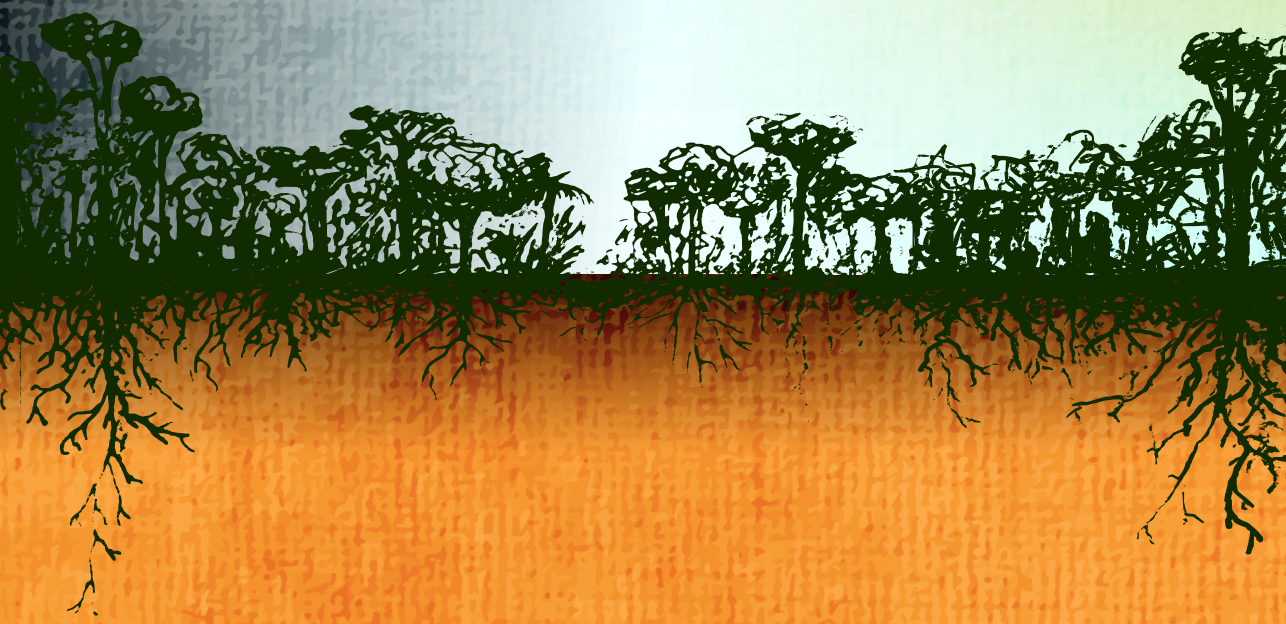
Seasonality of C, N and P cycling in a tropical Terra Firme forest soil



Karst Schaap

Propositions

1. The seasonality of biogeochemical processes in tropical forest soils is more pronounced, and therefore more consequential, than generally recognized. (this thesis)
2. The tropical soil P-cycle depends more on the N-cycle than vice versa. (this thesis)
3. The scientific value of a work increases with each contributing co-author.
4. With the growing presence of technology and computation in science, experimentation becomes more valuable.
5. We do not need more information to make better decisions.
6. Connecting cultures leads to societal progress.

Propositions belonging to the thesis, entitled

Seasonality of C, N and P cycling in a tropical Terra Firme forest soil

Karst Jacob Schaap
Wageningen, 28 May 2024

Seasonality of C, N and P cycling in a tropical Terra Firme forest soil

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Seasonality of C, N and P cycling in a tropical Terra Firme forest soil

Karst Schaap

Thesis

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Chapter 1:

General Introduction

1.1 Background

1.1.1 Forests and soils of the Amazon basin

Tropical forests cover 14% of the Earth's land surface (FAO 2020), providing numerous ecosystem services ranging from sustaining local communities to regulating global climate (Foley et al. 2007; Brandon 2014; Brockerhoff et al. 2017). There are many uncertainties regarding their future as a source or sink of carbon (C), leading to increased scientific attention in the last decades. Tropical forests' net primary production (NPP), C storage, nitrogen (N) fixation, and nitrous oxide emissions have a disproportionately large effect on global biogeochemical cycles, considering the large area they cover and year-round plant production (Townsend et al. 2011). While tropical forests are among the most productive forests globally, with long residence times for C (Saugier et al. 2001; Cleveland et al. 2015), the low soil phosphorus (P) availability in many tropical forests is considered to limit plant production and other ecosystem processes (Vitousek 1984; Cleveland et al. 2011; Du et al. 2020; Hou et al. 2020; Cunha et al. 2022). This highlights the importance of understanding soil biogeochemical processes and interdependencies to the functioning of tropical forest ecosystems.

Soils underlying forests are paramount in their functioning; the soil not only serves as a physical medium for plants to grow on; soil chemistry defines to a large extent the availability of nutrients that plants can access. The interplay between abiotic factors (including climate) and the biotic effects of the ecosystem defines how soils develop over time (Hillel 2008). In wet tropical regions, the climate is characterized by high precipitation and high temperatures (18°C or higher average), both stimulating soil development. In combination with stable geological surfaces, this may yield well-developed highly-weathered soils. While in most temperate and boreal areas there were relatively recent glacial events that provided fresh parent material in the absence of tectonic renewal, such events did not occur in most tropical areas. Therefore, tropical soils often have geologically old parent material (Quesada et al. 2011). The interplay between a climate that stimulates soil development and relatively old parent material defines the nature of many tropical soils and has major implications for the nutrient cycles and ecosystems they sustain.

During soil development, a major feature is the persistent loss of nutrients that are released by weathering from the parent material, for instance P (Walker and Syers 1976). Phosphorus has several forms in the soil, which change during soil development; younger soils have a large mineral pool of P (e.g., apatite), which is gradually weathered into labile

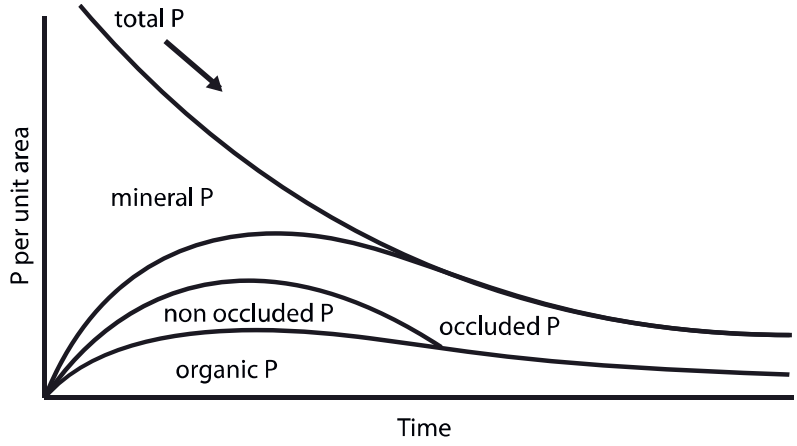

Figure 1.1: The development of soil P during pedogenesis. Adapted from Walker and Syers (1976)

(non-occluded) and organic soil P, but partially ends up as occluded P as well (Walker and Syers 1976). Throughout this soil development process, the total P pool decreases, and by the time mineral P sources are depleted, the non-occluded pools diminish rapidly (Fig. 1.1), increasing the relative importance of organic P fractions (Hoosbeek et al. 2023). Although this conceptual model is widely adopted, the distinction between pools that are occluded and non-occluded is poorly defined. It holds true however that older tropical soils generally have low P availability, conform the model.

The neotropical forests in the Amazon region make up about 40% of the remaining tropical forests worldwide (Laurance et al. 2001). These forests have a range of soils (Fig. 1.2), partially developed on pre-Cambrian Guyana and Brazilian shields, partially on sediments (Paleozoic in the central region with plinthosols, and Cretacious-Tertiary sediments closer to the Andes). In agreement with the above, many of these forests have highly-weathered soils with low available-P concentrations and are thus considered to be limited in their above-ground primary productivity (Aragão et al. 2009; Quesada et al. 2012; Cunha et al. 2022). Yet despite nutrient limitations, tropical forests are responsible for 55% of global forest C stocks (Pan et al. 2011). Some nutrients, like P, have to be sourced exclusively from the soil. The nutrient pools in soils are mediated by microbes. Microbes are crucial to the cycling of nutrients in the soil, through the excretion of enzymes that catalyze the breakdown of plant material. Through this mineralization of organic forms of nutrients, e.g., organic-P, they to a large extent regulate the availability of soil nutrients to plants. In this way, understanding the role of microbes in regulating soil nutrient availability is essential

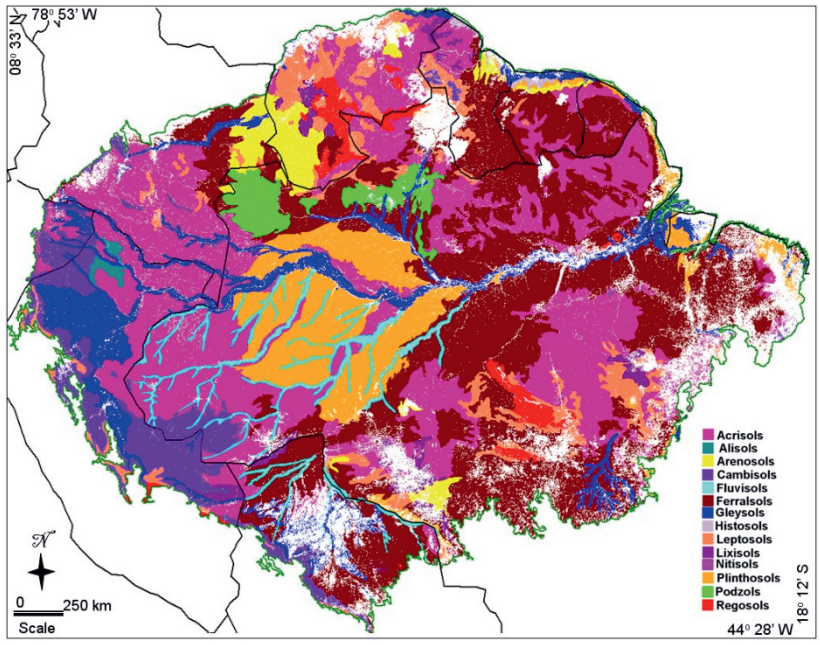

Figure 1.2: Distribution of soil types in the Amazon basin, from Quesada et al (2011)

for predicting the productivity and carbon storage potential of neotropical forests in the Amazon region.

1.1.2 Carbon and nutrients in plant and soil

Carbon is the common element present in and crucial to all known life. Plants produce organic compounds from atmospheric CO_2, through photosynthesis. These organic compounds are subsequently used by the plant for energy and as structural building blocks. Carbon assimilated by plants is the energy source for most other processes in the ecosystem; through decomposition microbes are able to extract sugars for their energy needs. However, nutrients such as N and P are needed in those processes and are crucial elements for all organisms; N is needed for proteins and nucleic acids, among other structural parts of cells, and P is an important nutrient for energy transport and is also structurally needed (e.g., DNA, RNA). To study the abundance of nutrients relative to carbon and each other, they can be expressed in stoichiometric ratios (i.e. C:N, C:P, N:P, etc.).

Deposition of N takes place naturally and can be a source for organisms active in the soil. In addition, some organisms have developed to fix atmospheric N. In the soil, apart from organic N, it is present as nitrite, nitrate, or ammonia. Although often scarce in younger soils, N tends to accumulate in soils as development progresses. While C and N are abundant

in the atmosphere, P is fundamentally different in that it originates from weathering of mineral P. Although on large timescales the origin of N in the ecosystem is atmospheric and the origin of P is through mineral weathering, on shorter timescales most of it is cycled within the ecosystem.

Hence, the main pathways through which nutrients enter the soil on short timescales are through plant litter, i.e., leaves, accompanied by branches, twigs, and reproductive material, along with roots (Vitousek 1982). Subsequently, decomposition of plant litter is needed before the nutrients are available to plants and microbes. To fulfill their nutritional needs, microbes produce enzymes that are crucial in this mineralization process. Microbes can be considered both consumers and suppliers of the nutrients they make available in inorganic form. Extracellular enzymes are considered to be indicative of microbial nutrient demand. Nutrients (or substrate) in the complex soil consumer web define microbial functioning, and hence changes in inputs to the soil change the composition of the microbial community (Nemergut et al. 2010). Stoichiometric ratios can aid in signaling metabolic constraints to biogeochemical processes and disentangle connections between resources, microbial interactions, and shifts in community dynamics that lead to feedbacks in nutrient availability (Mooshammer et al. 2014b; Zechmeister-Boltenstern et al. 2015). In this way, microbial decomposition of plant litter - or microbes and their interplay with substrate - largely defines the availability of nutrients to plants.

1.1.3 Global change and nutrients

The uptake of available nutrients by plants depends on soil nutrient concentration and stoichiometry. After plant uptake, plants use the nutrients for a range of vital processes. The anthropogenic increase of atmospheric availability of CO_2 may have implications for the nutrient economy and nutrient uptake from soil (see also Box 1.1). Changes in atmospheric CO_2 and nutrient deposition affect ecosystems through changes in their nutritional balance, or nutrient economy, with indirect effects throughout the ecosystem. It is generally assumed that increasing atmospheric CO_2 concentrations will affect the whole C cycle and increase forest production, but that it will potentially increase plant demand for nutrients (Box 1.1, Fig. 1.3). Plants take up CO_2 more efficiently under increasing atmospheric CO_2 concentrations. This could lead to decreases in evapotranspiration and increased net plant productivity, yet the latter requires increased uptake or efficiency in the use of nutrients as well (Fig. 1.3, Hofhansl et al. 2016). Since in a tropical context the predominant nutritional

Box 1.1: **The CO_2 fertilization effect**

Plants use photosynthesis to obtain energy and grow. During photosynthesis, plants absorb CO_2 and transform it to carbohydrates while also producing oxygen. Carbohydrates provide energy for the plant and serve as building blocks for plant growth. If the CO_2 concentration in the atmosphere rises, plants might be able to take it up more efficiently, leading to faster growth. This phenomenon is known as the **CO_2 fertilization effect**.

However, photosynthesis requires more than just CO_2 and light. Plants also depend on a supply of nutrients and water for the process to occur. This makes the future of the CO_2 fertilization effect uncertain. Although the CO_2 fertilization effect might be hampered by nutrient scarcity, plants could invest in extra energy for nutrient acquisition, which would have cascading effects in the respective nutrient cycles. This could impact the cycling and storage of C in tropical forests.

limitations concern P, especially the demand for P in the forest would increase (Lloyd et al. 2001). This would result in changed usage and acquisition strategies of plants; in low P soils this is argued to cause plant “mining” for P, through increased exudation of phosphatases and organic acids but also through increases in P resorption (Reichert et al. 2022).

Nutrient resorption from leaves before they are shed is a way for plants to deal with low nutrient content, in order to conserve the nutrient within the plant. This is then reflected in relatively nutrient-poor litter. Foliar N:P ratios vary between species, and evidence of variation between wet and dry seasons in some plants also exists (Townsend et al. 2007). When this litter reaches the soil, plants have developed strategies to take up the remaining nutrients as efficiently as possible. Many of those strategies are at least to some extent dependent on their timing; early resorption of nutrients would affect leaf functionality, while late resorption of nutrients would increase the risk of losing valuable nutrients. Also, investments in root growth or symbiosis must be synchronized with the availability of nutrients to generate the best (nutrient) return of the investment.

While tropical forests are considered a regulator of global climate, global climate also has its effect on them. Through the effect of anthropogenic emissions from a variety of sources, we can observe a range of changes to the biosphere, such as increased atmospheric CO_2 levels, increased nutrient deposition (N), changes in precipitation, and changes in

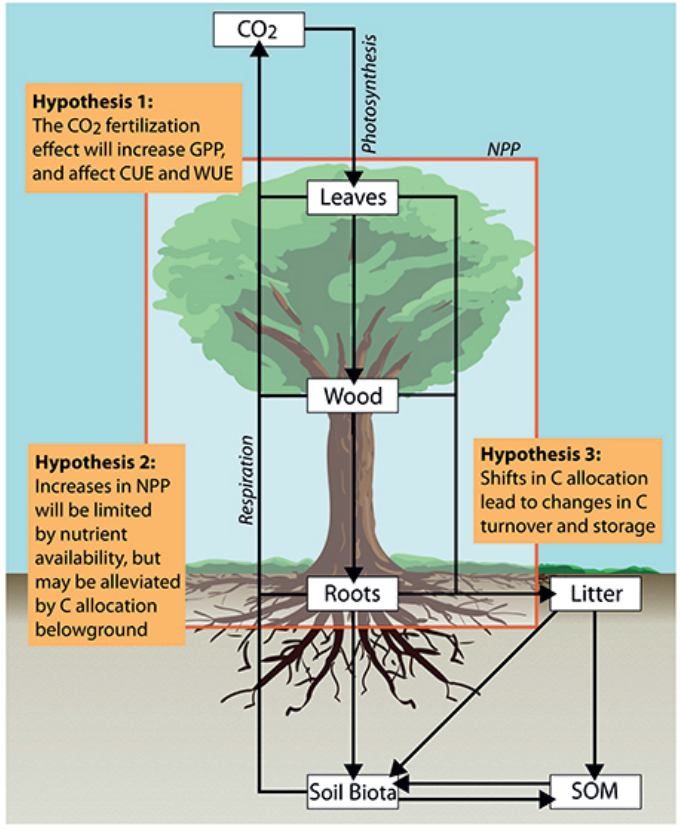

Figure 1.3: Simplified conceptual model depicting major pools and fluxes of carbon in tropical forests, from Hofhansl et al. 2016.

temperature (IPCC 2021). Changes in precipitation or temperature have the potential to affect many processes in a direct way, and those changes can present themselves through altered seasonality: different durations and intensities of seasons (Wang et al. 2021). This would affect forest phenology. Although there are many aspects of a changing climate that are worth investigating, the most consequential uncertainties to the functioning of tropical forests are those concerning the responses to increases in CO_2 concentration and changes in precipitation. Both processes have implications across scales and could lead to fundamental alterations to ecosystem functioning.

Since changes in CO_2 concentrations and precipitation both have the potential to fundamentally alter the biochemical cycles of tropical forests, the impact of those changes is investigated in several ways. On the ecosystem scale, experiments to study the complex impacts of environmental changes on biogeochemical cycles, ecosystem manipulation experiments (Fayle et al. 2015), can be paramount in reducing uncertainties in projections

about forest behavior under changes. For example, drought can be simulated by restricting throughfall, while effects of increases of atmospheric carbon are tested by manipulating its concentration. Nutrient addition (or fertilization) experiments serve partly to simulate enhanced nutrient deposition, but also to test hypotheses of nutrient limitation – i.e., if fertilization by a certain nutrient increases productivity, productivity is assumed to be limited by the respective nutrient in the control – or when fertilization does not take place (Cunha et al. 2022). Equally, for simulating elevated atmospheric CO_2 concentrations, FACE experiments locally increase CO_2 concentrations in plots, in order to study its effects on not only the individual plant, but the ecosystem as a whole.

