Biogeochemistry (2023) 163:1–15
https://doi.org/10.1007/s10533-022-01009-4

Seasonal fluctuations of extracellular enzyme activities are related to the biogeochemical cycling of C, N and P in a tropical terra-firme forest

Karst J. Schaap · Lucia Fuchslueger · Carlos Alberto Quesada · Florian Hofhansl · Oscar Valverde-Barrantes · Plínio B. Camargo · Marcel R. Hoosbeek

Received: 18 January 2022 / Accepted: 19 December 2022 / Published online: 21 January 2023

Abstract Extracellular enzymes (EE) play a vital role in soil nutrient cycling and thus affect terrestrial ecosystem functioning. Yet the drivers that regulate microbial activity, and therefore EE activity, remain under debate. In this study we investigate the temporal variation of soil EE in a tropical terra-firme forest. We found that EE activity peaked during the drier season in association with increased leaf litterfall, which was also reflected in negative relationships between EE activities and precipitation. Soil nutrients were weakly related to EE activities, although extractable N was related to EE activities in the top 5 cm of the soil. These results suggest that soil EE activity is synchronized with precipitation-driven substrate inputs and depends on the availability of N. Our results further indicate high investments in P acquisition, with a higher microbial N demand in the month before the onset of the drier season, shifting to higher P demand towards the end of the drier season. These seasonal fluctuations in the potential acquisition of essential resources imply dynamic shifts in microbial activity in coordination with climate seasonality and resource limitation of central-eastern Amazon forests.

Keywords Tropical forest soil · Nutrient stoichiometry · Leaf litter · Enzyme activity vectors · Extracellular enzymes · Soil nutrients

Responsible editor: Samantha R. Weintraub-Leff

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10533-022-01009-4.

K. J. Schaap · M. R. Hoosbeek
Soil Chemistry, Wageningen University, P.O. Box 47, 6700AA Wageningen, The Netherlands

K. J. Schaap (✉) · C. A. Quesada
Coordination of Environmental Dynamics (CODAM), National Institute of Amazonian Research (INPA), Av. André Araújo 2936, Petrópolis, Manaus 69067-375, Brazil
e-mail: karstsch@gmail.com

L. Fuchslueger
Division Terrestrial Ecosystem Research, Centre of Microbiology and Environmental Systems Science, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

F. Hofhansl
Biodiversity and Natural Resources Program, International Institute for Applied Systems Analysis (IIASA), Schlossplatz 1, A-2361 Laxenburg, Austria

O. Valverde-Barrantes
Department of Biological Sciences, Institute of Environment, International Center of Tropical Botany, Florida International University, Miami, FL 33199, USA

P. B. Camargo
CENA, University of São Paulo, Piracicaba, Brazil

Introduction

The activity of soil microbial communities plays a crucial role in the nutrient cycling of tropical lowland forests. Seasonality and subsequent variation in litter input affect soil microbes who are both consumers and suppliers, i.e., sink and source, of available nutrients in soil and ecosystem carbon (C), nitrogen (N) and phosphorus (P) cycling (Singh et al. 1989; Cavicchioli et al. 2019). Heterotrophic soil microbes depend on the supply of both labile and complex organic substrates from plants as their main energy (C) source (Soong et al. 2020). Nevertheless, soil microbial communities also depend on N and P for the synthesis of essential components—e.g., N for protein synthesis and P for DNA and energy transport and storage. Both plants and microbes need available forms of nutrients for uptake, which are largely provided through the conversion of more complex organic substrates to bioavailable products by breaking down larger polymers, a process catalyzed by extracellular enzymes (EE) (Skujiņš and Burns 1976; Baldrian 2009; Burns et al. 2013; Luo et al. 2017). Depolymerization of larger molecules by EE has often been considered the rate-limiting step in organic matter decomposition (Sinsabaugh and Follstad Shah 2012), and thus an important determinant of C and nutrient cycling potential in soil. Increased insight into the effects of seasonality on nutrient cycling may inform biogeochemical models.

Soil enzyme assays provide potential activities of enzymes, generally acting on the chain ends of polysaccharides, chitin and organic P, each specific substrate responsible for the rate limiting step in C, N and P decomposition. Commonly assayed enzymes include β-glucosidase, N-acetyl glucosamidase and phosphatase (liberating C, N, and P, respectively) (German et al. 2011). EE production depends on nutrient availability, and follows principles of resource or substrate supply and demand (Allison et al. 2011). Plant litter and soil nutrient contents can be used to characterize organic matter quality and to predict its respective turnover, with higher quality material (i.e. higher nutrient contents, lower molecular complexity) being turned over faster compared to more complex or lower quality organic matter (Zechmeister-Boltenstern et al. 2015). Stoichiometry of EE is used to assess nutrient limitations to microbial requirements (Sinsabaugh and Follstad Shah 2012; Moorhead et al. 2016). While N is considered to be the main limiting nutrient at higher latitudes, P-limitation is a prevalent characteristic in highly weathered tropical soils (Camenzind et al. 2018). Consequently, when compared to temperate ecosystems, tropical soil EE stoichiometries show high investments in phosphatases relative to enzymes targeting C and N (Waring et al. 2014).

Temperature and soil moisture may affect the activities of enzymes directly through reaction rates of EE (Nottingham et al. 2016) and EE and substrate diffusion within the soil. Temperature and moisture may also indirectly affect EE activities by affecting soil microbial community composition (Malik and Bouskill 2022). As a consequence, seasonal fluctuations in abiotic factors and litter input may affect soil microbial communities and their ability to take up or mineralize nutrients. There is evidence that litterfall and soil microbial biomass vary asynchronously, in association with seasonal shifts in nutrient availability in the wet tropics (Ruan et al. 2004). Seasonal fluctuations in various tropical ecosystem processes, such as the production of plant litter (Wu et al. 2016) and fine roots (Cordeiro et al. 2020) indicate seasonality in the cycling of C, N and P. Increasing our understanding of associated EE dynamics may provide insight into essential processes for sustained ecosystem functioning under future climatic conditions.

In this study we studied the effect of precipitation, temperature, litterfall and soil water content on microbial EE activities over the course of a seasonal cycle. Furthermore, we investigated seasonal dynamics of EE activities associated to C, N and P cycles in a tropical forest soil and used them as a proxy for soil microbial activity and nutrient demand. We hypothesized that: (1) litter inputs are the main driver of enzymatic activities, as opposed to temperature, precipitation, or soil water content; (2) total soil C and available C, N and P are negatively related to BG, NAG and AP activities, respectively (through increased microbial investments driven by low nutrient availability); and, (3) the P-related EE activities are higher than C and N related EE, due to low P availability in tropical soils.

Methods

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. The area is characterized by pristine old-growth tropical forests locally known as "Terra Firme" forests. These forests are situated on plateaus covered with nutrient poor and clay-rich soils classified as Geric Ferralsols. Soil texture consists on average of 68% clay, 20% sand and 12% silt and soil pH is on average 3.94 (Quesada et al. 2010). The mildly seasonal climate is defined by average annual rainfall of about 2400 mm, with a relatively drier period from June to November (months with at least 40% of days with <3 mm precipitation), while the average temperature fluctuates from 25.8 °C in April to 27.9 °C in September (Araújo et al. 2002).

Sample collection and processing

Soils were collected from 18 sampling points. At 6 locations along a 400 m north-south transect (every 80 m), we sampled three points in the east-west direction, with 10 m distance between the three 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 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.

