



Carbon, nitrogen and phosphorous contents, related enzyme activities and organic matter fractions of litter and soil in a *terra firme* forest in Central Amazonia

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Abstract

The response of lowland tropical forest on highly weathered P-limited soils to changes in atmospheric composition is likely to be regulated by P-availability from litter and soil. Our aim was to gain insight into possible mechanisms that may affect P-availability and C sequestration. We collected litter and soil samples along a transect in Central Amazonia. We examined litter and soil properties, determined C, N, and P contents and performed density fractionation to obtain organic-C, -N and -P fractions. To assess microbial demand and mineralization, we analysed C, N and P associated enzyme activities. The litter layer has an estimated turnover time of about one year or less and C, N and P contents were much smaller than in the mineral soil. Total soil C, N and P decreased with depth, while P was depleted relatively more. Most organic-C, -N and -P is stabilized by adsorption onto mineral surfaces. Non-adsorbed organic matter fractions were depleted of P. Nearly all organic-P appeared to be present in the mineral-associated fractions from where it, despite high phosphatase activities, appears not to be available for mineralization. Enzyme activities revealed that microbial C-demand in litter is higher than in soil, while P-demand was relatively high in soil and increased with depth. Hypothesized higher phosphatase activity under increasing atmospheric CO₂ concentrations may probably not alleviate P-limitation in these *terra firme* forests due to the limited availability of mineralizable organic-P.

Keywords Soil phosphorus · Litter and soil C:N:P stoichiometry · Organic matter fractionation · Soil enzyme activity · Nutrient limitation · Ferralsol

Introduction

The future response of lowland tropical forest growing on highly weathered P-limited soil may depend on the linkage between C, N and P cycling in litter and soil (Cleveland et al. 2011; Wieder et al. 2015). To gain insight into possible mechanisms that may affect P-availability and C sequestration, we collected litter and soil samples at 10 locations along a transect in Central Amazonia and examined relations between litter and soil properties. P-availability

and C sequestration may be affected by how organic matter is protected against mineralisation by several organic matter stabilization mechanisms (Von Lützw et al. 2006). Litter and the free light soil organic matter fraction are only protected by their own recalcitrance and are expected to be relatively small in this high turnover environment. The size of the physically protected occluded light fraction depends on pedogenic aggregate formation and is expected to be of intermediate size (Six et al. 2002). We expect that most organic matter is protected by adsorption onto mineral surfaces, which defers mineralization and therefore limits P-availability, but on the other hand, contributes to the long-term sequestration of C.

Next to stabilisation mechanisms, mineralisation and P-availability are also affected by extracellular enzyme (EE) activities. High tree species diversity may cause differences in litter quality along the transect which may affect associated EE activities in litter and soil (Sinsabaugh et al. 2002; Hobbie 2015; Cabugao et al. 2017). Relatively low N or P contents in

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litter and soil are expected to result in increased N- or P-related EE activities (Olander et al. 2000; Allison et al. 2005, 2011).

Furthermore, for C, N and P contents, organic matter stabilisation mechanisms, associated EE activities and C sequestration we expect to see a clear depth differentiation between aboveground litter and two mineral soil increments of 0–10 and 10–20 cm depth.

We hypothesize that due to the expected high litter turnover rates, C, N and P contents of litter are lower than of both soil increments. Moreover, due to uptake and adsorption of P we hypothesize C:P and N:P ratios to increase with depth. Based on soil information from nearby locations (Chauvel et al. 1987; Quesada et al. 2010), we expect high clay and Fe- and Al-hydroxides percentages for the mineral soil. Due to the large mineral surfaces facilitated by these clays and hydroxides, we hypothesize the mineral-associated heavy fraction to be the largest organic matter fraction, followed by the occluded and free light fractions, respectively.

Due to the expected P-limitation, we hypothesize phosphatase activities to be higher than C- and N-related EE activities. Moreover, because P-availability in litter and fresh soil organic matter is expected to be higher, we hypothesize phosphatase activities to increase with depth. Expected high phosphatase activities may deplete organic-P and subsequently mineralized-P may either be taken up by microbes and plants or become adsorbed onto Fe-hydroxides. We therefore postulate the P content of the occluded and free light organic matter fractions to be relatively small as compared to the mineral-associated P-fraction. This small mineralizable organic P-fraction, as a source of available P, may limit the response of this *terra firme* forest to environmental changes (Goll et al. 2018).

Methods

Litter and soil sampling

Samples were collected along the North–South transect in the Cuieiras Reserve of the Instituto Nacional de Pesquisas da Amazônia (INPA) which is located about 60 km NNW of Manaus, Amazonas (Andreae et al. 2002). This reserve has an area of 22,735 ha, is surrounded by a vast area of pristine tropical rainforest and is thought to be completely undisturbed due to the distance to rivers and difficulty of access. These old-growth tropical forests, locally known as *terra firme* forests, are located on plateaus with nutrient poor clay rich soils and were classified as Geric Ferralsols (Quesada et al. 2010). Average annual rainfall is about 2400 mm with the months November to March being relatively wet and May to September relatively dry (Araújo et al. 2002). The average temperature range is 26 °C (April) to 28 °C (September).

The sampled Ferralsols were formed in weathered sediments of the Barreiras formation which consists of erosion

products of the Guiana Shield (Chauvel et al. 1987; Hoorn et al. 2010). Due to repeated cycles of weathering, erosion and sedimentation, these parent materials are very poor in nutrients (P, Ca, K) and consist of kaolinite (> 80%), quartz (10%), gibbsite (5–8%) and Fe-hydrates (2%). The landscape is dissected by the hydrological system that drains towards the Rio Negro, leaving plateaus with Ferralsols, slopes with predominantly Acrisols and valley bottoms with Podzols (Chauvel et al. 1987). Quesada et al. (2011) estimated the area covered by Ferralsols across the Amazon Basin to be 31.6%.

Sampling took place at 10 locations along the existing ZF2 permanent-plot North–South transect at 15 m intervals in December of 2014. Litter samples were collected by placing a PVC ring with an inner diameter of 0.19 m on the forest floor, vertically cutting the litter along the inside of the ring while pressing the ring downwards until reaching the mineral soil and subsequently collecting the litter from within the ring while taking care not to include mineral soil. After the litter was collected, two 100 cm³ metal rings (Eijkelkamp) were vertically inserted and carefully excavated. Next, the mini-pit was further excavated to a level surface at 10 cm depth and again two rings were inserted and excavated to obtain deeper samples. The contents of each ring were emptied in zip-lock bags and labelled. All samples were placed in a cooler box for transport to the laboratory.