1.1.4 Seasonality and global change

Most climatic changes vary among regions, rendering generalized statements challenging. Although long-term trends in precipitation over the Amazon basin are not unidirectional (Almeida et al. 2017; IPCC 2021); average temperature is reported to increase, and most models predict a negative rainfall trend for the future (see, e.g., Marengo et al. 2018). A decrease in rainfall has many direct and indirect effects on the biogeochemical cycles of forests. Rainfall reduction can increase CO_2 fluxes from soil to atmosphere through increased oxygen (decreases in anoxic conditions) in soils and increased soluble C concentrations leached from decomposing litter (Cleveland et al. 2010). Drought has also been reported to cause large shifts in soil P-cycling, with organic pools increasing at the cost of inorganic soil P pools, attributed to several potential mechanisms such as increased litter (possibly with lower nutrient resorption), lower phosphatase activity through changes in microbial functioning, and through rapid microbial uptake of inorganic P (O'Connell et al. 2018). Furthermore, large effects of drought to microbial functioning and thus mediation of nutrient fluxes is implied by the effects on seasonality of enzyme activities and changes in the community structure (Buscardo et al. 2021).

It has been well described how seasonality affects the aboveground functioning of forests through changes in NPP and GPP (Potter et al. 2001; Saleska et al. 2003; Rowland et al. 2014; Xu et al. 2015; Girardin et al. 2016; Restrepo-Coupe et al. 2016). Consequently, litter production (Wieder and Wright 1995; Sanches et al. 2008a; Zhang et al. 2014; Wu et al. 2016; Paudel et al. 2020) and fine root growth (Cordeiro et al. 2020), both highly relevant for soil biogeochemical processes, show seasonality. Additionally, decomposition also shows seasonality (Wieder and Wright 1995). Seasonal effects are also reflected in soil CO_2 efflux, arguably related to the availability of P (Cusack et al. 2019).

Given the direct and indirect effects of rainfall reduction on biogeochemical cycles and microbial functioning, combined with the observed seasonality of other processes, it is important to also consider the potential for seasonality in tropical soil biochemistry. Some studies have been conducted on the effect of seasons on soil biogeochemistry, showing changes in microbial structure and function between seasons (Ruan et al. 2004; Eaton et al. 2011; Buscardo et al. 2018; Pajares et al. 2018), while others have shown seasonality in tropical soil nutrients (Yamashita et al. 2010; Mirabello et al. 2013). Considering those temporal dynamics to link above- and below-ground fluxes of nutrients is crucial in our understanding of ecosystem functioning (Bardgett et al. 2005). In light of the evidence for seasonality and its potential role in forest functioning, further research is needed to fully understand the temporal dynamics of soil biogeochemistry in tropical forests. In this way, belowground dynamics are crucial in predicting how tropical forests might develop in scenarios with a changed climate.

1.1.5 Microbial dynamics as seasonal driver

Since plant growth and plant litter production show seasonal variation, and decomposition of plant litter varies with season as well, it does not take a lot of imagination to hypothesize nutrient cycles as a whole, including their belowground components, also show seasonal variation. Through inputs of litter to the soil, the first way in which we would see seasonality belowground would be through variation in the stoichiometry of soil nutrient pools. Changes in microbial community composition can happen due to changing inputs of substrate (Nemergut et al. 2010) or drought (O'Connell et al. 2018; Buscardo et al. 2021). Through homeostasis, microbes are thought to have constrained stoichiometric ranges for their nutrient contents, yet storage of excess C or P is reported (Mooshammer et al. 2014b). Changes in the microbial community composition are often resulting in changes in different nutritional makeup of their biomass (Fanin et al. 2013) and might have implications for their function in soil.

Organic matter decomposition is mainly determined by moisture and temperature, along with the quality of substrate (Krishna and Mohan 2017). In tropical forests like the Amazon, soil moisture is the main driver of microbial activity (Meir et al. 2008). Although decomposition is dependent on several factors, extracellular enzymes (EE) are considered the proximate agents of organic matter decomposition (Sinsabaugh et al. 2008; Burns et al. 2013). Extracellular enzymes are mainly produced by microbes, with some contribution of plant roots exudating phosphatases (Skujiņš and Burns 1976). Stoichiometric approaches, often used to assess interactions between different (soil) nutrient pools and fluxes, have

recently been extended to enzymatic activity (see e.g., Moorhead et al. 2016). Older tropical soils show comparably low enzymatic C:P and N:P activity ratios (Waring et al. 2014). According to theory of supply and demand (Box 1.2, Allison et al. 2011), this would indicate that the P demand is high in tropical soils, resonating with observations of low P availability. Even so, some maintain that microbes are by definition only limited by C availability for their functioning (e.g., Mori 2020; Soong et al. 2020; Mori 2022). However, some microbial processes have been shown to be limited by P (Cleveland et al. 2002; Cleveland et al. 2006; Wieder et al. 2009; Camenzind et al. 2018).

Microbes have shown plasticity in their functioning in fertilization experiments in lowland tropical forests (Turner and Wright 2014), suggesting that enzymes are produced according to demand following the theory of enzymatic supply and demand (Box 1.2, Allison et al. 2011). In temperate forests, a distinct seasonality has been observed in enzyme activities and nutrient cycling (e.g., Smemo et al. 2021) and some first information on tropical seasonality of enzyme activities is emerging (Turner and Wright 2014; Nottingham et al. 2020). However, seasonal controls over microbial activity remain unknown to a large extent, especially in tropical forests in which enzymatic seasonality is often not explicitly considered.

Of special interest throughout the plant-soil system in tropical forests is the availability of P. Increased P mineralization and uptake might sustain increased growth under increased CO_2 concentrations (Hoosbeek 2016) and inclusion of the P cycle in vegetation models shows that P has a key role in forest productivity and may well dictate the capacity of the forest to respond to increased CO_2 concentrations (Fleischer et al. 2019; Terrer et al. 2019). In Walker and Syers (1976) model of P, the increasing relevance of soil organic P pool during soil development implies an increase in importance of biological activity. Since the fraction

Box 1.2: **Theory of enzymatic supply and demand**

Extracellular enzymes play a crucial role in soil carbon and nutrient cycling by allowing microbes and plant roots to access resources from complex organic molecules. Producers of these enzymes are thought to be under evolutionary pressure to minimize the cost-benefit ratio of enzyme production. This is supported by evidence that (a) enzyme producers allocate more resources to enzymes targeting limiting nutrients (demand driven enzyme activity) and (b) they have evolved regulation to increase enzyme production when substrate is abundant (supply driven enzyme activity).

of available P in well developed tropical forest soils is small, plants have evolved mechanisms to maintain a bioavailable P pool. These mechanisms concern the mineralization of P through enzymatic and microbial interactions with organic matter, among others such as the coupling of nutrient cycles (Reed et al. 2015). Exactly what controls the bioavailability of P is one of the urgent questions that need to be addressed. Understanding these controls over nutrient availability is of great importance to predicting the effects that global change will have on tropical forests.

1.1.6 Problem statement

Tropical forests provide numerous ecosystem services and are of great importance to global biogeochemical cycles. Anthropogenic changes in the biosphere will change the processes in these forests and understanding scenarios of forest functioning can aid in predicting how these changes will take place. Crucial to forest development is the role of soil biogeochemistry, as nutrient availability and its regulation by microbes already pose limitations to plant growth. These limitations might be exacerbated by global change. Insight into the current dynamics in the soil biogeochemistry can be refined; the seasonal dynamics of nutrient pools are poorly defined. For better insight in the future of tropical biomes, the need for more theory and observations on the seasonality of fluxes in litter and soil carbon and nutrient pools has been expressed (e.g., Wieder et al. 2015; Restrepo-Coupe et al. 2016). It is likely that global change will impact seasonality, e.g., by changes in the current (dry/wet) season length or intensity (IPCC 2021). Investigating the processes that induce temporal stresses on these forests can give us insight in how the forest will deal with those stresses in future scenarios as well. In this way, the effects of seasonality on soil biogeochemistry could give us more insight on how these forests might change under long-term shifts in climate.

Soil nutrient stoichiometry defines constraints on soil processes sustaining nutrient cycles, yet their seasonal dynamics are largely unknown. Considering global change scenarios where tropical forest responses to elevated atmospheric CO_2 assimilation by plants might be limited by soil P availability (Lloyd et al. 2001; Cernusak et al. 2013), investigating the existing temporal dynamics of soil C, N and P pools, as well as their interaction with each other can provide crucial benchmark information on how forest nutrient cycles might be affected in the future. Additionally, seasonal patterns of inputs and the dynamics of precipitation suggest shifts in microbial nutrient acquisition throughout the year are likely. Since forest nutrient cycles to a large extent depend on the enzymatic turnover of nutrients, investigating the dependencies of extracellular enzyme activities on moisture, temperature,

and especially substrate availability is of great importance (Henry 2012; Burns et al. 2013), and is especially relevant when future climatic changes to seasonality are considered. Ultimately, the productivity and thus carbon uptake of the Amazon forest, the largest tropical forest worldwide, is largely dependent on available-P, which is in turn dependent on P mineralization and mobilization. An improved understanding of the cycling of P in tropical forests is needed to accurately predict the future of tropical forests under elevated CO_2 concentrations (Cernusak et al. 2013). Specifically, the dynamics of and controls over P mineralization by phosphatase have been described as a research priority to reduce uncertainty about the response of the P cycle in scenarios with elevated atmospheric CO_2 (Achat et al. 2016; Fleischer et al. 2019).

1.2 Aims and Outlook of the thesis

The overarching objective of this thesis is to explore the extent to which there is seasonal variation in soil biogeochemistry, and we hypothesize that one of the main driving factors of this variation would be the seasonality of plant litter inputs. Changing climate and the increase of atmospheric CO_2 concentrations will affect many plant and soil processes and have great implications to the functioning of forests worldwide (Ainsworth & Long, 2005; Norby & Zak, 2011; Saxe et al., 1998). For understanding the fate of tropical forests, the commonly low-P soils sustaining year-round plant productivity are of fundamental importance. Yet they cannot be considered in isolation, since they are part of a complex web of other processes. To this end I investigated how the P-cycle is linked to other soil nutrient cycles. To study the effects of increases in CO_2 experimentally, the AmazonFACE project (Box 1.3) is being developed. In this thesis, I investigate the stoichiometric relations between nutrient pools, the relative abundance of the enzymes responsible for the turnover of nutrients and take a detailed look at the soil P cycle. Ultimately, these seasonal changes could form the basis for a soil-centric understanding of the impact of global changes in precipitation or increased nutrient demand due to increasing atmospheric CO_2 concentrations.

The first objective (Chapter 2) assesses the stoichiometric variation in total nutrient contents, available nutrients, and microbial biomass. There is quite some scientific information available about the forest and its function, including seasonality, but this information is predominantly about aboveground functioning. In a tropical context however, there is a need for more information about belowground processes, mainly about the extent of seasonality of soil nutrient stoichiometry and its interactions, as a way of assessing constraints on processes sustaining nutrient cycles. The second objective (Chapter 3) defines the drivers of enzyme activities and relative changes in nutrient demand. When nutrients are of limited supply in the soil, their supply depends on (re)cycling of organic material. This happens largely through turnover catalyzed by extracellular enzymes which are (mainly) produced by microbes. Seasonal shifts in inputs and the dynamics of precipitation suggest shifts in microbial nutrient acquisition throughout the year are likely. How and when those shifts take place, and the implications thereof, are not known. The third objective (Chapter 4) relates soil P pools to input through decomposition and transformations by enzymes. The availability of (inorganic) phosphorus, one of the crucial macronutrients for all organisms, is generally limited in tropical soils. The productivity and thus carbon uptake of the Amazon forest, the largest tropical forest worldwide, is largely

limited by this low soil available-P, yet there are uncertainties and unknowns in the dynamics of P and its availability and interactions.

1.2.1 Study site and general methodology

Data were collected at the AmazonFACE experimental site (2°35'40"S, 60°12'29"W) in Central Amazonia (see also https://amazonface.inpa.gov.br/, box 1.3), approximately 70 km north of Manaus, Brazil, in the experimental reserve "Cuieiras" (Estação Experimental de Silvicultura Tropical – EEST, see also Pereira et al. 2019), which is also the base for the LBA-K34 tower and several experimental observation stations. Characteristic of the study area are "Terra Firme" forests, located on plateaus with nutrient poor and clay rich soils (>70% clay) classified as Geric Ferralsols (Quesada et al. 2010). The biodiversity in those areas is high, partly due to the geological diversity at a regional level; the forest is intersected by streams and forms a landscape of "Terra Firme" plateaus and white sand valleys (de Oliveira and Mori 1999; Pupim et al. 2019). In the Amazon basin, where parent material for soils is generally old, highly weathered Acrisols and Ferralsols account for approximately 60% of all soils (Fig. 1.2, Quesada et al. 2011; Quesada et al. 2012), hence the study site is representative for large parts of the Amazon basin. Average annual rainfall is about 2,400 mm, with a relatively drier period from June to October, while average temperature fluctuates from 25.8°C in April to 27.9°C in September (Araújo et al. 2002). Modeling efforts have indicated a potential for high P-limitation at our study site (Fleischer et al. 2019; Mercado et al. 2021).

The AmazonFACE program aims to investigate forest dynamics in a Free Air Carbon Enrichment (FACE) experiment, evaluating the effect of elevated CO_2 in a tropical forest with soils representative for approximately 60% of the Brazilian Amazon (Quesada et al. 2011; Quesada et al. 2012; Lapola and Norby 2014). The data presented in this thesis contribute to a growing data set on the study site - prior to FACE experimentation - which can be considered be a baseline for future studies performed at the AmazonFACE site. By identifying and investigating the central questions of this thesis, the description of seasonality of soil biogeochemistry provides benchmark information to models and can inform studies into other aspects of the forest about the biogeochemical status quo throughout the year. As the ecosystem changes locally under FACE experimentation, the comparison of the FACE-data with the pre-experimental data from this thesis can uncover effects of elevated CO_2 on soil biogeochemistry, and by extent, biogeochemical cycles.

For each of the experimental chapters, I sampled soils during 2016 and 2017, and consistently analyzed them for several (bio)chemical properties, such as nutrient contents,

enzyme activities, and microbial biomass. The soils were sampled at two depths, 0-5 cm, and 5-15 cm, since I assumed that the expected variation of biogeochemical properties would be most pronounced in the upper layers. Additional data was obtained from the AmazonFACE project, such as temperature, precipitation, and litterfall data.

1.2.2 Is there seasonal stoichiometric variation?

In the second chapter, the central question is how the stoichiometries of soil nutrient pools (i.e., total, extractable and microbial pools) fluctuate over the course of a year. I hypothesize that:

- seasonal fluctuations are more pronounced in the upper, organic-rich soil layer, receiving more new organic matter litter inputs compared to deeper soil;
- soil C, N, and P concentrations respond dynamically to seasonal climate variations and increase in the wet season due to decomposition of fresh litter inputs from the preceding dry season.
- increased microbial substrate availability during the wet season should result in higher microbial C, N and P content, however, I would expect a relative constrained response of the stoichiometry due to stoichiometric homeostasis.

I collected soil samples quarterly for one year and analyzed them for total, extractable, and microbial C, N, and P. I assess whether there are significant differences between the sampling months and direct relations between the analyzed pools.

1.2.3 What controls microbial activity?

For the third chapter, my central question is how enzyme activities (as a proxy for microbial activity and nutrient demand) are related to the cycling of C, N, and P, and how these changes are related to climate, litter input, and soil nutrients. I hypothesize that:

- litter inputs are the main driver of enzymatic activities, as opposed to temperature, precipitation, or soil water content;
- total soil C and available C, N, and P are negatively related to the respective enzyme activities related to their mineralization (through increased microbial investments driven by low nutrient availability); and,
- P-related extracellular enzyme activities are higher than those that are related to C and N cycling, due to low P availability in these tropical soils.

I analyze monthly samples for extracellular enzyme activities.

1.2.4 How are P pools affected?

In the last experimental chapter (Chapter 4), the central question is if I can determine the drivers of changes in commonly analyzed Hedley P pools during a year. The Hedley sequential phosphorus fractionation procedure subjects soil samples to increasingly strong extractants, yielding P pools of decreasing availability (Tiessen and Moir 1993). I hypothesize:

- Fluctuations in P fractions to be most pronounced in the upper 5 cm of soil, where biological activity is highest.
- Seasonal variation in Hedley pools is driven by decomposition derived (organic and inorganic) P inputs to the soil, exchange between soil P fractions (catalyzed by phosphatase activities, among others), and outputs (i.e. uptake)

I aim to identify the relative importance of drivers controlling the fluctuation of different P pools, such as litterfall inputs, litter decomposition and phosphatase activity from either plant roots or microbes to degrade organic-P compounds.

1.2.5 Synthesis and conclusions

In the last chapter I combine and summarize the results of the previous chapters and reflect on the implications of the thesis. I also address limitations of the work and provide recommendations for future research.

Chapter 2:

Intra-annual dynamics of soil and microbial nutrient stoichiometry in a Central Amazon Terra Firme forest

Submitted for publication

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Abstract

Background: Seasonal fluctuations in precipitation significantly influence tropical ecosystem functioning, but the impact on soil and microbial nutrient cycling is not fully understood.

Aim: This study examines intra-annual fluctuations of C, N and P pools in tropical soil. The aim was to investigate the magnitude of intra-annual fluctuations in belowground biogeochemistry in nutrient availability and the microbial biomass in a tropical forest ecosystem.

Methods: We analyzed total, extractable, and microbial carbon (C), nitrogen (N), and phosphorus (P) content and stoichiometry in Terra Firme Ferralsols, which are representative of soils in the central Amazon basin.

Results: Our findings indicate intra-annual variations in resource availability, particularly between wet and dry seasons. Despite relatively constant total C, N and P stocks throughout the year, we found a decrease in extractable organic C and available (Olsen) P, and an increase in extractable N in the dry season compared to the wet season. Similarly, microbial biomass pools and stoichiometry varied across sampling dates and soil depths: relative to microbial-C and -N, microbial-P decreased in both wet and dry season, and was high in the transition from wet to dry season.

