Extracellular enzyme activities in tropical soils are driven by seasonal litter input

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Abstract

Background

It is relatively unknown if and how seasonal fluctuations of tropical microbial activity affect soil nutrient availability. In tropical forests, nutrient economics are often considered to be centered around phosphorus, which might be a limiting factor to sustain crucial ecosystem processes, such as primary production and decomposition of organic material, thus in turn affecting microbial processes and associated nutrient dynamics of the forest ecosystem.

Aims

We investigate seasonal fluctuations in extracellular hydrolytic soil enzyme activities and soil nutrients and its relationship with precipitation and litterfall input, in a lowland tropical forest in the Central Amazon region.

Methods

We analyzed data obtained from monitoring microbial enzyme activity and nutrient dynamics in litter and soil and use stoichiometric enzyme theory and proportional vectors for assessing relative nutrient limitation throughout a year.

Results

Our results show that precipitation seasonality was driving leaf litterfall, which was subsequently synchronized with extracellular enzyme activities in soil, such that both litterfall and enzyme activities peaked during the dry season.

Conclusions

Our study indicates that soil extractable nutrient concentrations were positively related to microbial enzyme activities, which thus highlights the importance of soil microbial processes for nutrient cycling in this phosphorus limited ecosystem. Our results suggest that projected shifts in climate seasonality that result in longer and more pronounced dry seasons, might desynchronize seasonal patterns of aboveground nutrient input and belowground microbial activity, and thus leading to a decoupling of nutrient cycling in tropical forest ecosystems.

1. Introduction

Primary productivity in terrestrial ecosystems is usually limited by nitrogen (N) or phosphorus (P) (Vitousek et al. 2010). In tropical forests, plant productivity is generally considered to be limited by P because of tropical soils' often highly weathered status and relatively old age (Walker and Syers 1976; Vitousek 1984; Cleveland et al. 2011; Du et al. 2020; Hou et al. 2020). Consequently, soil P is one of the strongest predictors of tropical forest productivity (Quesada et al. 2012). Microbial activity is considered a key process with a crucial role in ecosystem nutrient cycling (Cavicchioli et al. 2019). Microbes play an important role in coupled cycles of carbon (C), N, and P as both consumers and suppliers of available forms of nutrients, the latter of which facilitates plant production. Microbial foraging for nutrients is takes place largely through the secretion of extracellular enzymes (EE). Heterotrophic soil microbes are dependent on a supply of organic substrate from plants as their main energy (C) source (Soong et al. 2020), with N and P also playing a crucial role in sustaining microbial processes, with P-limitation as prevalent characteristic in tropical microbial communities (Camenzind et al. 2018). Vice versa, plant acquisition of N and P is dependent on the (largely microbial) EE, which convert complex organic substrate to digestible products by breaking down larger polymers into smaller compounds which can then be readily taken up by all kinds of organisms (Skujiņš and Burns 1976; Baldrian 2009; Burns et al. 2013; Luo et al. 2017).

Due to their significance, EE are often considered the rate limiting step in organic matter decomposition (Sinsabaugh and Follstad Shah 2012), and thus an important determinant of C and nutrient cycle potential in soil. Microbes, and plants to a lesser extent, secrete a range of EE (Skujiņš and Burns 1976; Burns 1982), but commonly studied soil hydrolytic enzymes act specifically on C, N, or P cycles (German et al. 2011). Roots and mycorrhizae are also contributing to the excretion of EE for P-acquisition (García-Garrido et al. 2002; Lugli et al. 2020). Evidence on the relative contribution of root-phosphatase is unconclusive; some indicate it only constitutes a small contribution to soil phosphatase (Cabugao et al. 2021), while others maintain that the relative contribution of roots on phosphatase is more substantial in litter decomposition (Martins et al. 2021). Phosphatases are linked to the availability of N in soils, since enzymes are proteins requiring C and N for their production (Allen et al. 2020), linking different nutrient cycles. Dijkstra et al (2013) argued that priming of the microbial biomass in a P-limited system might show different dynamics than N-limited systems. In P limited systems, P might be obtained by desorption and inorganic P-acquisition over organic sources of the nutrient. In contrast, N-limited systems might lean more to acquisition of nutrients from organic sources by priming. Moreover, temporal environmental fluctuations make this process even more complex, limiting different factors at different moments in time (Condit et al. 2013).

