan' (v. 0.6.11) and 'semPlot' R libraries (v. 1.1.5).

3 Results

The redundancy analysis explained 57% of the total variation in C-, N-, and P-acquiring enzymes, being distributed according to soil depth (33%) and land-use system (24%) (Fig. 2A). The analysis of ordination explained 84% of the total variation distributed in PC1 (52%) and PC2 (32%) and clustered C-, N-, and P-acquiring enzymes, and vectors A and L (Fig. 2B). In general, C-, N-, and P-acquiring enzymes correlated with 0−5 cm depth, mainly in ANA, TDF, and TAB sites.

The structural equation model (SEM) showed that both soil depth and land-use exerted significant influence on C-, N-, and P-acquiring enzymes (Fig. 3). Significant negative interaction was observed between soil depth and C-, N-, and P-acquiring enzymes (R^2 = −0.68, p < 0.01), which were explained through a latent variable (acquiring enzymes). Less significantly, land-use types influenced C-, N-, and P-acquiring enzymes (R^2 = −0.48, p < 0.01). This latent variable was positively and significantly weighted by C (R^2 = 0.91, p < 0.01) and N (R^2 = 0.36, p < 0.05), which were most pronounced at 0−5 cm depth.

At 0−5 cm depth, the values of the C-acquiring enzyme were highest in TDF and lowest in M; while at 5−10 cm depth the highest values were found in TDF and M, and the lowest in ANA and SCR (Fig. 4A). Regarding the N-acquiring enzyme, ANA showed the highest value found at 0−5 cm depth, while TAB and TDF showed the highest values at 5−10 cm depth (Fig. 4B). The values of P-acquiring enzyme were highest in TDF (0−5 cm depth), and ANA (5−10 cm depth) (Fig. 4C).

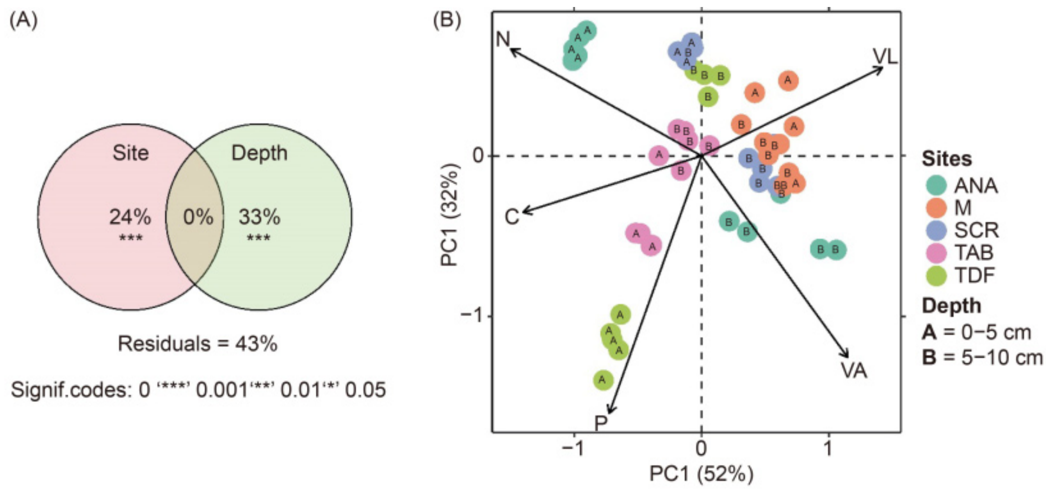

Fig. 2 Redundancy analysis ordination (RDA) for variables related to enzyme-acquired elements on sites in the rainy season. (A) Percentages of total variance explained by sampling site (Site) and soil profile (Depth) on soil enzyme-acquired C, N, and P. The function and p-values were calculated using the adjusted R-squared in redundancy analysis (RDA, type-2 scaling) ordination with Bray-Curtis dissimilarity index (B) First two dimensions (PC1 and PC2) of the ordination space from the RDA. Colors indicate five sites sampled: protected area with Angico (ANA), agricultural area with maize (M), scrub area (SCR), protected area with Ipê (TAB), tropical dry forest (TDF). The circles marked with capital letters A and B represent depths of 0−5 cm and 5−10 cm, respectively. The lines (vectors) indicate the correlations of the variables related to the acquisition of chemical elements by enzymes in the soil (P, C, and N) and the stoichiometric vectors A and L.