Litter and soil analysis

Representative subsamples of litter and soil were dried at 60 and 105 °C, respectively, to determine moisture content and to calculate total dry weight of each sample. Litter weight was expressed as g per m² and soil bulk density as g per cm³.

After removal of organic matter from subsamples, the particle size distribution was measured by laser diffraction (Coulter LS230 Grain Sizer)(Buurman et al. 1996). No carbonates were present in the soil. Soil pH was measured with a pH meter (Orion 701A) in a 1 M KCl solution suspension.

Sample pre-treatment for total organic C and N consisted of air-drying subsamples, crushing aggregates, removal of roots by hand and grinding. Homogenized subsamples were measured with an CN element analyser (Interscience EA 1108). For total P analysis, samples were digested by subsequent additions of a selenium-sulphuric acid mixture and peroxide while heated to 330 °C (Gerhardt Kjeldatherm digestion system)(Novozamsky et al. 1983). After dilution of the digest, P was determined colourimetrically (spectrophotometer Mechatronics Starrcol SC-60-S at 720 nm).

Soil density fractionation

Subsamples were subjected to density fractionation to obtain the following organic matter fractions: free light fraction (fLF), occluded light fraction (oLF) and mineral-associated heavy

fraction (maHF)(Six et al. 2002). Briefly, 10 g air dried soil (<2 mm) was shaken in a centrifuge tube with 50 ml sodium polytungstate ($3\text{Na}_2\text{WO}_4 \cdot 9\text{WO}_3 \cdot x\text{H}_2\text{O}$; SPT) solution of density 1.6 g cm^{-3} . The samples were centrifuged for 23 min at 4500 rpm (5900 g) (MSE Mistral 6000). The supernatant was poured over a Büchner funnel with a $0.7 \mu\text{m}$ Whatman GF/F glass filter using a Millipore vacuum filtration unit (Millipore, Bedford, MA). The free light fraction was rinsed off the filter into a glass beaker and oven dried at 40°C . Next, SPT solution was added to the residual soil material and shaken by hand to bring the precipitate into solution. An ultrasonic probe was used for 5 min at low intensity (6000 kJ) to break the soil aggregates and to disperse the occluded light fraction. The suspension was centrifuged for 23 min at 4500 rpm. The centrifuged tubes were decanted over a Büchner funnel with a $0.7 \mu\text{m}$ Whatman GF/F glass filter. The oLF fraction was rinsed off the filter into a glass beaker and oven dried at 40°C . The remaining precipitate, the maHF fraction, was rinsed thoroughly to remove all SPT. Total C, N and P contents of each fraction were determined similarly as for the whole soil samples.

Extracellular enzyme analysis

Enzyme activities of several hydrolytic enzymes related to C, N and P turnover in litter and soil were analysed (Table 1). Cellobiohydrolase (CB) catalyses the hydrolysis of cellulose into smaller polymers, while β -xylosidase (BX) catalyses the breakdown of hemicellulose. The sugars formed by both processes can be further broken down into monosaccharides (glucose) which is catalysed by β -glucosidase (BG) (Dick 2011). Leucine aminopeptidase (LAP) catalyses the hydrolysis of leucine and other hydrophobic amino acids from polypeptides and is used as an indicator for peptidase (i.e. protease, proteinase) activity (Sinsabaugh et al. 2008). Acid phosphatase (AP) catalyses the mineralization of phosphorus groups from organic matter.

Potential EE activities were assayed based on several method descriptions (Marx et al. 2001; Saiya-Cork et al.

2002; Wallenstein et al. 2008; DeForest 2009; German et al. 2011a, b, German et al. 2012). We used 4-Methylumbelliferone (MUB) and 7-Amino-4-methylcoumarin (AMC) as fluorescent compounds in combination with several substrates (Table 1). In short, 0.50 or 1.00 g dry weigh equivalent of fresh litter or soil subsample was added to 125 ml 50 mM sodium acetate buffer (pH 5.0) and sonicated for 60 s at low intensity (ca. 6000 kJ; VXC 130 Ultrasonic Processor, Sonics and Materials Inc., Connecticut, USA)(De Cesare et al. 2000) and continuously stirred upon further use. The following six combinations of solutions, with and without sample suspensions, were administered to 96-well microplates (3 replicates per sample): S1 250 μl buffer solution (Sodium acetate 50.0 mM pH 5.0); S2 50 μl buffer solution + 200 μl buffered sample suspension; S3 50 μl MUB- or AMC-standard series (0.25–50.0 μM) + 200 μl buffer solution; S4 50 μl MUB- or AMC-standard series (5.0–50.0 μM) + 200 μl buffered sample; S5 50 μl MUB- or AMC-substrate (200 μM) + 200 μl buffer solution; S6 50 μl MUB- or AMC-substrate (200 μM) + 200 μl buffered sample suspension. A FLUOstar Optima Fluorescence meter with combination optics (BMG LABTECH, Offenburg, Germany) was used to measure fluorescence (fluoS1–fluoS6) from each well at 5 min intervals for 1 h at 30°C with excitation at 360 and emission at 460 nm. Litter and soil particles may decrease the measured fluorescence by quenching. The difference in fluorescence between solutions S3 and S4, i.e. fluoS4–fluoS3, provided the magnitude of the quench for a specific sample.

