

RESEARCH ARTICLE

Soil enzyme responses to land use change in the tropical rainforest of the Colombian Amazon region

Adriana M. Silva-Olaya^{1*}, Dúber A. Mora-Motta¹, Maurício R. Cherubin², Daniel Grados³, Anil Somenahally⁴, Fausto A. Ortiz-Morea^{1*}

1 Amazonian Research Center CIMAZ-MACAGUAL, University of the Amazon, Florencia, Colombia, **2** Department of Soil Science, “Luiz de Queiroz” College of Agriculture, University of Sao Paulo, Sao Paulo, Brazil, **3** Instituto del Mar del Perú, Esquina Gamarra y General Valle s/n Chucuito, Callao, Perú, **4** Department of Soil and Crop Sciences, Texas A&M University, Overton, Texas, United States of America

* amsolayaa@gmail.com, adr.silva@udla.edu.co (AMSO); fau.ortiz@udla.edu.co (FAOM)



OPEN ACCESS

Citation: Silva-Olaya AM, Mora-Motta DA, Cherubin MR, Grados D, Somenahally A, Ortiz-Morea FA (2021) Soil enzyme responses to land use change in the tropical rainforest of the Colombian Amazon region. PLoS ONE 16(8): e0255669. <https://doi.org/10.1371/journal.pone.0255669>

Editor: Tunira Bhadauria, Feroze Gandhi Degree College, INDIA

Received: April 5, 2021

Accepted: July 21, 2021

Published: August 18, 2021

Copyright: This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the [Creative Commons CC0](https://creativecommons.org/licenses/by/4.0/) public domain dedication.

Data Availability Statement: All relevant data are within the manuscript.

Funding: This work was part of a research project studying the functional diversity of microbial communities of rainforest soil and litter in the Colombian Amazon region, supported by Sistema General de Regalías (grant number 201800100114). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Soil enzymes mediate key processes and functions of the soils, such as organic matter decomposition and nutrient cycling in both natural and agricultural ecosystems. Here, we studied the activity of five extracellular soil enzymes involved in the C, N, and P-mineralizing process in both litter and surface soil layer of rainforest in the northwest region of the Colombian Amazon and the response of those soil enzymes to land use change. The experimental study design included six study sites for comparing long-term pasture systems to native forest and regeneration practices after pasture, within the main landscapes of the region, mountain and hill landscapes separately. Results showed considerable enzymatic activity in the litter layer of the forest, highlighting the vital role of this compartment in the nutrient cycling of low fertility soils from tropical regions. With the land use transition to pastures, changes in soil enzymatic activities were driven by the management of pastures, with SOC and N losses and reduced absolute activity of soil enzymes in long-term pastures under continuous grazing (25 years). However, the enzyme activities expressed per unit of SOC did not show changes in C and N-acquiring enzymes, suggesting a higher mineralization potential in pastures. Enzymatic stoichiometry analysis indicated a microbial P limitation that could lead to a high catabolic activity with a potential increase in the use of SOC by microbial communities in the search for P, thus affecting soil C sequestration, soil quality and the provision of soil-related ecosystem services.

Introduction

The extracellular enzymes play important functions in the biogeochemical cycles, catalyzing the reactions involved in organic matter decomposition, becoming critical drivers of carbon (C) storage and the supply of nutrients to tropical forest ecosystems [1]. The activity of extracellular enzymes to degrade the litter layer is mostly regulated by soil microbial community, supplying energy and nutrients for microbial and plant growth [2]. Therefore, reducing in the

Competing interests: The authors have declared that no competing interests exist.

quantity and quality of the litter after land use change from native forests to agricultural uses could alter soil enzyme activity and nutrient biogeochemistry, including soil organic carbon sequestration [3].

Several studies revealed that soil enzyme production and activity are significantly impacted by land use change and agricultural practices [4–8]. However, the response of each soil enzyme is highly variable [8–11]. It was clear, to some extent, that soil enzyme responses are site specific depending on climate and soil conditions (e.g., nutrient availability), plant community type, and land management [8], with each factor impacting soil enzymes differently [5, 7]. Thus, understanding soil enzyme responses to land use changes in different soil conditions is critical for restoring soil health and increase land productivity.

In the Amazon region, intensive deforestation of native rainforest for expansion of livestock and agricultural activities has led to significant changes of soil C stocks, and degradation of soil physico-chemical properties in many regions [12–15]. Particularly, soil health degradation and soil C declines were substantial in pasture systems with low-fertility soils [14–16]. It is also well established that replacing native rainforest diminishes quantity and quality of organic matter input to the soil, the primary source of microbial resources (e.g., C, N, S, P), and, therefore, significantly shifting soil microbial communities [17–19]. Consequently, soil enzymatic activity could be affected, with implications on soil C storage and ecosystem functioning.

Extracellular enzyme dynamics in forest ecosystems and their responses after land use change, particularly for those farms situated in low-fertility soils is not clearly understood. Furthermore, there is a paucity of studies of soil enzyme activities in the litter layer, an essential component for that ecosystem functioning. Those ecosystems are experiencing rapid changes due to direct human disturbance, particularly in the Colombian portion of the Amazon region, which has become an important hotspot of deforestation of that biome [20]. In this region, very little is known about the effects of land use changes on enzyme activities, especially on the impact of the transition from natural forest to pastures for livestock production.

In this sense, we performed this study by leveraging several long-term field sites to evaluate the enzymatic activity in both litter and surface soil layer in the rainforest of the northwest Colombian Amazon region and the soil enzyme responses to land use change. The long-term study sites included native forests, pastures, and abandoned pastures under natural regeneration. Additionally, forest and pasture systems were compared separately within the mountain and hill landscapes. We also assessed soil C, N, and P contents to establish the relationships with soil enzymes. We hypothesized that the litter layer in tropical Amazon forests maintains high enzymatic activity responsible for C, N and P mineralization. This layer will be lost when the forest is replaced by pasture, but due to legacy effects maintaining higher mineralization potential and loss of organic matter. We also hypothesized that high N and P nutrient demand is a major driver for mineralization potential in pasture systems situated in low-fertile soil.

Material and methods

Study site and land uses

The study was performed in the Caquetá state of northwestern Colombian Amazon, which has experienced vast deforestation of Amazon Basin, ranking second globally after the arc of deforestation in Brazil [21]. Six sites were strategically selected on the two main landscapes of the Colombian Amazon region: hills and mountain landscape [22], guaranteeing the representability of the study area (Fig 1). The three sites on the hills landscape were located in the municipalities of i) San Vicente del Caguán (SVC); ii) Cartagena del Chairá (CTG), and iii) Solano (SLN). Those sites are located within a range of altitude between 235 to 290 m.a.s.l. The three sites in the mountain landscape were located in the municipality of Belén de Los

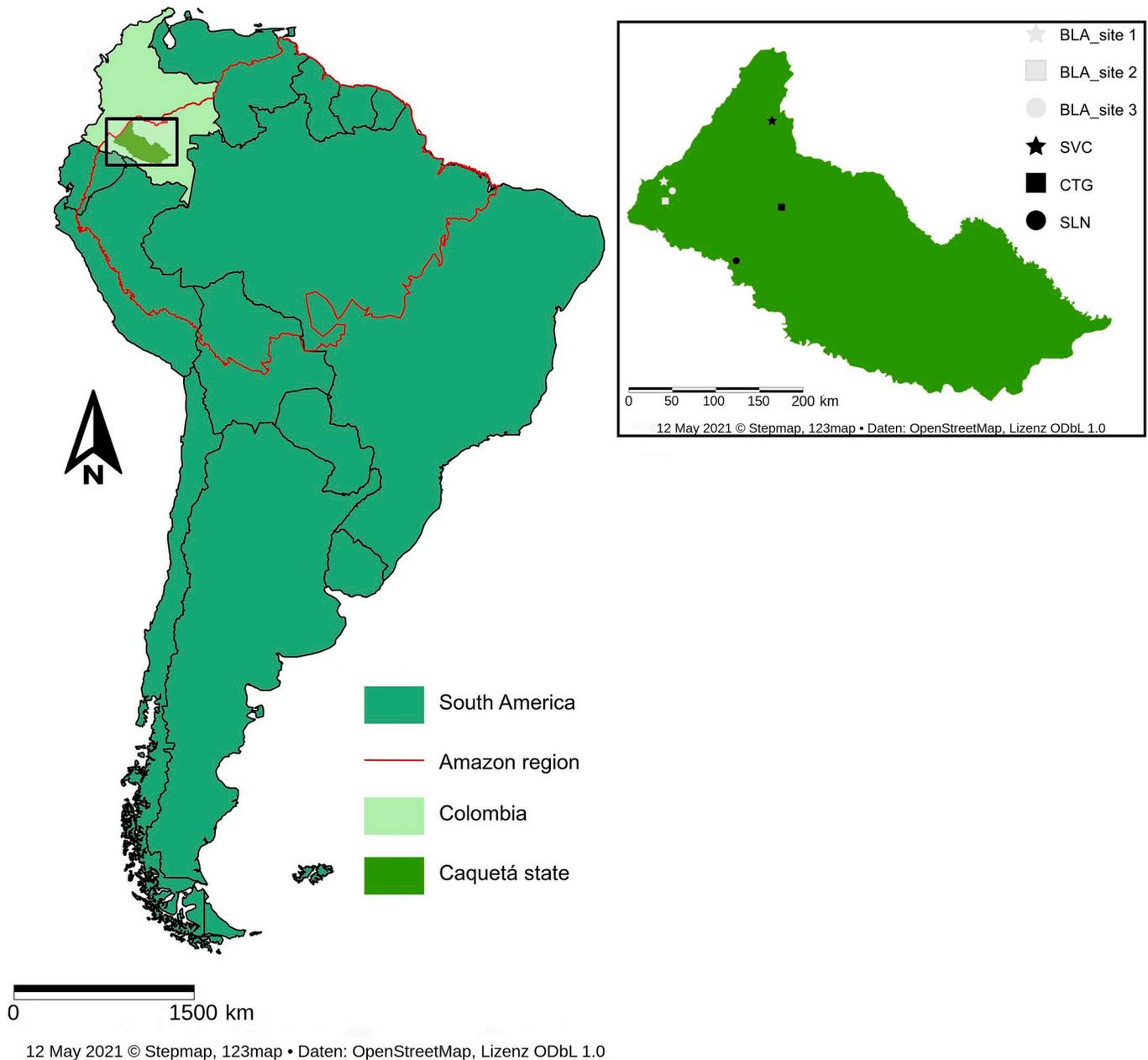


Fig 1. The geographic location of the study sites in the northwestern region of Colombian Amazon (BLA: Belén de los Andaquíes, SVC: San Vicente del Caguán, CTG: Cartagena del Chairá, SLN: Solano). Republished from [StepMap.com](https://www.stepmap.com/) under a CC BY license, with permission from StepMap GmbH, original copyright 2021.

<https://doi.org/10.1371/journal.pone.0255669.g001>

Andaquíes (BLA), in a region of Andes-Amazon transition with a maximum elevation of 1000 m.a.s.l. All selected sites respond to a significant rate of forest clearing for pasture establishments.