The timing of these limitations is increasingly relevant under global change scenarios. If climate and seasonality change, the nutritional stresses on the ecosystem might increase due to a de-synchronization of previously connected fluxes of nutrients, their transformations, their uptake, and subsequent losses from the ecosystem. Over the course of a year, however, nutrients such as P proceed through different forms and levels of availability (Cusack et al. 2019; Schaap et al. 2021). Leaf litterfall has been shown to follow a seasonal pattern in some tropical forests (Sanches et al. 2008; Wu et al. 2016; Restrepo-Coupe et al. 2016), arguably leading to changes in decomposition (Wieder and Wright 1995), soil microbial

community structure and function (Eaton et al. 2011; Buscardo et al. 2018; Pajares et al. 2018), soil nutrients (Yamashita et al. 2010), and fine root growth (Cordeiro et al. 2020). Synchronization of nutrient pulses takes place at different trophic levels and has a time lag between pulse and response (Yang et al. 2010). However, there is evidence that soil microbes react to litterfall asynchronously, preceding the litter input, indicating microbial regulation for nutrient competition in the wet tropics (Ruan et al. 2004), arguably because the temperature and humidity are less of a constraining factor to microbial nutrient cycling than in colder or drier ecosystems.

Temporal relationships among organic matter inputs, environmental conditions and EE expression are important for understanding soil nutrient dynamics. Seasonality in both nutrient uptake and mineralization might have implications for how limitations are dealt with by (micro)organisms and ultimately, how those limitations affect the forest nutrition on larger timescales. Most data of tropical soils and their nutritional balance comes from one-time surveys or single measurements, ignoring possible intra and inter-annual variation, hindering insight in the seasonal nutrient dynamics of the forest. In this study we investigate the dynamics of extracellular enzymes associated to C, N and P cycles in a tropical forest soil. We treat them as a proxy for soil microbial activity and indicator for (microbial) nutrient demand. We studied the EE over the course of a seasonal cycle, to dissect effects of rainfall seasonality versus substrate inputs in the form of litterfall. We hypothesized: 1) the system to be P limited, in our case reflected in relatively high enzymatic investments for P-acquisition 2) the enzymatic acquisition of mainly C, but also N should be relatively constant, 3) nutrients entering the soil through litter inputs, as opposed to variation in temperature and precipitation, to be to be the main driver for the enzymatic investments for P-acquisition, 4) soil nutrient status to be of limited effect on EE, since enzymatic expression is more indicative of demand than supply, and 5) enzyme vectors (calculated according to Moorhead et al. 2016) to be indicative of changes in nutrient investments through the year.

2. Methods

2.1 Site description and sampling strategy

The study was carried out at the AmazonFACE experimental site (2°35'40"S, 60°12'29"W) in Central Amazonia (more info on https://amazonface.inpa.gov.br/), approximately 70 km north of Manaus, Brazil, in the “Cuieiras” experimental reserve (Estação Experimental de Silvicultura Tropical – EEST, see also Pereira et al. 2019), which is also the base for the LBA-K34 tower and several experimental observation stations. Characteristic for the area are old-growth tropical forests locally known as “Terra Firme” forests, situated on plateaus with nutrient poor and clay rich soils (>70% clay) classified as Geric Ferralsols (Quesada et al. 2010). Average annual rainfall is about 2,400 mm, with a relatively drier period from June to October, while the average temperature fluctuates from 25.8°C in April to 27.9°C in September (Araújo et al. 2002).

2.2 Sample collection and processing

Soils were sampled from 18 sampling points. On 6 locations along a 400 m north-south transect (every 80m), we sampled 3 points in the east-west direction, with 10m distance between the 3 sampling points. The sampling scheme was adopted to consistently sample soils close to the AmazonFACE plots (for details, see Lapola and Norby, 2014), without disturbing soil within the plots. Soils were sampled in monthly between February 2016 and January 2017, using a custom-made steel soil corer (ø 10 cm). Soils were sampled at 0-5 cm and 5-15 cm depth and transported to the lab for sieving (2 mm), root and detritus removal and further processing. Litterfall was collected biweekly at the AmazonFACE plots. The total litter was dried, separated into leaf litter and other litter fractions and weighed (kg ha^{-1}).

Aliquots were stored after oven drying (48 h at 65°C) until further analysis, while selected measurements were performed in fresh soil within three days of sampling. Total soil P, extractable organic carbon, extractable nitrogen, and microbial biomass were analyzed every three months. Total soil C and N contents were determined monthly in composite samples of three (according to sampling location along the transect). Apart from the total C and N contents, all analyses were performed at the LTSP (Laboratório Temático de Solos e Plantas) laboratory at INPA (Instituto Nacional de Pesquisas da Amazônia) in Manaus, Brazil, nationally certified by Embrapa Soils (2016 Fertility Laboratory Quality Analysis Program, PAQLF, https://www.embrapa.br/en/solos/paqlf) and by the PIATV (Esalq/USP) inter-laboratorial program of vegetation tissue analysis (Grade A, http://piatv.com.br/).

Litterfall was collected biweekly at two of the AmazonFACE plots located along the transect (used in this study) starting in August 2015. Litter traps (0.5 × 0.5 m, n = 24) were installed 1 m above the ground, 12 traps per plot in a circular pattern. The total litter was dried, separated into leaf litter and other litter fractions, and weighed.