All calculations were made with blank corrected values, i.e. fluorescence data of S2–S6 were corrected for values obtained for S1. Regression lines were fitted to the fluorescence data yielding rates of fluorescence increase per well. The average emission and quench coefficients were calculated based on series of averaged standard concentrations of S3 and S4 according to (German et al. 2011a, b, German et al. 2012)

$$\text{emission coefficient}(\text{fluorescence nmol}^{-1}) = \frac{\text{slope fluoS3} \left(\frac{\text{fluorescence nmol}^{-1}}{\text{ml}} \right)}{\text{volume standard}(\text{ml})}$$

Table 1 Assayed enzymes and corresponding substrates

Enzyme	Substrate	Substrate code	EC number	
Cellobiohydrolase	CB	MUB- β -D-cellobioside	Sigma M 6018	3.2.1.91
β -xylosidase	BX	MUB- β -D-xylopyranoside	Sigma M 7008	3.2.1.37
β -glucosidase	BG	MUB- β -D-glucopyranoside	Sigma M 3633	3.2.1.21
Leucine aminopeptidase	LAP	L-Leucine-AMC	Sigma L 2145	3.4.11.1
Acid Phosphatase	AP	MUB-phosphate salt	Sigma M 8168	3.1.3.2

with, in our case, a *volume standard* of 0.050 ml; and

$$\text{quench coefficient} = \frac{\text{slope fluo}S4}{\text{slope fluo}S3}$$

The net fluorescence per interval was calculated as net fluorescence = $\left(\frac{\text{fluoS6}-\text{fluoS2}}{\text{quench coefficient}}\right) - \text{fluoS5}$ and the enzyme activity was calculated with the slope of the values of the measurement intervals over time as

$$\text{activity}(\text{nmol g}^{-1}\text{h}^{-1}) = \frac{\text{slope net fluorescence}(\text{h}^{-1}) \times \text{volume initial buffer}(\text{ml})}{\text{emission coef} \times \text{volume sample in S6}(\text{ml}) \times \text{sample weight}(\text{g})}$$

with, an initial buffer volume of 125 ml, sample volume in S6 of 0.200 ml and sample weight of the initial ~0.50 or ~1.00 g dry weigh equivalent of litter or soil, respectively. Finally, activities were expressed per gram of organic matter.

Statistical analysis

All data of 10 sample locations were plotted (histograms and Q–Q plots) and examined for outliers and normality (Field 2014). No data needed to be excluded or transformed. Pearson coefficients were calculated with IBM SPSS Statistics 25.

Results

Soil texture, bulk density and pH

The average differential particle size distribution showed two distinct peaks, one between 0.3 and 1.0 μm and another one between 1.5 and 3.0 μm (Fig. 1). Using the standard texture classes, i.e. clay (<2 μm) and silt (2–50 μm), divided the 1.5–3.0 μm peak into two classes. Instead, texture classes

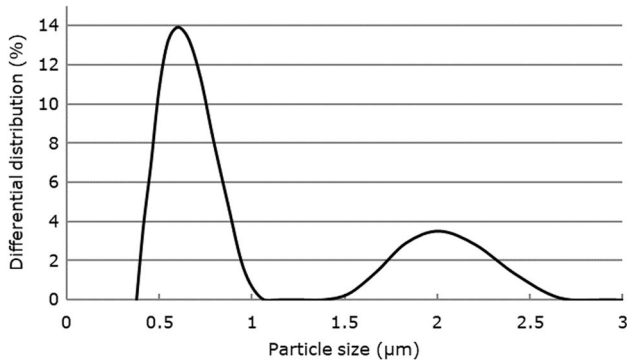


Fig. 1 Average ($n=10$) differential particle size distribution of the 0–10 cm soil depth increment

“fine clay” (<1 μm) and “fine silt” (1–3 μm) better represent both peaks. 86 and 90% of the soil particles at depth increments 0–10 and 10–20 cm were smaller than 1 μm (Table 2). At both depths the remainder of the particles classified as fine silt and no particles larger 3 μm were observed. The well aggregated soil structure yielded low soil bulk densities of 0.65 at 0–10 cm and 0.88 g cm^{-3} at 10–20 cm. Average soil pH was 4.6 for both depth increments.

Litter and soil C, N and P contents

The mean above ground litter weight was 748 g m^{-2} (se 76) and consisted primary of L (recent and almost undecomposed litter) and F (fragmented and partly decomposed) litter layers, while an H (humified) litter layer was either absent or mixed into the mineral soil. Average C contents of the litter, 0–10 and 10–20 cm depth compartments were 328, 4068 and 2797 g m^{-2} , respectively (Fig. 2). Also, N and P contents were highest at 0–10, followed by 10–20 cm and lowest in the litter.

C:N ratios were highest in the litter and decreased with increasing soil depth. However, C:P was high at 10–20, intermediate in the litter and low at 0–10 cm. N:P was also high at 10–20, but about equal in litter and at 0–10 cm.

Soil organic matter fractions C, N and P

Most C, N and P was found in the mineral-associated heavy fractions, followed by the free light and the occluded light fractions at both depths (Fig. 3). All C:N ratios of the light fractions are larger than 20, but C:N ratios of the mineral-associated fractions were on average 10.3 and 8.3 at 0–10 and 10–20 cm depth. P contents of both light fractions were very small as compared to C and N at both depths. C:P and N:P ratios of the light fractions were therefore very large (not shown). By far most P was found in the mineral-associated fractions.

Table 2 Soil texture, bulk density and pH of depth increments 0–10 and 10–20 cm

	0–10	($n=20$)	10–20	($n=20$)
	Mean	Se	Mean	Se
Fine clay % (<1 μm)	86.3	0.4	90.0	0.2
Clay % (<2 μm)	95.0	0.3	96.3	0.1
Fine silt % (1–3 μm)	13.7	0.4	10.1	0.2
Silt % (2–16 μm)	5.0	0.3	3.7	0.1
Bulk density (g cm^{-3})	0.65	0.02	0.88	0.01
pH (KCl)	4.57	0.03	4.59	0.04

Fig. 2 Litter and soil C, N and P contents and stoichiometric ratios (element contents in soil (g m^{-2}) per 0.1 m depth increment)