The regional climate is classified as a tropical rainforest—Af type (Koppen classification), with a mean annual temperature of 25.5°C and annual precipitation of 3793 mm. In the hills landscape, the soils are highly weathered, moderately deep, and classified as Typic Hapludults, which were originated from claystone. In contrast, the soils are less developed in the mountain

landscape, classified as Lithic Udorthents originated from granite and gneiss with sandstone inclusions [22]. Both hill and mountain soils are very acid, with high Al saturation and low-cation exchange capacity and basic cation contents (Ca²⁺, Mg²⁺, K⁺ and Na⁺), resulting in poor-chemically soils [22].

A paired-site comparison involving a forest and adjacent pasture, which was established after forest slash and burning, was assessed in each site. Additionally, mountain landscape areas with natural regeneration after pasture abandonment (a typical transition in land use in the Colombian Amazon region) were also evaluated, totalizing six study areas in the hills landscape and nine study areas in the mountain landscape. The chemical characteristics and granulometric composition of the soils in all study areas are presented in Table 1.

The forest areas correspond to a typical Amazon rainforest, constituted by a vegetation community dominated by regularly distributed arboreal elements representing approximately 80% of the total area, forming a discontinuous canopy with a height greater than 15 meters. The pasture areas correspond to pastures composed of *Brachiaria sp.* established >25 years ago after slash and burn of native forests in hills landscape and 15 years ago in mountain landscape; with an occupation of approximately one cattle head by hectare. The natural regeneration areas correspond to 10-year-old secondary vegetation, established over abandoned pastures. In that region, it is common, after the pasture yield decreases because of the absence of investments in soil and pasture management practices (e.g., fertilization, liming, control of soil erosion and weeds, and soil compaction management) the landowners abandon the pastures allowing secondary succession.

Soil sampling and analysis

In each area, soil sampling was done in four plots of 4 m² spaced 100 m apart following a completely randomized design. Since most of the biochemical transformations occur in the

Table 1. Soil granulometric fractions and chemical attributes in the study sites.

Landscape	Land use	Clay	Silt	Sand	pH	Ca	Mg	K	Al
		g kg ⁻¹				cmol kg ⁻¹			
		San Vicente del Caguán (SVC)							
Hills	Forest	292	246	462	4.19	4.94	3.36	0.22	2.52
	Pasture	261	267	472	3.94	0.40	0.24	0.12	2.16
		Solano (SLN)							
	Forest	319	320	361	4.10	0.18	0.15	0.44	4.40
	Pasture	331	260	409	4.08	0.49	0.21	0.31	2.35
		Cartagena del Chairá (CTG)							
	Forest	302	335	363	3.46	0.43	0.05	0.28	6.79
	Pasture	257	172	572	3.89	1.00	0.04	0.15	2.76
Mountain		Belén de los Andaquíes (BLA_site 1)							
	Forest	92	38	869	3.80	0.40	0.09	0.16	0.49
	Pasture	338	125	537	4.05	0.59	0.27	0.36	1.10
	Nat. Regeneration	228	120	651	3.83	0.96	0.26	0.30	1.99
		Belén de los Andaquíes (BLA_site 2)							
	Forest	251	118	631	3.84	0.48	0.18	0.34	1.20
	Pasture	209	133	658	4.62	0.70	0.26	0.60	0.60
	Nat. Regeneration	230	110	693	3.92	0.40	0.27	0.47	1.44
		Belén de los Andaquíes (BLA_site 3)							
	Forest	281.28	120.53	598.19	3.79	0.21	0.14	0.23	1.47
	Pasture	262.57	67.43	670.00	3.99	0.55	0.24	0.37	1.34
	Nat. Regeneration	264.45	83.36	652.19	3.83	1.00	0.19	0.19	1.42

<https://doi.org/10.1371/journal.pone.0255669.t001>

Table 2. Fresh matter and biochemical characteristics of forest litter layer from Colombian Amazon region.

Landscape	Study site	Total Fresh matter (Mg ha)	Biochemical characteristics (fraction < 2 mm)							
			C (%)	N (%)	P (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	C:N	C:P
Hills	SVC	24.70	23.59	1.27	0.13	19.20	15.09	11.81	18.82	185.05
	SLN	22.50	20.59	1.06	0.05	14.89	21.27	11.08	16.65	433.68
	CTG	21.50	26.01	1.42	0.10	18.80	18.34	10.49	18.22	266.79
Mountain	BLA_site 1	23.15	23.96	1.20	0.06	24.09	20.7	12.57	19.99	416.81
	BLA_site 2	30.40	24.16	1.23	0.09	9.86	16.21	11.96	19.60	259.10
	BLA_site 3	31.91	26.55	1.29	0.12	10.84	15.22	11.56	20.50	225.96

<https://doi.org/10.1371/journal.pone.0255669.t002>

topsoil layer, we collected ten soil cores (5-cm diameter x 10-cm depth) in 0–10 cm soil layer in each sampling plot. For that aim, all litter material was removed. Then all the individual samples were mixed to conform one composite sample per plot, which was placed in a sterile plastic bag, sealed, and transported (<24 h) under refrigeration (4°C) to the Biogeochemical Process Laboratory at the University of the Amazon (Florencia-Caqueta, Colombia).

In order to study the potential enzymatic activity of the litter layer, we also collected litter samples in the forest sites. For that, four quadrants of 25 x 25 cm were positioned around each soil sampling point, and all the litter inside it removed, weighed, and mixed into a composite sample, which was transported under refrigeration (4°C) to the laboratory. Subsequently, the litter samples at field-moist conditions were passed through a 2-mm sieve and all litter fraction lower than 2 mm was submitted to further analysis. No specific permits were required to collect soil and litter samples from the field locations, which were in non-protected land. The biochemical characterization of litter samples is presented in [Table 2](#).

Soil biochemical attributes such as phosphorus (P), nitrogen (N), and soil organic carbon (SOC) contents were quantified. For that aim, a portion of soil samples collected was air-dried and sieved to 2 mm. Then, soil P content was measured by extraction with the Bray II method [23] and determined the P-molybdate blue color on a visible spectrophotometer at 660 nm. For soil organic C and N quantification, samples were ground to a fine powder and sieved to 150 µm before determination by dry combustion [24] using a CN 802 carbon nitrogen elemental analyzer (furnace at 1000°C in pure oxygen).

The absolute activity of five extracellular hydrolytic enzymes involving P, N and C-mineralizing processes in soil and forest litter samples was assayed using standard fluorometric microplate methods [25]. The activity of acid phosphatase (P mineralization), β-1,4-N-acetylglucosaminidase (chitin degradation), β-1,4-glucosidase (sugar degradation), β-D-cellobiohydrolase (cellulose degradation), β-D-xylosidase (hemicellulose degradation) was examined as described by [25].

Briefly, soil or forest litter slurries were prepared in a sodium acetate buffer with a pH closely with the soil pH. Then, 800 µl of soil slurry was pipetted into 96-well deep plates. Separate plates were prepared for 4-methylumbelliferone standard curves for each sample. A dose of 200 µl of appropriate standards and substrates were added to the soil slurries. Samples and standards were incubated for 3 h at room temperature. Then supernatants were pipetted into black 96-well plates and the fluorescence was measured at 365 nm excitation wavelength and 450 nm emission wavelength in the VarioSkan Lux multimode microplate reader. For minimizing "well-to-well variation" [25], three assay replicates in each plate were prepared. The absolute activities of each type of enzyme were expressed in units of nmol of product per gram of oven-dry weight soil/litter per hour.

For each soil land use, the geometric mean of soil enzyme activities (*GMea*) was calculated as:

$$GMea = \sqrt[5]{P + N + C \text{ cycling enzymes}}$$

Where P denotes acid phosphatase, N corresponds to β -1,4-N-acetylglucosaminidase and C cycling enzymes denote the activity of β -1,4-glucosidase + β -D-cellobiohydrolase + β -D-xylosidase, respectively [26, 27].

Since absolute enzyme activity gives an estimate of the rate at which the product of activity is made available in the soil, we further calculated the specific enzyme activities ($\mu\text{mol mg C}^{-1} \text{ h}^{-1}$) by dividing the absolute enzyme activity ($\text{nmol g}^{-1} \text{ soil h}^{-1}$) by the soil total C (mg kg^{-1}). The specific enzyme activity allows reliable comparison of soil with different land-use types and can give an insight into the nutritional status of the organic matter regarding soil microorganisms [5, 7]. Microbial C:N, C:P and N:P acquisition (E_{CN} , E_{CP} and E_{NP} , respectively) were calculated as:

$$E_{CN} = \ln C \text{ cycling enzymes} / \ln N \text{ cycling enzyme}$$

$$E_{CP} = \ln C \text{ cycling enzymes} / \ln P \text{ cycling enzyme}$$

$$E_{NP} = \ln N \text{ cycling enzyme} / \ln P \text{ cycling enzyme}$$

Where *C cycling enzymes* denote the absolute activity of β -1,4-glucosidase + β -D-cellobiohydrolase + β -D-xylosidase, *N cycling enzyme* corresponds to the absolute activity of β -1,4-N-acetylglucosaminidase and *P cycling enzyme* denotes the absolute activity of acid phosphatase, respectively.

Statistical analyses

Changes in the variables assessed between forest litter and soil, as well as between land uses (forest and pastures), were analyzed using the Student's Test ($p < 0.05$ and $p < 0.01$). In the mountain landscape, the effect of land use on the different variables was studied by adjusting a linear mixed effect model (lmer), which considered the land-use as a fixed factor and plots as random factors. The assumptions of normality and homogeneity of variance were evaluated using an exploratory residual analysis. When significant, the means of the variables were compared according to Tukey's test ($p < 0.05$). All analyses were conducted in statistical software R version 4.0.3 [28], using integrated development environment RStudio version 1.3.1. [29].

Results

Comparison of SOC, N and P content in the soil layer of different land use practices

Land use change from forest to pasture induced a decline in soil C and N content in the hills landscape at SVC and CTG sites (Table 3). In mountain landscape, the transition zones within forest, pasture and natural regeneration did not significantly alter SOC and N content at BLA_site 2 and site 3. Whereas significantly higher SOC and N content were observed in pasture and natural regeneration sites than forest sites at the BLA_site 1. Soil C:N ratio did not significantly differ among land use systems in both types of landscapes.

In general, low soil P contents were observed in all sites evaluated. The P content did not change due to pasture establishment in CTC and SVC sites in hills landscape as either in BLA_site 2 and site 3 of the mountain landscape. Subtle differences in P content between forest and pasture were only detected in SLN site and BLA_site 1 mountain landscape.

Table 3. Soil C, N, C:N ratio, and P in forest, pasture, and natural regeneration in the Colombian Amazon region.