2.3 Total C, N and P

Total soil C and N were determined in milled dry aliquots by mass spectrometry (Finnegan Delta Plus) for elemental C and N (Carlo-Erba, 1110). Total P was determined in dry (unmilled) 0.5 g aliquots with the molybdate blue method (Murphy and Riley 1962) after acid digestion using concentrated sulphuric acid solution (H_2SO_4, 18 M), followed by H_2O_2 (Quesada et al. 2010; see also Schaap et al. 2021).

2.4 Extractable C, N and P

Extractable organic carbon (eoC) and extractable nitrogen (eN) were obtained from extracts of 2 grams of fresh soil in 20 ml 1M KCl solution, shaken for one hour and subsequently filtered. The filtered extract was then analyzed in a TOC/TN analyzer (TOC-V CPH E200V⁄TNM-1 220V; Shimadzu, Vienna, Austria). Extractable P (Olsen et al. 1954) was determined from extractants of 2g of soil in 20 ml 0.5M bicarbonate solution ($NaHCO_3$, pH 8.5), shaken for one hour and filtered. Extractant was analyzed following the photometrical Murphy-Riley molybdate blue method (712 nm) (Murphy and Riley 1962). All analyses were accompanied by two method blanks (no soil) to account for contamination or background signal, and possible lab variation was accounted for by analyzing standards during each batch of photometric extract reading.

2.5 Potential soil extracellular enzyme activities

Potential extracellular enzyme activities (EEA) of three common hydrolytic enzymes relevant to C, N and P cycling were assayed using a fluorescence method. We followed a lab protocol based on Marx et al. (2001) and calculations from German et al. (2011). 4-Methylumbelliferyl β-D-glucopyranoside (M3633 Sigma), 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (M2133) and 4-methylumbelliferyl phosphate disodium salt (M8168 Sigma) were used as substrates for β-glucosidase (BG), N-acetyl glucosaminidase (NAG) and acid phosphatase (AP), respectively. All are widely used in soil enzyme assays as they can be considered a proxy for microbial demand of C, N and P. 4-methylumbelliferone standards were used and substrate controls, sample controls and blanks were measured to control any background signal. All enzymes were assayed in soil slurries of 0.5 g of fresh soil dissolved in 50 ml sodium acetate buffer (pH 5.5) and vortexed for one minute before pipetting aliquots in a black microplate (96 well polystyrene, flat bottom). Standard curves were generated in the soil slurry using Methylumbellliferyl as a standard with appropriate standard curves (M1381 Sigma). In addition, we measured substrate controls, sample controls, and blanks to account for potential background signal. Microplates were incubated for 1 hour at 20°C, whereafter fluorescence measurements were performed with an Infinite F200 Pro plate reader (Tecan Austria GMBH, Grödig, Austria), with fluorescence intensity measured from the top ($\lambda_{excitation}$ = 360 and $\lambda_{emission}$ = 440 nm). Enzyme activities were calculated as μmol g C^{-1} day^{-1}.

2.6 Quantitative analyses

Extracellular enzymatic stoichiometry (EES) and vectors were calculated according to Moorhead et al. (2016). Enzyme activity ratios and proportional activities were calculated using the natural logarithm. The enzyme and nutrient ratios for C:N, C:P and N:P were calculated in each sample as with ln transformed ratios (e.g., ln(C:N), ln(BG:NAG), etc.), while proportional ratios were calculated as

$$C{:}N_{proportional} = \ln\frac{BG}{BG + NAG}$$

and

$$C{:}P_{proportional} = \ln\frac{BG}{BG + AP}$$

Vectors were calculated using both of those ratios, their length as

$$Vectorlength = \sqrt{C{:}P_{proportional}{}^{2} + C{:}N_{proportional}{}^{2}}$$

and their angle in degrees as

$$Vectorangle = \tan^{-1}\left(\frac{C{:}N_{proportional}}{C{:}P_{proportional}}\right)$$

Means were calculated according to the stoichiometric mean recommended by Isles (2020) as the mean of each natural logarithm (i.e. mean = $(\ln(ratio_1) + \ln(ratio_2) + \ldots + \ln(ratio_n))/n$), all values are reported ± their standard error. Data processing and statistical tests were performed in R 4.1.2 (R Core Team 2020). Linear regression models were applied to assess relations between variables (lm function, base R). Relations between enzyme activities were performed with ln transformed values.