Conclusions: Our research highlights the significant intra-annual variation in nutrient pools, particularly dynamic microbial carbon and nutrient fractions, in weathered tropical forest soils. These variations suggest shifts in microbial community composition due to fluctuations in soil nutrient availability, which can be influenced by climate or land use changes, thereby impacting terrestrial carbon and nutrient cycles.

Keywords: intra-annual variability, nutrient ratios, microbial biomass, tropical forest soil

2.1 Introduction

Soils are crucial for sustaining forest productivity and ecosystem functioning by facilitating the cycling of carbon (C) and water (Crowther et al. 2019; Quesada et al. 2020). The formation, stabilization and long-term sequestration of soil organic C (SOC) is determined by the physical and chemical properties of the soil mineral matrix, organic matter inputs, and microbial decomposer activity (Cotrufo et al. 2015; Liang et al. 2017). Minor changes in SOC stocks could substantially impact atmospheric CO_2 concentration (Stockmann et al. 2013). However, the impact of a changing climate on SOC and soil nutrient dynamics are uncertain, highlighting the need for better understanding of those dynamics for climate feedback projections (Bradford et al., 2016; Wieder et al., 2015).

The main inputs to SOC and soil nutrients are derived from above- and below-ground plant litter and root exudation, but these inputs vary over the course of a year. In tropical forests, seasonality has been observed in tree growth (Hofhansl et al. 2014) and leaf production (Wagner et al. 2016), which subsequently affects C assimilation and litter return to the soil (Chave et al. 2010; Wu et al. 2016). Although limited data is available, there seems to be a similar variation in belowground plant productivity in tropical forests over the course of a year, with an increase of fine root productivity and labile C inputs during wetter conditions and a decrease in periods of lower water availability (Yavitt and Wright 2001; Green et al. 2005; Cordeiro et al. 2020). Hence, both plant leaf and root turnover are driven by fluctuations in precipitation resulting in variation in organic matter inputs and turnover in tropical forest soils.

Decomposition of plant litter into simpler compounds is catalyzed by microbial activity (Cotrufo et al. 2015; Veen et al. 2019). Globally, organic matter decomposition is mainly determined by moisture and temperature, along with the quality of substrate (Krishna and Mohan 2017). In tropical forests like the Amazon, where temperature is less of a constraining factor, moisture is the main driver of soil microbial activity (Meir et al. 2008). Accordingly, (heterotrophic) soil respiration (Chambers et al. 2004) and decomposition have been reported to show seasonal variation (Paudel et al., 2020; Sanches et al., 2008; Wieder & Wright, 1995). Heterotrophic microbes require C as primary energy source (Soong et al. 2020), but to maintain growth and activity they also depend on the supply of nutrients, such as N and P, hence fluctuations in resource availability have implications for microbial functioning. For instance, soil microbial activity was found to dynamically adapt to changes in resource availability over the course of a year, by switching from labile inputs from plant roots during the peak vegetation period towards decomposing litter and more stable soil

organic matter (SOM) resources in the absence of labile C inputs (Kaiser et al. 2010). Therefore, soil nutrient availability for both microbes and plants may be limited by microbial decomposition and affected by the rate of mineralization of leaf litter.

While P is lost from soils over geological time (Walker and Syers 1976), intra-annual P availability in weathered tropical soils depends largely on the microbially mediated mineralization of organic forms of P (Chapter 4, Schaap et al. 2021). In contrast, N is usually relatively abundant also in highly weathered soils, since it can be assimilated from the atmosphere by N_2 fixation and increases with soil age (see e.g., Hedin et al., 2009a). In soils, microbial N is often correlated with microbial C content, while the relationship between microbial C and P is less predictable (Hartman and Richardson 2013), since some microbial groups are able to build up polyphosphates and store excess P for later use (Kulakovskaya 2014). Nevertheless, over large gradients, soil microbial biomass P is related to microbial growth rates (Elser et al. 2003), crucial for nucleic acids and membrane components and as important energy resource (Ehlers et al. 2010) underlining the dependence of microbes on P availability.

Stoichiometric ratios of resources and consumers can be used to explore intra-annual metabolic constraints on ecological processes (Mooshammer et al. 2014b). Substrates with higher C:N or C:P ratios and complex carbon compounds decompose slower than substrates with more favorable quality, suggesting a potential N- or P-limitation on decomposition processes or decomposer communities (Wieder et al., 2009; Zechmeister-Boltenstern et al., 2015). Generally, C:N and C:P ratios of SOM decrease with microbial turnover as C is respired in the process. Microbes can maintain relatively homeostatic stoichiometric ratios of C:N (Spohn 2016); in general bacteria have a tighter, lower C:N range compared to fungi (Xu et al. 2013; Zechmeister-Boltenstern et al. 2015). Therefore, changes in soil microbial biomass C:N:P ratios have been attributed to shifts in microbial community composition, for instance to changes in fungal relative to bacterial abundance in soil (Fanin et al. 2013; Soong et al. 2020).

In temperate biomes, intra-annual fluctuations in litter-microbe-soil interactions are well-documented, however, in tropical forests understanding of these interactions is emerging. Insight into the controls and interactions that drive soil microbial carbon and nutrient dynamics allows improved predictions related to climate change. This is particularly relevant as changes in precipitation patterns are predicted for the Amazon forest (Douville et al. 2021). This study therefore examines the connection between tropical soil nutrient dynamics, litter input, and microbial stoichiometry over the course of a year. Since most

short term SOM cycling takes place in the topsoil (Balesdent et al. 2018) we focused our campaign on shallow soil depths (0-5 cm and 5-15 cm) in a highly weathered Ferralsol. In these soils, we investigated variation in total, extractable, and microbial C, N, and P and their relative stoichiometry. We hypothesized that:

a) soil extractable C, N, and P concentrations dynamically respond to intra-annual climate variations and increase during the wet season resulting from the decomposition of freshly deposited litter from the preceding dry season;

b) increased resource availability in the wet season results in higher microbial C, N and P contents. However, due to stoichiometric homeostasis, we expect a relatively constrained response of microbial stoichiometry;

c) intra-annual fluctuations in total nutrient concentration are more pronounced in the upper soil, due to exposure to seasonal organic matter inputs.

2.2 Methods

2.2.1 Site description

This study was carried out at the AmazonFACE experimental site (2°35'40"S 60°12'29"W) in Central Amazonia (more information available at amazonface.inpa.gov.br/), approximately 70 km north of Manaus, Brazil, in the experimental reserve Cuieiras (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 meteorology of the area is well described and reported (Araújo et al. 2002; Saleska et al. 2013; Fuentes et al. 2016) and additional data can be obtained from nearby meteorological stations or the Amazon Tall Tower Observatory (e.g., inmet.gov.br or Andreae et al., 2015). Average annual rainfall is about 2,400 mm, with a relatively drier period from June to November (hereafter: dry season), while average temperature fluctuates from 25.8°C in April to 27.9°C in September (Araújo et al., 2002,). The average daily precipitation during this study at the AmazonFACE site is compared with the 2001-2022 average daily precipitation in Manaus (Fig. 2.1). The precipitation pattern at the study site followed the long-year average but with slightly wetter summer months. This differences might be due to increased interception by tower-based rain gauges above the canopy, while Manaus data were collected at ground level at a standardized meteorological station.

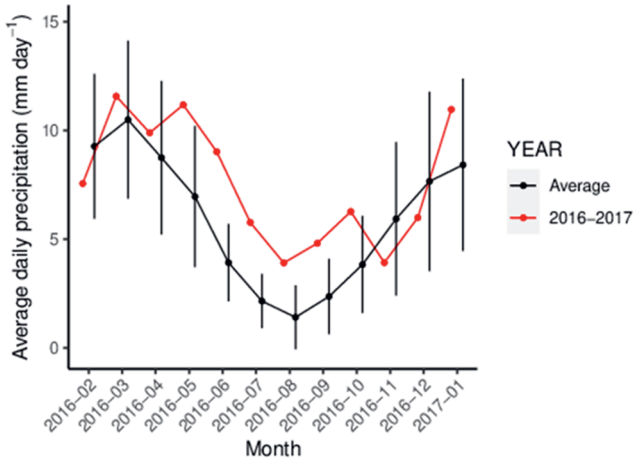

Figure 2.1: Precipitation at the AmazonFACE plots, daily average (red line and dots ±SE) per month. For reference, the black line is the average precipitation in Manaus for 2001-2022, calculated from publicly available data from INMET (https://portal.inmet.gov.br/).

The area is characterized by old-growth tropical forests, locally known as “Terra Firme” forests. These forests are located on plateaus with nutrient poor and clay rich soils (>70% clay) classified as Geric Ferralsols (Quesada et al. 2010). Across the Amazon basin, approximately 60% of the soils are described as Ferralsols or Acrisols. They are dominated by Iron-(hydr)oxides, highly weathered, and depleted in P and other mineral nutrients (Quesada et al. 2011). These soils usually have a thin (approx. 3-5 cm thick) A horizon with a higher organic matter content that is more organic, followed by AC and C horizons with decreasing organic matter contents with depth. The primary productivity of forests growing on such soils is assumed to be limited by the availability of P (Hou et al. 2020; Cunha et al. 2022) and microbial processes are considered to be limited by P in tropical soils as well (Camenzind et al. 2018).

2.2.2 Sample collection and processing

Soils were collected from 18 sampling points distributed close to the AmazonFACE plots (for details, see Lapola & Norby, 2014). In 2016, soil samples were collected every three months between February and November, using a steel soil corer (ø 10 cm). The first two sampling campaigns took place during the wet season, the other two during the dry season. The soils were sampled at depths of 0-5 and 5-15 cm and transported to the lab for sieving (2 mm), removal of roots and debris and further processing. Soil aliquots for further analysis were stored after oven drying (48 h at 65°C), measurements in fresh soils were conducted within three days after collection. Except for total C and N content, all analyses were performed at the LTSP laboratory (Laboratório Temático de Solos e Plantas) at INPA (Instituto Nacional de Pesquisas da Amazônia) in Manaus, Brazil.

2.2.3 Total, extractable and microbial C, N and P

Total soil C (C_t) and N (N_t) content were determined in dry milled composited samples (3 replicate samples pooled by sampling location, separated by layer) using an EA (IRMS). Total P (P_t) was determined in dry 0.5 g aliquots with the molybdate blue method (Murphy and Riley 1962) after acid digestion using a concentrated sulfuric acid solution (H_2SO_4, 18 M), followed by H_2O_2 (Quesada et al., 2010; see also Chapter 4 or Schaap et al., 2021).

Extractable organic carbon (C_e) and extractable nitrogen (N_e) were determined in KCl extracts (2 g of fresh soil in 20 ml of 1M KCl) and analyzed on a TOC/TN analyzer (TOC-V CPH E200V TNM-1 220V; Shimadzu, Vienna, Austria). For extractable inorganic P (P_e), we used the Olsen-P method (Olsen et al. 1954) where extracts were obtained from 2 g of fresh soil shaken for 1 h in 20 ml of 0.5 M $NaHCO_3$ before filtering. The filtered extract was then analyzed colorimetrically with the molybdate blue method (Murphy and Riley 1962).

The same procedure as for C_e, N_e, and P_e was followed for microbial biomass, but with a 24 h chloroform fumigation prior to extraction with KCl or $NaHCO_3$ (Vance et al. 1987). Filtered extracts (2 g of $CHCl_3$ fumigated fresh soil in 20 ml of 1M KCl) were analyzed using a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V; Shimadzu, Vienna, Austria), and the difference between these extracts and C_e/N_e extracts was calculated as microbial C (C_m) and N (N_m) on a dry soil basis. Similarly, microbial P (P_m) concentrations were calculated from the difference between P_e and the fumigated soil extractable P concentrations determined in $NaHCO_3$ extracts (2 g of $CHCl_3$ fumigated fresh soil shaken for 1 h in 20 ml of 0.5 M $NaHCO_3$). The resulting C_m, N_m, and P_m values were not adjusted by any extraction efficiency factor.

2.2.4 Statistical analyses

All calculations were performed in R (version 4.2.1, R Core Team, 2022). Soil stoichiometric ratios were calculated as molar ratios. Whenever their mean is expressed, we used the mean of the natural log of the individual ratios (as recommended by Isles, 2020). To facilitate comparison with other studies, the mean of the log transformed ratios were transformed back to their natural exponent.

To assess if parameters varied over time (i.e. between collection dates), we applied linear mixed-effect models using sampling location as a random effect ("lme" function from the "nlme" package version 3.1-160, Pinheiro et al., 2022). Subsequently, model residuals were evaluated for violation of normality, heteroskedasticity, and temporal autocorrelation. Some variables needed the removal of outliers or the application of the varIdent variance structure, as is indicated in the respective results. The most parsimonious model structure was selected by comparing the AIC of the respective models. When significant differences between sampling campaigns were established, we calculated the least squares model means and performed Tukey's HSD test for significant differences between groups ("lsmeans" from the "emmeans" package, version 1.8.2, Lenth, 2022). Spearman's correlation was used to assess direct relations between variables ("cor.test" function, Supplementary Fig. S2.1).

2.2.5 Microbial fractions

Previous studies have reported the "microbial quotient" of soils, which is commonly calculated from the microbial biomass carbon divided by the total organic carbon contents (e.g., Anderson, 2003; Sparling, 1992); in other words, microbial carbon as a fraction of total carbon. Here we extended that line of thought by calculating this quotient not only for microbial carbon, but also for N and P. This resulted in three distinct (but related) microbial fractions: C_m/C_t, N_m/N_t and P_m/P_t.

Since the C, N and P are important indicators of soil nutrient status and are usually related we visualized microbial fractions (or quotients) of the respective C, N or P pool in relation to each other. More specific, we plotted monthly average ratios of C_m/C_t to N_m/N_t, and fractions of C_m/C_t to P_m/P_t and N_m/N_t to P_m/P_t fractions (Fig. 2.5). This allows to interpret (i) the distance from the origin is an indication of the size of the microbial biomass relative to total C and nutrient contents; and to investigate (ii) divergence from the 1:1 stoichiometric equilibrium of bulk soil and microbial biomass nutrient ratios. Points below (i.e., to the right of) the 1:1 equilibrium line would indicate higher stoichiometric ratios in the microbial biomass as compared to the bulk soil; equally, lower microbial ratios relative to the soil would result in points above (or to the left of) this 1:1 line.

2.3 Results

2.3.1 Intra-annual dynamics of extractable C, N and P

Extractable organic C (C_e) was on average 1047.56 ± 71.79 µg g^{-1} in the top 5 cm, peaking in the wet months (February and May) and with lower concentrations in August and November (Supplementary Table S2.1, Fig. 2.2b). At 5-15 cm C_e averaged 927.31 ± 74.60 µg g^{-1}, were highest in May (1727.64 ± 126.43) and lowest in August (455.73 ± 11.53 µg g^{-1}), yet those differences could not be assessed with our model due to heteroskedasticity even with outlier removal and addition of a variance structure. In contrast, total extractable N (N_e) was 105.58 ± 3.57 µg g^{-1} in the upper 5 cm, with a maximum in August (135.3 ± 5.05 µg g^{-1}), while the lowest value was acquired in February with 92.52 ± 5.82 µg g^{-1} (Fig. 2.2e). At 5-15 cm soil depth the average total N_e was lower compared to the top 5 cm with 79.18 ± 2.51 µg g^{-1} but followed the same pattern with peaks in August (95.92 ± 2.39 µg g^{-1}) and lowest values in February (62.02 ± 2.66 µg g^{-1}). Extractable inorganic P (P_e) was 2.29 ± 0.17 µg g^{-1} on average in the top 5 cm, peaking in May with 2.94 ± 0.26 µg g^{-1}, and was lowest in November with 1.37 ± 0.14 µg g^{-1} (Fig. 2.2h). At 5-15 cm depth average P_e was 1.10 ± 0.08 µg g^{-1}, highest in the wet season, and lowest in the dry season.