Part of the samples were stored after drying at 65 °C for 48 h until further analysis, while fresh soil was used for selected measurements within 3 days of sampling. Soil enzymes were analyzed monthly for each of the sampling locations in fresh soil. Total soil P, extractable organic carbon, extractable nitrogen, and microbial biomass were analyzed every 3 months. Total soil C and N contents were determined monthly in composite samples consisting of the three east-west samples collected at each location along the north-south transect. Apart from the total C and N contents, all analyses were performed at the LTSP laboratório temático de solos e plantas (LTSP) laboratory at Instituto Nacional de Pesquisas da Amazônia (INPA) 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.

Total C, N and P

Total soil C and N were determined in milled dry aliquots by an EA (IRMS). 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).

Extractable C, N and P

Extractable organic carbon (eoC) and extractable nitrogen (eN) were obtained from extracts of 2 g of fresh soil in 20 ml 1 M 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-1 220 V; Shimadzu, Vienna, Austria). Extractable P (Olsen P, Olsen et al. 1954) was determined from extractants of 2 g of soil in 20 ml 0.5 M 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 method blanks (no soil) to account for contamination or background signal, and lab variation was accounted for by analyzing standards during each batch of photometric extract reading.

Potential soil extracellular enzyme activities

Potential EE activities of three common hydrolytic enzymes relevant to C, N and P cycling were assayed using a fluorescence method based on Marx et al. (2001) and German et al. (2011). 4-Methylumbelliferyl β-D-glucopyranoside (M3633 Sigma, substrate concentration 200 µM), 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (M2133, substrate concentration 200 µM) and 4-methylumbelliferyl phosphate disodium salt (M8168 Sigma, substrate concentration 1 mM) 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. Substrate concentrations were established in preliminary experiments to ensure reaction rates at substrate saturation (and thus V_{max}). 4-methylumbelliferone standards (M1381 Sigma) 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 microplate (96 well polystyrene, black flat bottom). 200 µl soil slurry was used with 50 µl substrate. Microplates were incubated for 1 h at 23 °C, then 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).

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 proportional 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 per sampling date (n = 18, at two depths) according to the stoichiometric mean recommended by Isles (2020) as the mean of each natural logarithm, all values are reported ± their standard error. Data processing and statistical tests were performed in R 4.2.1 (R Core Team 2022).

We used linear regression models to assess direct relations between precipitation, temperature and litterfall. Linear mixed-effect models were applied to assess relations between enzymes and the other variables using the "lme" function from the "nlme" package (version 3.1–157, Pinheiro et al. 2022); sampling location was included as random effect, with data ln transformed for normality where indicated. For the linear mixed-effect models shown in the graphs, only one fixed effect was included per model, for the reported mixed-effect models in the table the depth was included as a fixed effect as well. Additionally, for the models shown in the table the "varIdent" variance structure was used to allow for different variances per stratum (sampling depth) and additionally the model residuals were checked for autocorrelation (no significant temporal autocorrelation was found). All models' residuals were checked for homogeneity and normality. Conditional R^2 values for the linear mixed-effect models shown in graphs were obtained with the "r.squaredGLMM" function from the "MuMIn" package (version 1.46.0, Bartoń 2022) (Fig. 1).

Results

Precipitation showed a distinct drier period (at least 40% of days with < 3 mm, Fig. 1b) between July and November, during which it was also a few degrees

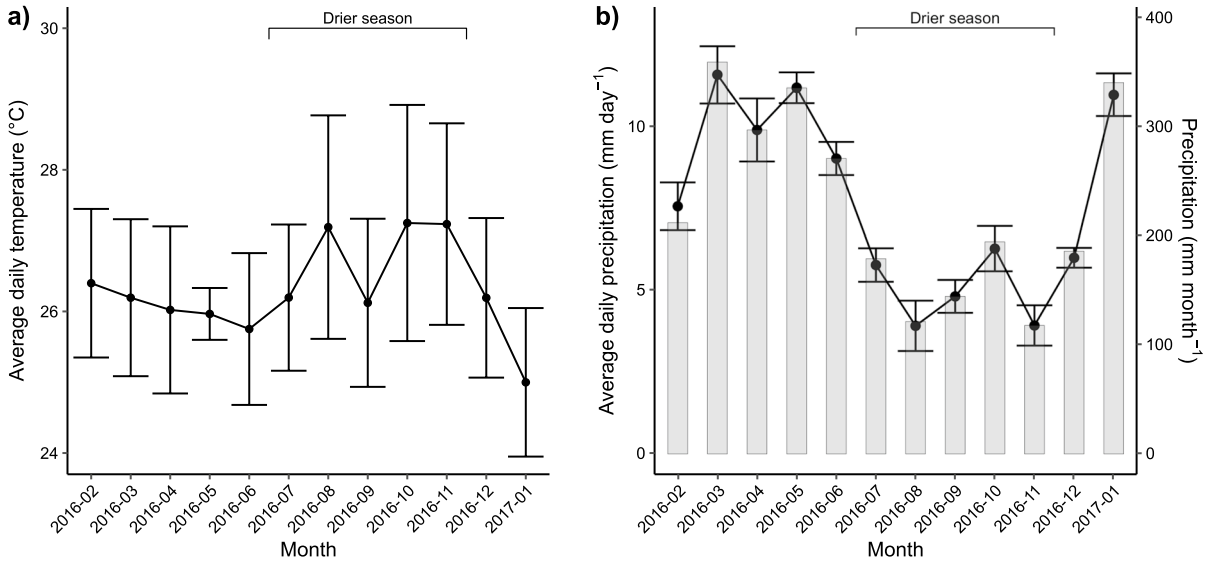


Fig. 1 **a** daily average air temperature (at 34.6 m, above forest canopy), and **b** rainfall at the AmazonFACE plots, daily average (line and dots ± SE) and the monthly sum (bars). Both temperature and precipitation were measured every 30 min (n = 48 per day) and calculated per day. The "Drier season" bracket indicates which months are treated as the drier months of the year in the manuscript, defined as the months with at least 40% of days with < 3 mm precipitation

warmer, but average temperature varied little and stayed within a 24.5–27.5 °C range (daily average) (Figs. 1a, S1a). Annual leaf litterfall amounted to 5565 ± 55 kg ha^{-1} $year^{-1}$, with a distinct peak during the drier months (Fig. 2a). Leaf litterfall was significantly correlated to the average monthly temperature ($F_{(1;9)} = 5.3$, $p = 0.047$, Fig. S1b) and showed a negative relation with the average rainfall ($F_{(1;9)} = 42$,