((Figure 1))

3. Results

Precipitation showed a distinct drier period (at least 40% of days with < 3 mm, Figure 1b) between July and November, during which it was also some degrees warmer, but average temperature varied little and stayed within a 24.5-27.5°C range (on average) during the measurement interval (Figure 1a, Supplementary figure S1a). During those drier months, the leaf litterfall peaked (Figure 2a). Annual leaf litterfall amounted to 5565 ± 55 kg ha^{-1} $year^{-1}$, with a distinct peak during the drier months. Leaf litterfall was not significantly correlated to the average monthly temperature (Supplementary figure S1b) but showed a negative relation with the average rainfall ($F_{(1;9)}$ = 18.9, P = 0.002, Figure 2b) indicating clearly higher leaf litterfall when drier.

((Figure 2))

Average soil C concentration at 0-5 cm was 5.53 ± 0.02%, ranging from 4.19 ± 0.09 in January to 7.28 ± 0.27% in May (Table S1). N concentration was 0.35 ± 0.00% on average, peaking in May with 0.42 ± 0.01% and reaching the lowest concentration in January with 0.29 ± 0.00%. Total P averaged 156.39 ± 0.69 µg g^{-1}, ranging from 141.8 ± 2.13 µg g^{-1} in August to 204.52 ± 3.46 µg g^{-1} in February, with the note that the measurement frequency of total P was lower than for C and N (Table S2). For 5-15 cm, the average total C, N and P contents were 2.84 ± 0.01%, 0.21 ± 0.00%, and 118.22 ± 0.52 µg g^{-1}; C ranged from 2.03 ± 0.03% (January) to 3.58 ± 0.07% (June), N ranged from 0.17 ± 0.00% (January) to 0.26 ± 0.01% (June) and P ranged from 107.86 ± 1.61 µg g^{-1} (May) to 143.24 ± 3.24 µg g^{-1} (February)(Table S1, Table S2). The eoC, eN and Olsen P in the top 5 cm of soil were 1034.06 ± 6.17 µg C g^{-1} dry soil, 101.41 ± 0.34 µg N g^{-1} dry soil and 2.08 ± 0.01 µg P g^{-1} dry soil. In 5-15 cm 10 cm, those values were lower with 916.86 ± 6.41 µg g^{-1} soil, 76.78 ± 0.24 µg g^{-1} and 1.19 ± 0.00 µg g^{-1} dry soil, respectively (Table 1). At both analyzed soil depths, the highest values for eoC were found in May (1806.59 ± 31.33 µg g^{-1} and 1727.64 ± 31.61 µg g^{-1} respectively), while the lowest values were measured in August (594.69 ± 5.98 µg g^{-1} and 455.73 ± 2.80 µg g^{-1} respectively). In contrast, the eN values were lowest in February at both depths (82.1 ± 1.54 µg g^{-1} and 58.24 ± 0.74 µg g^{-1} respectively), while reaching their highest values in August (135.3 ± 1.19 µg g^{-1} and 95.92 ± 0.58 µg g^{-1} respectively. Olsen P peaked in March at both depths, while in the top 5 cm the lowest concentration was found in April (1.16 ± 0.02 µg g^{-1}), and in the lower soil increment the lowest value was reached in January (0.52 ± 0.02 µg g^{-1}).

EE activity on a dry soil basis in the top soil (0-5 cm) amounted to 1.13 ± 0.00 μmol g^{-1} day^{-1} for BG, 4.84 ± 0.02 μmol g^{-1} day^{-1} for NAG and 109.88 ± 0.26 μmol g^{-1} day^{-1} for AP, while in the 5-15 cm soil increment those values dropped to 0.64 ± 0.00 μmol g^{-1} day^{-1}, 1.76 ± 0.00 μmol g^{-1} day^{-1} and 70.34 ± 0.19 μmol g^{-1} day^{-1} respectively (Supplementary Figure S2). Consequently, EE activities as expressed per gram soil C were determined to be 0.21 ± 0.00 μmol g C^{-1} day^{-1} for BG, 0.87 ± 0.00 μmol g C^{-1} day^{-1} for NAG and 20.21 ± 0.04 μmol g C^{-1} day^{-1} for AP, while in 5-15 cm those activities were 0.23 ± 0.00, 0.63 ± 0.00, and 26.26 ± 0.08 μmol g soil C^{-1} day^{-1} for BG, NAG and AP respectively (Figure 3a, c, e). The highest values for the EE activities in the top 5 cm were in the drier season, or just before, with BG peaking in August (0.34 ± 0.02 μmol g C^{-1} day^{-1}), and NAG and AP peaking in September (1.22 ± 0.06 μmol g C^{-1} day^{-1} and 44.61 ± 0.90 μmol g C^{-1} day^{-1} respectively) while the lowest values were measured in the wetter months; in January for BG and NAG (0.12 ± 0.00 μmol g C^{-1} day^{-1} and 0.20 ± 0.01 μmol g C^{-1} day^{-1} respectively), and in June for AP (15.52 ± 0.35 μmol g C^{-1} day^{-1}). This pattern was generally reflected at 5-15 cm, but BG and NAG peaked just before the drier season (in June, 0.31 ± 0.01 μmol g C^{-1} day^{-1} and 1.37 ± 0.04 μmol g C^{-1} day^{-1} respectively) while AP peaked in September (31.91 ± 0.57 μmol g C^{-1} day^{-1}). The lowest EE activities at 5-15 cm depth were all in January (BG 0.13 ± 0.00 μmol g C^{-1} day^{-1}, NAG 0.40 ± 0.01 μmol g C^{-1} day^{-1} and AP 12.80 ± 0.19 μmol g C^{-1} day^{-1}).