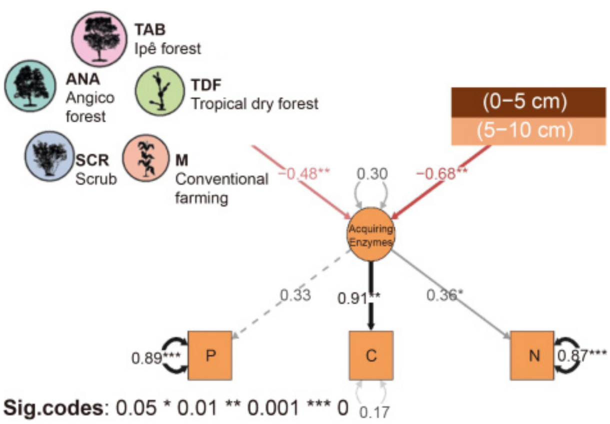

Fig. 3 Representation of multivariate regression between C-, N-, and P-acquiring enzymes and independent variables (land-use types and soil depth). Numbers represent the adjusted correlations between variables based on the structural equation modeling (SEM). Black and red lines indicate positive and negative correlations, respectively.

The stoichiometry of enzymes showed variation between soil depth and land-use system. The values of C:P and N:P ratios were higher at 0−5 cm depth, while no significant variation, between soil depth, was observed for C:N ratio. The values of the C:N ratio did not vary between land-uses at 0−5 cm depth, while that at 5−10 cm depth the highest C:N ratio was found in ANA (Fig. 5A). Regarding the C:P and N:P ratios, the values were highest in SCR and lowest in TDF and TAB, at 0−5 cm depth (Fig. 5B, C). Interestingly, TDF showed the highest C:P and N:P ratios at 5−10 cm depth. The values of vectors varied between soil depth and land-use (Table 3). At 0−5 cm depth, the vector A showed higher values in M, TAB, and TDF; while the values did not vary between land-uses at 5−10 cm depth, except for TDF which showed the lowest value. The vector L showed higher values in M, SCR, and TAB, at 0−5 cm depth, while it did not vary at 5−10 cm depth.

4 Discussion

This study evaluated the responses of C-, N-, and P-acquiring enzymes in different land-use types from Brazilian semiarid. Here, we addressed the hypothesis that different land-use types could influence the stoichiometry of enzymes and the acquisition of C, N, and P in soils. In line with this hypothesis, we found distinct responses of C-, N-, and P-acquiring enzymes and their stoichiometry as influenced by soil depth and land-use types. This study observed higher values of acquiring enzymes at 0−5 cm depth compared to 5−10 cm depth, mainly in land-use type with the presence of trees, such as TDF, ANA, TAB, and SCR. First, it is expected that soil enzymes decrease in depth due to decreased availability of organic sources and nutrients in deeper soil layers (Ge et al., 2010; Medeiros et al., 2017). Second and most important, land-use types with the presence of trees contribute to the higher amount of plant litter on the soil surface, which promotes higher microbial activity by C-, N-, and P-acquiring enzymes at 0−5 cm depth (Piotrowska-Długosz et al., 2022). Therefore, these assumptions could explain the higher influence of soil depth, mainly 0−5 cm depth, on the variation of C-, N-, and P-acquiring enzymes.