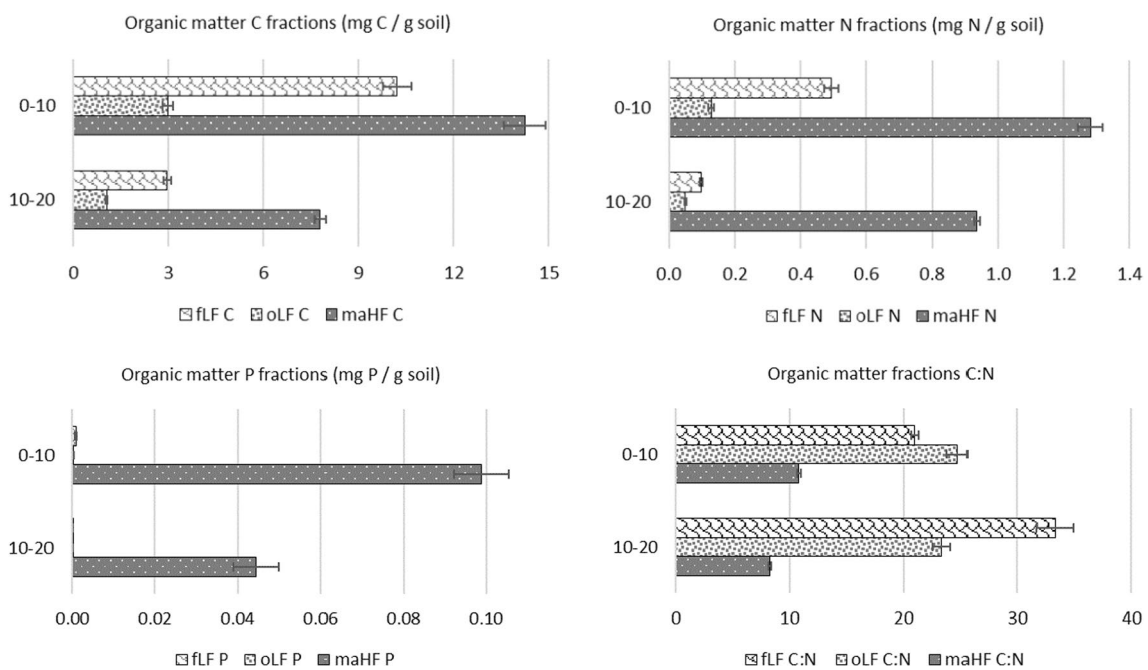
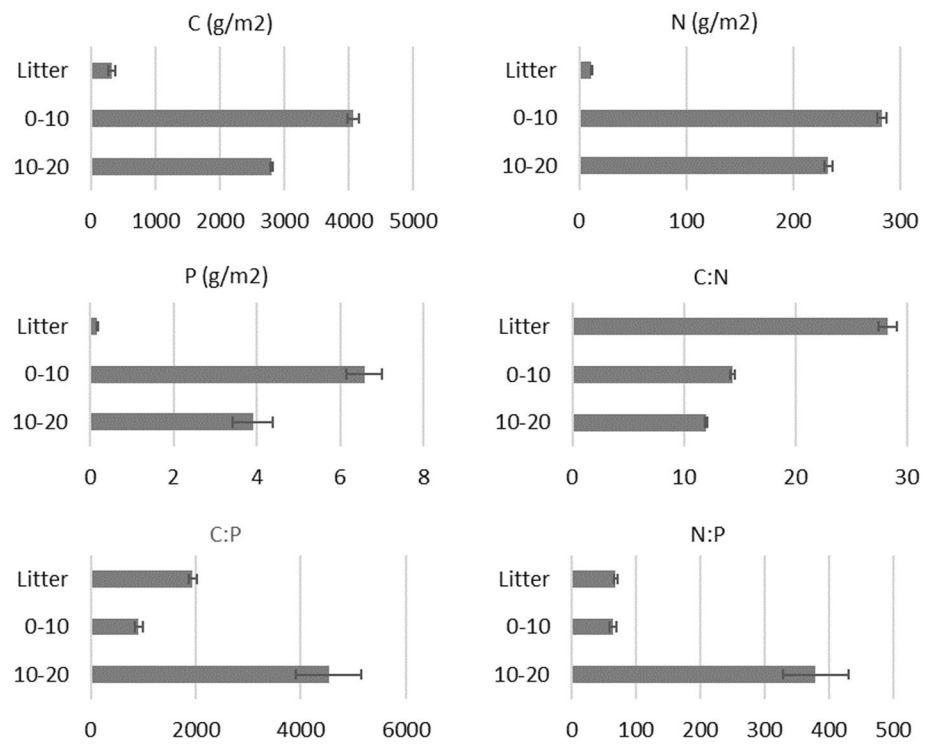


Fig. 3 Soil organic matter C, N and P contents of the free light (fLF), occluded light (oLF) and mineral associated heavy (maHF) fractions (soil depth 0–10 and 10–20 cm)

Enzyme activities in litter and soil

BG activities were 4.4 and 2.9 times higher in litter than in soil at 0–10 and 10–20 cm (Table 3). CB and BX activities in litter were also higher than at 0–10 cm, but lower

than at 10–20 cm. LAP activities were about equal with depth. AP activities were much higher as compared to the other enzyme activities, and showed increasing activities with depth, i.e. 10–20 > 0–10 > litter. BG:LAP ratios in

Table 3 Extracellular enzyme activities in litter and soil at depth increments 0–10 and 10–20 cm

	Litter Mean	(n = 10) Se	0–10 Mean	(n = 20) Se	10–20 Mean	(n = 20) Se
CB (nmol g ⁻¹ h ⁻¹)*	143.1	21.3	69.1	10.0	312.3	73.1
BX (nmol g ⁻¹ h ⁻¹)*	262.9	28.5	176.7	30.1	393.8	88.8
BG (nmol g ⁻¹ h ⁻¹)*	1630.3	181.3	368.5	69.0	567.8	94.3
LAP (nmol g ⁻¹ h ⁻¹)*	630.6	128.1	593.2	85.9	726.9	78.5
AP (nmol g ⁻¹ h ⁻¹)*	8188.6	467.8	17,174.7	1420.7	38,054.9	3078.3
BG:LAP	3.6	0.6	0.6	0.1	0.9	0.2
BG:AP	0.200	0.020	0.020	0.002	0.015	0.002
LAP:AP	0.078	0.016	0.034	0.003	0.022	0.003

*activities per gram of organic matter

litter were higher than at 0–10 and 10–20 cm. BG:AP and LAP:AP ratios decreased with depth.