2.3.2 Intra-annual dynamics of microbial C, N, and P

Microbial C, N and P were highly dynamic. On average, the microbial C (C_m) concentration in the top 5 cm was 826.85 ± 48.99 µg g^{-1}, with no significant differences in averages between sampling campaigns (Fig. 2.2c). At 5-15 cm depth C_m was on average 674.98 ± 56.17 µg g^{-1} (5-15 cm), highest in May (1026.12 ± 167.96) and significantly lower in the dry season. Microbial N (N_m) was on average 81.33 ± 7.22 µg g^{-1} in the top 5 cm, showing no significant differences between the first three months, but reaching a low in November with 42.43 ± 3.81 µg g^{-1} (Fig. 2.2f). At 5-15 cm N_m was lower with an average of 47.10 ± 4.13 µg g^{-1} following the same temporal pattern as in the top 5 cm. Microbial P (P_m) was on average 2.49 ± 0.27 µg g^{-1} at 0-5 cm and ranged from the highest values in February with 4.49 ± 0.65 µg g^{-1} to lowest

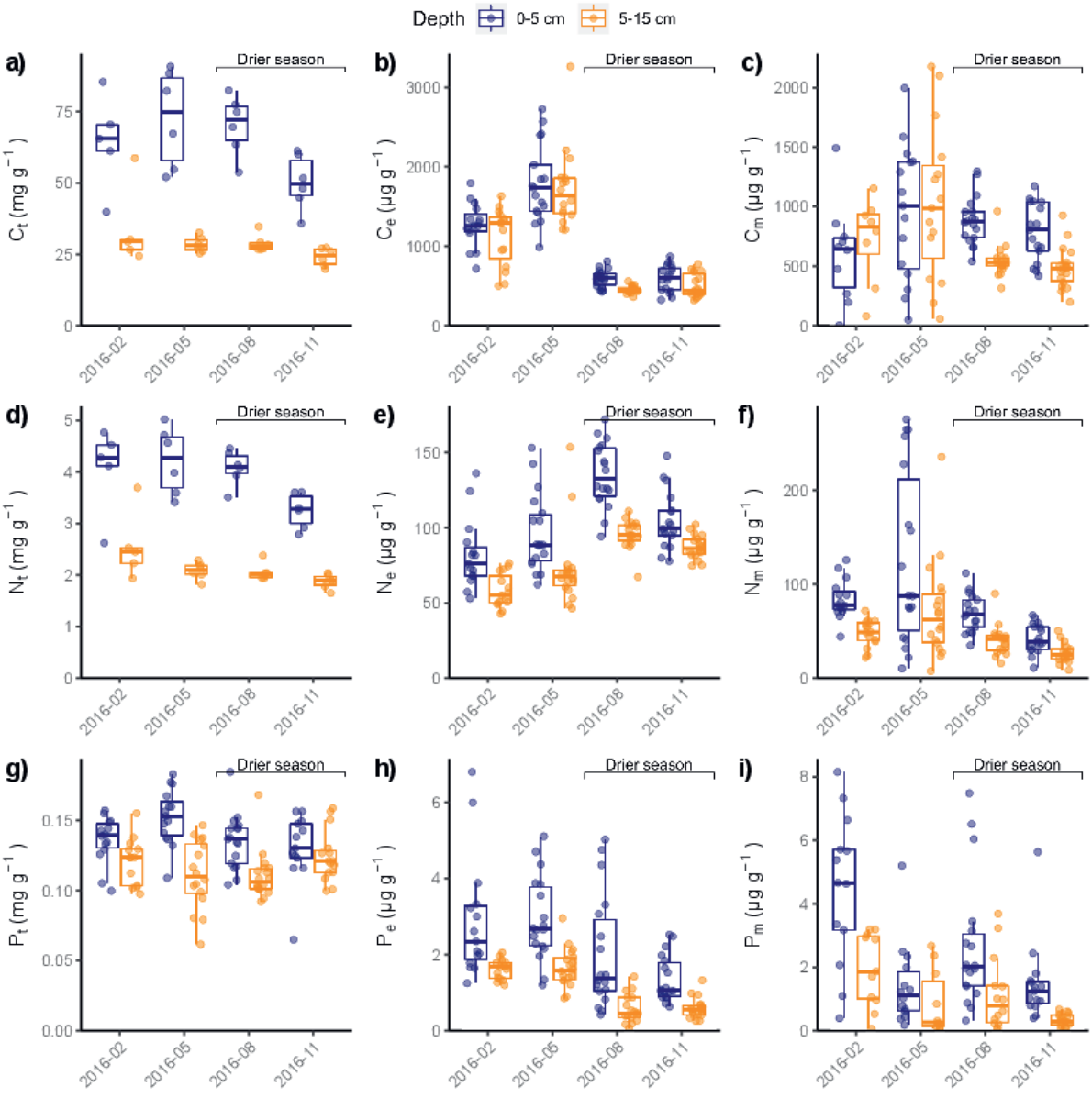

Figure 2.2: Concentrations of soil a) total Carbon (C_t, n = 6, per timepoint), b) KCl extractable C (C_e, n = 18), c) microbial C (C_m, n = 18), d) total Nitrogen (N_t, n = 6), e) KCl extractable N (N_e, n = 18), f) microbial N (N_m, n = 18), g) total Phosphorus (P_t, n = 18), h) inorganic bicarbonate-extractable P (P_e, n = 18), and i) microbial P contents (P_m, n = 18) at 0-5 cm and 5-15 cm soil depth over the course of a year. Boxplots are showing the minimum, first quartile, median, third quartile, maximum, and all observations on a dry soil basis.

values in November with 1.50 ± 0.33 µg g^{-1}. At 5-15 cm depth P_m was even lower with on average 1.04 ± 0.15 µg g^{-1}, and was highest in February (1.95 ± 0.35) and lowest in November (0.36 ± 0.05 µg g^{-1}) (Fig. 2.2i).

2.3.3 Intra-annual variation of total soil C, N and P concentrations

Total soil C (C_t) was on average 64.33 ± 3.25 mg g^{-1} at 0-5 cm soil depth and was lowest in November (dry season) and significantly increased in the two preceding sampling campaigns in May and August (Fig. 2.2a, Supplementary Table S2.1). At 5-15 cm depth C_t concentration was significantly lower, with on average 28.52 ± 1.53 mg g^{-1}, but showed the same temporal pattern as in the top 5 cm. Total soil N (N_t) was on average 3.25 ± 0.14 mg g^{-1} at 0-5 cm depth, remained constant for the first three sampling dates, but significantly dropped in November (Fig. 2.2d). At 5-15 cm soil depth, N_t was 2.13 ± 0.08 mg g^{-1} and was significantly higher in February (2.57 ± 0.30) compared to lowest values in November (1.88 ± 0.06 mg g^{-1}). Total soil P (P_t) amounted on average to 0.14 ± 0.00 mg g^{-1} in the top 5 cm, peaked in May with 0.15 ± 0.01 mg g^{-1}, and was lowest in November with 0.13 ± 0.01 mg g^{-1} (Fig. 2.2g). At 5-15 cm depth, P_t was 0.12 ± 0.00 mg g^{-1} on average, with no significant differences between months.

2.3.4 Intra-annual dynamics of soil and microbial stoichiometry (C:N:P ratios)

The average ratio for extractable C_e:N_e at the top sampling depth was 10.3, while in the soil below this ratio increased to 11.6 (Fig. 2.3b, e, h). The average C_e:P_e ratio was 1202 at 0-5 cm depth, while at 5-15 cm this ratio was 2290. The N_e:P_e ratio was 116.3 on average in the top 5 cm, while in the ten cm below this ratio rose to 189.8.

The ratios for microbial C_m:N_m at the upper 5 cm were 11.87 on average, while in the soil below this ratio was 18.2 (Fig. 2.3c, f, i). The average C_m:P_m ratio was 1094, while at 5-15 cm this ratio rose to 2333. The average N_m:P_m ratio was 86.3 in the top 5 cm, while in the 5-15 cm below this ratio dropped to 147.4.

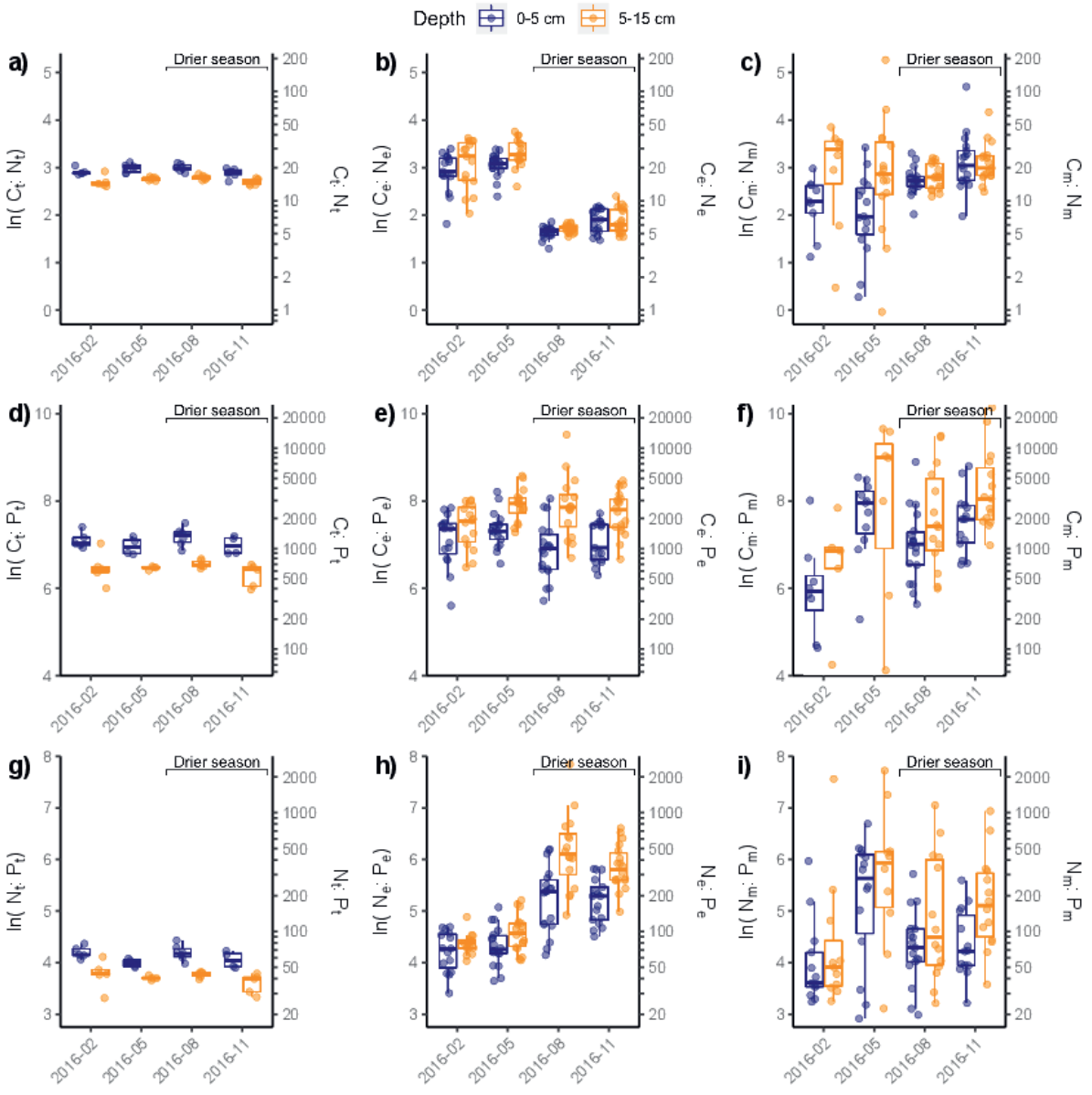

Figure 2.3: Soil a) total C:N (n = 6), b) extractable C:N (n = 18), and c) microbial C:N (n = 18), d) total C:P (n = 6), e) extractable C:P (n = 18), f) microbial C:P (n = 18) g) total N:P (n = 6), h) extractable N:P (n = 18) and microbial N:P ratios (n = 18). The secondary axis shows the natural exponent of the ln transformed values. Boxplots are showing the minimum, first quartile, median, third quartile and maximum of natural log transformed values. For both the C_m:N_m and C_m:P_m, one observation in February is omitted (outside of plot range).

The C_t:N_t ratio in the top 5 cm was on average 19.0, and in the 5-15 cm layer this average was 15.5 (Fig. 2.3). The average C_t:P_t ratio in the upper 5 cm was 1186, and at 5-15 cm it was 635. An average N_t:P_t ratio of 61.9 was found in the top 5 cm, while at 5-15 cm depth this ratio decreased to 41.0. For C_t, N_t, and P_t contents at 5-15 cm, the first and last sampling campaigns respectively showed higher and lower ratios of C_t:N_t and C_t:P_t, indicating fluctuating SOM quality during the year.

2.3.5 Correlations between soil total, extractable and microbial C, N, and P.

At both depths across all samplings, different nutrient pools correlated with each other (Supplementary Fig. S2.1). At the 5-15 cm depth we found more significant relations than in the top 5 cm. Relations (significance level $p < 0.05$) were generally positive, with a notable exception for relations involving N_e. Strongest correlations were found between C_t and N_t (0.97 and 0.86 for 0-5 cm and 5-15 cm respectively), as well as C_e and N_e (-0.3 and -0.52) and C_m and N_m (0.29 and 0.36), but also of N_m correlated with both C_e (0.33 and 0.39) and negatively with N_e (-0.34 and -0.43). In the top 5 cm, P_t was related to C_e (0.31) and N_e (-0.32), while at 5-15 cm P_m significantly correlated with C_t (0.47), N_t (0.65), and P_t (0.31).

2.3.6 Intra-annual dynamics of microbial to soil total C, N and P fractions.

To gain insight into the dynamics of the microbial pools we related microbial C, N and P to soil total C, N and P during a year (Fig. 2.4). In addition, we followed the stoichiometry of these fractions throughout the year (Fig. 2.5). In the top 5 cm, the average C_m/C_t fraction

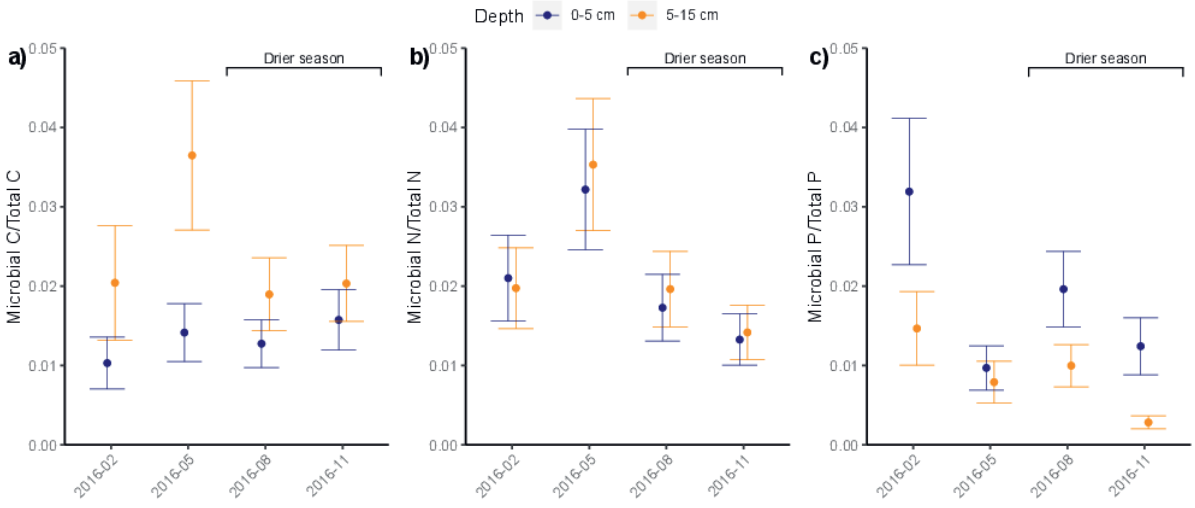

Figure 2.4: Microbial C, N and P as a fraction of the bulk soil C, N and P contents at 0-5 cm and 5-15 cm depth. Error bar indicates standard error.

remained stable throughout the year (Fig 2.3a), while N_m/N_t increased during the wet period and sharply declined with the onset of the drier period (Fig 2.3b), and P_m/P_t declined during the wet period and remained stable during the drier period (Fig 2.3c). At 10-15 cm soil depth, N_m/N_t followed a similar pattern as compared to 0-5 cm depth, while C_m/C_t increased during the wet period and decreased towards the drier period. This is in contrast with P_m/P_t, decreasing during the wet period.

We found a stronger increase of N_m/N_t than C_m/C_t at 0-5 cm soil depth in the wet season (Fig. 2.3a and 2.3b). As a result, from February to May, N_m/N_t versus C_m/C_t moved up (Fig. 2.4a), indicating a relative enrichment of N_m as compared to C_m during the wet period. However, towards and during the drier period (May – August – November) N_m/N_t decreased more than C_m/C_t (Fig. 2.3a and 2.3b), resulting in Nm/Nt versus Cm/Ct moving down (Fig. 2.4a), indicating a relative loss of N_m. At 5-15 cm soil depth, C_m/C_t versus N_m/N_t stayed close to the 1:1 line throughout the year, indicating uniform temporal changes between C_t/N_t and C_m/N_m. When considering the distance to the origin, the microbial biomass increased during the wet period (Feb – May) and decreased during the drier period (May – Aug – Nov).

While C_m/C_t at 0-5 cm remained constant during the year, P_m/P_t decreased during the wet season, increased towards the drier season, and tended to decrease during the drier season (Fig. 2.3a and 2.3c). This resulted in high variation of P_m/P_t versus C_m/C_t (Fig. 2.4b) indicating a relative decrease of P_m during the wet and drier season, but an increase during the

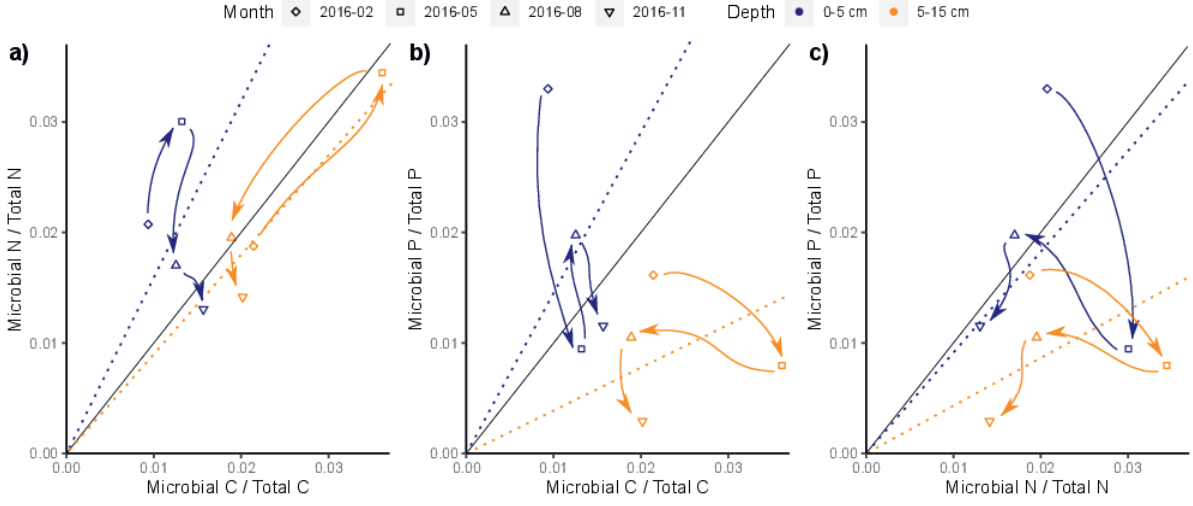

Figure 2.5: Pattern of microbial community C, N, and P as a fraction of bulk soil C_t, N_t and P_t at 0-5 cm (blue) and 5-15 cm (orange). Arrows follow the chronological order to emphasize seasonal shifts in microbial stoichiometry. Dotted lines show the averages for the respective depths.

transition from the wet towards the drier season. At 5-15 cm soil depth, P_m/P_t followed a similar, but less pronounced pattern as observed for the top soil (Fig. 2.3c). Again, C_m/C_t versus P_m/P_t at 5-15 cm (Fig. 2.4b) showed a loss of P_m during both the wet and dry season, while during the transition from wet to drier season P_m increased.

Comparing N and P dynamics we found that at 0-5 cm depth, N_m/N_t increased while P_m/P_t decreased during the wet season. However, during the drier season these nutrients showed a similar pattern. At 5-15 cm depth, the nutrients followed similar patterns, albeit less pronounced. This resulted in relative losses of P_m as compared to N_m and the bulk soil N_t and P_t contents during the wet season at both depths (Fig. 2.4c). Subsequently, P_m increased towards the drier season at both depths. At both depths we found relative decreases of P_m during the wet season, increases towards the drier season and decreases during the dry season. At both depths, relative N_m and P_m decrease during the dry season. However, during the wet period and the following transition towards the drier period, N_m and P_m showed opposing trends.