Landscape	Land use	San Vicente del Caguán (SVC)			
		C (g kg ⁻¹)	N (g kg ⁻¹)	C:N	P (mg kg ⁻¹)
Hills	Forest	24.76 (2.01) a*	2.76 (0.23) a	9.03 (0.41) a	1.24 (0.15) a
	Pasture	12.05 (2.71) b	1.46 (0.17) b	8.72 (1.76) a	1.25 (0.11) a
		Cartagena del Chairá (CTG)			
	Forest	30.50 (1.63) a	3.12 (0.13) a	9.82 (0.52) a	2.24 (0.27) a
	Pasture	21.38 (2.32) b	2.31 (0.23) b	9.22 (0.15) a	1.65 (0.26) a
		Solano (SLN)			
	Forest	26.30 (1.94) a	2.53 (0.15) a	10.36 (0.15) a	3.25 (0.58) b
	Pasture	21.74 (0.99) a	2.17 (0.13) a	10.09 (0.45) a	5.51 (0.36) a
Mountain		Belén de los Andaquíes (BLA_site 1)			
	Forest	13.95 (1.57) b	1.30 (0.18) b	10.98 (0.38) a	8.93 (0.83) a
	Pasture	29.75 (1.28) a	2.80 (0.10) a	10.61 (0.15) a	3.17 (0.74) b
	Natural Regeneration	25.96 (3.07) a	2.88+0.32 a	8.98 (0.15) b	7.31 (0.81) a
		Belén de los Andaquíes (BLA_site 2)			
	Forest	35.08 (3.89) a	3.31 (0.33) a	10.59 (0.43) a	5.03 (0.99) ab
	Pasture	37.02 (3.75) a	3.56 (0.42) a	10.53 (0.31) a	3.17 (0.69) b
	Natural Regeneration	36.50 (2.36) a	4.44 (0.59) a	9.44 (0.90) a	8.36 (1.58) a
		Belén de los Andaquíes (BLA_site 3)			
	Forest	29.81 (2.47) a	3.27 (0.32) a	9.22 (0.42) a	4.71 (0.96) a
	Pasture	27.59 (2.87) a	2.67 (0.22) a	10.24 (0.37) a	3.26 (0.27) a
	Natural Regeneration	30.32 (3.37) a	2.90 (0.20) a	10.33 (0.50) a	2.90 (0.46) a

*Means and standard errors (in parenthesis) followed by the same lowercase letter in each study site did not differ among themselves according to the Student's Test in hills landscape and Tukey's HSD test and mountain landscape ($p < 0.05$).

<https://doi.org/10.1371/journal.pone.0255669.t003>

Absolute enzyme activities in litter and soil layer of the tropical forest

The absolute activity of all five enzymes assessed was higher in the litter than in the topsoil (Figs 2 and 3). The most substantial changes were observed for β -1,4-glucosidase, β -1,4-N-acetylglucosaminidase, exhibiting, for some cases, an activity more than 10 times higher in the litter than in the soil. The activities of β -D-cellobiohydrolase and β -D-xylosidase were higher in the litter than in soil in CTG and SLN in hills landscape sites (Fig 2) and in BLA_site 1 and BLA_site 2 in mountain landscape sites (Fig 3).

Influence of land use change on the absolute activity of soil enzymes

In the hills landscape, the transition from forest to pasture reduced the absolute activity of C, N, and P cycling enzymes, with values, in average, 20% lower in pasture than in forest for β -1,4-glucosidase, β -D-cellobiohydrolase, and β -D-xylosidase, and 33% and 51%, respectively, for β -1,4-N-acetylglucosaminidase and acid phosphatase (Fig 4). Similar trends were noted for enzyme activity based on the *GMea* index (Fig 4).

In the mountain landscape, an increase in the absolute activity of soil C and N cycling enzymes was noted in pasture soil compared to forest soil at BLA_site 1 and BLA_site 2. Abandonment of pastures and regeneration practices caused a decrease in the potential activity of those enzymes in BLA_site 2 (Fig 5). In BLA_site 3, significant differences were detected in β -D-xylosidase and acid phosphatase, with forest showing higher values than pastures.

The *GMea* index indicated an increase in soil enzymatic activity in pasture soil compared to forest soil at BLA_site 1 and BLA_site 2. For the BLA_site 3 no differences were observed among land use types (Fig 5).

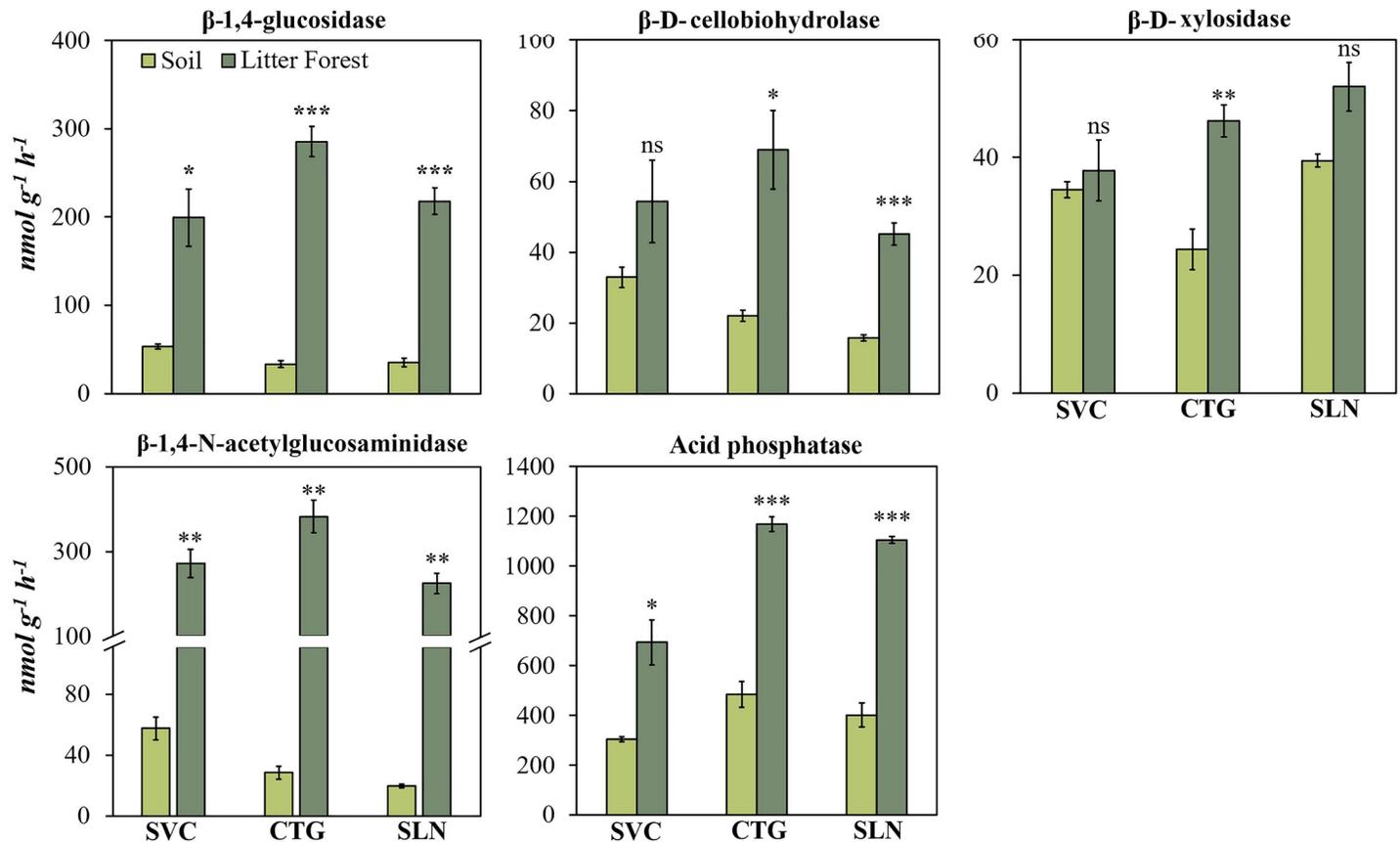


Fig 2. β -1,4-glucosidase, β -D-cellobiohydrolase, β -D-xylosidase, β -1,4-N-acetylglucosaminidase, acid phosphatase enzymes activity in soil and litter of the forest located in hill landscape sites (SVC: San Vicente del Caguan, CTG: Cartagena del Chaira, SLN: Solano) of Colombian Amazon region. Error bars denote standard error. Asterisks indicate that the means values differ significantly between themselves to the level $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) for each study site, according to the Student's Test.

<https://doi.org/10.1371/journal.pone.0255669.g002>

Influence of land use change on soil enzymatic activities weighted by soil C content

Soil enzyme activity normalized to per unit of SOC to reveal a relative comparison between the land use type. Under this approach, no changes were observed between forest and pasture soil in hills landscape for the activity of soil enzymes associated with C and N cycling (Fig 6). On the other hand, differences in enzymatic activity of acid phosphatase ($p < 0.05$) were verified in all the study areas, indicating a potential decrease (average ~34%) of that enzyme activity with the land use change.

In the mountain landscape, land use change did not alter the activity of β -1,4-glucosidase, β -D-xylosidase, and β -1,4-N-acetylglucosaminidase at BLA_site 1 and BLA_site 3. However, there were significant differences at BLA_site 2, with higher values found in pasture soil than those found in forest and natural regeneration.

Changes in the activity of β -D-cellobiohydrolase and acid phosphatase were detected in BLA_site 1 and BLA_site 2, with a higher specific activity of β -D-cellobiohydrolase in pasture than forest, while higher acid phosphatase activity was observed in forest than pastures (Fig 7).