Discussion

Soil texture, bulk density and pH

The sampled Ferralsols are characterized by a unique combination of high clay contents and low bulk densities. Chauvel et al. (1987), Luizão et al. (2004) and Quesada et al. (2010) reported topsoil clay percentages of 65–75, 65 and 68%, respectively, for Ferralsols in Central Amazonia. These percentages are lower than our mean of 95%, which is probably due to methodological differences between the sedimentation-pipette method used for the cited studies and the laser-diffraction method used in this study (Muggler et al. 1997). The distinct particle size distribution of our samples may be explained by its mineralogical composition, where the large peak (0.3–1.0 μm ; Fig. 1) primarily represents kaolinite and the smaller peak (1.5–3.0 μm) primarily represents Fe- and Al-hydroxides and quartz (Chauvel et al. 1991). Observed soil pH values were well within range of values reported by Chauvel et al. (1987), Correa et al. (1989) and Quesada et al. (2010).

The relatively low bulk densities may be explained by the sequence of soil forming processes as described by Chauvel et al. (1987). The combination of removal of material through clay neof ormation and quartz dissolution and the concurrent reinforcement of the matrix structure by the redistribution of Fe-(hydr-)oxides around contact points, may allow, with sufficient time, the formation of a stable well-aggregated soil with low bulk density. Addition of SOM to the topsoil and biological activity may explain the lower bulk density at 0–10 as compared to 10–20 cm soil depth.

Litter C, N and P

At a comparable site, Klinge et al. (1968a, b), Klinge et al. (1968a, b) reported a mean litterfall dry weight of 730 g m⁻² with C, N and P contents of 379, 10.6 and 0.21 g m⁻², respectively. These values for litterfall are about similar to our litter stock data, i.e. 1.15, 0.92 and 1.20 times the C, N and P litter stocks. At another nearby site with a comparable Ferralsol, Luizao (1989) recorded an 3-year average annual litter fall of 825 g m⁻² with mean annual litter C, N and P inputs of 388, 15.1 and 0.31 g m⁻² y⁻¹, respectively. These inputs are 1.18, 1.30 and 1.78 times the average C, N and P litter stocks of our study. With the annual litter input being equal or slightly larger than the litter pool, we may derive that the litter layer turnover time is about one year or less. Based on inputs and stocks we infer that the loss rate of C, N and P from the litter layer is in the order P >> N > C.

Soil C, N and P contents

Soil C, N and P contents decreased with depth which coincides with field observations in which the A horizon (darker colour, many roots, bioturbation) is primarily confined to the top 10 cm of the mineral soil. C:N ratios decreased as expected with depth in the order of litter, 0–10 and 10–20 cm and are well within range with studies at comparable sites (Luizão et al. 2004; Quesada et al. 2010). Plant tissue with relatively high C:N ratios is increasingly decomposed with depth causing the C:N ratios to approach values typical of microbial tissue at 10–20 cm. High C:P and N:P ratios at 10–20 cm are likely due to plant uptake of P after mineralization of organic-P (see below).

Soil organic matter fractions C, N and P

Most soil organic matter is chemically protected by adsorption onto mineral surfaces (Fig. 3). This stabilization mechanism defers the mineralization of SOM and contributes to

the long term sequestration of C in these soils (Von Lützwow et al. 2006). The second largest fraction is the “unprotected” free light fraction, which includes fresh and recent above and belowground litter. Recalcitrance to decomposition of this litter depends largely on the inherited chemical structure of plant tissues. The occluded light fraction is the smallest fraction, which indicates that physical protection against decomposition is a relative minor mechanism in these soils (von Lützwow et al. 2007). Although minor, this physical protection, which is largely due to the formation of aggregates, is largely confined to the A horizon in the top 10 cm of the mineral soil.

For both soil depths C:N ratios of the light fractions were above 20 but decreased to around 10 for the mineral-associated fractions due to microbial decomposition of the initially plant derived litter. More striking are the low P contents of the light fractions at both soil depths. Both free and occluded light organic matter fractions are depleted of P which is probably due to active mining of P for uptake by microbial communities and plant roots. Nearly all P appeared to be present in the mineral-associated fractions from where it, despite a high demand (high AP activities), appears not to be available for mineralization (Soong et al. 2020).

Litter and soil enzyme activities

Cellobiohydrolase (CB) and β -xylosidase (BX) catalyze the microbial breakdown of respectively cellulose and hemicellulose into sugars. The activities of CB and BX in litter were higher than in 0–10 cm soil but lower than in deeper soil. So, the demand for sugars is relatively high in the sub-soil, intermediate in litter and lower in the topsoil. However, for the final break down of sugars into glucose, β -glucosidase (BG) activities were 3 to 4 times higher in litter as compared to mineral soil at both depths suggesting that microbial communities in litter are more C-limited than in soil (Table 3).

The N-related enzyme (LAP) activity did not differ with depth. Phosphatase (AP) activities, however, are relatively low in litter and increase in soil with depth, suggesting relatively less P-limitation in litter and increasing P-limitation with depth in soil.

To be able to compare nutrient limitation at different sites it may be more informative to look at ratios of EE activities rather than to look at absolute values (Arnosti et al. 2014). The mean BG:AP ratio reported by Turner et al. (2014) in no-P-addition plots in a lowland tropical rainforest in central Panama was 0.039, which is higher than the mean BG:AP ratios of 0.020 (0–10) and 0.015 (10–20 cm) observed in our study. This difference indicates a relatively larger investment in the production of AP, which points at a more severe P-limitation in the mineral soil at our site. Weintraub et al. (2013) observed a mean soil BG:AP ratio of 0.22 in control plots of a litter manipulation experiment in a lowland

wet tropical forest in southwestern Costa Rica. Again, this ratio is higher as observed in this study which suggests relatively severe P-limitation at our site. Moreover, Waring et al. (2014) performed a meta-analysis of enzyme activities and found the lowest BG:AP ratios to be associated with old or acid soils that are P-limited. Our BG:AP ratios of 0.020 (0–10) and 0.015 (10–20 cm) are at the very low end of the “most weathered soil types” distinguished by Waring et al. (2014).