((Figure 3))

Although total nutrient contents of the soil did not seem to show a clear seasonal pattern (Table S1, S2), the EE activities were higher in the drier months from July to November. To test whether climatic factors (temperature, moisture) or leaf litter inputs to the soil were related to enzyme activities, we applied linear regression models to assess their relationship. In the top 5 cm the tested relation with BG proved not significant (Figure 3b), whereas the average BG activity at 5-15 cm was significantly related to the litterfall ($F_{(1, 9)}$ = 10.4, p = 0.011, Figure 3b), but not to the precipitation or temperature (Supplementary Figure S3a, b). The NAG activity was not significantly related to any of the three examined factors (Figure 3d, Supplementary Figure S3c, d). In contrast, average AP activity was positively related to leaf litterfall in the 5-15 depth ($F_{(1, 9)}$ = 9.54; p = 0.013), and a positive tendency in the top 5 cm ($F_{(1, 9)}$ = 4.64, p = 0.06; Figure 3f), but negatively to precipitation ($F_{(1, 9)}$ = 10.57, p = 0.014 for the top 5 cm; $F_{(1, 9)}$ = 9.26, p = 0.01 for 5-15 cm; Supplementary Figure S3f) at both studied soil depths.

Soil C:N and the corresponding enzyme activity ratio showed a negative relationship (for 0-5 cm: $F_{(1, 192)}$ = 22.59, p < 0.01; for 5-15 cm: $F_{(1, 192)}$ = 5.09, p = 0.03), while the N:P ratio and corresponding ln(NAG:AP) showed a positive relation ($F_{(1, 56)}$ = 8.15, p < 0.01 for the top 5 cm; $F_{(1, 57)}$ = 8.13, p < 0.01, for the 5-15 cm depth), C:P and associated enzyme activities (ln(BG:AP)) were not significantly related (Figure 4a, c, e). Among the three tested enzymes all relations were positive and significant (p < 0.01, Figure 4b, d, f). The relation of the enzymes to the extractable C, N and P were dominated by the significant relations that eN and the enzyme activities showed, together with the significant relations between the AP activity and the eoC, eN and Olsen P (Figure S4).

((Figure 4))

While the EE activities showed some seasonality when considered separately (Figure 3), their activity ratios and proportional activities show less variation (Table 2). We also calculated vectors of those proportional activities, useful for distinguishing effects in relative nutrient demand. However, the vector lengths and angles of the proportional activities decrease after the drier months with higher litter inputs, indicating a shift from C and P acquisition (lower vector length) towards N acquisition enzymes at the start of the rain season. Moreover, the angles and the lengths of the vectors show a significant positive relationship (Figure 5, $F_{(1, 191)} = 6.55$, $p = 0.01$ for the top 5 cm; $F_{(1, 192)} = 25$, $p < 0.01$ for 5-15 cm, Supplementary figure S4), indicating overall relations in relative nutrient demand; a tendency that links enzyme activities for C and P acquisition if we consider vector angle as a proxy for P over N demand and vector length as an indicator of a relative investment in C acquisition.

((Figure 5))

4. Discussion

4.1 Overview

In this study, we provide insight in the shifts of dynamics of nutrient limitations during the year through enzymes as a proxy for (microbial) nutrient demand. Overall, the enzyme activities point toward a strong P demand; BG and NAG activities were low compared to AP. We provide evidence that the natural litter cycle is synchronized with activities of enzymes used for the acquisition of P. This is also reflected in the enzyme vectors, which showed pre-drier season shift towards relatively more N acquisition, and the shift towards more P-acquisition at the end of the drier season.

4.2 Microbial activity

Our data shows a chronic high investment in AP, suggesting the system to be P limited as hypothesized. Phosphorus, when it has limited bioavailability in soil, must be cycled tightly, warranting both enzymatic production when available P is low and strong linkage between the enzyme activities and litter inputs to the system (Schaap et al. 2021). Our EE data is in the same range as compared to enzyme activities measured along an altitude gradient in the Andes, albeit lower than on lower altitudes reported (Nottingham et al. 2016); Tischer et al. (2014) also reported similar values, although in neither study seasonality was included. In our study the soil extracellular enzyme stoichiometry and vectors were strongly influenced by the high activities of AP in the system and the relatively low values of both BG and NAG.