The soil under tropical forest (TDF) showed the highest

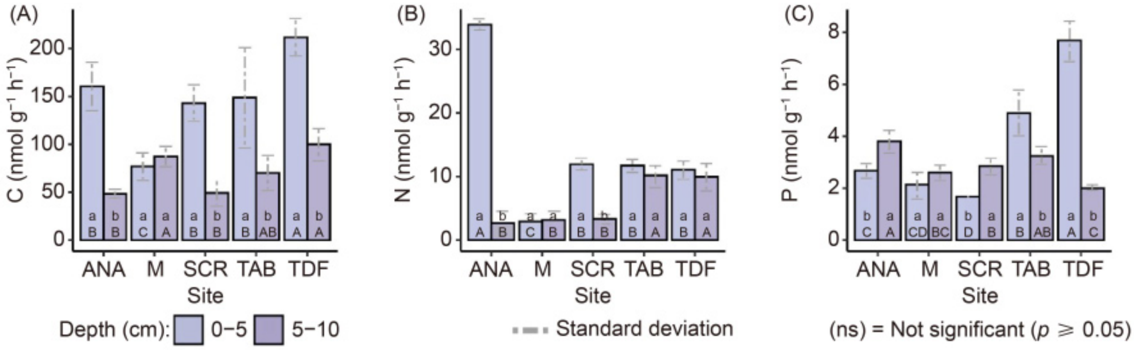

Fig. 4 C-, N-, and P-acquiring enzymes in soil. TDF = tropical dry forest, ANA = Angico, TAB = Ipê, SCR = scrub area (SCR), M = agricultural area with maize. The means were compared with each other according to Fisher's LSD (Least Significant Difference) post-hot test with Bonferroni correction at a 5% significance level. Different letters at the base of the bars indicate that the means are different from each other: lower case − for depths within the same site; upper case − for sites within the same depth.

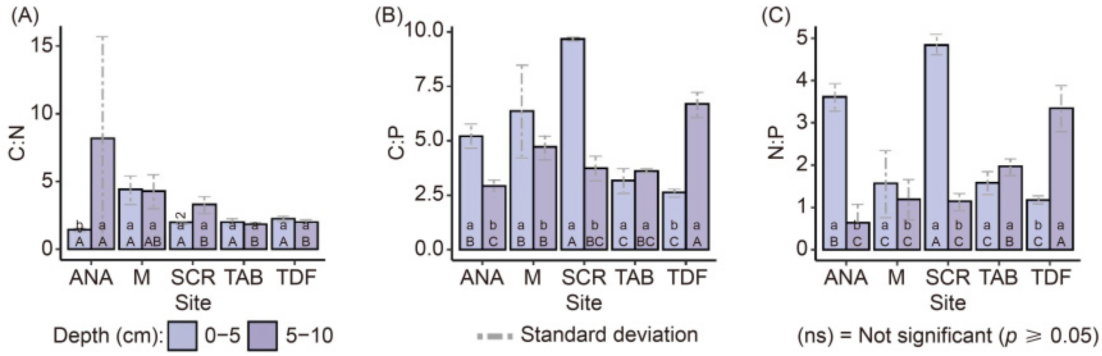

Fig. 5 C:N, C:P, and N:P ratios in soil. TDF = tropical dry forest, ANA = Angico, TAB = Ipê, SCR = scrub area (SCR), M = agricultural area with maize. The means were compared with each other according to Fisher's LSD (Least Significant Difference) post-hot test with Bonferroni correction at a 5% significance level. Different letters at the base of the bars indicate that the means are different from each other: lower case−for depths within the same site; upper case−for sites within the same depth.

Table 3 Vector analysis between soil depth and land-use types.

Land-use	Vector A (degree)		Vector L (unitless)	
	0−5 cm	5−10 cm	0−5 cm	5−10 cm
ANA	0.46 ± 0.01bB	0.88 ± 0.02aA	1.02 ± 0.01bB	1.12 ± 0.08aA
M	0.73 ± 0.09aA	0.76 ± 0.07aB	1.18 ± 0.02aA	1.15 ± 0.02aA
SCR	0.50 ± 0.01bB	0.76 ± 0.04aB	1.13 ± 0.01aA	1.10 ± 0.03aA
TAB	0.66 ± 0.03aA	0.60 ± 0.03aC	1.01 ± 0.04aB	1.02 ± 0.01aB
TDF	0.74 ± 0.01aA	0.53 ± 0.03bC	1.00 ± 0.02bB	1.10 ± 0.01aA

[a] means were compared with each other according to Fisher's LSD (Least Significant Difference) post-hot test with Bonferroni correction at 5% significance level. Different letters indicate that means are different from each other: lower case − for depths within the same site; upper case − for sites within the same depth. TDF = tropical dry forest, ANA = Angico, TAB = Ipê, SCR = scrub area (SCR), M = agricultural area with maize.