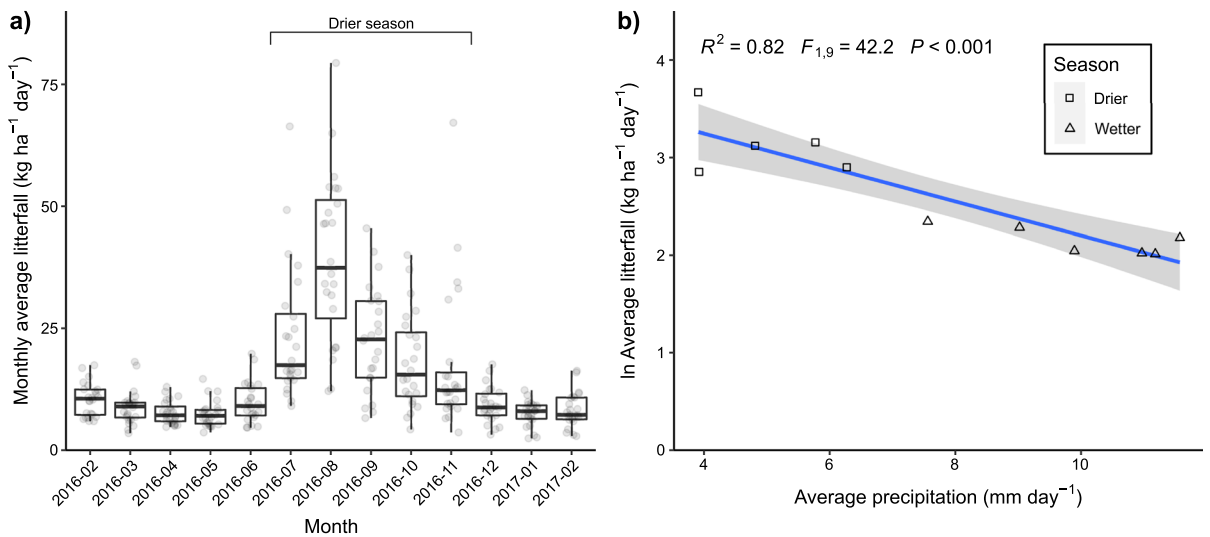
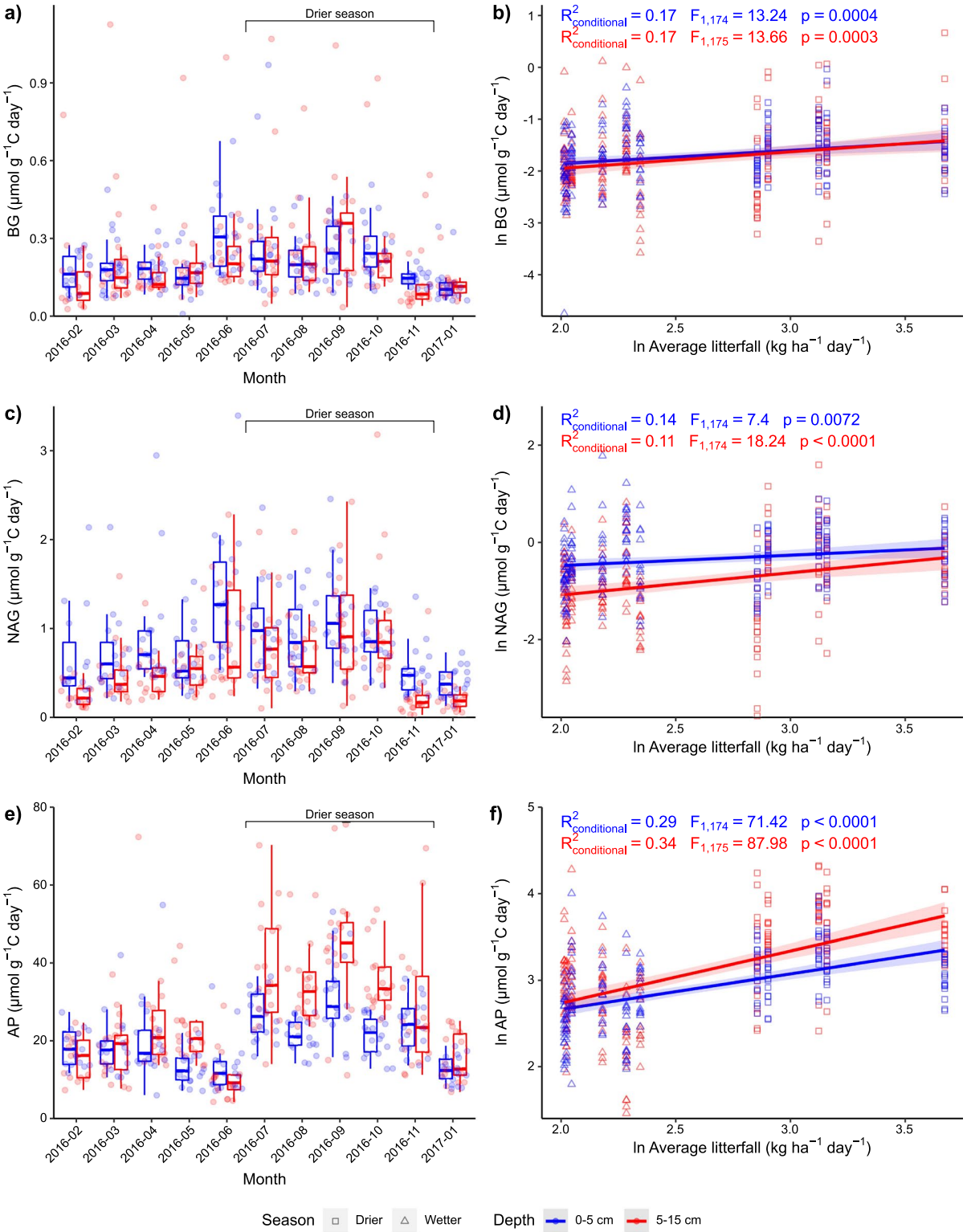


Fig. 2 **a** leaf litter collected (biweekly) at the AmazonFACE plots, recalculated for average daily litter quantity (average per month, each observation represents a different littertrap, n = 24. Boxplot shows median and quartiles), and **b** the relation between average daily leaf litterfall and average daily precipitation (natural logarithm) per month. Drier season indicates the drier months as established in Fig. 1


a)
Drier season
BG (μmol g−1C day−1)
Month
b)
R2conditional = 0.17 F1,174 = 13.24 p = 0.0004
R2conditional = 0.17 F1,175 = 13.66 p = 0.0003
ln BG (μmol g−1C day−1)
ln Average litterfall (kg ha−1 day−1)
c)
Drier season
NAG (μmol g−1C day−1)
Month
d)
R2conditional = 0.14 F1,174 = 7.4 p = 0.0072
R2conditional = 0.11 F1,174 = 18.24 p < 0.0001
ln NAG (μmol g−1C day−1)
ln Average litterfall (kg ha−1 day−1)
e)
Drier season
AP (μmol g−1C day−1)
Month
f)
R2conditional = 0.29 F1,174 = 71.42 p < 0.0001
R2conditional = 0.34 F1,175 = 87.98 p < 0.0001
ln AP (μmol g−1C day−1)
ln Average litterfall (kg ha−1 day−1)
2016-02
2016-03
2016-04
2016-05
2016-06
2016-07
2016-08
2016-09
2016-10
2016-11
2017-01
Season Drier Wetter
Depth 0-5 cm 5-15 cm

◂**Fig. 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 (plots **a**, **c** and **e**). The text in plots **b**, **d** and **f** shows the relation between enzymes and litterfall estimated with a linear mixed effects model, with the sampling location as a random effect

$p < 0.001$, Fig. 2b) indicating higher leaf litterfall during the drier months. Soil water content showed limited variation (Fig. S2), and had no significant relation to either precipitation, temperature or litterfall.