BG activity was low in general, and while all enzyme activities seemed to dip at the end of the drier period, the NAG activity (catalyzing the depolymerization of C and N rich compounds, Luo et al. 2017) seemed to be more affected than the others. To evaluate the second hypothesis about the constant enzymatic investment in C and N acquisition by microbes, we reported activities of BG and NAG. Usually enzymes

show seasonal variation (Baldrian et al. 2013; Zuccarini et al. 2020; Bai et al. 2021), but in the referred studies annual temperature variations were larger. In the current study, the microbial investment in BG and NAG is relatively small compared to AP, but there is still an observable seasonality contrary to our second hypothesis. The positive relationship between the BG activity and litter inputs in the top 5 cm of soil indicates a synchronization between new substrate and the investments of microbes. While substrate is available, enzymatic expression increases, possibly to fulfill microbial energy needs which could be connected to the demand for P.

4.3 Leaf litter and its relation to EE

We hypothesized that litter inputs would be related to P acquisition, measured by enzyme activity. Litterfall indeed is a strong predictor of AP activity, although precipitation plays a role as well. This relation is contrasted by the lack of coupling between litter and NAG, which is illustrative of the differences between nutrient cycles and the relative limitation of the two. NAG principally catalyzes the breakdown of chitin present in for example cell walls, and a relatively higher activity could also indicate higher microbial turnover (Zeglin et al. 2013). Others also encountered difficulty relating the NAG activity to environmental factors and soil nutrients in a tropical context (Waring et al. 2014). The positive relation between BG activity in the 5-15 cm depth and the new inputs of substrate could signal an increase in microbial energetic needs while producing AP - a notion that is also supported by the pre-drier season increase in BG and NAG activities. Since temperature and precipitation were not significantly related to most enzymes and nutrients, the soil microbial activity and nutrient cycling seem to be mainly driven by inputs of substrate. In a study with differing litter input treatments in a different tropical forest (Weintraub et al. 2013), inputs were found to exert strong controls over the enzyme activities, but enzyme activities were also related to soil nutrient status.

The leaf litterfall seems to be comparable to earlier studies in the area (Lucas et al. 1993; Luizão et al. 2004; Wu et al. 2016), with slightly lower annual litter production. Possibly, the lower observed litterfall was a consequence of relatively higher litterfall in the preceding year, which had an El Niño event. Litterfall and forest productivity in general has been shown earlier to be strongly determined by precipitation and El Niño events (Hilker et al. 2014; Hofhansl et al. 2014); this is also in line with the relation between litter and precipitation we report. In other tropical forests, seasonality in litterfall has also been described, often attributed to a drier or a wetter season (Chave et al. 2010; Sayer and Tanner 2010; Parsons et al. 2014). Temperature can also drive leaf litterfall in other tropical systems (Kitayama et al. 2020), but at our site this relation was not as strong as the relation between precipitation and litter. Aboveground phenology and litterfall is well established to be seasonal in the tropics (Chave et al. 2010; Wu et al. 2017), and evidence is emerging that this is reflected in soil microbial communities (Buscardo et al. 2018).

4.4 Substrate availability and enzyme activity

Our study suggests that the soil extractable nutrient status might not always be related to EE's in the soil, partially confirming our fourth hypothesis. However, significant relations of eN with the enzyme activities

suggests an importance of a supply of available N for microbial enzyme production, while the significant negative relation between AP and Olsen P signals a higher demand when the nutrient is in short supply. The observed negative relation between the enzyme C:N stoichiometry and the soil C:N ratio, together with the lack of a relation between NAG and litterfall, is indicative of a relative decoupling of nutrient availability and demand. Other studies point to the soil nutrient status as an important source of differences between EE activities (e.g., Olander and Vitousek 2000), which might still hold up if seasonality in the system is more visible in the quicker cycles, and to a lesser extent in more recalcitrant nutrient fractions. It is likely that our study shows a relative status quo where a) microbes (with possible contributions of AP root exudation) seem to be synchronized with seasonality by producing enzymes when the litter falls, b) available nutrients (measured here as eoC, eN and Olsen P) in the mineral soil are taken up quickly (hours-days) by plants and microbes, therefore not clearly related to their corresponding enzyme (with an exception for eN) and c) the soil nutrients that are less available (represented partially by the total soil nutrient contents) follow a different pattern. However, if nutrients are cycling through the system at a rate that was not completely captured by our measurements; the enzyme activities might hold important cues about the timing of nutrient demand.