Plotting the C:N and C:P ratios of the EE activities on the vertical and horizontal axis yields a “Moorhead-graph” in which the length of a vector (connecting origin and plotted point) is a measure of microbial C-limitation and the angle of the vector indicates the degree of P- versus N-limitation (Moorhead et al. 2013, 2016). Vectors above the 1:1 line indicate relatively more P-limitation and vectors below the 1:1 line represent relative more N-limitation. We summed the C-related activities and plotted $(BG + BX + CB)/LAP$ versus $(BG + BX + CB)/AP$ (Fig. 4). The length of the litter vector is much larger than the length of the soil vectors indicating that the microbial C-demand in litter is larger than in mineral soil. This coincides with the negative relation of BG and C% in litter (Fig. 5a). Based on the ‘economic rule of enzyme production’ as described by Olander et al. (2000), Allison et al. (2005) and Allison et al. (2011), this inverse relationship shows that the microbial communities in the litter layer are C-limited. The positive relationships observed between N% and LAP and P% and AP (Fig. 5b and c), indicate that N and P are not limiting to the microbial communities in the litter. But still, in the Moorhead-graph (Fig. 4) the litter vector is well above the 1:1 line which indicates that microbial demand for P is larger than for N.

The 0–10 and 10–20 cm soil depth vectors have larger angles as compared to the litter vector (Fig. 4), indicating more pronounced P-limitation in the mineral soil. Of both soil compartments, 10–20 cm is more P-limited. This

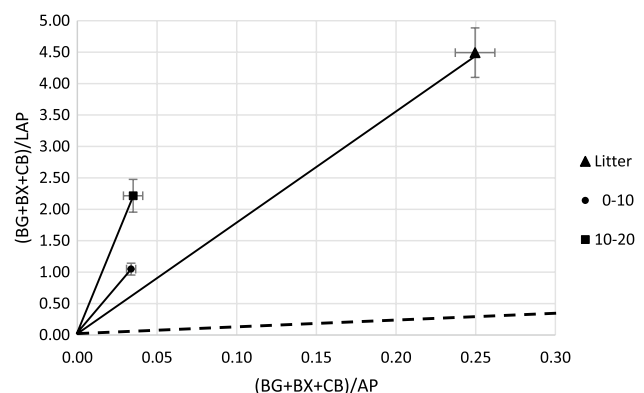
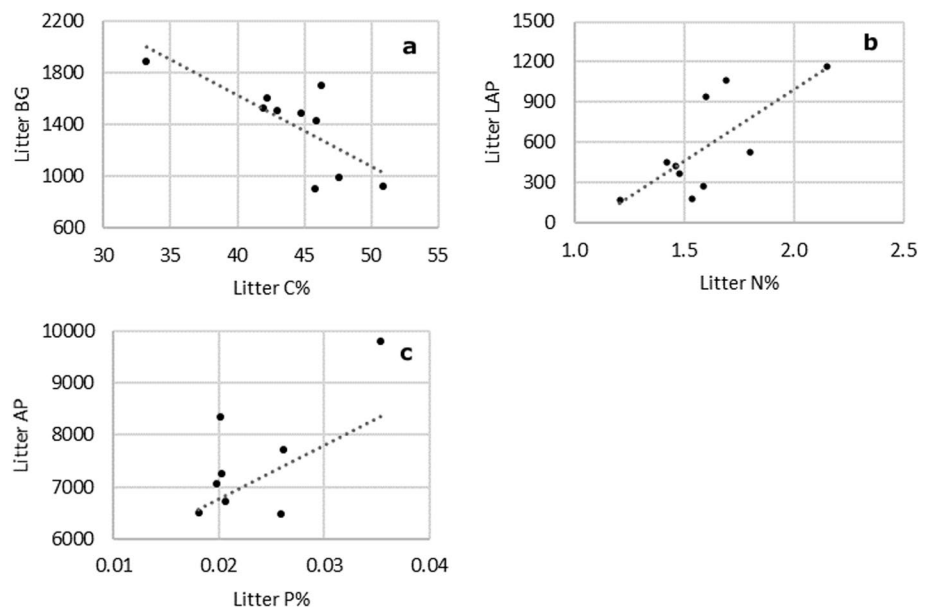


Fig. 4 Mean enzyme activity ratios in litter ($n=10$), soil 0–10 ($n=20$) and soil 10–20 cm ($n=20$). The dashed line depicts the 1:1 line

Fig. 5 a, b and c. Extracellular enzyme activities ($\text{nmol g}^{-1} \text{h}^{-1}$) in the litter layer related to C, N and P%. Pearson coefficients (pc) and significances (sig); 5a pc = -0.753 , sig = 0.012 ; 5b pc = 0.736 , sig = 0.015 ; 5c pc = 0.456 , sig = 0.185



increasing P-limitation with depth (litter-0–10–10–20 cm) was also reflected in the increasing AP activities with depth (Table 3).

Depth differentiation

Herrera et al. (1978) and Cerri et al. (1991) observed root activity to be concentrated near the surface of the mineral soil and argued nutrient uptake to occur mainly from the aboveground decomposing litter (Schubart et al. 1984). This coincides well with our own observations while sampling litter layers, i.e. many fine roots sticking upwards from the mineral soil into the decomposing litter. Plants invest in fine absorptive roots to increase P uptake (Lugli et al. 2020; Martins et al. 2021), which diminishes the leaching of mineralized-P from litter into the mineral soil and subsequent adsorption onto Fe-(hydr)-oxides in these Ferralsols.

Litter stocks of C, N and P were small as compared to total C, N and P content of mineral soil due to the estimated litter turnover times of about 1 year or less. C:N ratios decreased with depth as expected, but C:P and N:P ratios increased at 10–20 cm depth due to very low P contents. Most C, N and P is preserved in the mineral-associated organic matter fractions at both depths, while the free and occluded light organic matter fractions were depleted of P. These light organic-P fractions have likely largely been mineralized with the help of high phosphatase activities in mineral soil and subsequently produced inorganic-P has been taken up by plants and microbes.

Microbial communities in the litter layer are relatively more C-limited as compared to the mineral soil. Still, microbes in the litter layer invest in N- and P-related EE where the demand for P is clearly larger than for N.

Phosphatases may have also been produced in part by plant roots (Nannipieri et al. 2011; Martins et al. 2021). As discussed, the increase in phosphatase activity with depth resulted in depletion of non-adsorbed organic-P in the mineral soil.

Future response

A global quantification of experimental elevated CO_2 effects on biomass showed that CO_2 fertilization is primarily driven by soil N or P availability for 65 and 25% of global vegetation, respectively (Terrer et al. 2019). The response of lowland tropical forests to increasing atmospheric CO_2 concentrations is likely to be regulated by P-availability (Cleveland et al. 2011; Wieder et al. 2015). Based on an ensemble of 14 terrestrial ecosystem models including C, N and P, Fleischer et al. (2019) showed that P-availability reduces simulated CO_2 -induced biomass C growth by about 50% over 15 years as compared to estimates by only C or C and N models. Results suggested that the response among the P-enabled models depends on contrasting plant P use and acquisition strategies considered among the models.