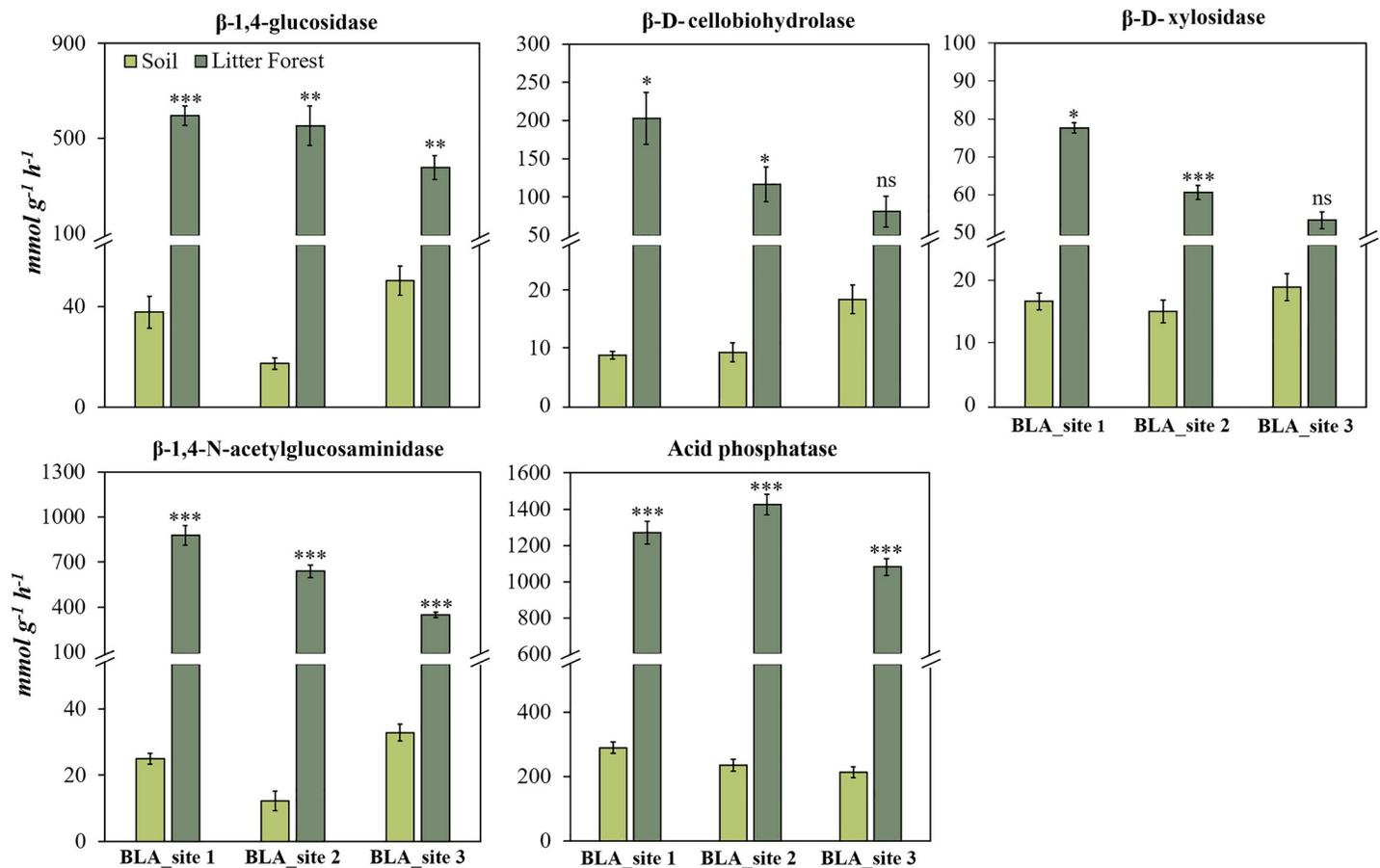


Fig 3. β-1,4-glucosidase, β-D-cellobiohydrolase, β-D-xylosidase, β-1,4-N-acetylglucosaminidase, acid phosphatase enzymes activities in soil and litter of forest mountain landscape sites over an Andes-Amazon transition in Colombian Amazon region. Error bars denote standard error. Asterisks indicate that the means values of soil and litter differ significantly between themselves to the level $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) for each study site, according to the Student's Test.

<https://doi.org/10.1371/journal.pone.0255669.g003>

Stoichiometry enzymes activity

Stoichiometric analysis (relative quantity) of C, N and P enzymes indicated differences in ratios between the soil and litter layer of the forest from all sites (Table 4). Overall, ECN ratios were higher than one in both layers, and lower in the litter layer compared to soil layer. Opposite trends were detected for ECP and ENP ratios, which were generally lower in soil layer compared to litter. Moreover, both ratios were mostly lower than one in all study sites indicating a higher abundance of P-enzymes compared to C and N enzymes (Table 4).

The transition from forest to pasture and later natural regeneration of pastures did not cause changes in the E_{CN} -ratios. However, land use change impacts were observed in the E_{CP} and E_{NP} -ratios, as both ratios were generally highest in pasture systems than those in forest (Table 5).

Discussion

Potential enzyme activities in soil and litter of the tropical forest

Enzymes are essential components within the litter layer of forest ecosystems for catalyzing litter decomposition and nutrient cycling. Our results confirmed higher abundance and activity of C, N and P cycling enzymes in the litter layer and soil, as previously reported in the literature [30, 31]. The microbial production of extracellular enzymes generally is associated with

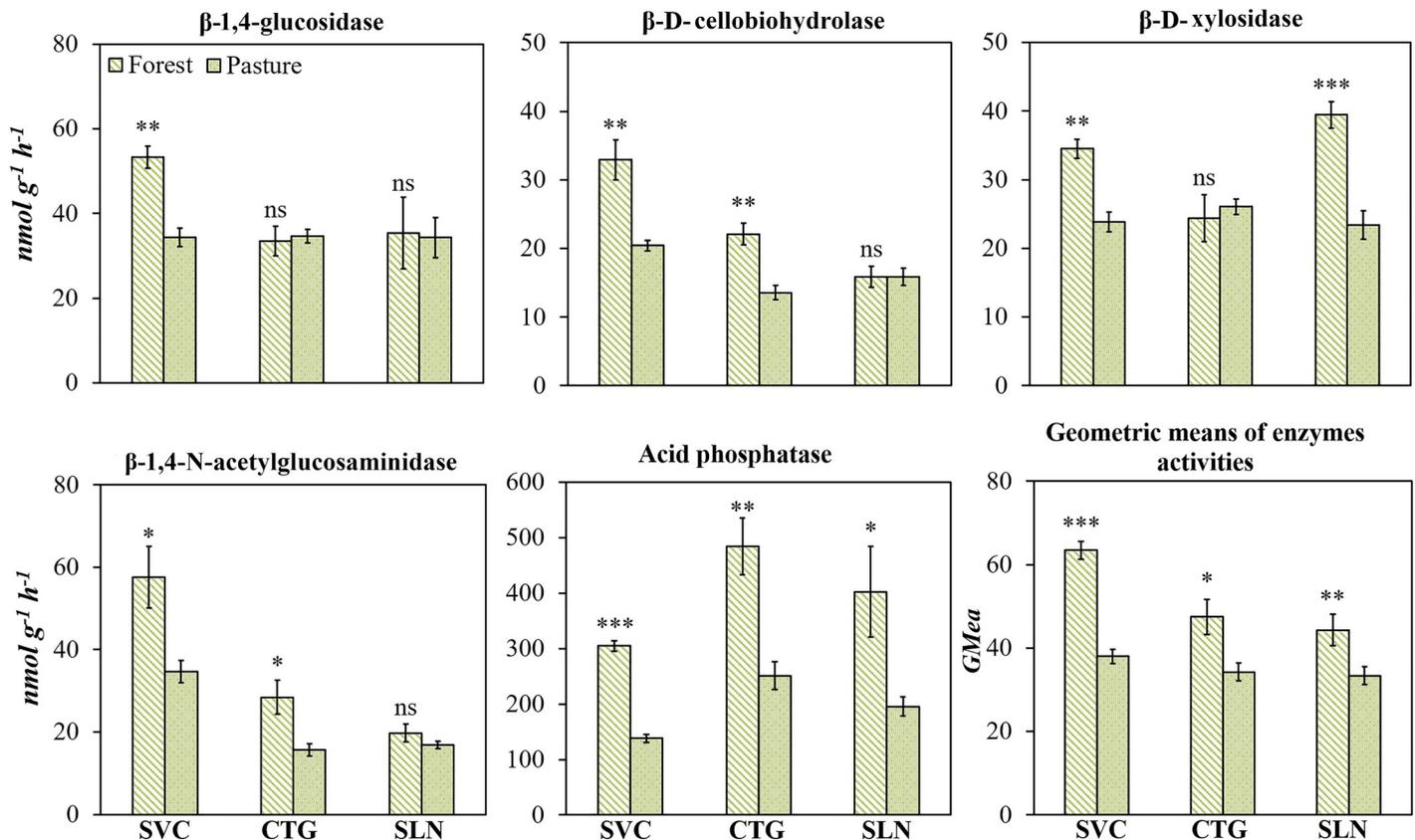


Fig 4. β -1,4-glucosidase, β -D-cellobiohydrolase, β -D-xylosidase, β -1,4-N-acetylglucosaminidase, acid phosphatase enzymes activities in the soil of forest and pastures located in hills landscape (SVC: San Vicente del Caguan, CTG: Cartagena del Chaira, SLN: Solano) of Colombian Amazon region; as well as the index of geometric mean enzyme activity. Error bars denote standard error. The asterisk indicates that the means values of soil and litter differ significantly between themselves to the level $p < 0.05$.

<https://doi.org/10.1371/journal.pone.0255669.g004>

organic C supply and nutrient needs [32], and is primarily stimulated by the plant litter as substrate for microbial growth and activity [33–35].

Several organic compounds of litter may trigger the higher microbial production of enzymes compared to soil. According to the substrate stimulation model, the enzyme activity could be stimulated by the presence of the substrate that it degrades [2]. In this sense, C-degrading enzymes may have been stimulated in the litter when its substrates, e.g., glucosides, disaccharides and cellobioses, increased due to the degradation of cellulose and hemicellulose (Table 2). Furthermore, the glucose, a non-substrate but simple compounds of litter that is readily available and assimilated by microbes, may also cause microbial production of β -1,4-glucosidase [2] by stimulating the microbial growth, which leads to a demand for labile C and hence the production of C-acquiring enzymes [32].

Alternatively, to the substrate stimulation model, soil microbes synthesize many enzymes to facilitate the acquisition of most limiting nutrients in the environment [2]. This could explain the high activity of acid phosphatase and the β -1,4-N-acetylglucosaminidase activity observed in the litter layer at our study sites. An increase in the production of acid phosphatase enzyme was expected since the C:P ratio of the litter was higher than the critical value of 186 (Table 2), above which the conditions are considered to be P-limited for microbial growth and thus, soil microbes generally produce additional P-acquiring enzymes to mineralize organic matter [36].