4.5 Enzyme vectors and timing of microbial demand

EE vector analysis, as conceptualized by Moorhead (2016), is increasingly used to distinguish between relative demand of C, N and P acquiring enzymes. Although we do not use the exact same set of enzymes, we uncovered patterns that can be related to the phenology of soil microbial biomass and activity, related to the last hypothesis. Transforming the enzyme data to vectors of proportional activities indicated an increasing relative N-demand in the months leading up to the drier season (lower vector angle) but saw this demand dropping during the drier season. During the drier season the P-enzymes were relatively more abundant (higher vector angle). Once leaf litter reaches the soil, there are different pathways to the inclusion in the soil as soil organic matter (SOM), where the labile components are released first, and particulate recalcitrant matter is incorporated in later stages (Cotrufo et al. 2013; Cotrufo et al. 2015). This time lag is a possible explanation for the vectors to show a relative trend towards more P-acquisition towards the end of the drier season; P-loss from litter is not immediate (Martins et al. 2021) and this might cause this delay in enzymatic response (Schaap et al. 2021). In both the drier and the wetter season, the vector angles are indicating a rather strong indication of P-limitation (Moorhead et al. 2016), especially at the lower depth. This is in line with a meta-analysis on enzymatic stoichiometry in the tropics (Waring et al. 2014). The vector lengths, indicating a relative shift towards C-acquisition (Moorhead et al. 2016), showed a less conclusive pattern, with a notable increase after the drier season in the 5-15 cm depth. Apart from the demand, this could also indicate higher availability of C compounds (e.g., cellulose, hemicellulose) available from litter.

Conclusion

The aim of this study was to investigate seasonal soil nutrient dynamics through the interaction between litterfall, soil nutrients and EE. This study showed how leaf litterfall is connected to biochemical changes in the soil EE activity and stoichiometry. In line with our expectations the soil nutrients at the site are

hinting towards a limitation of P, which was further corroborated by a relatively high AP activity compared to NAG and BG, indicating that the soils microbial community indeed had a high P demand suggesting chronic P limitation. Both BG and AP activities at the 5-15 cm depth were related to the leaf litter inputs, displaying a synchronization between nutrient inputs to the soil system and nutrient acquisition by EE, which arguably points towards substrate availability as a driver for those EE activities, rather than (microbial) demand. The weaker relations found between enzymes and litter in the topsoil would suggest microbial demand is a more important driver there. Vectors of proportional enzyme activities uncovered a relative increase of P demand towards the end of the drier period and seem to indicate an increase of relative N-demand in the months leading up to the drier season.

The study contributes to our understanding of the complex interactions in the dynamic tropical soil system. The study has observations from a single year, and future studies should address inter-annual variation in nutrient fluxes and the activity of the microbial biomass. Moreover, the study takes place in a single study area within the vastness of the tropical forest, warranting replication of the study in areas with different edaphic conditions and forest structure. Generally, greater efforts to understand effects of tropical seasonality on the fluxes of nutrients are needed to identify dependencies between components of the ecosystem. Studies of biochemical seasonality in soils remain relatively rare, even though they could be crucial in defining the response of nutritional cycles in tropical forests to gradual shifts in seasonality, such as a longer dry season. Information on tropical nutritional dynamics might prove critical when nutrient cycles are dependent on climate as they appear to be in this study; synchronization of seasonal patterns of nutrient inputs through litterfall and microbial activity are paramount through maintaining the nutrition of the tropical forest. This study identifies several dynamical interactions between seasonality, microbial activity and nutrient status of tropical soils that ought to be confirmed and expanded to better represent nutritional implications under future change scenarios.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data availability statement

Data will be made available in a public repository upon publication

Author Contributions

KJS, MRH, LF, and CAQ conceptualized and designed the research. Experiments were carried out by KS and LF, with field support from FH and OVB. PBC's laboratory performed total C and N analyses. Statistical analyses were performed by KJS, with support from MRH, LF and FH. KJS prepared the manuscript, with contributions from LF, CAQ, FH, OVB and MRH.