Increasing atmospheric CO_2 concentration may alleviate P-limitation through the availability of extra photosynthetically obtained C (Hungate et al. 1997; Richardson et al. 2009; Hoosbeek 2016). The extra labile C may be used by microbial communities and plants to increase phosphatase production (Allison et al. 2011; Margalef et al. 2021) which enhances the mineralization of organic-P from litter and soil (Sinsabaugh et al. 1994; Turner et al. 2011). But will there be enough mineralizable litter and soil organic matter to meet the increased P-demand due to increased NPP under increased atmospheric CO_2 ? Litter turnover is one year or

less and therefore this possible P-supply is limited and will most likely not meet increased P-demand. Moreover, periods of drought may diminish litter mineralization and C-cycling which further limits P-availability (Goll et al. 2018).

Available soil organic-P, i.e. non-adsorbed organic-P, was found to be depleted at both soil depths in this study. Therefore, we postulate that increased phosphatase activity has no effect on P mineralization from these light fractions. Nearly all organic-P appeared to be present in the mineral-associated fractions from where it, despite high phosphatase activities, appears not to be available through enzymatic hydrolysis and subsequent mineralization. Therefore, these *terra firme* forests may show limited response to increasing atmospheric CO₂ concentration.

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Data availability All data will be available at the DANS-EASY repository (<https://easy.dans.knaw.nl>).

Declarations

Conflict of interests The authors declare no competing interests.

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References

- Allison SD, Weintraub MN, Gartner TB, Waldrop MP (2011) Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. *Soil Enzymol* 22(229):243
- Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol Biochem* 37(5):937–944
- Andreae MO, Artaxo P, Brandão C, Carswell FE, Ciccioli P, da Costa AL, Culf AD, Esteves JL, Gash JHC, Grace J, Kabat P, Lelieveld J, Malhi Y, Manzi AO, Meixner FX, Nobre AD, Nobre C, Ruivo MdLP, Silva-Dias MA, Stefani P, Valentini R, von Jouanne J, Waterloo MJ (2002) Biogeochemical cycling of carbon, water, energy, trace gases, and aerosols in Amazonia: the LBA-EUS-TACH experiments. *J Geophys Res Atmos* 107(D20):LBA 33 1-LBA333
- Araújo AC, Nobre AD, Kruijt B, Elbers JA, Dallarosa R, Stefani P, von Randow C, Manzi AO, Culf AD, Gash JHC, Valentini R, Kabat PC (2002) Comparative measurements of carbon dioxide fluxes from two nearby towers in a central Amazonian rainforest: the Manaus LBA site. *J Geophys Res Atmos* 107(D20):LBA 58 51-LBA5858
- Arnosti C, Bell C, Moorhead DL, Sinsabaugh RL, Steen AD, Stromberger M, Wallenstein M, Weintraub MN (2014) Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. *Biogeochemistry* 117(1):5–21
- Buurman P, Van Lagen B, Velthorst EJ (1996) Manual for soil and water analysis. Backhuys publishers, The Netherlands, Leiden
- Cabugao KG, Timm CM, Carrell AA, Childs J, Lu T-YS, Pelletier DA, Weston DJ, Norby RJ (2017) Root and rhizosphere bacterial phosphatase activity varies with tree species and soil phosphorus availability in Puerto Rico tropical forest. *Front Plant Sci* 8:1834
- Cerri CC, Volkoff B, Andreaux F (1991) Nature and behaviour of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus. *Forest Ecol Manag* 38(3):247–257
- Chauvel A, Lucas Y, Boulet R (1987) On the genesis of the soil mantle of the region of Manaus, Central Amazonia Brazil. *Experientia* 43(3):234–241
- Chauvel A, Grimaldi M, Tessier D (1991) Changes in soil pore-space distribution following deforestation and revegetation: an example from the Central Amazon Basin Brazil. *For Ecol Manag* 38(3):259–271
- Cleveland CC, Townsend AR, Taylor P, Alvarez-Clare S, Bustamante MM, Chuyong G, Dobrowski SZ, Grierson P, Harms KE, Houlton BZ (2011) Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecol Lett* 14(9):939–947
- Correa JC, Reichardt K (1989) The spatial variability of Amazonian soils under natural forest and pasture. *GeoJournal* 19(4):423–427
- De Cesare F, Garzillo AMV, Buonocore V, Badalucco L (2000) Use of sonication for measuring acid phosphatase activity in soil. *Soil Biol Biochem* 32(6):825–832
- DeForest JL (2009) The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. *Soil Biol Biochem* 41(6):1180–1186
- Dick RP (2011) Methods of soil enzymology. WI, Soil Science Society of America, Madison
- Field A (2014) Discovering statistics using IBM SPSS statistics. Sage Publications Ltd, London
- Fleischer K, Rammig A, De Kauwe MG, Walker AP, Domingues TF, Fuchslueger L, Garcia S, Goll DS, Grandis A, Jiang M, Haverd V, Hofhansl F, Holm JA, Kruijt B, Leung F, Medlyn BE, Mercado LM, Norby RJ, Pak B, von Randow C, Quesada CA, Schaap KJ, Valverde-Barrantes OJ, Wang Y-P, Yang X, ZaehleZhu SQ, Lapola DM (2019) Amazon forest response to CO₂ fertilization dependent on plant phosphorus acquisition. *Nat Geosci* 12(9):736–741
- German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes, ZL-Allison, SD, (2011a) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biol Biochem* 43(7):1387–1397
- German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison, SD (2012b) Corrigendum to “Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies.” *Soil Biol Biochem* 44(1):151