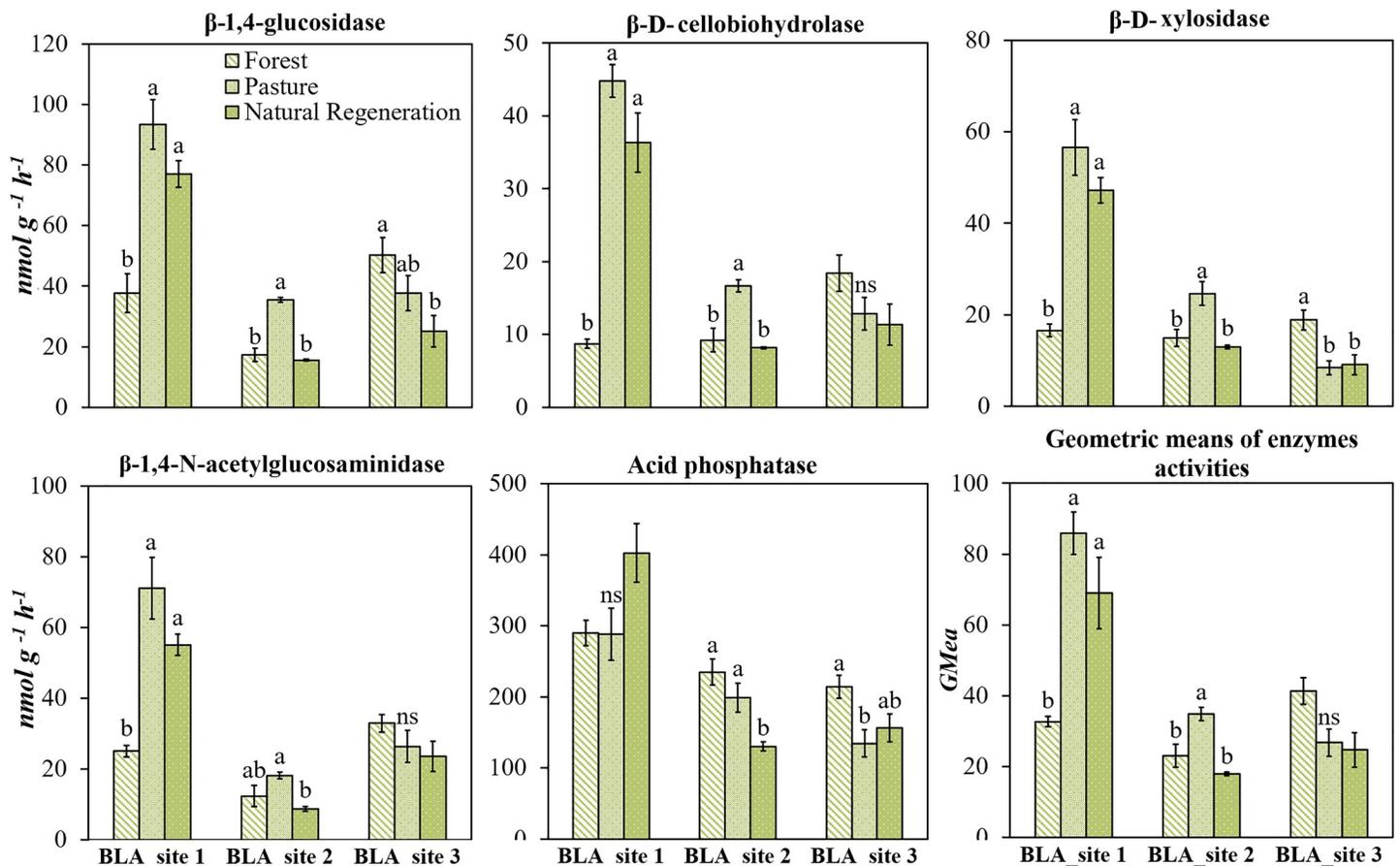


Fig 5. β -1,4-glucosidase, β -D-cellobiohydrolase, β -D-xylosidase, β -1,4-N-acetylglucosaminidase and acid phosphatase enzymes activities, and *GMea* in the soil of forest, pasture and natural regeneration in mountain landscape sites over an Andes-Amazon transition in Colombian Amazon region. Error bars denote standard error. Means followed by the same letters, above the bars, did not differ significantly according to Tukey's HSD test ($p < 0.05$), using "lmer" model.

<https://doi.org/10.1371/journal.pone.0255669.g005>

Regarding the N cycling enzyme, the lower enzymatic acquisition ratios C:N in litter than in soil suggests that in litter the microbial processes were probably limited by N, and thus microbial communities allocated more resources to produce N-acquiring enzymes rather than C.

Overall, the results reported here reflect the importance of litter layer in SOM cycling in tropical rainforest, highlighting its relevance to C sequestration and as a source of nutrients, mainly P and N, to the soil biota and plants. This compartment is usually burned during land preparation for establishing pasture, with long-term implications to restore soil health in those low-fertility soils.

Changes in SOC, N, and P content and enzyme activities due to transition from forest to pastures

Long-term land use change from forest to pasture within hill landscape led to soil C and N depletion, whereas no significant changes were detected in the mountain landscape.

Those variable soil C and N responses to land transition from forest to pasture corroborate previous studies in the Amazon region [14, 16, 37–41]. In hill landscape the pasturelands are under long-term continuous grazing and poor pasture/soil management practices resulting in

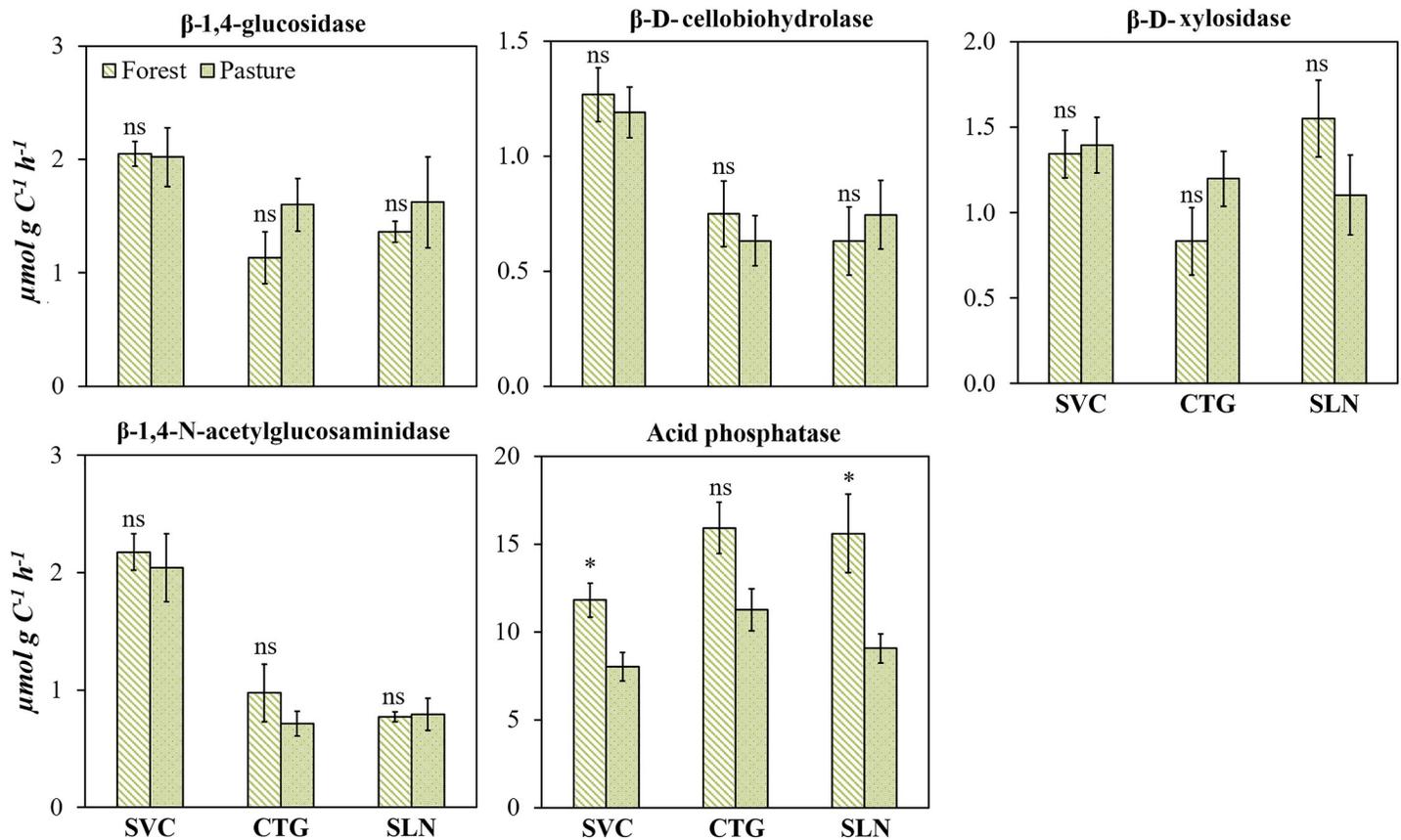


Fig 6. Specific activity per unit of SOC of soil enzymes β -Glucosidase, β -D-Cellobiosidase, β -D-Xylosidase, N-acetyl- β -D-Glucosaminidase, acid phosphatase in pristine forest and pastures located in hills landscape (SVC: San Vicente del Caguan, CTG: Cartagena del Chaira, SLN: Solano) of Colombian Amazon region. Error bars denote error deviation. The asterisk indicates that the means values of soil and litter differ significantly between themselves ($p < 0.05$) for each study site, according to the Student's Test.

<https://doi.org/10.1371/journal.pone.0255669.g006>

reduced C inputs to soil and soil health degradation as previously reported in the Colombian Amazon region [14].

In contrast in less-deep soils such as those studied in the mountain landscape, the shorter period under pasture use and management characterized by a long resting time of grasses have contributed to maintain soil C and N content at similar levels to those observed in native vegetation. Grasses of the genus *Brachiaria* present the ability to add a higher amount of C because of the activity of its root system [42–44]. Thus, site specific land management practices are important factors for impacting SOC storage and must be understood clearly to minimize the losses.

Higher C and N values found in the pasture and natural regeneration areas in BLA_site 1 are likely associated with higher clay content compared to forest soil (Table 1) and not due to land use change. A positive relationship between soil clay and soil C has been pointed out by several studies [45–47].

Regarding P, our study revealed that soil P is limiting in those tropical soils. Overall, low plant-available P contents were also reported by Fernandes *et al.* [48], Soltangheisi *et al.* [49] and Olaya-Montes *et al.* [14] in Amazon soils. Highly-weathered tropical soils, rich in Fe and Al oxides in clay fraction, can adsorb large amounts of P as insoluble phosphate [50, 51], limiting the availability of inorganic P for plants [48].