Total soil C at 0–5 cm was on average 5.53 ± 0.02%, with the lowest value of 4.19 ± 0.09% in January to highest value of 7.28 ± 0.27% in May (Fig. S3). Total soil N was 0.35 ± 0.00% on average, following roughly the same pattern as total C. Total P averaged 156.39 ± 0.69 mg kg^{-1}, ranging from 141.8 ± 2.13 mg kg^{-1} in August to 204.52 ± 3.46 mg kg^{-1} in February, with the note that the measurement frequency of total P was lower than for C and N (Fig. S3). For 5–15 cm, the average total C (2.84 ± 0.01%), total N (0.21 ± 0.00%), and P contents (118.22 ± 0.52 mg kg^{-1}) were lower as compared to the top 5 cm but followed the same temporal trend as in the top 5 cm (see Fig. S3). The eoC, eN and Olsen P in the top 5 cm of soil were 1034.0 ± 6.2 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. At 5–15 cm those values were lower with 916.86 ± 6.41 mg C kg^{-1} soil, 76.78 ± 0.24 mg N kg^{-1} and 1.19 ± 0.00 mg P kg^{-1} dry soil, respectively (Fig. S4). At both soil depths, we found the highest average values for eoC in May (1806.6 ± 31.3 mg kg^{-1} and 1727.6 ± 31.6 mg kg^{-1} respectively), while the lowest average was measured in August (594.7 ± 6.0 mg kg^{-1} and 455.7 ± 2.8 mg kg^{-1} respectively). In contrast, the eN values were lowest in February at both depths (82.1 ± 1.54 mg kg^{-1} and 58.24 ± 0.74 mg kg^{-1} respectively), while reaching their highest values in August (135.3 ± 1.19 mg kg^{-1} and 95.92 ± 0.58 mg kg^{-1} respectively. Olsen P peaked in March at both depths, while in the top 5 cm showed the lowest concentration in April (1.16 ± 0.02 mg kg^{-1}), while in the lower soil increment the lowest value was reached in January (0.52 ± 0.02 mg kg^{-1}).

Average EE activities (as expressed per gram soil C; for values per dry soil see Fig. S5) were 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 at 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 (Fig. 3a, c, e). In the top 5 cm EE activity rates peaked just before and during drier season and were lowest in the wetter season, with BG showing highest rates 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) 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 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}).

We applied linear mixed effect models to assess relationships between climatic factors (temperature, moisture), leaf litter inputs and soil enzyme activities, (Table 1, Fig. 3). We found that BG activities at both soil depths were significantly positively related to litterfall inputs (Fig. 3b), but not to temperature (Fig. S6a), and to precipitation only in the top 5 cm (Fig. S6b), while potential NAG activity rates were significantly related to only the litterfall (Figs. 3d, S6c, d). In contrast, potential AP activity was significantly related to all studied drivers, with litter and temperature having a positive, and precipitation a negative relationship (Figs. 3f, S6e, f). Based on linear mixed effect models including sampling depth as a fixed factor, we identified leaf litterfall as the strongest driver for potential EE rates while we found no significant effects of precipitation, temperature, and soil water content (except for NAG), respectively (Table 1).

Although the EE activities were higher overall in the drier months from July to November, total soil nutrient contents did not seem to show a clear seasonal pattern (Fig. S3). To assess if nutrients provided ecological constraints on the microbial activity, we investigated relations between nutrients and enzymes. Although the total soil C, N, and P contents provided limited significant relations with EE (Fig. S7), the relations of enzymes with the extractable C, N, and

Table 1 Analysis of variance F statistics, with p values in parentheses, for enzyme responses to either litterfall, precipitation, temperature, or soil water content, combined with sampling depth. Sampling location included as a random factor in all models. Asterisks (*) indicate a significant improvement over the null models AIC

		BG		NAG		AP	
Model	Factors	df	F (p)	df	F (p)	df	F (p)
Null	Intercept	1, 368	9048 (< 0.0001)	1, 368	11,871 (< 0.0001)	1, 368	118,217 (< 0.0001)
	Depth	1, 368	1 (0.3156)	1, 368	39 (< 0.0001)	1, 368	12 (0.0006)
	AIC:	728		854		579	
	Factors	df	F	df	F	df	F
Litterfall	Intercept	1, 367	9099 (< 0.0001)	1, 367	12,060 (< 0.0001)	1, 367	124,096 (< 0.0001)
	ln(Litter)	1, 367	26 (< 0.0001)	1, 367	23 (< 0.0001)	1, 367	149 (< 0.0001)
	Depth	1, 367	1 (0.3143)	1, 367	41 (< 0.0001)	1, 367	17 (< 0.0001)
	AIC:	704*		834*		455*	
	Factors	df	F	df	F	df	F
Precipitation	Intercept	1, 367	9119 (< 0.0001)	1, 367	11,921 (< 0.0001)	1, 367	125,110 (< 0.0001)
	Precip.	1, 367	12 (0.0006)	1, 367	7 (0.0102)	1, 367	138 (< 0.0001)
	Depth	1, 367	1 (0.3224)	1, 367	40 (< 0.0001)	1, 367	17 (< 0.0001)
	AIC:	718*		850*		462*	
	Factors	df	F	df	F	df	F
Temperature	Intercept	1, 367	9076 (< 0.0001)	1, 367	11,875 (< 0.0001)	1, 367	122,614 (< 0.0001)
	Temp.	1, 367	4 (0.0409)	1, 367	4 (0.0579)	1, 367	70 (< 0.0001)
	Depth	1, 367	1 (0.3230)	1, 367	39 (< 0.0001)	1, 367	15 (0.0001)
	AIC:	726*		853		516*	
	Factors	df	F	df	F	df	F
Soil water content	Intercept	1, 367	8713 (< 0.0001)	1, 367	11,202 (< 0.0001)	1, 367	108,993 (< 0.0001)
	SWC	1, 367	4 (0.0545)	1, 367	43 (< 0.0001)	1, 367	0.2 (0.6526)
	Depth	1, 367	0 (0.9517)	1, 367	14 (0.0003)	1, 367	14 (0.0002)
	AIC:	727		841*		580	

P were dominated by the significant positive relations that eN and the enzyme activities showed overall (especially in the top 5 cm), while also showing significant negative relations between the AP activity and eoC and Olsen P (Fig. 4).

While the EE activities showed an observable drier-season effect when considered separately (Fig. 3), their activity ratios and proportional activity ratios showed less distinct patterns (Table S1). From these proportional activity ratios we calculated enzyme vectors, useful for distinguishing relative nutrient demand (Fig. 5). Although at a first glance the vectors indicate a persistent high demand of P, the vector angles decreased after the drier months, indicating a relative shift from P acquisition towards N acquisition enzymes during the rainy season; especially in June, just before the onset of the drier season, the relative N-demand was high. Moreover, the vector lengths peaked in June, indicating an increased C demand. The angles and the lengths of the vectors showed a weak yet significant negative relationship (Fig. S8), indicating a weak relation between relative C and N demand.

Discussion

In this study, we report on the dynamics of EE in tropical soils, which highlights seasonal fluctuations in soil microbial nutrient demand and relative