2.4 Discussion

We reported significant intra-annual variation in most soil C, N, and P pools. During the wet season, extractable C and P increased, while extractable N decreased. Microbial nutrient pools showed a divergence between N and P dynamics. This resulted in a dynamic microbial stoichiometry, with variation across sampling dates and soil depths, suggesting a highly dynamic microbial community. This interaction between inputs, nutrient availability and microbes resulted in higher nutrient concentrations in the top 5 cm of soil as compared to the soil below. Overall, our results underscore a surprisingly high intra-annual variation in nutrient pools in highly weathered tropical forest soils, in response to wet-dry seasonal dynamics.

2.4.1 Dynamics of extractable C and nutrient pools within a year

Our first hypothesis postulated extractable C and nutrient concentrations would increase during the wet season, due to decomposition of litter deposited in the preceding dry season. We confirmed this hypothesis for soil extractable C_e and P_e, which increased in the wet season, but N_e peaked in the dry season.

In the wet season, higher substrate leaching from accumulated plant litter and easier substrate diffusion likely increased microbial activity, increasing C and P availability. Soil moisture is an important control over soil C-cycling and nutrient availability (Cusack et al. 2019), and despite constant soil moisture during our campaign (Supplementary Fig. S2.2) increased leaching of dissolved material in the wet season could be a mechanism for C to reach deeper layers. Precipitation events can result in pulses of soil CO_2 emission (Meir et al. 2008), litter C leachate losses (Schwendenmann and Veldkamp 2005) and river C discharge (Waterloo et al. 2006). Leaf litter leachate can also desorb P in tropical soils (Schreeg et al. 2013), possibly explaining the increase in P_e during the wet season.

In contrast, during the dry season, microbial access to, and turnover of, C_e and nutrients could have been reduced. Moreover, in months with less precipitation decreased root presence was found (Cordeiro et al. 2020) which could result in lower P_e uptake. This suggests that even small intra-annual shifts and a change in the frequency of climate anomalies could lead to substantial changes in plant nutrient uptake and thus critically affect the synchrony of resource availability and plant acquisition capacity (Nord and Lynch 2009). The simultaneous decrease in C_e and P_e, but not N_e, from the wet to dry season could indicate a change in the nutrient decomposition and mineralization dynamics by the microbial community.

Canopy leaching of dry deposited N (Hinko-Najera Umana and Wanek 2010) could further explain the contrasting pattern of N_e. Organic N is depolymerized to amino acids and other N forms, then further mineralized into NH_{4+} (Geisseler et al. 2010). After NH_4^+ nitrification, NO_3^- may be lost in wet conditions due to leaching, while soluble N compounds may accumulate in the dry season. Dry conditions can increase adsorbed ammonium concentrations (Homyak et al. 2017). Despite N's importance for plants and soil microbes it is not considered a limiting nutrient in tropical forests; highly weathered tropical soils are sometimes considered N-saturated (Hedin et al. 2009b). Nitrogen saturation could explain the contrasting pattern of N_e, if the N cycle is an open cycle, as opposed to the "closed" P cycle forcing plants to (re)cycle P_e efficiently.

2.4.2 Non-homeostatic stoichiometry indicates dynamic microbial community.

We hypothesized to find little variation in microbial stoichiometry between sampling dates, since microbes can adjust their functioning and nutrient use efficiency to substrate quality to regulate homeostasis (Mooshammer et al. 2014a; Spohn 2016). Since we could not establish such homeostatic behavior of the microbial biomass, we expected substrate to exert a strong control over microbial functioning and therefore expected variation of total C:N:P stoichiometry to be reflected in microbial C:N:P stoichiometry. Yet C, N and P content as fractions of total nutrient contents (C_m/C_t, N_m/N_t and P_m/P_t ratios) also varied significantly over the course of a year (Fig. 2.4).

Throughout the year, the microbial C fraction at 0-5 cm was smaller than at 5-15 cm (Fig. 2.4a), while the opposite was observed for the microbial P fraction (Fig. 2.4c); the microbial N fractions were similar between depths (Fig. 2.4b). This results in stoichiometric differentiation in the microbial biomass between depths. Increased abundance of microbial P in the top 5 cm could be the result of new labile inputs being absorbed quickly into the microbial biomass. Phosphorus is less mobile in the soil matrix than dissolved organic C, which could maintain the P-rich microbial biomass in the top layer through fresh inputs, but not easily reaching the soil below. Surprisingly, the microbial N fraction did not show differences between sampling depths. We showed a pattern in which May has a relative peak in the microbial N fractions. The large microbial N fraction in the wet season could be due to a shift in microbial composition. Previous reports have noted non-homeostatic behavior of microbial stoichiometry in (tropical) soils, with especially C:P and N:P ratios showing high variation (Soong et al. 2018). Interestingly, microbial nutrient demand seems to shift towards N acquisition at the end of the wet season and towards P acquisition during the dry season (Chapter 3, Schaap et al. 2023).

Our findings show a relative decrease in N_m compared to C_m during the dry period, potentially due to increased enzyme production in the dry season (Chapter 3, Schaap et al. 2023) leading to elevated N demands from microbes. We observed relative decreases of P_m compared to N_m during the wet season. This could be related to shifts in microbial community structure, as exemplified by Fanin et al. (2013), where the ratio of fungi:bacteria increased with the N:P ratio of the microbial biomass. Lower microbial N:P ratios in the top 5 cm as compared to the soil below (Fig. 2.4c) might thus suggest a higher concentration of bacterial decomposers. We also noted fluctuations in P_m levels throughout the year, with increases in the transition from wet to dry season and decreases during both seasons. These dynamics could be partially explained by the storage of P in microbial reserves as alternative to shifts in microbial community composition. Such findings underscore the need for further examination of seasonality in the structure and functioning of the microbial community, particularly in relation to the relative abundance of saprotrophic and mycorrhizal fungi and their impact on nutrient demand and turnover in tropical soils.

2.4.3 Highly dynamic nutrient cycling in the upper soil layers.

In line with our third hypothesis, we found that the top soil layer (upper 5 cm) exhibited more pronounced fluctuations of nutrients, their stoichiometric ratios also showed this pattern. C_t and N_t concentrations in the top 5 cm were roughly double those in the 10 cm below. In tropical forests, litter decomposes fast, with a turnover time of about a year (Powers et al. 2009; Martins et al. 2021). These litter inputs also strongly control organic C inputs at the soil surface, often dominated by particulate fractions (Lavallee et al. 2020). In more mineral soil, SOC is bound to the mineral soil matrix and Fe/Al-oxides (Souza et al. 2017) and undergoes less temporal fluctuations (Lavallee et al. 2020; Cotrufo and Lavallee 2022). In contrast to the sharp decline with depth in the C_t and N_t content, P_t was more similar between depths. Soil P is comprised of several fractions, including an occluded (adsorbed) fraction, and organic fractions related to the inputs of litter (Chapter 4, Schaap et al. 2021); it is plausible that the large recalcitrant pool would mask intra-annual patterns when observing total P.

The C:N ratio for fresh litter was 35.6 (Martins et al. 2021), and this study reports a soil C_t:N_t ratio of 19.0 and 15.5 in the top 5 cm and 5-15 cm depth, respectively. At our site, the average soil C:N ratio of 15.5 at 5-15 cm is similar to that reported for tropical forest soils by Xu et al. (2013), while others reported markedly higher values for tropical forests (Silver et al. 2000). Total C:N ratios remained largely similar throughout the year. The observed N_t dynamics dominate stoichiometric changes for the C:N and N:P ratios. The depth

differentiation between the total C:N, C:P and N:P ratios followed the same pattern for all those ratios; they were slightly lower at 5-15 cm depth. This is in line with earlier reports that found a relative decrease in nutrient ratios with depth (Chen et al. 2022b), suggesting that C is increasingly lost with depth since at deeper depths the organic material is older (Balesdent et al. 2018). The average reported soil C:P was 1078.4, higher than those of tropical soils in general (169.4, Xu et al., 2013), suggesting a pronounced P limitation of the NPP in the studied forest (Menge et al. 2012; Cunha et al. 2022). The higher C:P and N:P found in the topsoil further signals a higher turnover of new inputs compared to the deeper soil.

We found a microbial C:N of 14.4 while the C:P was 2248; both substantially higher than the global averages of 9.0 (C:N) and 56.3 (C:P), especially for microbial C:P (Xu et al., 2013). The relations between the extractable C and P and the microbial biomass C and N at 5-15 cm suggest C and P availability is a driver for microbial biomass nutrients in the mineral soil. Microbial P-contents were positively related to the total C, N and P contents, which could reflect organic material driving microbial P nutrition. Earlier studies revealed P-limitation based on microbial investments in enzymes, especially during the dry season (Chapter 3, Schaap et al. 2023) which, together with the temporal fluctuations found here, suggests a C and P co-limitation of the soil microbial community. Limitation of tropical microbial productivity by P availability has previously been reported for other tropical areas (Cleveland et al. 2002; such as Turner and Wright 2014; Camenzind et al. 2018), while some also reported a limitation by N (Nottingham et al. 2015).

Our results suggest increased microbial biomass size during the wet season. Enzyme activities indicate an increased nutrient demand synchronized with increased litterfall, which is related to precipitation (so that the dry season has more litterfall and higher enzyme activities, see Chapter 3 or Schaap et al. 2023). Therefore, it is likely that microbial incorporation of enzymatic products primarily occurs in the wet season. The lack of accumulation of the extractable fractions of C and P in the dry season suggests high microbial and plant demand for C_e and P_e.

2.5 Conclusions

Intra-annual climate variability affects total, available, and microbial C, N, and P pools in a tropical terra firme forest soil; total and extractable soil C and nutrient pools decrease during the dry season, while extractable N increases. We reported variation of microbial stoichiometry using an approach to highlight the intra-annual dynamics of the microbial stoichiometry as fractions of the soil resources. Microbial biomass N and P show a strikingly large fluctuation in stoichiometry, which could reflect changes in microbial community structure or temporary microbial nutrient storage, in particular of P. Capturing intra-annual shifts in nutrient availability and microbial biomass provides a better understanding of the belowground biogeochemical functioning of tropical forests.

Supplementary Material to Chapter 2

Table S2.1 (next page): Mixed effect model outcomes, with the model type (fixed or mixed effect), intercept F value (and significance), the F value of the sampling month (with significance) and Tukey post hoc estimations of the least squares mean per month. The models included the sampling location as a random factor if this significantly improved the model fit according to the AIC (mixed), if not the fixed model structure was kept. Remarks show if a variance structure or outlier removal was needed to meet the model assumptions of homoskedasticity. Different letters after the means indicate significant differences. Ratios were ln transformed prior to analysis.

0-5 cm

	Model description					Post hoc estimations (Tukey)			
Variable	Model	df	Intercept	Month	Remarks	02-2016 (Wetter)	05-2016 (Wetter)	08-2016 (Drier)	11-2016 (Drier)
Total C	Mixed	3, 14	216.99 (p < 0.0001)	6.51 (p = 0.0055)	-	65.0 ab	72.5 a	70.2 a	50.2 b
Total N	Mixed	3, 14	466.85 (p < 0.0001)	7.17 (p = 0.0038)	-	4.07 a	4.22 a	4.08 a	3.25 b
Total P	Fixed	3, 55	2816.63 (p < 0.0001)	3.37 (p = 0.0249)	-	0.136 ab	0.152 a	0.135 ab	0.130 b
Extractable C	Mixed	3, 58	508.19 (p < 0.0001)	59.08 (p <0.0001)	VarIdent*	1257 a	1783 b	595 c	590 c
Extractable N	Fixed	3, 64	1498.05 (< 0.0001)	16.97 (p < 0.0001)	-	82.1 a	96.2 ac	135.3 b	104.8 c
Extractable P	Fixed	3, 65	235.59 (p < 0.0001)	6.52 (p = 0.0006)		2.91 a	2.94 a	2.03 ab	1.37 b
Microbial C	Mixed	3,39	582.03 (p < 0.0001)	1.81 (p = 0.161)	VarIdent*	-	-	-	-
Microbial N	Mixed	3, 46	527.01 (p < 0.0001)	18.41 (p < 0.0001)	VarIdent*	84.4 a	126.8 a	69.4 a	42.4 b
Microbial P	Fixed	3, 56	114.47 (p < 0.0001)	8.48 (p = 0.0001)	-	4.49 a	1.44 b	2.67 b	1.50 b
Total C:N	Mixed	3, 14	11287 (p < 0.0001)	3.83 (p = 0.0341)	-	2.92 a	2.98 a	2.99 a	2.88 a
Total C:P	Fixed	3, 15	22257 (p < 0.0001)	1.21 (p = 0.3391)	-	-	-	-	-
Total N:P	Fixed	3, 15	17335 (p < 0.0001)	2.64 (p = 0.0875)	-	-	-	-	-
Extractable C:N	Mixed	3, 57	5863.00 (p < 0.0001)	200.71 (p < 0.0001)	1 outlier removed, VarIdent*	2.97 a	3.05 a	1.63 b	1.87 c
Extractable C:P	Fixed	3, 63	11210 (p < 0.0001)	2.30 (p < 0.0861)	-	-	-	-	-
Extractable N:P	Fixed	3, 64	6747.48 (p < 0.0001)	22.19 (p < 0.0001)	-	4.21 a	4.32 a	5.19 b	5.24 b
Microbial C:N	Fixed	3, 54	1003.80 (p < 0.0001)	9.88 (p < 0.0001)	-	2.20 ab	2.01 a	2.72 bc	3.11 c
Microbial C:P	Fixed	3, 48	2223.27 (p < 0.0001)	8.60 (p = 0.0001)	-	5.45 a	7.63 b	6.99 b	7.51 b
Microbial N:P	Fixed	3, 54	1516.95 (p < 0.0001)	5.06 (p = 0.0037)	-	3.97 a	5.21 b	4.29 a	4.36 ab

(continued on next page)

5-15 cm

	Model description					Post hoc estimations (Tukey)			
Variable	Model	df	Intercept	Month		02-2016 (Wetter)	05-2016 (Wetter)	08-2016 (Drier)	11-2016 (Drier)
Total C	Mixed	3, 13	723.13 (p < 0.0001)	8.89 (p = 0.0018)	1 outlier removed	27.5 ab	28.4 a	28.7 a	24.0 b
Total N	Fixed	3, 19	912.36 (p < 0.0001)	4.07 (p = 0.0217)	-	2.57 a	2.09 ab	2.06 ab	1.88 b
Total P	Mixed	3, 58	2205.28 (p < 0.0001)	2.01 (p = 0.1229)	-	-	-	-	-
Extractable C	Persistent violations of assumptions**					-	-	-	-
Extractable N	Fixed	3, 62	4475.27 (p < 0.0001)	59.63 (p < 0.0001)	2 outliers removed	58.2 a	64.5 a	87.4 b	95.9 c
Extractable P	Fixed	3, 64	602.41 (p < 0.0001)	45.50 (p < 0.0001)	-	1.624 a	1.664 a	0.584 b	0.603 b
Microbial C	Mixed	3, 37	452.75 (p < 0.0001)	4.14 (p = 0.0126)	VarIdent*	721 ab	1026 a	543 b	485 b
Microbial N	Mixed	3, 45	368.78 (p < 0.0001)	12.05 (p < 0.0001)	1 outlier removed, VarIdent*	48.2 a	62.3 a	40.2 a	26.6 b
Microbial P	Mixed	3, 29	73.47 (p < 0.0001)	9.44 (p = 0.0002)	VarIdent*	1.953 a	0.886 ab	1.160 ab	0.361 b
Total C:N	Fixed	3, 19	31147.13 (p < 0.0001)	2.15 (p = 0.1277)	-	-	-	-	-
Total C:P	Fixed	3, 16	15356.85 (p < 0.0001)	1.13 (p = 0.3675)	-	-	-	-	-
Total N:P	Fixed	3, 16	8713.42 (p < 0.0001)	1.11 (p = 0.3732)	-	-	-	-	-
Extractable C:N	Persistent violations of assumptions**					-	-	-	-
Extractable C:P	Fixed	3.61	13486.10 (p < 0.0001)	2.48 (p = 0.0691	-	-	-	-	-
Extractable N:P	Mixed	3, 44	14428.65 (p < 0.0001)	80.04 (p < 0.0001)	2 outliers removed, VarIdent*	4.37 a	4.57 a	5.85 b	6.17 b
Microbial C:N	Mixed	3, 35	3005.92 (p < 0.0001)	1.99 (p = 0.1339)	VarIdent*	-	-	-	-
Microbial C:P	Fixed	3, 37	1438.55 (p < 0.0001)	2.38 (p = 0.0853)	-	-	-	-	-
Microbial N:P	Fixed	3, 45	873.61 (p < 0.0001)	2.53 (p = 0.0691)	-	-	-	-	-

* VarIdent variance structure used to allow for different variances between months

** Model residuals did not meet assumptions of homoscedasticity or normality, even with outlier removal and/or variance structure

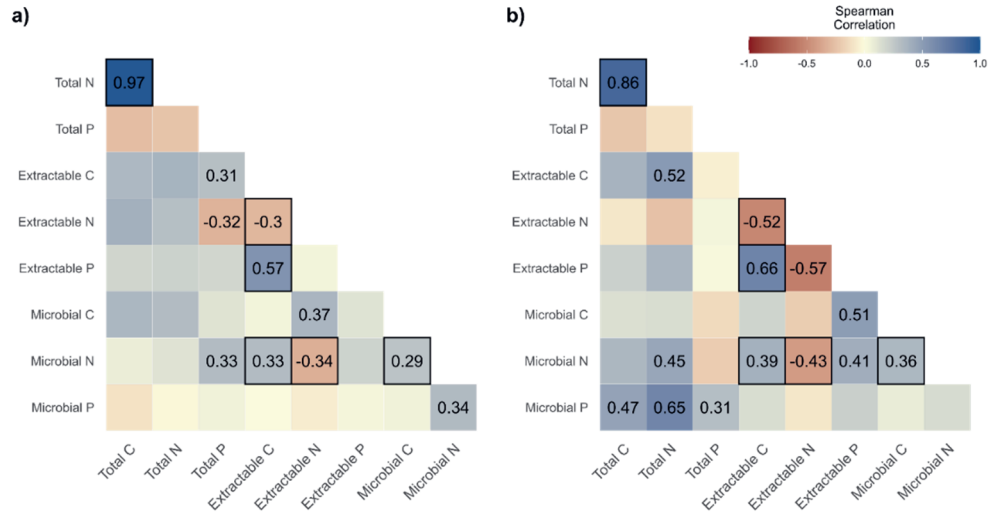

Figure S2.1: Spearman's correlations between soil total, available and microbial C, N, and P at a) 0-5 cm and b) 5-15 cm depth. Color according to the correlation coefficient, which is only shown if significant ($p < 0.05$). Black outlines indicate where correlation was significant at both depths.