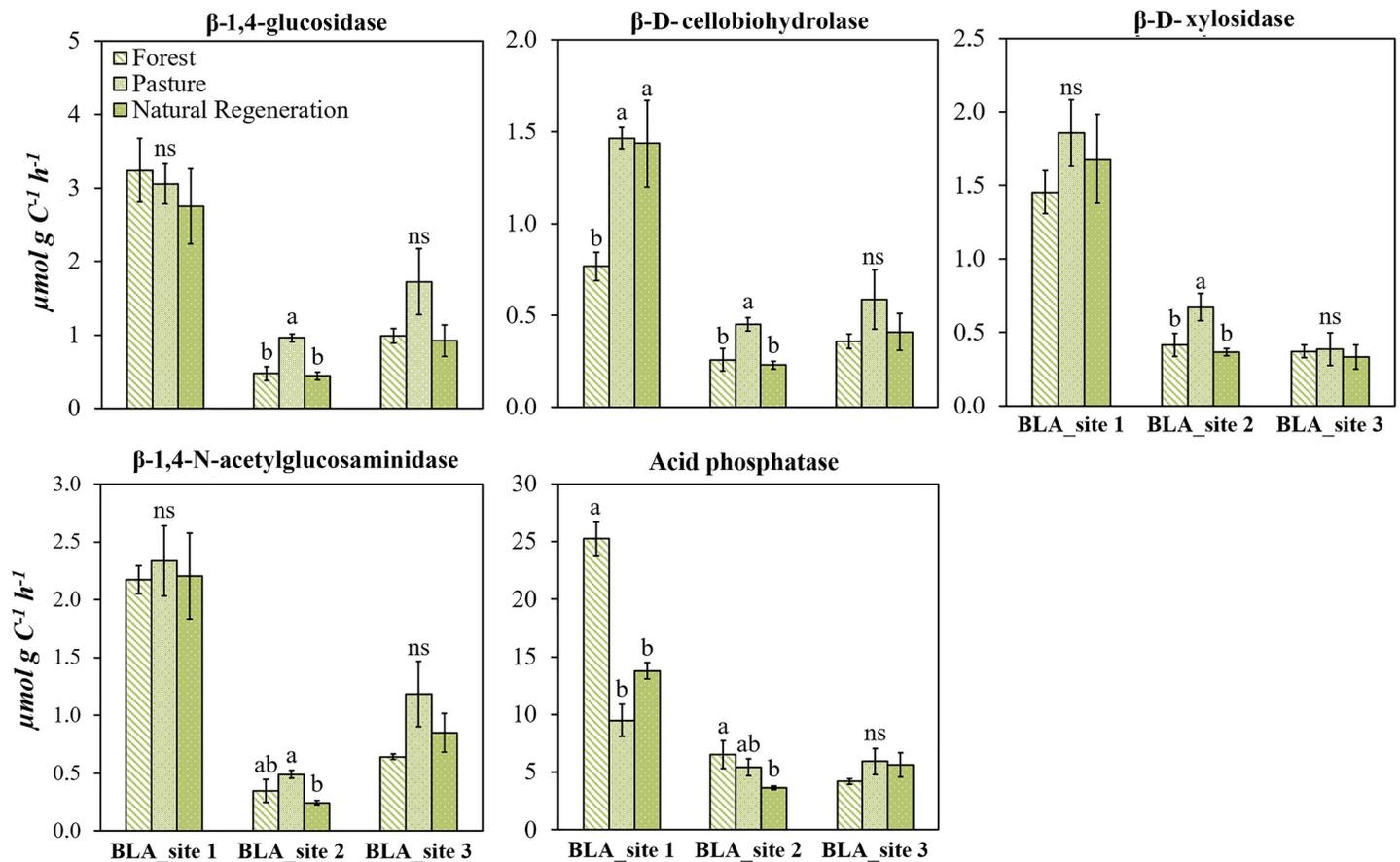


Fig 7. Specific activity per unit of SOC of soil enzymes β-Glucosidase, β-Celobiosidase, β-Xylosidase, N-acetyl-β-Glucosaminidase, and acid phosphatase in the soil of the forest, pasture, and natural regeneration in mountain landscape sites over an Andes-Amazon transition in Colombian Amazon region. Error bars denote error deviation. Means followed by the same lowercase letter in each study site did not differ among themselves according to Tukey's HSD test ($p < 0.05$).

<https://doi.org/10.1371/journal.pone.0255669.g007>

Those changes in soil C, N and P induced by the land transition from Amazon forest to pastures seem to be the main driver of soil enzyme activity alterations. It was also reported in tropical forests in Ecuador and Costa Rica by Tischer *et al.* [52] and Cleveland *et al.* [53], respectively. The range of absolute enzyme activity values found in this study are similar to those reported in Brazil and Panama [11, 54–57] but they were lower than those observed in

Table 4. Stoichiometry enzymes activity in forest litter and soil in Colombian Amazon region.

Landscape	Study site	E _{CN}		E _{CP}		E _{NP}	
		Litter forest	Soil	Litter forest	Soil	Litter forest	Soil
Hill	San Vicente del Caguan	1.03 (0.03) b*	1.19 (0.04) a	0.87 (0.02) a	0.84 (0.01) a	0.86 (0.01) a	0.70 (0.02) b
	Cartagena del Chairá	1.01 (0.01) b	1.33 (0.03) a	0.85 (0.01) a	0.68 (0.03) b	0.84 (0.01) a	0.54 (0.03) b
	Solano	1.08 (0.02) b	1.51 (0.02) a	0.82 (0.01) a	0.75 (0.02) b	0.77 (0.02) a	0.50 (0.01) b
Mountain	BLA_site 1	1.00 (0.004) b	1.29 (0.02) a	0.95 (0.02) a	0.73 (0.01) b	0.95 (0.02) a	0.57 (0.01) b
	BLA_site 2	1.02(0.03) b	1.55 (0.10) a	0.90 (0.02) a	0.68 (0.02) b	0.89 (0.01) a	0.44 (0.04) b
	BLA_site 3	1.06 (0.03) b	1.28 (0.04) a	0.89 (0.02) a	0.83 (0.01) a	0.84 (0.002) a	0.65 (0.01) b

*Means and standard error (SE) followed by the same letter did not differ among themselves according to the T-Student's test ($p < 0.05$).

<https://doi.org/10.1371/journal.pone.0255669.t004>

Table 5. Stoichiometry enzymes activity in soil under forest and pastures in Colombian Amazon region.

Landscape	Land use	Enzymes		
		C:N	C:P	N:P
Hills		San Vicente del Caguán (SVC)		
	Forest	1.19 (0.04) a*	0.84 (0.01) b	0.70 (0,02) a
	Pasture	1.23 (0.01) a	0.89 (0.01) a	0.72 (0.02) a
		Solano (SLN)		
	Forest	1.50 (0.01) a	0.75 (0.02) b	0.50 (0.01) b
	Pasture	1.52 (0.01) a	0.81 (0.01) a	0.54 (0.01) a
		Cartagena del Chairá (CTG)		
	Forest	1.33 (0.03) b	0.68 (0.03) b	0.51 (0.03) a
	Pasture	1.58 (0.04) a	0.78 (0.01) a	0.50 (0.01) a
	Mountain		Belén de los Andaquíes (BLA_site 1)	
Forest		1.28 (0.02) a	0.73 (0.01) c	0.57 (0.01) c
Pasture		1.24 (0.03) a	0.93 (0.01) a	0.75 (0.02) a
Nat. Regeneration		1.24 (0.03) a	0.84 (0.02) b	0.68 (0.02) b
		Belén de los Andaquíes (BLA_site 2)		
Forest		1.55 (0.10) a	0.68 (0.02) b	0.44 (0.04) b
Pasture		1.47 (0.03) a	0.80 (0.02) a	0.55 (0.01) a
Nat. Regeneration		1.68 (0.06) a	0.74 (0.01) ab	0.44 (0.01) b
		Belén de los Andaquíes (BLA_site 3)		
Forest		1.35 (0.05) a	0.81 (0.01) a	0.60 (0.01) a
Pasture		1.39 (0.07) a	0.81 (0.03) a	0.58 (0.03) a
Nat. Regeneration		1.30 (0.04) a	0.74 (0.03) a	0.57 (0.03) a

*Means and standard errors (SE) followed by the same letter in each study site did not differ among themselves according to the Student's Test in hills landscape and Tukey's HSD test in mountain landscape ($p < 0.05$).

<https://doi.org/10.1371/journal.pone.0255669.t005>

Puerto Rico and Ecuador [52, 58–60], revealing some regional variations even within tropical rainforests.

Overall, the activity of the enzymes studied followed the order acid phosphatase > β -1,4-N-acetylglucosaminidase = β -1,4-glucosidase = β -D-xylosidase = β -D-cellobiohydrolase. The highest acid phosphatase activity levels observed in all the study sites might be a response of plant and microbial communities to the severe P deficiency in these areas (Table 3), as also reported by Soltangheisi *et al.* [49]. Under limited nutrients conditions, the production of enzymes is stimulated by plant roots and microorganisms; therefore, phosphatase could likely be exuded in order to enhance the release of inorganic P from organic P compounds [61], demonstrating in this way the relevance of this enzyme in the mineralization of organic P in tropical Amazon region, as well as the role of soil organic matter in the provision of P for plant growth.

High P degrading enzyme activities have also been reported in both temperate [5, 62] and tropical ecosystems [4, 63, 64]. On the other hand, since chitin is considered the inducer of the activity of β -1,4-N-acetylglucosaminidase, the alterations in the activity of that enzyme can be related to differences in the content of this component (chitin) between soil from forest and pasture areas, more specifically to alterations in macrofauna and fungal biomass, in which chitin is a major structural component [65, 66]. Although we did not measure those variables, previous studies have indicated a reduction in microbial biomass and macrofauna as a response to extensive pastures in tropical regions [67–70].

Compared to global surveys, values of stoichiometry enzymatic activity C:P and N:P lower than 1 observed in all studied soils indicated greater investment toward P acquisition relative to C and N acquisition (Table 4), ratifying the primary microbial P limitation exposed before [36, 71]. Since enzymes control the turnover of soil organic matter by degrading organic molecules to assimilate C, N and P [72], subsequent consequences of organic P-mineralization could lead to further decline of SOC and N [73]. We believe this to be the case in the pastures studied here with chronic soil-P infertility. Thus, pasture management must consider increasing crop diversity to enhance mycorrhizal interactions, which are capable of acquiring inorganic soil-P and contribute to meet the P needs of plants.

Ratios of C:N acquiring enzymes were greater than one, which indicated that microbes allocated more resources to produce C-acquiring enzymes rather than β -1,4-N-acetylglucosaminidase. Although those results could suggest that a C-limiting condition is present in all the areas, preliminary studies pointed out that enzymes degrading SOM to provide energy (C) versus nutrients (N and P) may be regulated by resource availability [1], with C-degrading enzymes being stimulated by elevated concentrations of their substrates and end-products [2]. Furthermore, since some microbes can use labile C to decompose recalcitrant organic matter to obtain N (N-mining theory) the microbial N-acquisition can be alleviated by C-acquisition [74], which is consistent with a soil C:N ratio in all sites below the threshold of 14.3 [75].

As with individual enzyme activity per unit of soil mass, the *GMea* index was altered with land use change (Figs 4 and 5) in all the study sites, proving to be a suitable indicator for estimating the soil microbial community response due to pasture establishment. To decouple the changes in soil enzyme activities from SOC changes, we calculated weighted enzymatic activity based on SOC base. In contrast to the absolute enzyme activities, although land use transition from forest to pastures decreases the SOC and those enzyme activities when considered individually in hills landscape, enzyme activities as a proportion of SOC are maintained. Therefore, after a long-term pasture establishment, the rate of reduction in SOC is greater than enzyme activity, thereby maintaining high metabolic activity likely promoted by N and P limitations.

This pattern was also found in mountain landscape, with increases in the activity of β -1,4-glucosidase, β -D-cellobiohydrolase and β -D-xylosidase in pastures of BLA_site 2, which could also provide insight into how suitable is the SOM for degradation by C and N cycling enzymes in pasture areas, thus providing a measure of organic matter quality [71]. Both the turnover of roots and root exudation are important sources of SOM in pastures, supplying a variety of labile carbon compounds to soil [76]. Experiments studying the effect of litter and root plants on soil enzyme activity have reported an increase in C and N-degrading enzyme concentrations when root growth is allowed due to active transport of labile carbon from roots to associated microbial communities [77, 78].