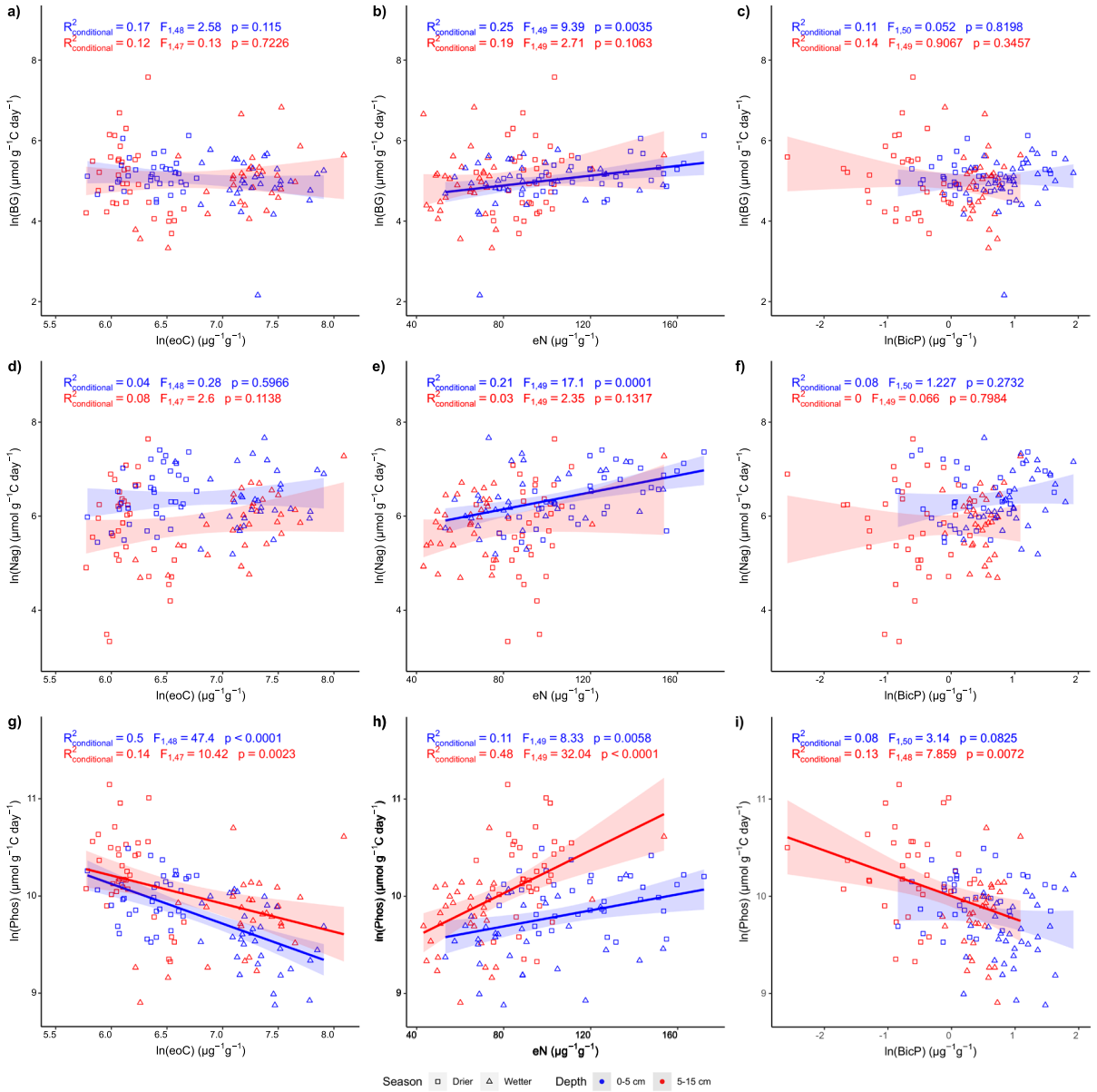


Fig. 4 Relations between EE and extractable soil nutrients at 0–5 cm and 5–15 cm. Conditional R^2, F and p values for linear mixed effect models with sampling location as a random effect. **a**, **b**, and **c** β-glucosidase as related to eoC (natural logarithm), eN and Olsen P (natural logarithm); **d**, **e** and **f** N-acetyl glucosamidase as related to eoC (natural logarithm), eN and Olsen P (natural logarithm); **g**, **h**, and **i** Phosphatase as related to eoC (natural logarithm), eN and Olsen P (natural logarithm)

investments in respective EE. We found that EE activities follow the seasonal signal of leaf litterfall. Furthermore, we found a significant positive relationship between eN and most enzyme activities. BG and NAG activities were relatively low compared to AP, which may indicate a strong demand for P in these highly weathered tropical forest soils. Our results suggest that microbial resource limitation shifts from relatively more N-demand at the end of the rainy season, to increased P-demand during the drier season, indicating seasonal changes in the relative microbial investment in EE.

The reported leaf litterfall seems to be comparable to earlier studies in the area (Lucas et al. 1993;

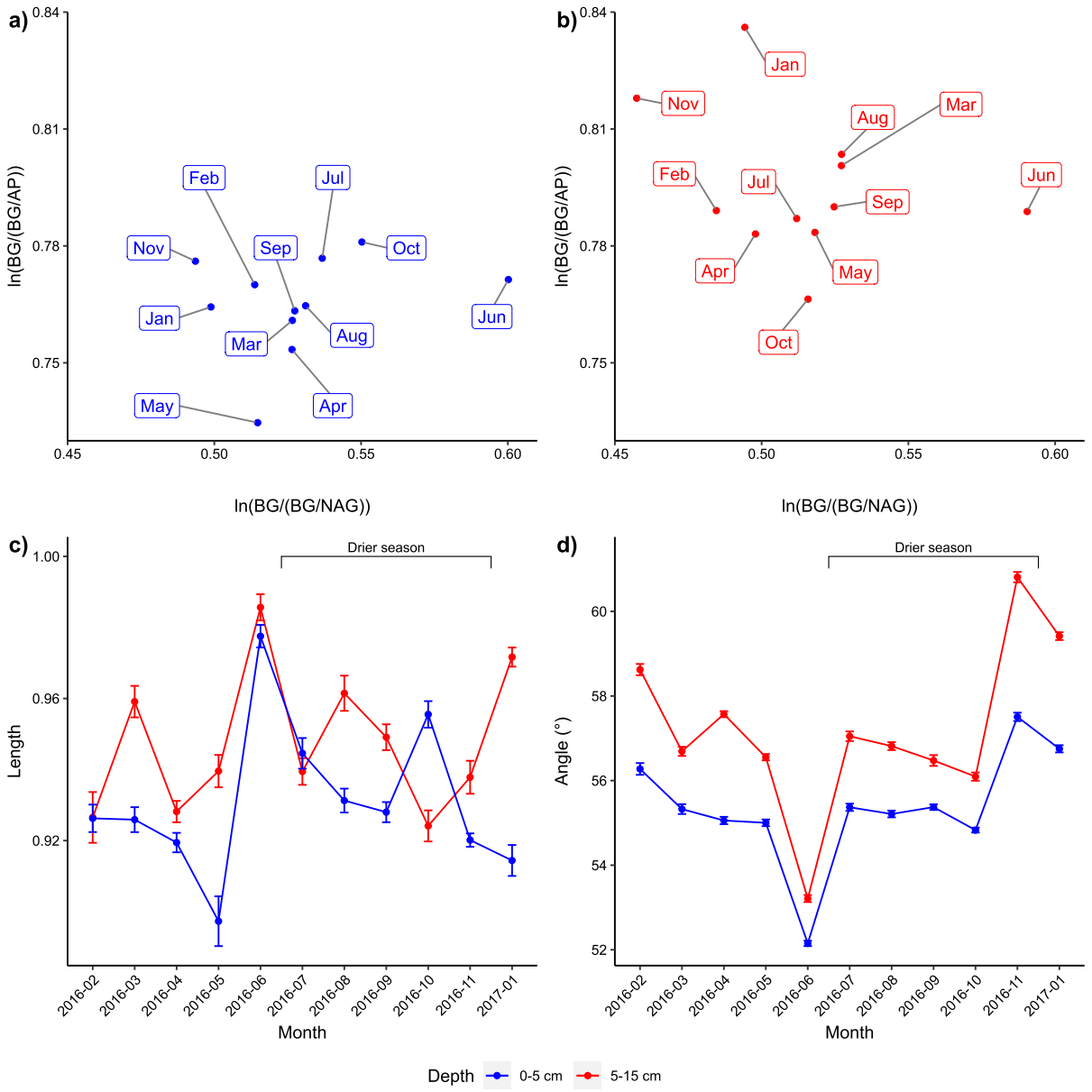


Fig. 5 Average monthly vectors of proportional enzyme activities at **a** 0–5 cm and **b** 5–15 cm, and average vector properties **c** length (unitless), and **d** angle (in degrees) of the monthly average vectors. The error bar in **c** and **d** represents the standard error)

Luizao 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 association with an El Niño event observed in the preceding year (e.g. Hilker et al. 2014). 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 these patterns are reflected also in soil microbial communities, with more decomposers and anaerobic saprophytes present in the wetter season (Buscardo et al. 2018). The positive relationship between enzyme activity and litter inputs therefore also indicates a synchronization and a link between new substrate, microbial community changes and changing investments of microbes in enzymes.