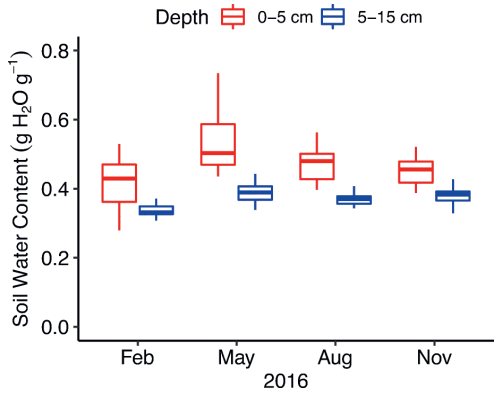

Figure S2.2. Gravimetric soil water content at the two sampling depths

Chapter 3:

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

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A correction to this chapter has been submitted to Biogeochemistry. This correction has been added to the end of this thesis chapter (pages 69-75).

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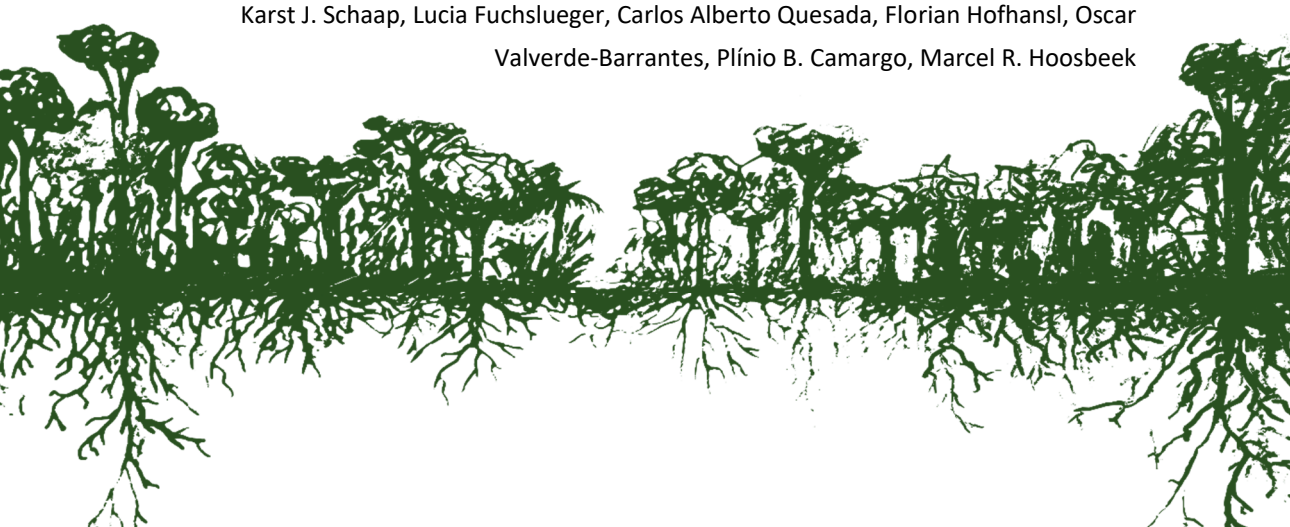


Abstract

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

3.1 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.

3.2 Methods

3.2.1 Site description and sampling strategy

The study was carried out at the AmazonFACE experimental site (2°35'40"S 60°12'29"W) in Central Amazonia (more info on https://amazonface.inpa.gov.br/), approximately 70 km north of Manaus, Brazil, in the "Cuieiras" experimental reserve (Estação Experimental de Silvicultura Tropical – EEST, see also Pereira et al. 2019), which is also the base for the LBA-K34 tower and several experimental observation stations. 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 2,400 mm, with a relatively drier period from June to November (months with at least 40% of days with <3mm precipitation), while the average temperature fluctuates from 25.8°C in April to 27.9°C in September (Araújo et al. 2002).

3.2.2 Sample collection and processing

Soils were collected from 18 sampling points. At 6 locations along a 400 m north-south transect (every 80m), we sampled 3 points in the east-west direction, with 10m distance between the 3 sampling points. The sampling scheme was adopted to consistently sample soils close to the AmazonFACE plots (for details, see Lapola and Norby, 2014), without disturbing soil within the plots. Soils were sampled 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 three 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 three 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) laboratory at INPA (Instituto Nacional de Pesquisas da Amazônia) in Manaus, Brazil, nationally certified by Embrapa Soils (2016 Fertility Laboratory Quality Analysis Program, PAQLF,

https://www.embrapa.br/en/solos/paqlf) and by the PIATV (Esalq/USP) inter-laboratorial program of vegetation tissue analysis (Grade A, http://piatv.com.br/). Litterfall was collected biweekly at two of the AmazonFACE plots located along the transect (used in this study) starting in August 2015. Litter traps (0.5 × 0.5 m, n = 24) were installed 1 m above the ground, 12 traps per plot in a circular pattern. The total litter was dried, separated into leaf litter and other litter fractions, and weighed.

3.2.3 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 Chapter 4 or Schaap et al. 2021).

3.2.4 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 1M KCl solution, shaken for one hour and subsequently filtered. The filtered extract was then analyzed in a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V; Shimadzu, Vienna, Austria). Extractable P (Olsen P, Olsen et al. 1954) was determined from extractants of 2 g of soil in 20 ml 0.5M bicarbonate solution ($NaHCO_3$, pH 8.5), shaken for one hour and filtered. Extractant was analyzed following the photometrical Murphy-Riley molybdate blue method (712 nm) (Murphy and Riley 1962). All analyses were accompanied by 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.

3.2.5 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 Vmax). 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 hour 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).

3.2.6 Quantitative analyses

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

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

and

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

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

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

and their angle in degrees as

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

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 (Table 3.1) 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).

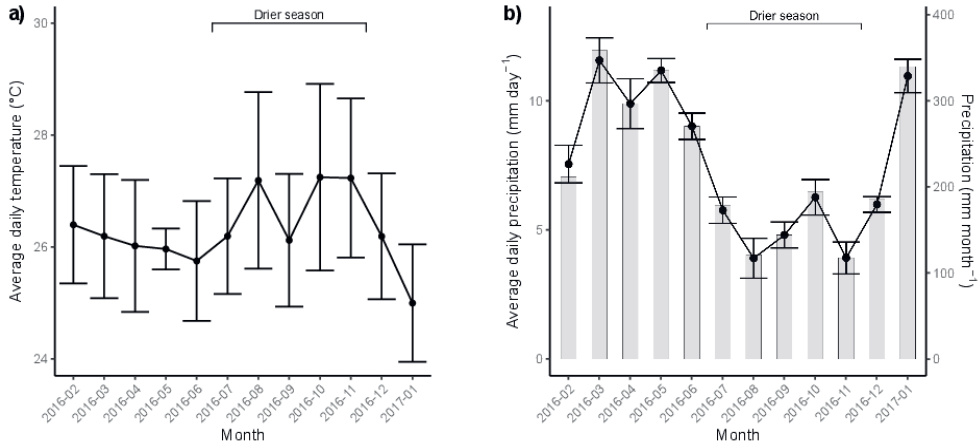


Figure 3.1: a) daily average air temperature (at 34.6 m, above forest canopy), and b) rainfall at the AmazonFACE plots, daily average (line and dots ± SE) and the monthly sum (bars). Both temperature and precipitation were measured every 30 minutes (n = 48 per day) and calculated per day. The "Drier 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 <3mm precipitation.

3.3 Results

Precipitation showed a distinct drier period (at least 40% of days with < 3 mm, Fig. 3.1b) between July and November, during which it was also a few degrees warmer, but average temperature varied little and stayed within a 24.5-27.5°C range (daily average) (Fig. 3.1a, Fig. S3.1a). . Annual leaf litterfall amounted to 5565 ± 55 kg ha^{-1} $year^{-1}$, with a distinct peak during the drier months (Fig 3.2a). Leaf litterfall was significantly correlated to the average monthly temperature ($F_{(1;9)}$ = 5.3, p = 0.047, Fig. S3.1b) and showed a negative relation with the average rainfall ($F_{(1;9)}$ = 42, p < 0.001, Fig. 3.2b) indicating higher leaf litterfall during the drier months. Soil water content showed limited variation (Fig. S3.2), 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. S3.3). 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. S3.3). 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. S3.3). 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

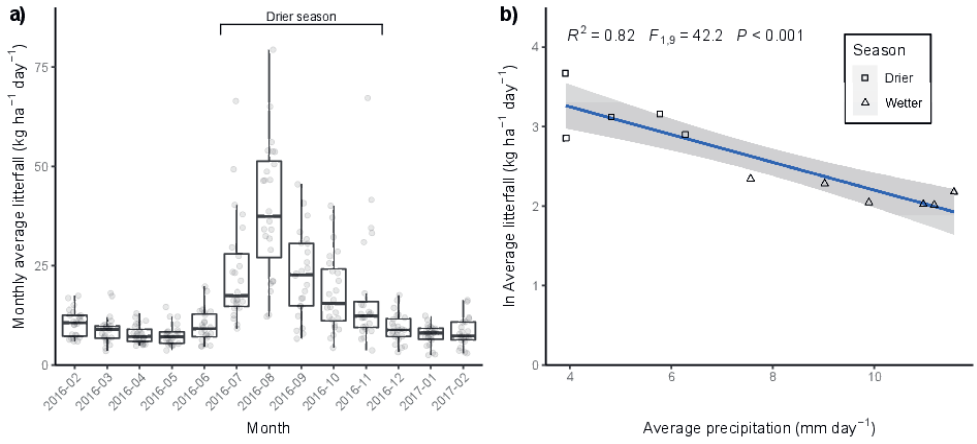


Figure 3.2: a) leaf litter collected (biweekly) at the AmazonFACE plots, recalculated for average daily litter quantity (average per month, each observation represents a different littertrap, n = 24. Boxplot shows median and quartiles), and b) the relation between average daily leaf litterfall and average daily precipitation (natural logarithm) per month. Drier season indicates the drier months as established in Fig. 3.1.

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. S3.4). 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. S3.5) 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 in 5-15 cm those activities were 0.23 ± 0.00, 0.63 ± 0.00, and 26.26 ± 0.08 µmol g soil C^{-1} day^{-1} for BG, NAG and AP respectively (Fig. 3.3 a, 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, (Fig. 3.3). We found that BG activities at both soil depths were significantly positively related to litterfall inputs (Fig. 3.3b), but not to temperature (Fig. S3.6a), and to precipitation only in the top 5 cm (Fig. S3.6b), while potential NAG activity rates were significantly related to only the litterfall (Fig. 3.3d, Fig. S3.6c, 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 (Fig. 3.3f, Fig. S3.6e, f). Based on linear mixed effect models including

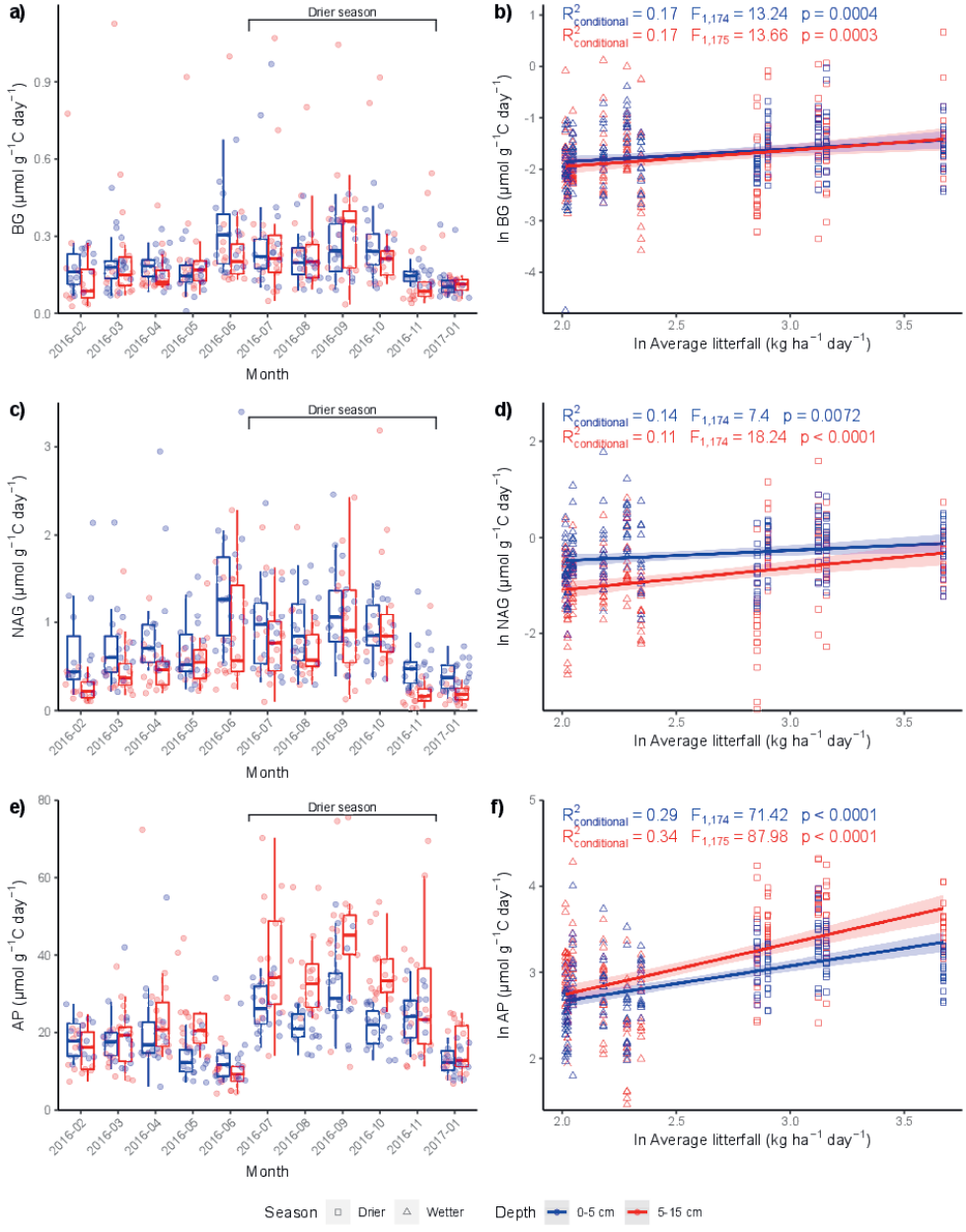


Figure 3.3: C, N and P related extracellular enzyme activities (BG, NAG and AP per soil C) from February 2016 till January 2017, and their relation to the average monthly leaf litterfall. Boxplots are showing the median, the lower and upper hinges correspond to the first and third quartiles (plots a, c and e). The text in plots b, d and f shows the relation between enzymes and litterfall established with linear mixed effects model, with the sampling location as a random effect.

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 3.1).

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. S3.3). 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. S3.7), the relations of enzymes with the extractable C, N, and 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. 3.4).

While the EE activities showed an observable drier-season effect when considered separately (Fig. 3.3), their activity ratios and proportional activity ratios showed less distinct patterns (Table S3.1). From these proportional activity ratios we calculated enzyme vectors, useful for distinguishing relative nutrient demand (Fig. 3.5). 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. S3.7), indicating a weak relation between relative C and N demand.

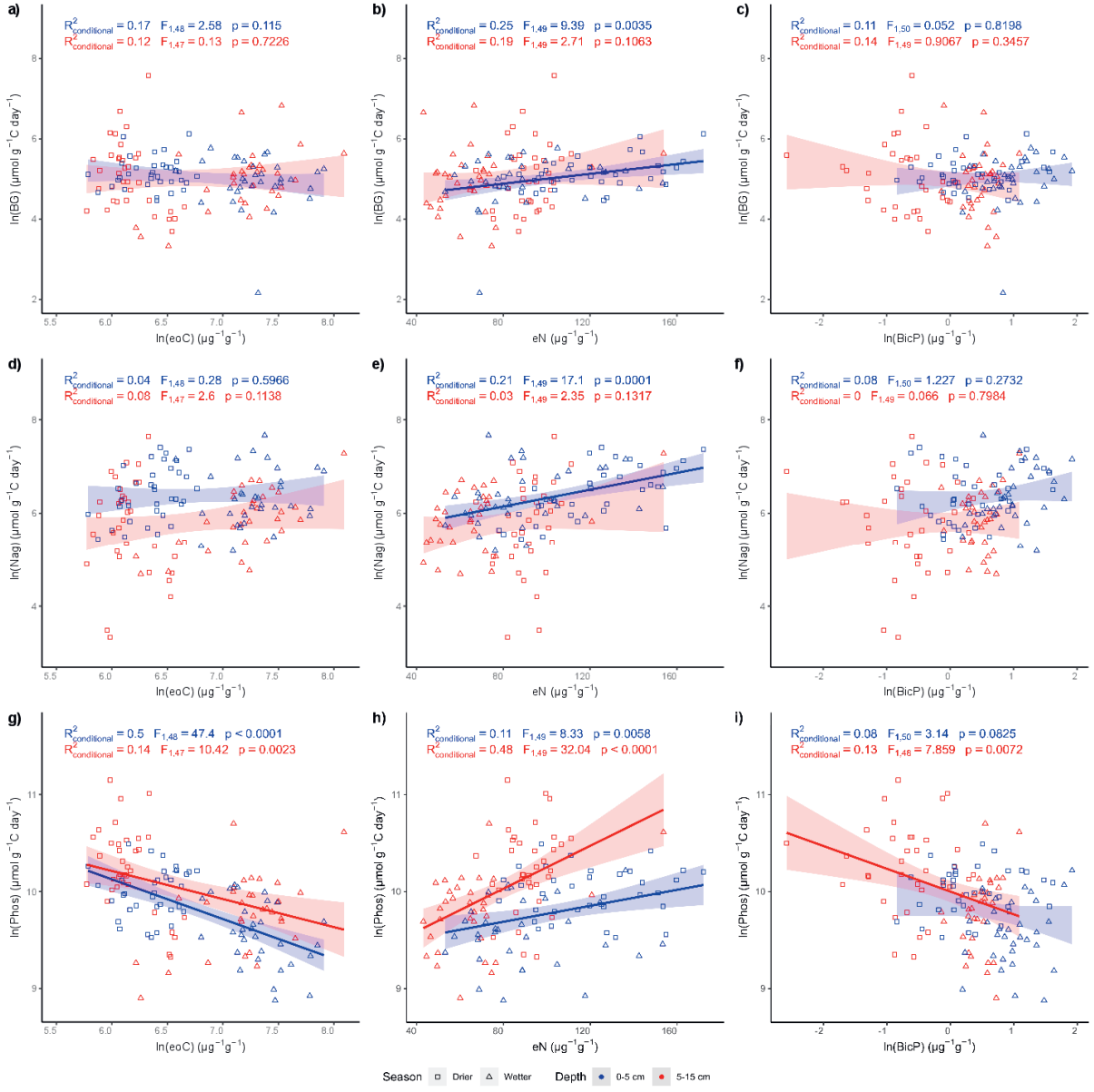


Figure 3.4: Relations between EE and extractable soil nutrients at 0-5 cm and 5-15 cm. Conditional R^2, F and p values for linear mixed effect models with sampling location as a random effect. a), b), and c) ß-glucosidase as related to eoC (natural logarithm), eN and Olsen P (natural logarithm); d), 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).