- Goll DS, Joetzjer E, Huang M, Ciais P (2018) Low phosphorus availability decreases susceptibility of tropical primary productivity to droughts. *Geophys Res Lett* 45(16):8231–8240
- Herrera R, Jordan C, Klinge H, Medina E (1978) Amazon ecosystems. Their structure and functioning with particular emphasis on nutrients. *Interciencia* 3(4):223–232
- Hobbie SE (2015) Plant species effects on nutrient cycling: revisiting litter feedbacks. *Trends Ecol Evol* 30(6):357–363
- Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330(6006):927–931
- Hoosbeek MR (2016) Elevated CO₂ increased phosphorous loss from decomposing litter and soil organic matter at two FACE experiments with trees. *Biogeochemistry* 127(1):89–97
- Hungate BA, Holland EA, Jackson RB, Chapin FS, Mooney HA, Field CB (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* 388(6642):576–579
- Klinge H, Rodrigues WA (1968a) Litter production in an area of Amazonian terra firme forest. Part I. Litter-fall, organic carbon and total nitrogen contents of litter. *Amazoniana* 1(4):287–302
- Klinge H, Rodrigues WA (1968b) Litter production in an area of Amazonian terra firme forest. Part II. Mineral nutrient content of the litter. *Amazoniana* 1(4):303–310
- Lugli LF, Andersen KM, Aragão LEOC, Cordeiro AL, Cunha HFV, Fuchsluger L, Meir P, Mercado LM, Oblitas E, Quesada CA, Rosa JS, Schaap KJ, Valverde-Barrantes O, Hartley IP (2020) Multiple phosphorus acquisition strategies adopted by fine roots in low-fertility soils in Central Amazonia. *Plant Soil* 450(1):49–63
- Luizao FJ (1989) Litter production and mineral element input to the forest floor in a Central Amazonian forest. *GeoJournal* 19(4):407–417
- Luizão RCC, Luizão FJ, Paiva RQ, Sousa LS, Kruijt B (2004) Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest. *Glob Change Biol* 10(5):592–600
- Margalef, O, Sardans, J, Maspons, J, Molowny-Horas, R, Fernández-Martínez, M, Janssens, IA, Richter, A, Ciais, P, Obersteiner, M, Peñuelas, J (2021). The effect of global change on soil phosphatase activity. *Global Change Biology* n/a(n/a).
- Martins NP, Fuchsluger 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* 469:287–303
- Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol Biochem* 33(12–13):1633–1640
- Moorhead D, Rinkes Z, Sinsabaugh R, Weintraub M (2013) Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. *Front Microbiol* 4:223
- Moorhead DL, Sinsabaugh RL, Hill BH, Weintraub MN (2016) Vector analysis of coenzyme activities reveal constraints on coupled C, N and P dynamics. *Soil Biol Biochem* 93:1–7
- Muggler CC, Pape T, Buurman P (1997) Laser grain-size determination in soil genetic studies 2. Clay content, clay formation, and aggregation in some Brazilian oxisols. *Soil Sci* 162(3):219–228
- Nannipieri P, Giagnoni L, Landi L, Renella G (2011). Role of phosphatase enzymes in soil. phosphorus in action: biological processes in soil phosphorus cycling. E Bünemann, A Oberson, E Frossard. Berlin, Heidelberg, Springer Berlin Heidelberg: 215–243
- Novozamsky I, Houba VJG, Van Eck R, Van Vark W (1983) A novel digestion technique for multi-element plant analysis. *Commun Soil Sci Plant Anal* 14(3):239–2486
- Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49(2):175–191
- Quesada CA, Lloyd J, Anderson LO, Fyllas NM, Schwarz MC, Zimczik CI (2011) Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences* 8(6):1415–1440
- Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, Santos AJB, Hodnett MG, Herrera R, Luizão FJ, Arneith A, Lloyd G, Dezzio N, Hilke I, Kuhlmann I, Raessler M, Brand WA, Geilmann H, Moraes Filho JO, Carvalho FP, Araujo Filho RN, Chaves JE, Cruz Junior OF, Pimentel TP, Paiva R (2010) Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* 7(5):1515–1541
- Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimise access to soil phosphorus. *Crop Pasture Sci* 60(2):124–143
- Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biol Biochem* 34(9):1309–1315
- Schubart, OHR, Franken, W, Luizão, FJ (1984). Uma floresta sobre solos. *Ciência Hoje*. 2: 26-32
- Sinsabaugh RL, Carreiro MM, Repert DA (2002) Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* 60(1):1–24
- Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. *Soil Biol Biochem* 26(10):1305–1311
- Six J, Callewaert P, Lenders S, De Gryze S, Morris SJ, Gregorich EG, Paul EG, Paustian, K, K (2002) Measuring and understanding carbon storage in afforested soils by physical fractionation. *Soil Sci Soc A J* 66:1981–1987
- Soong JL, Janssens IA, Grau O, Margalef O, Stahl C, Van Langenhove L, Urbina I, Chave J, Dourdain A, Ferry B, Freycon V, Herault B, Sardans J, Peñuelas J, Verbruggen E (2020) Soil properties explain tree growth and mortality, but not biomass, across phosphorus-depleted tropical forests. *Sci Rep* 10(1):2302
- Terrer C, Jackson RB, Prentice IC, Keenan TF, Kaiser C, Vicca S, Fisher JB, Reich PB, Stocker BD, Hungate BA, Peñuelas J, McCallum I, Soudzilovskaia NA, Cernusak LA, Talhelm AF, Van Sundert K, Piao S, Newton PCD, Hovenden MJ, Blumenthal DM, Liu YY, Müller C, Winter K, Field CB, Viechtbauer W, Van Lissa CJ, Hoosbeek MR, Watanabe M, Koike T, Leshyk VO, Polley HW, Franklin O (2019) Nitrogen and phosphorus constrain the CO₂ fertilization of global plant biomass. *Nat Clim Chang* 9(9):684–689
- Turner BL, Engelbrecht BMJ (2011) Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* 103(1):297–315
- 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(1):115–130
- Von Lützw M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *Eur J Soil Sci* 57:426–445
- von Lützw M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol Biochem* 39(9):2183–2207

Wallenstein, MD, Weintraub, MN, (2008) Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. *Soil Biol Biochem* 40(9):2098–2106

Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. *Biogeochemistry* 117(1):101–113

Weintraub SR, Wieder WR, Cleveland CC, Townsend AR (2013) Organic matter inputs shift soil enzyme activity and

allocation patterns in a wet tropical forest. *Biogeochemistry* 114(1):313–326

Wieder WR, Cleveland CC, Smith WK, Todd-Brown K (2015) Future productivity and carbon storage limited by terrestrial nutrient availability. *Nat Geosci* 8(6):441–444

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