values of C-acquiring enzyme in both soil depth. It indicates that systems with great richness and diversity of plants, such as the tropical native forest, contribute with high amount of C sources to microbes, increasing the values of C-acquiring enzymes (Gunina and Kuzyakov, 2015). In addition, the highest activities of soil acquiring enzymes under TDF are due to the highest biomass and diversity of root types that increase C inputs, being source of energy to enzyme production (Medeiros et al., 2015; Li et al., 2020). The increase in the amount and diversity of litter and root exudates found in forest, such as TDF compared to M, stimulated the activities of hydrolytic enzymes (Bastida et al., 2019). Indeed, in a tropical native Cerrado, Araujo et al. (2022) observed higher values of C-acquiring enzymes in Cerradao (the most diverse physiognomy). In contrast, the area under Angico forest (ANA) showed higher values of N-acquiring enzyme at 0−5 cm depth and it can be explained due to the inputs of plant litter with high N content since Angico is a N-fixing plant. The introduction of legumes into cropping systems (Sekaran et al., 2019), the permanence of plant residues in the soil (Malobane et al., 2020), and the high temperatures found in tropical dry regions are factors that increase urease activity. It is well known that the N-acquiring enzyme is influenced by inputs of plant litter (Mukumbareza et al., 2016) as well as the presence of N-fixing tree species (Pereira et al., 2023). On the other hand, the land uses with Ipê forest (TAB) and tropical forest (TDF) showed the highest values of N-acquiring enzyme at 5−10 cm depth. It could suggest a contribution of organic matter on N-acquiring enzyme along with soil profile in these forest systems (Levinski-Huf and Klein, 2018).

Tropical forest (TDF) at 0−5 cm and Angico (ANA) at 5−10 cm showed higher values of P-acquiring enzyme, and it suggests that these land-use types present high activity in P cycling in soils, thus contributing to increase primary productivity (Margalef et al., 2017). In addition, the soil samples from TDF (0−5 cm depth), and ANA (5−10 cm depth) presented low availability of P, it could explain the increase in P-acquiring enzyme (Piotrowska-Długosz and Charzyński, 2015) due to the increase of microbial biomass and demand for P (Sarto et al., 2020). According to Chen et al., 2020, when the availability of P is lows, microorganisms invest metabolic resources to acquire P, so increasing the activity of P-enzyme.

In SCR, P-acquiring enzymes decreased and it contributed to increase C:P and N:P ratios at 0−5 cm depth. Since SCR presented high availability of P, it contributed to decrease P-acquiring enzyme and consequently increase the C:P and N:P ratios. In contrast, C:N ratio did not vary between areas and indicates that soil microorganisms had similar availability of C and N in soils, mainly in native and protected area. Indeed, Araujo et al. (2022) found higher C:P and N:P, and similar C:N in native tropical Cerrado, which could explain no variation in C:N ratio in this study. It suggests that soils under forests have more contribution of litterfall to microbial activity.

Although the values of vectors A and L have shown variation between land-use types and soil depth, all values are within the same range, i.e., vectors L and A were higher than 1° and 45°, respectively. Vector L indicates P limitation to soil microorganisms, while vector A indicates N (< 45) and P (> 45) limitation (Chen et al., 2018). Although the vector analysis is an indirect estimation of C, N, and P limitations, our results suggests that soils under different land-use types in the Brazilian semiarid are P-limited. It corroborates with a previous study in the Brazilian Cerrado, which indicates that all native soils are P-limited to microorganisms (Araujo et al., 2022).

5 Conclusions

In this study, we evaluated if different land-use types could influence the stoichiometry of enzymes and the acquisition of C, N, and P in soils from tropical dry areas in the Brazilian semiarid region. To address this hypothesis, this study evaluated the stoichiometry of C-, N-, and P-acquiring enzymes in soils under a tropical dry forest (TDF), a protected area with Angico (ANA), a protected area with Ipê (TAB), a scrub area (SCR), and an agricultural area with maize (M). Our analysis revealed that the enzymatic stoichiometry varied between land-use types and soil depth. In general, C- and N-acquiring enzymes were higher at soil surface (0−5 cm depth) in area under native (TDF) and protected (ANA) systems, respectively. It indicates that native and protected system receive more inputs of C and N, so stimulating higher enzymes activities related to C and N. In contrast, the lower P availability found usually in tropical soils contributes to increase the P limitation. These results suggests that soils under different land-use types in the Brazilian semiarid are P-limited. However, the use of vectors A and L present limitation as they are estimators of nutrients acquiring by microbes. As further studies, we could suggest the evaluation of microbial community composition, and functionality related to C, N, and P cycling in semiarid conditions. In addition, it needs to clarify all possible mechanisms underlying the responses of C-, N-, and P-acquiring enzymes (Chen et al., 2018).

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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