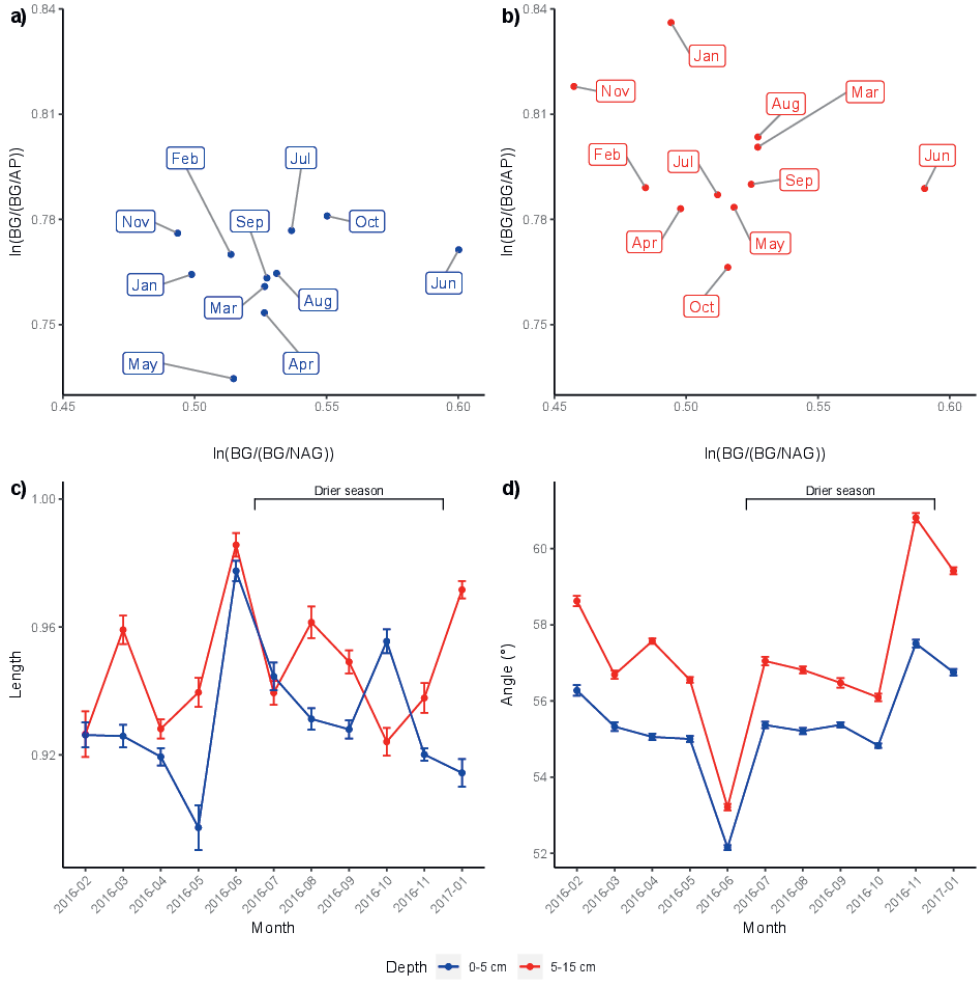


Figure 3.5: Average monthly vectors of proportional enzyme activities at a) 0-5 cm and b) 5-15 cm, and average vector properties c) length (unitless), and d) angle (in degrees) of the monthly average vectors. The error bar in c) and d) represents the standard error).

Table 3.1. 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.

Model	Factors	BG		NAG		AP	
		df	F (p)	df	F (p)	df	F (p)
Null	Intercept	1, 368	9048 (<0.0001)	1, 368	11871 (<0.0001)	1, 368	118217 (<0.0001)
	Depth	1, 368	1 (0.3156)	1, 368	39 (<0.0001)	1, 368	12 (0.0006)
	AIC:		728		854		579
Litterfall	Factors	df	F	df	F	df	F
	Intercept	1, 367	9099 (<0.0001)	1, 367	12060 (<0.0001)	1, 367	124096 (<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*
Precipitation	Factors	df	F	df	F	df	F
	Intercept	1, 367	9119 (<0.0001)	1, 367	11921 (<0.0001)	1, 367	125110 (<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*
Temperature	Factors	df	F	df	F	df	F
	Intercept	1, 367	9076 (<0.0001)	1, 367	11875 (<0.0001)	1, 367	122614 (<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*
Soil Water Content	Factors	df	F	df	F	df	F
	Intercept	1, 367	8713 (<0.0001)	1, 367	11202 (<0.0001)	1, 367	108993 (<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

3.4 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 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; 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 the 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 P-acquisition would imply N limitation on AP production. This suggests further investigation into the seasonal dynamics of the tropical N-cycle, especially in relation to

precipitation, which 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-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; Cotrufo et al. 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 (Chapter 4, 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.

Supplementary Material to Chapter 3

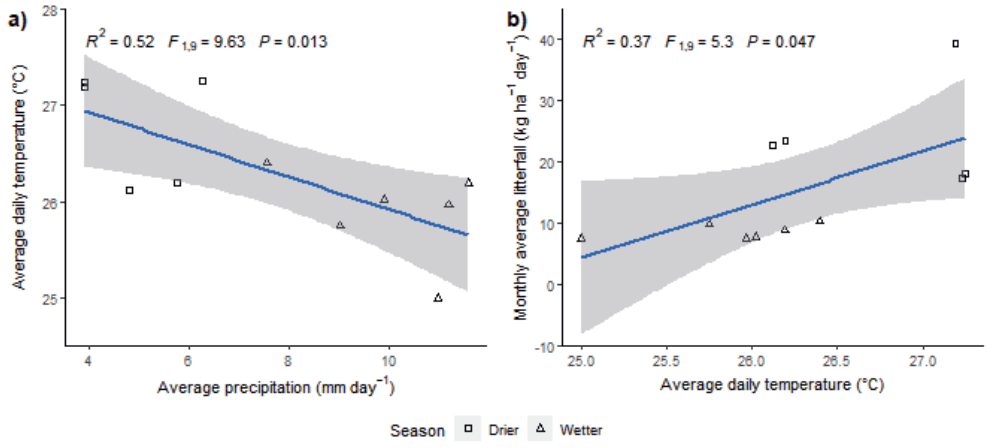


Figure S3.1: The relation between a) average temperature and average precipitation, and b) the relation between average temperature and average litterfall.

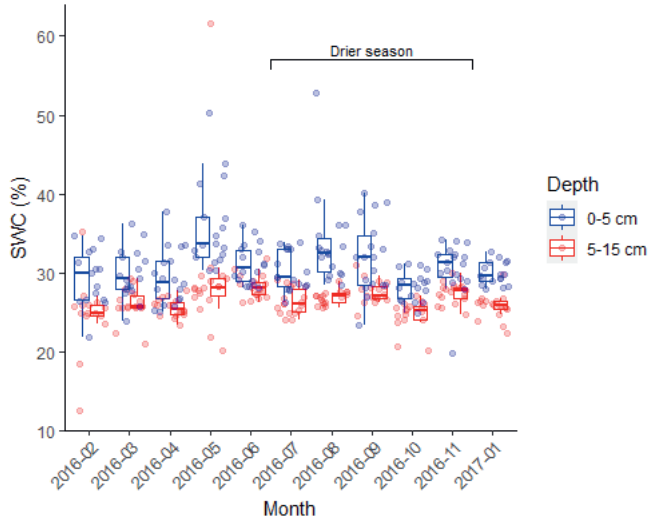


Figure S3.2: Gravimetric soil water contents (SWC) during the sampling period. Boxplots showing the median, the lower and upper hinges correspond to the first and third quartiles.

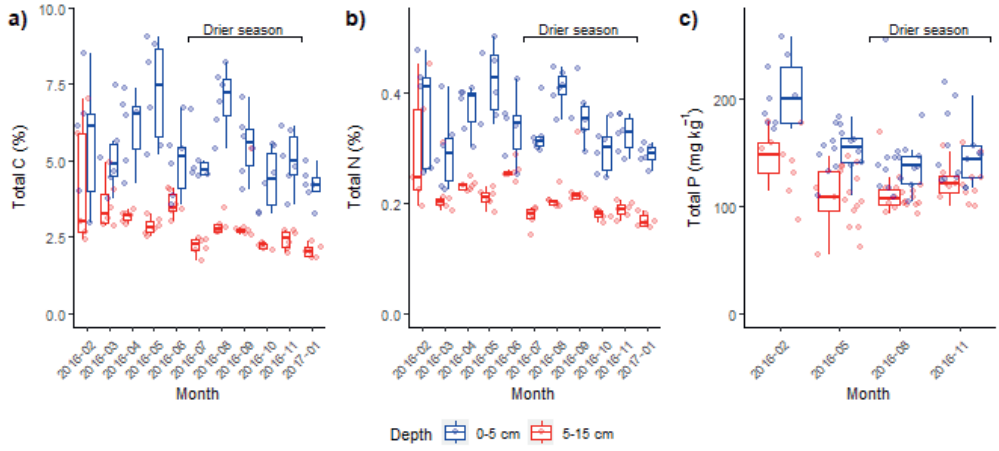


Figure S3.3: Soil nutrient data for the respective sampling dates. Total C and total N in mass percent of dry soil. Boxplots showing the median, the lower and upper hinges correspond to the first and third quartiles.

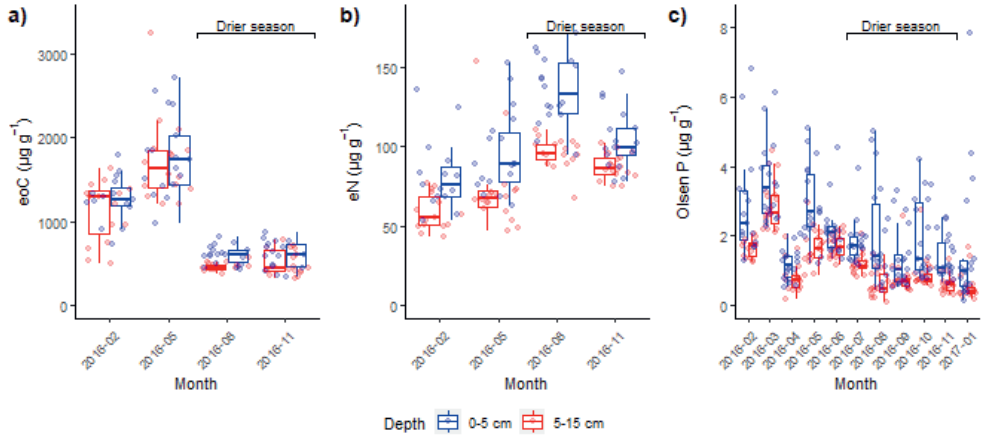


Figure S3.4: eoC, eN and Olsen P in µg g^{-1} dry soil. Boxplots showing the median, the lower and upper hinges correspond to the first and third quartiles.

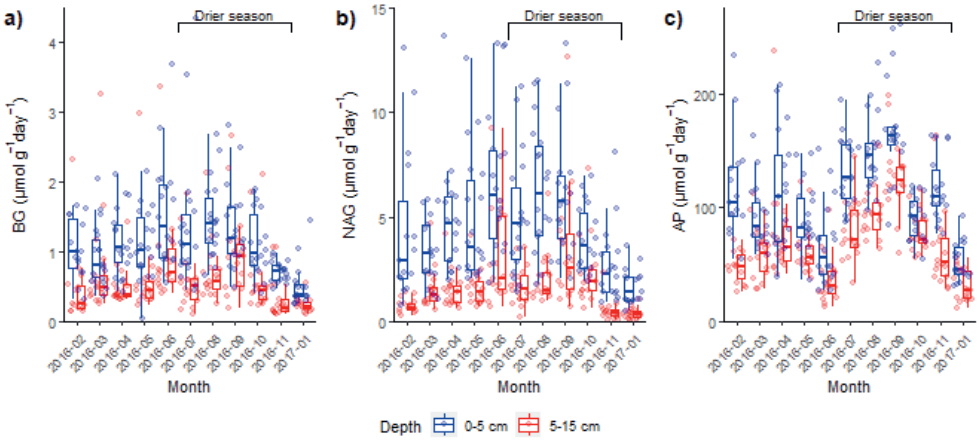


Figure S3.5: C, N and P related extracellular enzyme activities (BG, NAG and AP per gram dry soil) from February 2016 till January 2017. Boxplots showing the median, the lower and upper hinges correspond to the first and third quartiles.

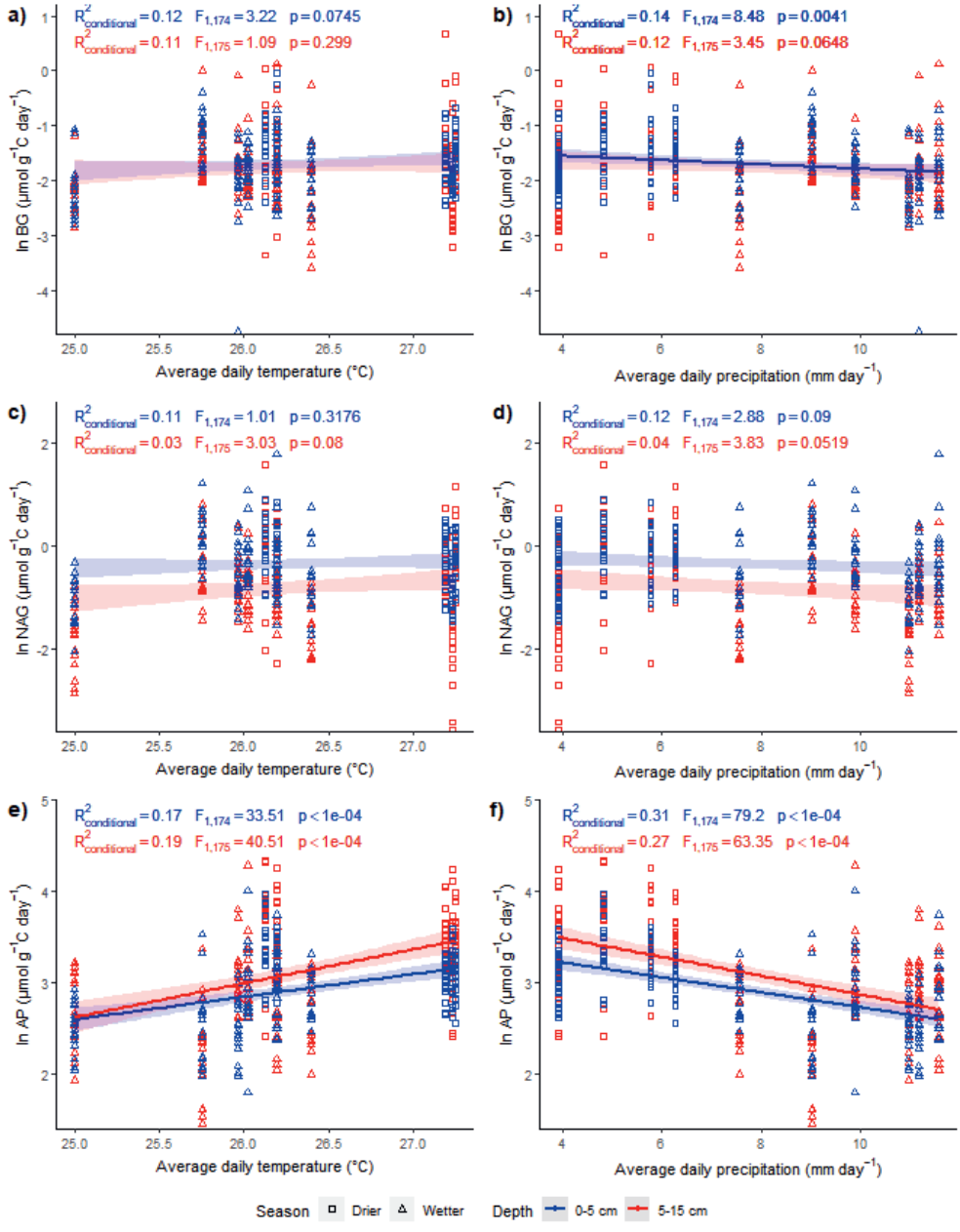


Figure S3.6: Relation between average enzyme activities and temperature and precipitation. Regression line only shown for significant relationships

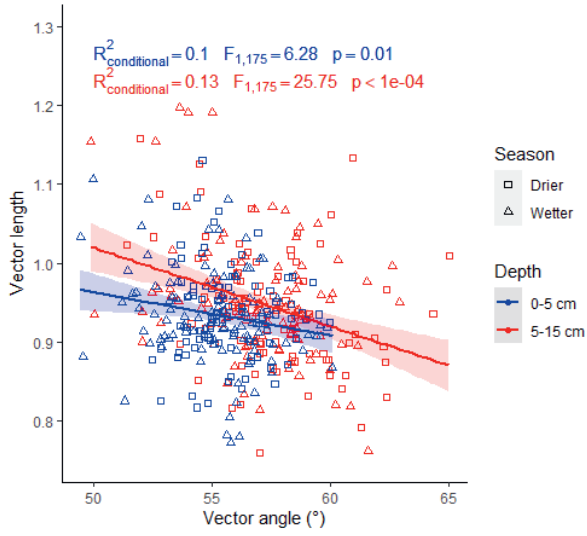


Figure S3.7: Relation between enzyme vector length (unitless) and vector angle (°)

Table S3.1: Proportional enzyme activities as calculated according to Moorhead (2016). All means ± SE.