On the other hand, the phosphatase activity per unit of carbon followed the same trend observed in absolute activity, with the soil C losses in the hills landscape being accompanied by a decrease in the abundance of P-acquiring enzyme per unit of C. Moreover, non-changes in SOC in mountain landscape were associated with the absence of alterations in the specific activity of acid phosphatase. This could be explained by the decrease of soil N due to pasture establishment, which may have a determinant role in regulating acid phosphatase activity according to the results obtained by [79], who analyzed measurements of phosphatase in natural soils around the world. Overall, the development of a soil microbial community in pastures with enhanced catabolic activity and greater biomass turnover could have led to the synthesis of C and N cycling enzymes comparable to the forest and natural regeneration sites. Our results also support the hypothesis that enhanced soil organic matter quality, specifically the presence of substrates, may trigger microbial production of studied enzymes related to C, N and P cycles.

Conclusions

The litter layer plays a key role in the nutrient budget of the Amazon rainforest. By harboring a considerable pool of extracellular enzymes responsible for regulating its decomposition, this compartment becomes an essential component for C sequestration and the biogeochemical cycling of elements in that ecosystem. With the land transition from forest to pastures, the litter layer is lost. Then, the direction and magnitude of changes in soil enzymatic activities depended largely on the management of pastures, with SOC and N losses and reduced absolute activity of soil enzymes induced by long-term pastures under continuous grazing (25 years). Values of stoichiometry enzymatic activity indicated a primary microbial P limitation in the study areas, which could lead to an increased use of soil C by microbial metabolism in the search for P causing a further decline of SOC and N in pastures.

With the land use transition from forest to pastures a soil microbial community with high catabolic activity per C unit is developed, which could affect SOM cycling with implications in soil health and the provision of soil-related ecosystem services.

Overall, this study provides the first regional data on soil enzymatic activity in tropical forests and pastures of the Colombian Amazon region, providing new insights to a better understanding of land use change effects on the soil biological activity and the dynamic of biogeochemical cycles.

Acknowledgments

The authors thank the farmers for allowing us to research in their lands.

Author Contributions

Conceptualization: Adriana M. Silva-Olaya, Maurício R. Cherubin, Anil Somenahally, Fausto A. Ortiz-Morea.

Data curation: Daniel Grados.

Formal analysis: Adriana M. Silva-Olaya, Daniel Grados.

Funding acquisition: Adriana M. Silva-Olaya.

Investigation: Adriana M. Silva-Olaya, Dúber A. Mora-Motta.

Methodology: Adriana M. Silva-Olaya, Dúber A. Mora-Motta, Fausto A. Ortiz-Morea.

Project administration: Adriana M. Silva-Olaya.

Software: Daniel Grados.

Supervision: Adriana M. Silva-Olaya, Fausto A. Ortiz-Morea.

Writing – original draft: Adriana M. Silva-Olaya, Dúber A. Mora-Motta, Fausto A. Ortiz-Morea.

Writing – review & editing: Maurício R. Cherubin, Anil Somenahally, Fausto A. Ortiz-Morea.

References

1. Weintraub SR, Wieder WR, Cleveland CC, Townsend AR. Organic matter inputs shift soil enzyme activity and allocation patterns in a wet tropical forest. *Biogeochemistry*. 2013 Jul 1; 114(1–3):313–26.
2. Hernández DL, Hobbie SE. The effects of substrate composition, quantity, and diversity on microbial activity. *Plant Soil*. 2010 May 21; 335(1):397–411.

3. Shi W. Agricultural and Ecological Significance of Soil Enzymes: Soil Carbon Sequestration and Nutrient Cycling. In Springer, Berlin, Heidelberg; 2010. p. 43–60.
4. Acosta-Martínez V, Cruz L, Sotomayor-Ramírez D, Pérez-Alegría L. Enzyme activities as affected by soil properties and land use in a tropical watershed. *Appl Soil Ecol.* 2007 Jan 1; 35(1):35–45.
5. Trasar-Cepeda C, Leirós MC, Gil-Sotres F. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. *Soil Biol Biochem.* 2008 Sep 1; 40(9):2146–55.
6. Chaer G, Fernandes M, Myrold D, Bottomley P. Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. *Microb Ecol.* 2009 Jul 31; 58(2):414–24. <https://doi.org/10.1007/s00248-009-9508-x> PMID: 19330551
7. Wang B, Bin Liu G, Xue S, Zhu B. Changes in soil physico-chemical and microbiological properties during natural succession on abandoned farmland in the Loess Plateau. *Environ Earth Sci.* 2011 Mar 1; 62(5):915–25.
8. Raiesi F, Riahi M. The influence of grazing enclosure on soil C stocks and dynamics, and ecological indicators in upland arid and semi-arid rangelands. *Ecol Indic.* 2014 Jun 1; 41:145–54.
9. Dinesh R, Ghoshal Chaudhuri S, Sheeja TE. Soil biochemical and microbial indices in wet tropical forests: Effects of deforestation and cultivation. *J Plant Nutr Soil Sci.* 2004 Feb 1; 167(1):24–32.
10. An S, Zheng F, Zhang F, Van Pelt S, Hamer U, Makeschin F. Soil quality degradation processes along a deforestation chronosequence in the Ziwuling area, China. *Catena.* 2008 Nov 15; 75(3):248–56.
11. Sarto MVM, Borges WLB, Sarto JRW, Pires CAB, Rice CW, Rosolem CA. Soil microbial community and activity in a tropical integrated crop-livestock system. *Appl Soil Ecol.* 2020 Jan 1; 145:103350.
12. Melo VF, Orrutéa AG, Motta ACV, Testoni SA. Land use and changes in soil morphology and physical-chemical properties in southern Amazon. *Rev Bras Cienc do Solo.* 2017; 41:170034.
13. Nóbrega RLB, Guzha AC, Torres GN, Kovacs K, Lamparter G, Amorim RSS, et al. Effects of conversion of native cerrado vegetation to pasture on soil hydro-physical properties, evapotranspiration and streamflow on the Amazonian agricultural frontier. *PLoS One.* 2017 Jun 1; 12(6):e0179414. <https://doi.org/10.1371/journal.pone.0179414> PMID: 28609462
14. Olaya-Montes A, Llanos-Cabrera MP, Cherubin MR, Herrera-Valencia W, Ortiz-Morea FA, Silva-Olaya AM. Restoring soil carbon and chemical properties through silvopastoral adoption in the Colombian Amazon region. *L Degrad Dev.* 2020 Dec;ldr.3832.
15. Polanía-Hincapié KL, Olaya-Montes A, Cherubin MR, Herrera-Valencia W, Ortiz-Morea FA, Silva-Olaya AM. Soil physical quality responses to silvopastoral implementation in Colombian Amazon. *Geoderma.* 2021 Mar 15; 386:114900.
16. Navarrete D, Sitch S, Aragão LEOC, Pedroni L. Conversion from forests to pastures in the Colombian Amazon leads to contrasting soil carbon dynamics depending on land management practices. *Glob Chang Biol.* 2016 Oct 1; 22(10):3503–17. <https://doi.org/10.1111/gcb.13266> PMID: 26929394
17. Maharning AR, Mills AAS, Adl SM. Soil community changes during secondary succession to naturalized grasslands. Vol. 41, *Applied Soil Ecology.* Elsevier; 2009. p. 137–47.
18. Pedrinho A, Mendes LW, Merloti LF, De Cassia Da Fonseca M, De Souza Cannavan F, Tsai SM. Forest-to-pasture conversion and recovery based on assessment of microbial communities in Eastern Amazon rainforest. *FEMS Microbiol Ecol.* 2019 Mar 1; 95(3):236.
19. Pedrinho A, Mendes LW, Merloti LF, Andreote FD, Tsai SM. The natural recovery of soil microbial community and nitrogen functions after pasture abandonment in the Amazon region. *FEMS Microbiol Ecol.* 2020 Sep 1; 96(9):149. <https://doi.org/10.1093/femsec/fiaa149> PMID: 32710756
20. Finer M, Mamani N. MAAP Synthesis: 2019 Amazon Deforestation Trends and Hotspots. MAAP Synth [Internet]. 2020;4. Available from: <https://www.amazonconservation.org/tag/synthesis/>
21. Murad CA, Pearse J. Landsat study of deforestation in the Amazon region of Colombia: Departments of Caquetá and Putumayo. *Remote Sens Appl Soc Environ.* 2018 Aug 1; 11:161–71.
22. IGAC IGAC. Estudio General de Suelos y Zonificación de tierras: Departamento de Caquetá, Escala 1:100.000. Bogotá: Imprenta Nacional de Colombia; 2014. 412 p.
23. Bray RH, Kurtz LT. Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 1945; 59(1):39–45.
24. Nelson DW, Sommers LE. Total Carbon, Organic Carbon, and Organic Matter. In John Wiley & Sons, Ltd; 1996. p. 961–1010.
25. Bell CW, Fricks BE, Rocca JD, Steinweg JM, McMahon SK, Wallenstein MD. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *J Vis Exp.* 2013;(81):50961. <https://doi.org/10.3791/50961> PMID: 24299913