The pattern of increased potential EE activity during the drier season was evident for all studied enzymes, and we hypothesized this was mainly driven by increases in litter inputs in these months. Litterfall showed significant (positive) relations to all enzyme activities, while precipitation showed weaker or insignificant (negative) relations towards EE, and temperature only showed consistent (positive) relations with AP activity. The pattern of increased potential EE activity in drier months has been observed by others as well; Smith et al. (2015) found increased EE activity during the dry season in a Puerto Rican subtropical forest, as did Singh et al. (2020) in a dry tropical ecosystem. They attributed these dry season increases in EE to reduced access of microbes to resources—reducing microbial assimilation and triggering enzyme production—and decreased enzyme turnover and clay-mineral interactions causing immobilization, all attributed to reduced soil moisture. We reported a similar pattern, yet soil water content did not seem to be a big constraint in our studied soil system, even in the drier season. Similarly, the observed temperature range was limited at our site, which could explain why we observed relations between temperature and AP activity, but not consistently to the other EE activities.

Even though our hypothesis of litter driving EE investments is supported by our analysis, it remains a challenge to untangle the effects of litter, precipitation, and to a lesser extent, temperature. Precipitation was strongly related to litterfall, indicating that indirectly or directly, precipitation drives changes in belowground biochemistry. Most likely, they are both determining soil EE expression through different mechanisms—with decreases in precipitation stimulating litterfall and thus substrate (mainly C), while soil moisture limits enzymatic mobility to some extent. Both could be reflected in increased potential activity; increased labile substrate would stimulate microbial enzyme production through increased return of investments from produced enzymes, yet decreased moisture would increase immobilization—the last one being more of a methodological artefact than something which would be reflected in in-situ turnover.

We hypothesized total soil nutrient contents to be negatively related to EE, however, we found weak relations between total nutrients and enzymes. Moreover, we expected the relation between the available nutrient contents and the related enzyme activity to be negative, since low nutrient availability would stimulate investments in acquisition, yet relations between eoC, eN, Olsen P, respectively and EE activities in the soil were not always significant. BG and eoC were not related, while eN and NAG even showed a positive relation in the top 5 cm. Only Olsen P and AP activity showed the expected relation, albeit weak, suggesting some degree of demand driven AP investments by microbes (Sinsabaugh and Follstad Shah 2012) with possible contributions from roots (Guilbeault-Mayers et al. 2020). However, as an alternative driver of EE activity, we found significant positive relations of eN with most enzyme activities, which suggests that soil microbial communities depend on a supply of eN to maintain EE production.

Fertilization studies in tropical areas indicate that N addition can stimulate organic matter turnover and EE activities (Marklein and Houlton 2012; Wang et al. 2018), although others did not find such effect (Turner and Wright 2014). Nitrogen fixation can be linked to the acquisition of P by AP production (Allison et al. 2006; Nasto et al. 2014). Our study indicates that this stimulation of EE by available N can be observed in short timespans as well, suggesting the production of enzymes is dependent on the available N-supply. This is of interest to the functioning of P-limited tropical forests, i.e. the link between N availability and AP production would imply N limitation on P acquisition. This suggests further investigation into the seasonal dynamics of the tropical N-cycle, especially in relation to precipitation, would be important to improve our understanding of microbial functioning and the P-cycle.

The EE activity rates at our site were in the same range as compared to enzyme activities measured in a tropical mountain rainforest (Tischer et al. 2014) and as reported along an altitudinal gradient in the Andes, albeit lower than on lowest altitudes reported (Nottingham et al. 2016). BG activity, liberating glucose (available C) as last step in the breakdown of cellulose, was low overall, yet with an observable increase during the drier season synchronized with litterfall. NAG and AP activity, used as a proxy for N and P demand, also showed increases during the drier season and synchronization with litterfall. Notably, NAG activity, breaking down chitin and possibly indicative of microbial turnover, increased just before the drier

season (June); in contrast, AP activity showed a relative decrease in the same month.

The measured enzyme activities may shed light on microbial nutrient demand or allocation to resource acquisition. Our results suggest a relatively higher investment of AP compared to BG and NAG; which according to our third hypothesis would indicate that central-eastern Amazon forest soils are limited by P availability. Using EE activity vector analysis, as conceptualized by Moorhead (2016), we identified temporal changes in microbial nutrient demand that are likely related to the phenology of soil microbial biomass and activity. We found an increased N-demand at the end of the wet season (lower vector angle), which decreased during the drier season, in favor of the P-demand (larger vector angle). This was mainly driven by the different pattern of NAG activity compared to the other enzymes. NAG catalyzes the breakdown of chitin present in for example fungal cell walls, and a relatively higher activity could also indicate higher microbial turnover (Zeglin et al. 2013). This has been observed by Mori (2020; see also Mori et al. 2021) as well, who challenged the idea that low enzymatic C:N activity ratios (proportional activities in our case) reflect microbial N limitation if the dominant substrate is not cellulose. An alternative to the microbial limitation-hypothesis could be changes in substrate, such as a switch from more plant derived substrate to higher turnover of the microbial biomass, driving NAG-dynamics. Indeed, inputs of C-rich plant material to subtropical soils can shift the fungal community to N-limitation, while the bacterial community shifts towards a co-limitation by C and N (Rosinger et al. 2019).

During the drier season, P demand was relatively more pronounced (larger vector angle). Once leaf litter reaches the soil, there are different pathways for the incorporation of organic matter (SOM) into the soil, where the labile components are released first, and particulate recalcitrant matter is incorporated in later stages (Cotrufo et al. 2013, 2015). This time lag is a possible explanation for the trend indicated by the vectors towards more P-acquisition towards the end of the drier season; P-loss from litter does not occur immediately (Martins et al. 2021), which might cause a delay in enzymatic response (Schaap et al. 2021). However, we found no evidence of a significant lag effect between litter inputs and AP dynamics in our data (no significant autocorrelation of model residuals).

Vectors of proportional enzyme activities showed a relative increase in microbial P demand towards the end of the drier season and seem to indicate an increase of relative N-demand at the end of the rainy season—before litterfall increases. Changes in microbial biomass size a month prior to litter inputs have been reported for tropical forests, which were attributed to plant mediated shifts in belowground C and nutrient inputs and decreased nutrient uptake related to seasonal leaf senescence (Ruan et al. 2004). An equal mechanism could be at play here. A possible link that might explain our findings is that the litterfall is driven by the seasonality of precipitation, and consequently, N—required for enzyme production—is mobilized (into an accessible form, measured here as eN) rather quickly from substrate through increased activity of NAG, allowing for increased EE production in the drier season for all enzymes. This might also explain the comparably low AP activity before the drier season (June), and the relative dip in the vector angles—and thus a stronger N-demand. This suggests enzyme dynamics are mainly controlled by microbial access to available N, which is also in line with the observed relations between eN and EE activities.