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Figures

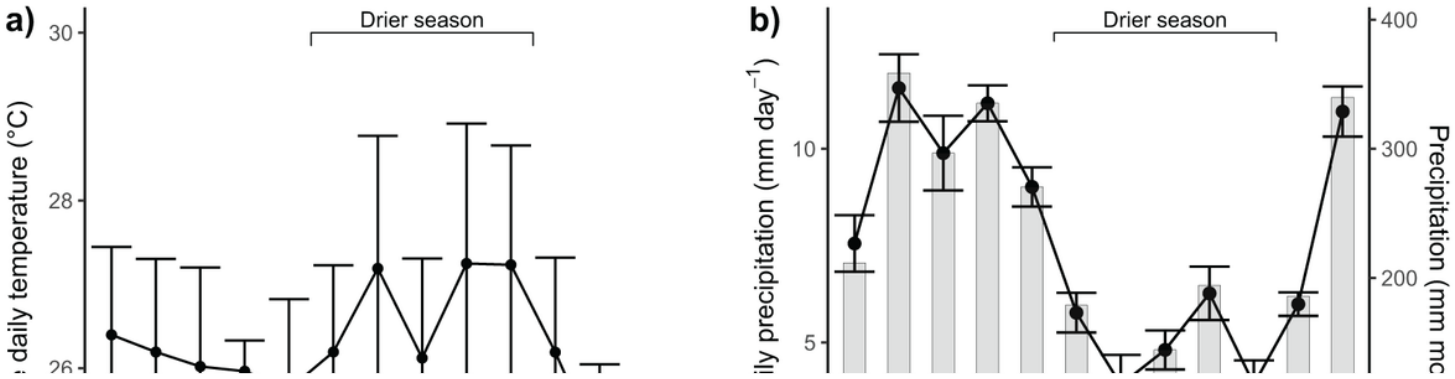

Figure 1

a) daily average air temperature (at 34.6 m, above forest canopy), and b) rainfall at the AmazonFACE plots, daily average (line and dots ± SE) and the monthly sum (bars). Both temperature and precipitation were measured every 30 minutes and calculated per day (n = 48 per day). The “Dry season” bracket indicates which months are treated as the drier months of the year in the rest of the manuscript, defined as the months with less than 40% of days with <3mm precipitation.

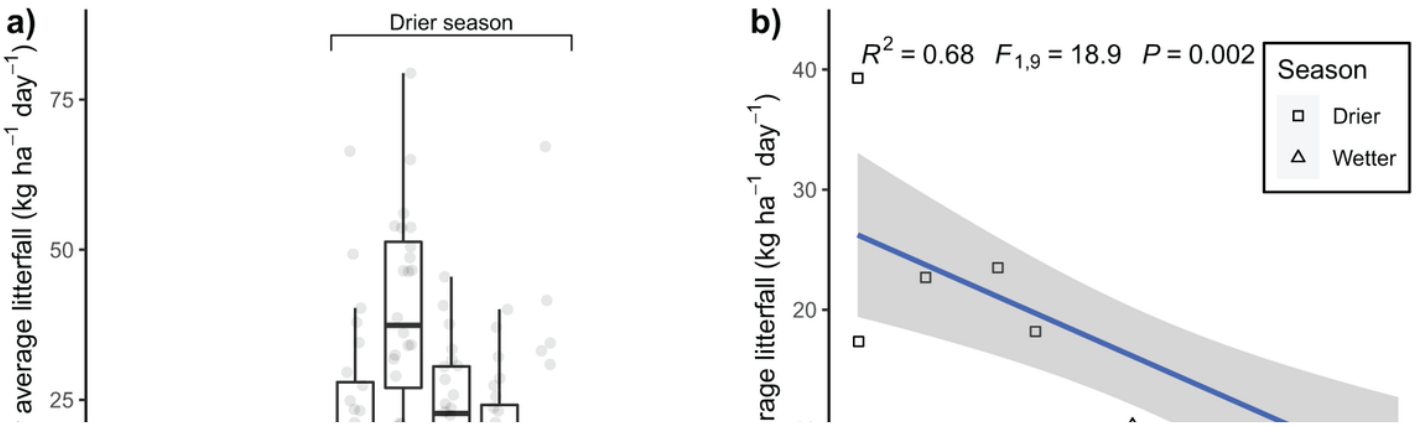

Figure 2

a) leaf litter collected (biweekly) at the AmazonFACE plots, recalculated for an average daily litter quantity per trap (each observation represents a different trap, n = 24. Boxplot shows median and quartiles), and b) the relation between average daily leaf litterfall and average daily precipitation per month. Dry season indicates the dry months as established in Fig. 1.

Figure 3

C, N and P related extracellular enzyme activities (BG, NAG and AP per soil C) from February 2016 till January 2017, and their relation to the average monthly leaf litterfall. Boxplots are showing the median, the lower and upper hinges correspond to the first and third quartiles

Figure 4

Relations between EES and soil nutrient stoichiometry, and enzyme activities as related to each other, at 0-5 cm and 5-15 cm. a) relation between C:N ratios of soil nutrients and enzymes, b) relation between C and N enzyme activities (BG and NAG), c) relation between C:P ratios of soil nutrients and enzymes, d) relation between C and P enzyme activities (BG and AP), e) relation between N:P ratios of soil nutrients and enzymes, d) relation between N and P enzyme activities (NAG and AP).

Figure 5

Average monthly vectors of proportional enzyme activities at a) 0-5 cm and b) 5-15 cm, and average vector properties c) length (unitless), and d) angle (in degrees) of the monthly average vectors. Vectors above the 1:1 line in a) and b) are P-limited according to the vectors, below the line are considered N-limited. The error bar in c) and d) represents the standard error).

Supplementary Files

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