26. Hinojosa MB, García-Ruíz R, Viñegla B, Carreira JA. Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. In: *Soil Biology and Biochemistry*. Pergamon; 2004. p. 1637–44.
27. Paz-Ferreiro J, Gascó G, Gutiérrez B, Méndez A. Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. *Biol Fertil Soils*. 2012 Jul 21; 48(5):511–7.
28. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria; 2020. Available from: <http://www.r-project.org/>
29. RStudio Team. RStudio: Integrated Development Environment for R [Internet]. Boston, MA; 2021. Available from: <http://www.rstudio.com/>
30. Ma Y, Zhu B, Sun Z, Zhao C, Yang Y, Piao S. The effects of simulated nitrogen deposition on extracellular enzyme activities of litter and soil among different-aged stands of larch. *J Plant Ecol*. 2014 Jun 1; 7(3):240–9.
31. Ge X, Xiao W, Zeng L, Huang Z, Zhou B, Schaub M, et al. Relationships between soil–litter interface enzyme activities and decomposition in *Pinus massoniana* plantations in China. *J Soils Sediments*. 2017 Apr 1; 17(4):996–1008.
32. Tian L, Shi W. Short-term effects of plant litter on the dynamics, amount, and stoichiometry of soil enzyme activity in agroecosystems. *Eur J Soil Biol*. 2014 Aug 22; 65:23–9.
33. Pan F, Zhang W, Liang Y, Liu S, Wang K. Increased associated effects of topography and litter and soil nutrients on soil enzyme activities and microbial biomass along vegetation successions in karst ecosystem, southwestern China. *Environ Sci Pollut Res*. 2018 Jun 1; 25(17):16979–90. <https://doi.org/10.1007/s11356-018-1673-3> PMID: 29627959
34. Wang W, Page-Dumroese D, Lv R, Xiao C, Li G, Liu Y. Soil Enzyme Activities in *Pinus tabuliformis* (Carrière) Plantations in Northern China. *Forests*. 2016 May 26; 7(12):112.
35. Waring BG. Exploring relationships between enzyme activities and leaf litter decomposition in a wet tropical forest. *Soil Biol Biochem*. 2013 Sep 1; 64:89–95.
36. Sinsabaugh RL, Hill BH, Follstad Shah JJ. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*. 2009 Dec 10; 462(7274):795–8. <https://doi.org/10.1038/nature08632> PMID: 20010687
37. Fearnside PM, Barbosa RI. Soil carbon changes from conversion of forest to pasture in Brazilian Amazonia. *For Ecol Manage*. 1998 Aug 20; 108(1–2):147–66.
38. Desjardins T, Barros E, Sarrazin M, Girardin C, Mariotti A. Effects of forest conversion to pasture on soil carbon content and dynamics in Brazilian Amazonia. *Agric Ecosyst Environ*. 2004 Jul 1; 103(2):365–73.
39. Fujisaki K, Perrin A-S, Desjardins T, Bernoux M, Balbino LC, Brossard M. From forest to cropland and pasture systems: a critical review of soil organic carbon stocks changes in Amazonia. *Glob Chang Biol*. 2015 Jul 1; 21(7):2773–86. <https://doi.org/10.1111/gcb.12906> PMID: 25726833
40. Houghton RA, Skole DL, Nobre CA, Hackler JL, Lawrence KT, Chomentowski WH. Annual fluxes of carbon from deforestation and regrowth in the Brazilian Amazon. *Nature*. 2000 Jan 20; 403(6767):301–4. <https://doi.org/10.1038/35002062> PMID: 10659847
41. Durigan M, Cherubin M, de Camargo P, Ferreira J, Berenguer E, Gardner T, et al. Soil Organic Matter Responses to Anthropogenic Forest Disturbance and Land Use Change in the Eastern Brazilian Amazon. *Sustainability*. 2017 Mar 7; 9(3):379.
42. Baptistella JLC, de Andrade SAL, Favarin JL, Mazzafera P. *Urochloa* in Tropical Agroecosystems. Vol. 4, *Frontiers in Sustainable Food Systems*. Frontiers Media S.A.; 2020. p. 119.
43. McSherry ME, Ritchie ME. Effects of grazing on grassland soil carbon: a global review. *Glob Chang Biol*. 2013 May 1; 19(5):1347–57. <https://doi.org/10.1111/gcb.12144> PMID: 23504715
44. Oliveira J de M, Madari BE, Carvalho MT de M, Assis PCR, Silveira ALR, de Leles Lima M, et al. Integrated farming systems for improving soil carbon balance in the southern Amazon of Brazil. *Reg Environ Chang*. 2018 Jan 1; 18(1):105–16.
45. Singh M, Sarkar B, Sarkar S, Churchman J, Bolan N, Mandal S, et al. Stabilization of Soil Organic Carbon as Influenced by Clay Mineralogy. In: *Advances in Agronomy*. Academic Press Inc.; 2018. p. 33–84.
46. Soong JL, Janssens IA, Grau O, Margalef O, Stahl C, Van Langenhove L, et al. Soil properties explain tree growth and mortality, but not biomass, across phosphorus-depleted tropical forests. *Sci Rep*. 2020 Dec 1; 10(1):1–13. <https://doi.org/10.1038/s41598-019-56847-4> PMID: 31913322
47. Zhou W, Han G, Liu M, Zeng J, Liang B, Liu J, et al. Determining the Distribution and Interaction of Soil Organic Carbon, Nitrogen, pH and Texture in Soil Profiles: A Case Study in the Lancangjiang River Basin, Southwest China. *Forests*. 2020 May 10; 11(5):532.

48. Fernandes SAP, Bernoux M, Cerri CC, Feigl BJ, Piccolo MC. Seasonal variation of soil chemical properties and CO₂ and CH₄ fluxes in unfertilized and P-fertilized pastures in an Ultisol of the Brazilian Amazon. *Geoderma*. 2002 Jun 1; 107(3–4):227–41.
49. Soltangheisi A, Withers PJA, Pavinato PS, Cherubin MR, Rossetto R, Do Carmo JB, et al. Improving phosphorus sustainability of sugarcane production in Brazil. *GCB Bioenergy*. 2019 Dec 18; 11(12):1444–55. <https://doi.org/10.1111/gcbb.12650> PMID: 31894185
50. Cherubin MR, Franco ALC, Cerri CEP, Oliveira DM da S, Davies CA, Cerri CC. Sugarcane expansion in Brazilian tropical soils—Effects of land use change on soil chemical attributes. *Agric Ecosyst Environ*. 2015 Dec 5; 211:173–84.
51. Gérard F. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils—A myth revisited. Vol. 262, *Geoderma*. Elsevier; 2016. p. 213–26.
52. Tischer A, Potthast K, Hamer U. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. *Oecologia*. 2014 May 1; 175(1):375–93. <https://doi.org/10.1007/s00442-014-2894-x> PMID: 24532178
53. Cleveland CC, Townsend AR, Schmidt SK, Constance BC. SOIL MICROBIAL DYNAMICS AND BIOGEOCHEMISTRY IN TROPICAL FORESTS AND PASTURES, SOUTHWESTERN COSTA RICA. *Ecol Appl*. 2003 Apr 1; 13(2):314–26.
54. Nottingham AT, Turner BL, Chamberlain PM, Stott AW, Tanner EVJ. Priming and microbial nutrient limitation in lowland tropical forest soils of contrasting fertility. *Biogeochemistry*. 2012 Nov 1; 111(1–3):219–37.
55. Sarto MVM, Borges WLB, Bassegio D, Pires CAB, Rice CW, Rosolem CA. Soil microbial community, enzyme activity, C and N stocks and soil aggregation as affected by land use and soil depth in a tropical climate region of Brazil. *Arch Microbiol*. 2020 Dec 1; 202(10):2809–24. <https://doi.org/10.1007/s00203-020-01996-8> PMID: 32747999
56. Steinweg JM, Jagadamma S, Frerichs J, Mayes MA. Activation Energy of Extracellular Enzymes in Soils from Different Biomes. Mormile MR, editor. *PLoS One*. 2013 Mar 25; 8(3):e59943. <https://doi.org/10.1371/journal.pone.0059943> PMID: 23536898
57. Turner BL. Variation in pH optima of hydrolytic enzyme activities in tropical rain forest soils. *Appl Environ Microbiol*. 2010 Oct 1; 76(19):6485–93. <https://doi.org/10.1128/AEM.00560-10> PMID: 20709838
58. Cusack DF, Torn MS, McDowell W, Silver WL. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Glob Chang Biol*. 2010 Jan 1; 16(9):2555–72.
59. Cusack DF, Torn MS, McDowell WH, Silver WL. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Glob Chang Biol*. 2012 Jan 1; 18(1):400–400.
60. Tischer A, Blagodatskaya E, Hamer U. Extracellular enzyme activities in a tropical mountain rainforest region of southern Ecuador affected by low soil P status and land-use change. *Appl Soil Ecol*. 2014 Feb 1; 74:1–11.
61. Sinsabaugh RL, Antibus RK, Linkins AE, McClaugherty CA, Rayburn L, Repert D, et al. Wood Decomposition: Nitrogen and Phosphorus Dynamics in Relation to Extracellular Enzyme Activity. *Ecology*. 1993 Jul 1; 74(5):1586–93.
62. Saviozzi A, Levi-Minzi R, Cardelli R, Riffaldi R. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. *Plant Soil*. 2001; 233(2):251–9.
63. Smith AP, Marín-Spiotta E, Balsler T. Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: a multiyear study. *Glob Chang Biol*. 2015 Sep 1; 21(9):3532–47. <https://doi.org/10.1111/gcb.12947> PMID: 25873563
64. Stone MM, DeForest JL, Plante AF. Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biol Biochem*. 2014 Aug 1; 75:237–47.
65. Griffiths B. *Soil Microbiology and Biochemistry*, Second Edition. By E. A. Paul and F. E. Clark, San Diego: Academic Press (1996), pp. 340, £29.50. ISBN 0-12-546806. *Exp Agric*. 1997 Jul; 33(3):385–7.
66. Olander LP, Vitousek PM. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*. 2000; 49(2):175–90.
67. De Barros JA, De Medeiros EV, Da Costa DP, Duda GP, De Sousa Lima JR, Dos Santos UJ, et al. Human disturbance affects enzyme activity, microbial biomass and organic carbon in tropical dry sub-humid pasture and forest soils. *Arch Agron Soil Sci*. 2020 Mar 20; 66(4):458–72.
68. Franco ALC, Sobral BW, Silva ALC, Wall DH. Amazonian deforestation and soil biodiversity. *Conserv Biol*. 2019 Jun 4; 33(3):590–600. <https://doi.org/10.1111/cobi.13234> PMID: 30306643

69. Marichal R, Grimaldi M, Feijoo MA, Oszwald J, Praxedes C, Ruiz Cobo DH, et al. Soil macroinvertebrate communities and ecosystem services in deforested landscapes of Amazonia. *Appl Soil Ecol*. 2014 Nov 1; 83:177–85.
70. Melo VS, Desjardins T, Silva ML Jr, Santos ER, Sarrazin M, Santos MMLS. Consequences of forest conversion to pasture and fallow on soil microbial biomass and activity in the eastern Amazon. *Soil Use Manag*. 2012 Dec 1; 28(4):530–5.
71. Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, et al. Stoichiometry of soil enzyme activity at global scale. *Ecol Lett*. 2008 Nov 1; 11(11):1252–64. <https://doi.org/10.1111/j.1461-0248.2008.01245.x> PMID: 18823393
72. Waring BG, Weintraub SR, Sinsabaugh RL. Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. *Biogeochemistry*. 2014 Jan 18; 117(1):101–13.
73. Joan R, José Manuel BM, Xavier SF. Phosphorus mobilization in low-P arable soils may involve soil organic C depletion. *Soil Biol Biochem*. 2017 Oct 1; 113:250–9.
74. Craine JM, Morrow C, Fierer N. MICROBIAL NITROGEN LIMITATION INCREASES DECOMPOSITION. *Ecology*. 2007 Aug 1; 88(8):2105–13. <https://doi.org/10.1890/06-1847.1> PMID: 17824441
75. Cleveland CC, Liptzin D. C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry*. 2007 Sep; 85(3):235–52.
76. Smith FA. Plant roots. Growth, activity and interaction with soils. *Ann Bot*. 2007 Mar 13; 100(1):151–2.
77. Averill C, Finzi A. Plant regulation of microbial enzyme production in situ. *Soil Biol Biochem*. 2011 Dec 1; 43(12):2457–60.
78. Kotroczó Z, Veres Z, Fekete I, Krakomperger Z, Tóth JA, Lajtha K, et al. Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biol Biochem*. 2014 Mar 1; 70:237–43.
79. Margalef O, Sardans J, Fernández-Martínez M, Molowny-Horas R, Janssens IA, Ciais P, et al. Global patterns of phosphatase activity in natural soils. *Sci Rep*. 2017 Dec 1; 7(1):1–13. <https://doi.org/10.1038/s41598-016-0028-x> PMID: 28127051