In summary, our study shows that microbial activity is synchronized with litter seasonality, as shown by the relation between leaf litter and EE activities. Moreover, our results suggest available forms of nutrients (measured here as eoC, eN and Olsen P) in the mineral soil were taken up quickly (hours-days, e.g., Menge et al. 2009; Helfenstein et al. 2018) and did therefore not show strong relations to the corresponding enzymes in the monthly measurement intervals, yet available N facilitates enzymatic activity. Microbial activity showed a permanent high demand for P, although before the drier season, an increased N-demand was observed. We conclude that in the studied tropical forest ecosystem, soil nutrient availability is an important determinant of dynamic changes in EE mediated nutrient acquisition capacity, which is in turn related to plant phenology and climate seasonality. Our results suggest a supply of available N is paramount to EE activity to maintain microbial enzyme production, yet also demonstrate a persistently high P demand. Future research should untangle the temporal dependencies between nutrient

cycles—such as the N and P cycle—to address the timing of constraints and limitations to microbial functioning under different biotic and abiotic conditions. This may increase insight into the response of nutritional cycles in tropical forests to shifts in seasonality, such as more prolonged and more severe dry seasons through climate change.

Acknowledgements The authors thank Luciano Castilho for logistic support, Erison Gomes and Adriana Grandis for assistance with lab work and Alessandro Araújo and Mariana Gonçalves dos Reis for preparing and providing meteorological data. We are thankful to the AmazonFACE-team, the crew at the ZF2 field site, and the LTSP laboratory for general support. We are grateful to the editor (Samantha Weintraub-Leff) and two anonymous reviewers for insightful recommendations which improved the manuscript substantially.

Author contributions KJS, MRH, LF, and CAQ conceptualized and designed the research. Experiments were conducted 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.

Funding We would like to acknowledge the AmazonFACE program of the National Institute of Amazonian Research (INPA), which was funded by the Inter-American Development Bank (IADB) through a technical cooperation agreement with the MCTI Grant BR-T1284, CAPES Grant 23038.007722/2014-77, CAPES-INPA Grant 88881.154644/2017-01, and by FAPEAM Grant 2649/2014. Additional funding was provided by the CNPq Grant CNPq/LBA 68/2013. KS was supported by a CNPq/LBA 68/2013 PCI grant and by the AmazonFACE program with a CAPES scholarship Finance Code 001. LF was supported by the European Union's Horizon 2020 research and innovation program under the Marie Sklodovska-Curie grant agreement No 847,693 (REWIRE).

Data availability The soil data is publicly available on https://doi.org/10.5281/zenodo.7239239.

Declarations

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

References

Allison SD, Nielsen C, Hughes RF (2006) Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biol Biochem 38:1537–1544. https://doi.org/10.1016/j.soilbio.2005.11.008

Allison SD, Weintraub MN, Gartner TB, Waldrop MP (2011) Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. In: Shukla G, Varma A (eds) Soil enzymology. Springer, pp 229–243

Araújo AC, Nobre AD, Kruijt B, Elbers JA, Dallarosa R, Stefani P, Von Randow C, Manzi AO, Culf AD, Gash JHC, Valentini R, Kabat P (2002) Comparative measurements of carbon dioxide fluxes from two nearby towers in a central amazonian rainforest: the Manaus LBA site. J Geophys Res 107:1–20. https://doi.org/10.1029/2001JD000676

Baldrian P (2009) Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ 55:370–378. https://doi.org/10.17221/134/2009-pse

Bartoń K (2022) MuMIn: multi-model inference. https://cran.r-project.org/package=MuMIn

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234. https://doi.org/10.1016/j.soilbio.2012.11.009

Buscardo E, Geml J, Schmidt SK, Freitas H, Da Cunha HB, Nagy L (2018) Spatio-temporal dynamics of soil bacterial communities as a function of Amazon forest phenology. Sci Rep 8:1–13. https://doi.org/10.1038/s41598-018-22380-z

Camenzind T, Hättenschwiler S, Treseder KK, Lehmann A, Rillig MC (2018) Nutrient limitation of soil microbial processes in tropical forests. Ecol Monogr 88:4–21. https://doi.org/10.1002/ecm.1279

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW, Danovaro R, Foreman CM, Huisman J, Hutchins DA, Jansson JK, Karl DM, Koskella B, Mark Welch DB, Martiny JBH, Moran MA, Orphan VJ, Reay DS, Remais JV, Rich VI, Singh BK, Stein LY, Stewart FJ, Sullivan MB, van Oppen MJH, Weaver SC, Webb EA, Webster NS (2019) Scientists' warning to humanity: microorganisms and climate change. Nat Rev Microbiol 17:569–586. https://doi.org/10.1038/s41579-019-0222-5

Chave J, Navarrete D, Almeida S, Álvarez E, Aragão LEOC, Bonal D, Châtelet P, Silva-Espejo JE, Goret J-Y, von Hildebrand P, Jiménez E, Patiño S, Peñuela MC, Phillips OL, Stevenson P, Malhi Y (2010) Regional and seasonal patterns of litterfall in tropical South

America. Biogeosciences 7:43–55. https://doi.org/10.5194/bg-7-43-2010

Cordeiro AL, Norby RJ, Andersen KM, Valverde-Barrantes O, Fuchslueger L, Oblitas E, Hartley IP, Iversen CM, Gonçalves NB, Takeshi B, Lapola DM, Quesada CA (2020) Fine-root dynamics vary with soil depth and precipitation in a low-nutrient tropical forest in the Central Amazonia. Plant Environ Interact 1(1):3–16. https://doi.org/10.1002/pei3.10010

Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E (2013) The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob Chang Biol 19:988–995. https://doi.org/10.1111/gcb.12113

Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix ML, Wall DH, Parton WJ (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat Geosci 8:776–779. https://doi.org/10.1038/ngeo2520

German DP, Weintraub MN, Lauber S, Rinkes CL, Allison ZL (2011) SD optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

Guilbeault-Mayers X, Turner BL, Laliberté E (2020) Greater root phosphatase activity of tropical trees at low phosphorus despite strong variation among species. Ecology 101:1–9. https://doi.org/10.1002/ecy.3090

Helfenstein J, Jegminat J, McLaren TI, Frossard E (2018) Soil solution phosphorus turnover: derivation, interpretation, and insights from a global compilation of isotope exchange kinetic studies. Biogeosciences 15:105–114. https://doi.org/10.5194/bg-15-105-2018

Hilker T, Lyapustin AI, Tucker CJ, Hall FG, Myneni RB, Wang Y, Bi J, Mendes de Moura Y, Sellers PJ (2014) Vegetation dynamics and rainfall sensitivity of the Amazon. Proc Natl Acad Sci 111:16041–16046. https://doi.org/10.1073/pnas.1404870111

Isles PDF (2020) The misuse of ratios in ecological stoichiometry. Ecology. https://doi.org/10.1002/ecy.3153

Lapola DM, Norby RJ (2014) AmazonFACE: assessing the effects of increased atmospheric CO2 on the ecology and resilience of the Amazon forest. Science plan and implementation strategy. AmazonFace

Lucas Y, Luizao FJ, Chauvel A, Rouiller J, Nahon D (1993) The relation between biological activity of the rain forest and mineral composition of soils. Sci (80-) 260:521–523. https://doi.org/10.1126/science.260.5107.521

Luizao RCC, Luizao FJ, Paiva RQ, Monteiro TF, Sousa LSLS, Kruijt B, Luizão RCC, Luizão FJ, Paiva RQ, Monteiro TF, Sousa LSLS, Kruijt B (2004) Variation of carbon and nitrogen cycling processes along a topographic gradient in a central amazonian forest. Glob Chang Biol 10:592–600. https://doi.org/10.1111/j.1529-8817.2003.00757.x

Luo L, Meng H, Gu JD (2017) Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J Environ Manage 197:539–549. https://doi.org/10.1016/j.jenvman.2017.04.023

Malik AA, Bouskill NJ (2022) Drought impacts on microbial trait distribution and feedback to soil carbon cycling. Funct Ecol. https://doi.org/10.1111/1365-2435.14010

Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol 193:696–704. https://doi.org/10.1111/j.1469-8137.2011.03967.x

Martins NP, Fuchslueger L, Fleischer K, Andersen KM, Assis RL, Baccaro FB, Camargo PB, Cordeiro AL, Grandis A, Hartley IP, Hofhansl F, Lugli LF, Lapola DM, Menezes JG, Norby RJ, Rammig A, Rosa JS, Schaap KJ, Takeshi B, Valverde-Barrantes OJ, Quesada CA (2021) Fine roots stimulate nutrient release during early stages of leaf litter decomposition in a Central Amazon rainforest. Plant Soil. https://doi.org/10.1007/s11104-021-05148-9

Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol Biochem 33:1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7

Menge DNL, Pacala SW, Hedin LO (2009) Emergence and maintenance of nutrient limitation over multiple timescales in terrestrial ecosystems. Am Nat 173:164–175. https://doi.org/10.1086/595749

Moorhead DL, Sinsabaugh RL, Hill BH, Weintraub MN (2016) Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biol Biochem 93:1–7. https://doi.org/10.1016/j.soilbio.2015.10.019

Mori T (2020) Does ecoenzymatic stoichiometry really determine microbial nutrient limitations? Soil Biol Biochem 146:107816. https://doi.org/10.1016/j.soilbio.2020.107816

Mori T, Aoyagi R, Kitayama K, Mo J (2021) Does the ratio of β-1,4-glucosidase to β-1,4-N-acetylglucosaminidase indicate the relative resource allocation of soil microbes to C and N acquisition? Soil Biol Biochem 160:108363. https://doi.org/10.1016/j.soilbio.2021.108363

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC (2014) Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. Ecol Lett 17:1282–1289. https://doi.org/10.1111/ele.12335

Nottingham AT, Turner BL, Whitaker J, Ostle N, Bardgett RD, McNamara NP, Salinas N, Meir P (2016) Temperature sensitivity of soil enzymes along an elevation gradient in the peruvian Andes. Biogeochemistry 127:217–230. https://doi.org/10.1007/s10533-015-0176-2

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Dep Agric Circ 939:1–19

Pereira IS, do Nascimento HEM, Vicari MB, Disney M, DeLucia EH, Domingues T, Kruijt B, Lapola D, Meir P, Norby RJ, Ometto JPHB, Quesada CA, Rammig A, Hofhansl F, (2019) Performance of laser-based electronic devices for structural analysis of amazonian terra-firme forests. Remote Sens. https://doi.org/10.3390/rs11050510

Pinheiro J, Bates D, R Core Team (2022) nlme: linear and nonlinear mixed effects models. https://CRAN.R-project.org/package=nlme

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, Santos a JB, Hodnett MG, Herrera R, Luizão FJ, Arneth A, Lloyd G, Dezzeo N, Hilke I, Kuhlmann I, Raessler M, Brand W, Geilmann H, Filho JOM, Carvalho FP, Filho RNA, Chaves JE, Cruz OF, Pimentel TP, Paiva R, (2010) Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. Biogeosciences 7:1515–1541. https://doi.org/10.5194/bg-7-1515-2010

R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rosinger C, Rousk J, Sandén H (2019) Can enzymatic stoichiometry be used to determine growth-limiting nutrients for microorganisms?—a critical assessment in two subtropical soils. Soil Biol Biochem 128:115–126. https://doi.org/10.1016/j.soilbio.2018.10.011

Ruan HH, Zou XM, Scatena FN, Zimmerman JK (2004) Asynchronous fluctuation of soil microbial biomass and plant litterfall in a tropical wet forest. Plant Soil 260:147–154. https://doi.org/10.1023/B:PLSO.0000030177.20951.94

Schaap KJ, Fuchslueger L, Hoosbeek MR, Hofhansl F, Martins NP, Valverde-Barrantes OJ, Hartley IP, Lugli LF, Quesada CA (2021) Litter inputs and phosphatase activity affect the temporal variability of organic phosphorus in a tropical forest soil in the Central Amazon. Plant Soil 469:423–441. https://doi.org/10.1007/s11104-021-05146-x

Singh JS, Raghubanshi AS, Singh RS, Srivastava SC (1989) Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. Nature 338:499–500. https://doi.org/10.1038/338499a0

Singh AK, Jiang XJ, Yang B, Wu J, Rai A, Chen C, Ahirwal J, Wang P, Liu W, Singh N (2020) Biological indicators affected by land use change, soil resource availability and seasonality in dry tropics. Ecol Indic 115:106369. https://doi.org/10.1016/j.ecolind.2020.106369

Sinsabaugh RL, Follstad Shah JJ (2012) Ecoenzymatic stoichiometry and ecological theory. Annu Rev Ecol Evol Syst 43:120913143848009. https://doi.org/10.1146/annurev-ecolsys-071112-124414

Skujiņš J, Burns RG (1976) Extracellular enzymes in soil. Crit Rev Microbiol 4:383–421. https://doi.org/10.3109/10408417609102304

Smith AP, Marín-Spiotta E, Balser T (2015) Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: a multiyear study. Glob Chang Biol 21:3532–3547. https://doi.org/10.1111/gcb.12947

Soong JL, Fuchslueger L, Marañon-Jimenez S, Torn MS, Janssens IA, Penuelas J, Richter A (2020) Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. Glob Chang Biol 26:1953–1961. https://doi.org/10.1111/gcb.14962

Tischer A, Blagodatskaya E, Hamer U (2014) 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 74:1–11. https://doi.org/10.1016/j.apsoil.2013.09.007

Turner BL, Wright SJ (2014) The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. Biogeochemistry 117:115–130. https://doi.org/10.1007/s10533-013-9848-y

Wang C, Lu X, Mori T, Mao Q, Zhou K, Zhou G, Nie Y, Mo J (2018) Responses of soil microbial community to continuous experimental nitrogen additions for 13 years in a nitrogen-rich tropical forest. Soil Biol Biochem 121:103–112. https://doi.org/10.1016/j.soilbio.2018.03.009

Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117:101–113. https://doi.org/10.1007/s10533-013-9849-x

Wu J, Albert LP, Lopes AP, Restrepo-Coupe N, Hayek M, Wiedemann KT, Guan K, Stark SC, Christoffersen B, Prohaska N, Tavares JV, Marostica S, Kobayashi H, Ferreira ML, Campos KS, da Silva R, Brando PM, Dye DG, Huxman TE, Huete AR, Nelson BW, Saleska SR (2016) Leaf development and demography explain photosynthetic seasonality in Amazon evergreen forests. Sci (80-) 351:972–976. https://doi.org/10.1126/science.aad5068

Wu J, Serbin SP, Xu X, Albert LP, Chen M, Meng R, Saleska SR, Rogers A (2017) The phenology of leaf quality and its within-canopy variation are essential for accurate modeling of photosynthesis in tropical evergreen forests. Glob Chang Biol 38:42–49. https://doi.org/10.1111/gcb.13725

Zechmeister-Boltenstern S, Keiblinger KM, Mooshammer M, Penuelas J, Richter A, Sardans J, Wanek W (2015) The application of ecological stoichiometry to plant-microbial-soil organic matter transformation. Ecol Monogr 85:133–155. https://doi.org/10.1890/14-0777.1

Zeglin LH, Kluber LA, Myrold DD (2013) The importance of amino sugar turnover to C and N cycling in organic horizons of old-growth Douglas-fir forest soils colonized by ectomycorrhizal mats. Biogeochemistry 112:679–693. https://doi.org/10.1007/s10533-012-9746-8