	Month	n	Proportional BG:AP	Proportional BG:NAG
0-5 cm	2016-02	15	0.514 ± 0.003	0.770 ± 0.004
	2016-03	18	0.527 ± 0.003	0.761 ± 0.003
	2016-04	18	0.526 ± 0.002	0.753 ± 0.002
	2016-05	18	0.515 ± 0.004	0.735 ± 0.006
	2016-06	18	0.600 ± 0.003	0.771 ± 0.002
	2016-07	18	0.537 ± 0.003	0.777 ± 0.004
	2016-08	18	0.531 ± 0.002	0.765 ± 0.003
	2016-09	18	0.527 ± 0.002	0.763 ± 0.002
	2016-10	18	0.550 ± 0.002	0.781 ± 0.003
	2016-11	18	0.494 ± 0.001	0.776 ± 0.002
	2017-01	17	0.501 ± 0.003	0.764 ± 0.004
5-15 cm	2016-02	15	0.485 ± 0.005	0.789 ± 0.005
	2016-03	18	0.527 ± 0.003	0.801 ± 0.003
	2016-04	18	0.498 ± 0.002	0.783 ± 0.002
	2016-05	18	0.518 ± 0.003	0.783 ± 0.004
	2016-06	18	0.590 ± 0.003	0.789 ± 0.003
	2016-07	18	0.512 ± 0.003	0.787 ± 0.003
	2016-08	18	0.527 ± 0.004	0.804 ± 0.004
	2016-09	18	0.525 ± 0.003	0.790 ± 0.003
	2016-10	17	0.516 ± 0.003	0.766 ± 0.003
	2016-11	18	0.457 ± 0.003	0.818 ± 0.004
	2017-01	18	0.494 ± 0.002	0.836 ± 0.002

Correction to Chapter 3

These corrections have been submitted to *Biogeochemistry*.

In the published article, there is a mistake in the calculated enzyme activities and the related vectors. The enzyme activities are a factor 100 too small, affecting the values reported in the text and in figures 3.3 and 3.4. Furthermore, the equations used for the calculation of the proportional activities indicate ln-transformations while ln-transformations should not have been applied. This affected figure 3.5. The correct equations, in-text corrections and correct figures are shown below.

The correct equations for the proportional activities are without a ln-transformation:

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

and

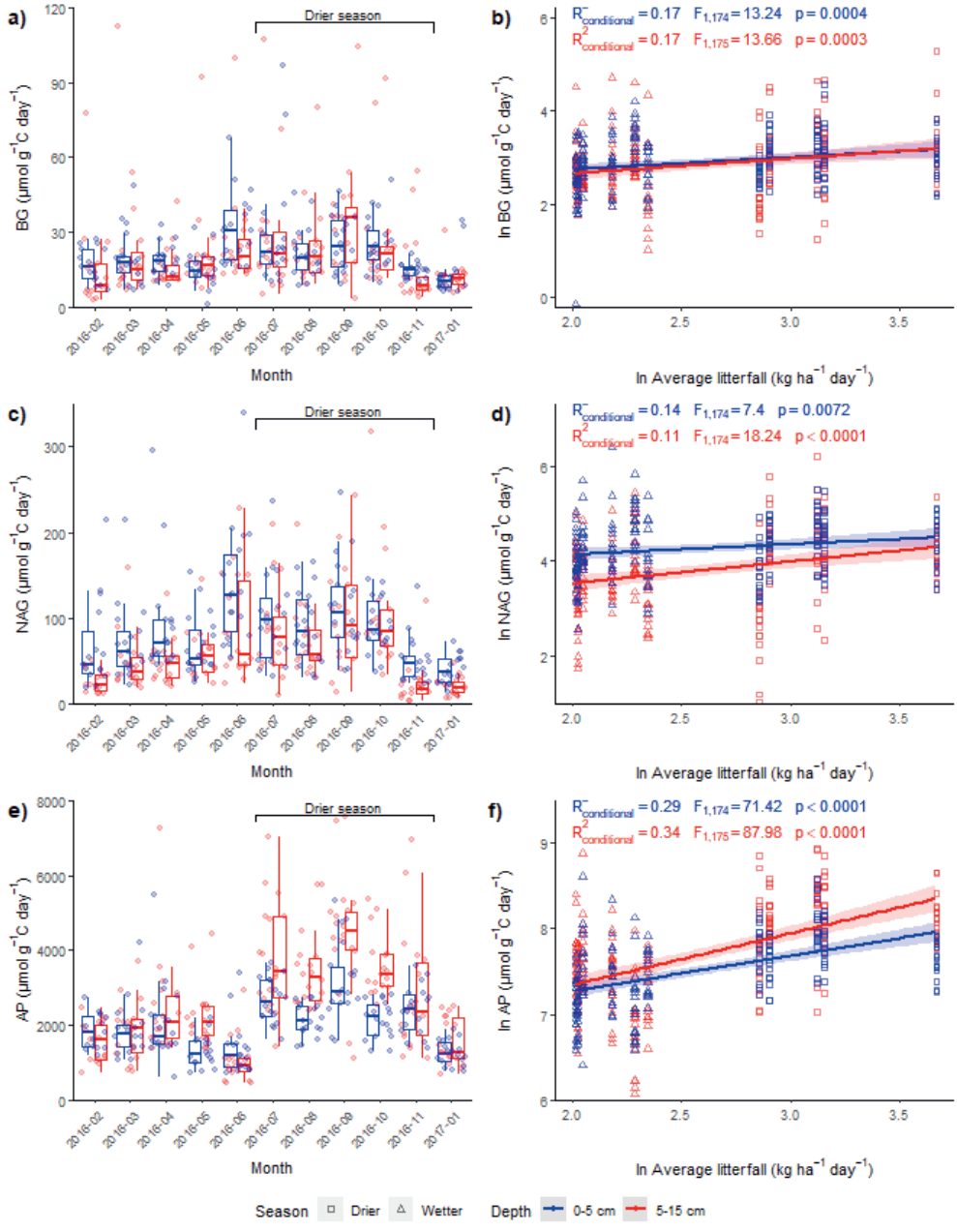
$$C{:}P_{proportional} = \frac{BG}{BG + AP}$$

The correct text in the results section with respect to enzyme activities is:

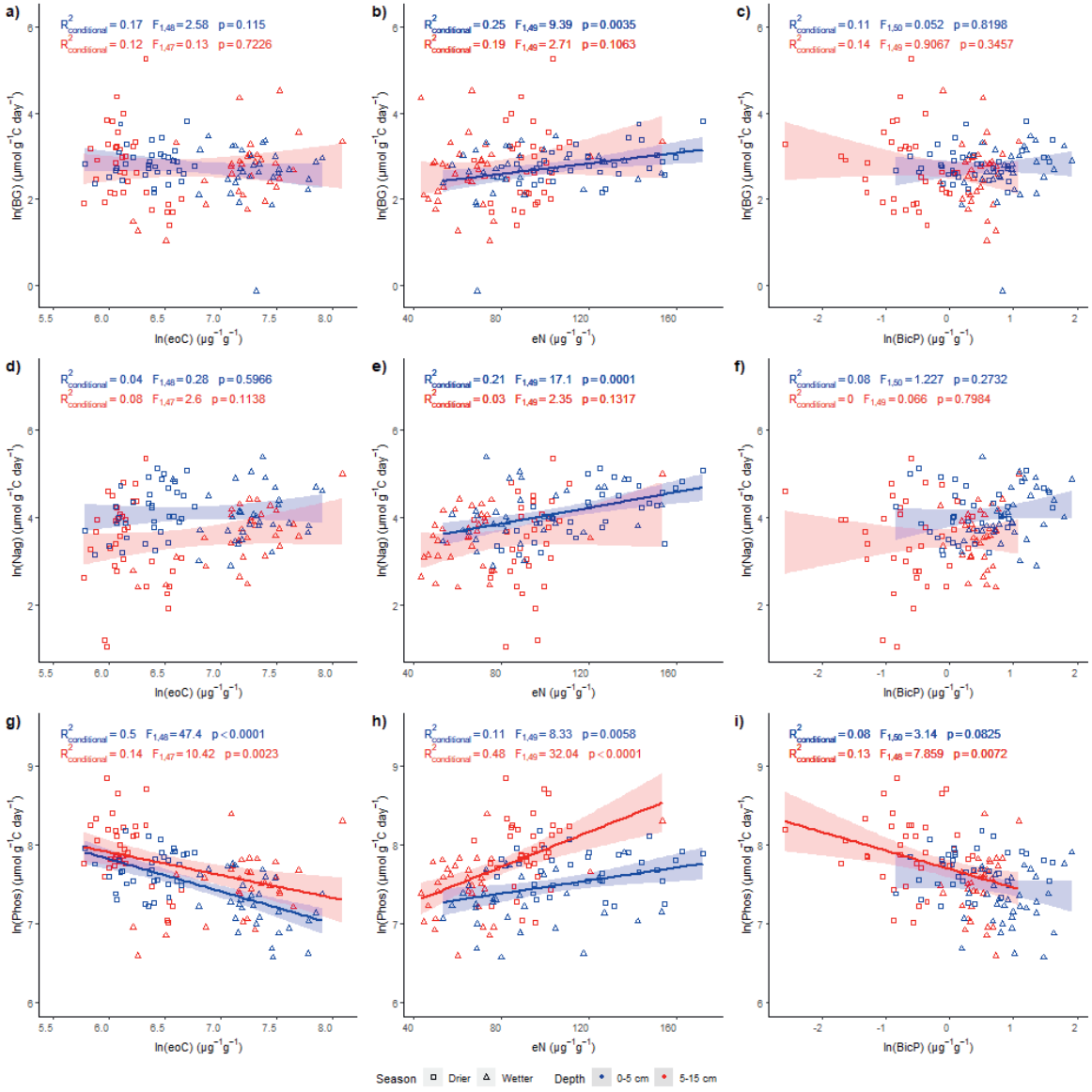
Average EE activities (as expressed per gram soil C; for values per dry soil see Fig. S3.5) were 21.13 ± 0.06 µmol g C^{-1} day^{-1} for BG, 86.82 ± 0.33 µmol g C^{-1} day^{-1} for NAG and 2020.98 ± 4.49 µmol g C^{-1} day^{-1} for AP, while in 5-15 cm those activities were 23.01 ± 0.12, 63.21 ± 0.31, and 2626.40 ± 7.74 µmol g soil C^{-1} day^{-1} for BG, NAG and AP respectively (Fig. 3.3 a, c, e). In the top 5 cm EE activity rates peaked just before drier season and were lowest in the wetter season: BG and NAG peaked just before the drier season (in June, 31.34 ± 0.78 µmol g C^{-1} day^{-1} and 137.31 ± 3.87 µmol g C^{-1} day^{-1} respectively) while AP peaked in September (3190.60 ± 57.30 µmol g C^{-1} day^{-1}). The lowest EE activities at 0-5 cm depth were all in January (BG 12.54 ± 0.45 µmol g C^{-1} day^{-1}, NAG 39.80 ± 0.97 µmol g C^{-1} day^{-1} and AP 1254.93 ± 18.69 µmol g C^{-1} day^{-1}).

This pattern was reflected at 5-15 cm, but with BG showing highest rates in August (34.47 ± 2.40 µmol g C^{-1} day^{-1}), and NAG and AP peaking in September (121.58 ± 6.13 µmol g C^{-1} day^{-1} and 4461.28 ± 89.68 µmol g C^{-1} day^{-1} respectively). The lowest EE activities at this depth were in January for BG and NAG (12.10 ± 0.29 µmol g C^{-1} day^{-1} and 19.61 ± 0.60 µmol g C^{-1} day^{-1} respectively), and in June for AP (1051.54 ± 33.51 µmol g C^{-1} day^{-1}).

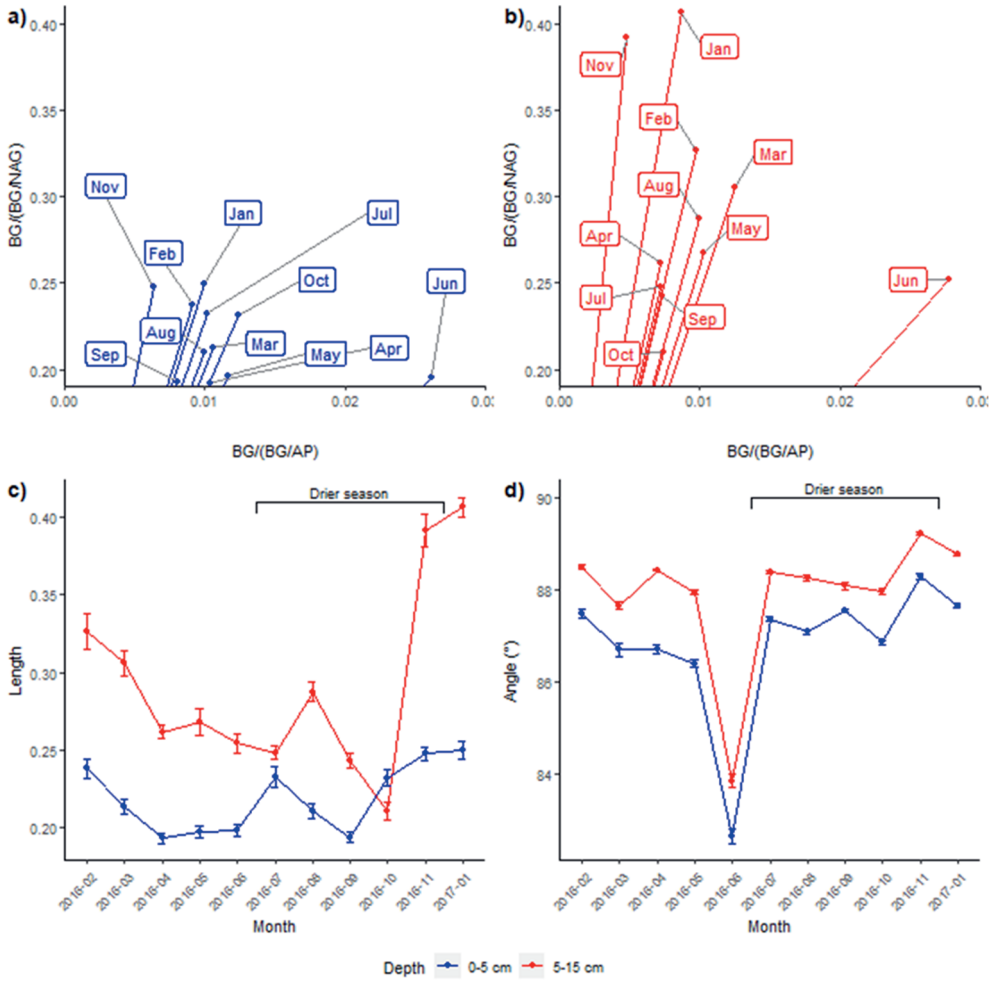
The correct figures:



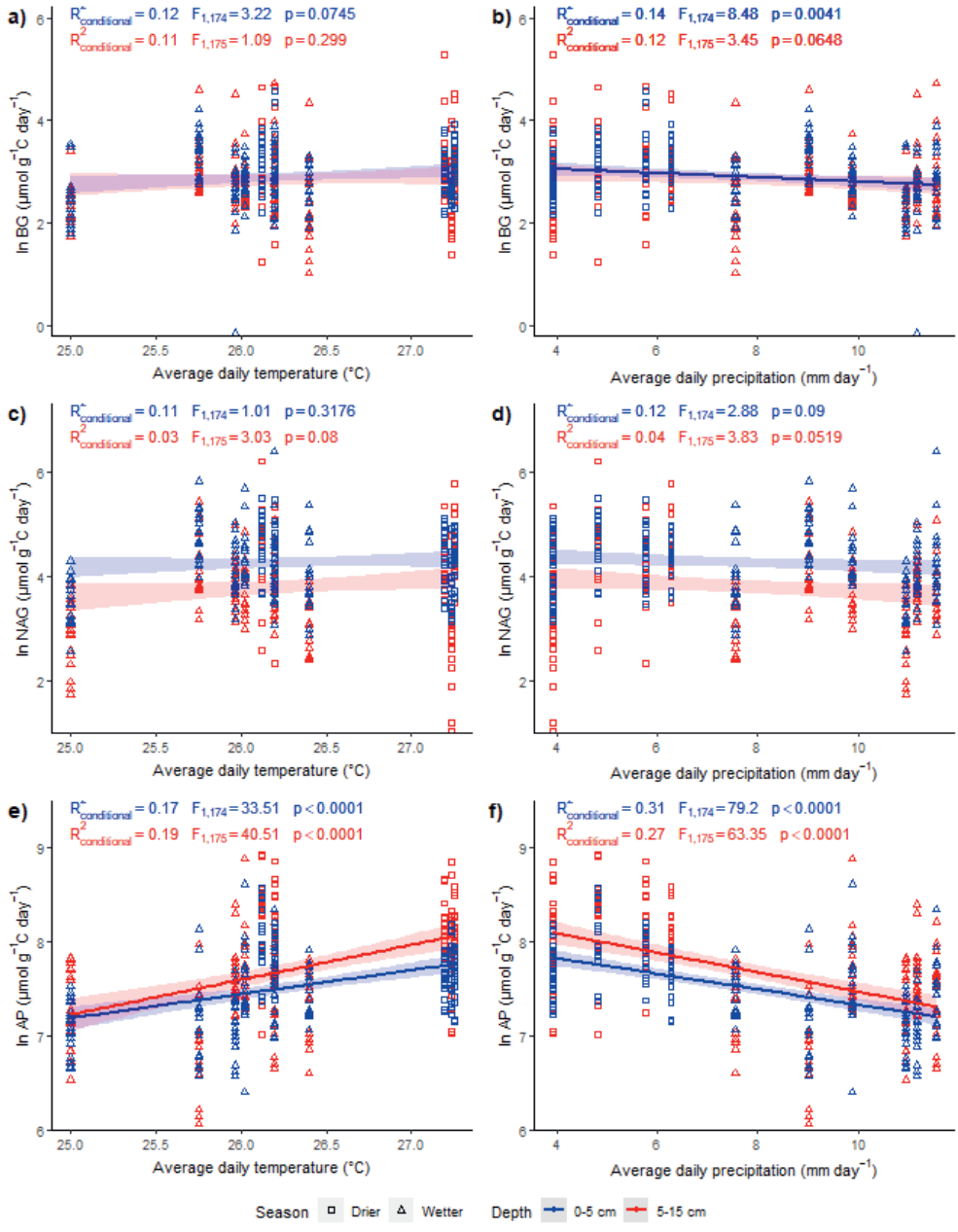
Corrected Figure 3.3: C, N and P related extracellular enzyme activities (BG, NAG and AP per soil C) from February 2016 till January 2017, and their relation to the average monthly leaf litterfall. Boxplots are showing the median, the lower and upper hinges correspond to the first and third quartiles (plots a, c and e). The text in plots b, d and f shows the relation between enzymes and litterfall established with linear mixed effects model, with the sampling location as a random effect.



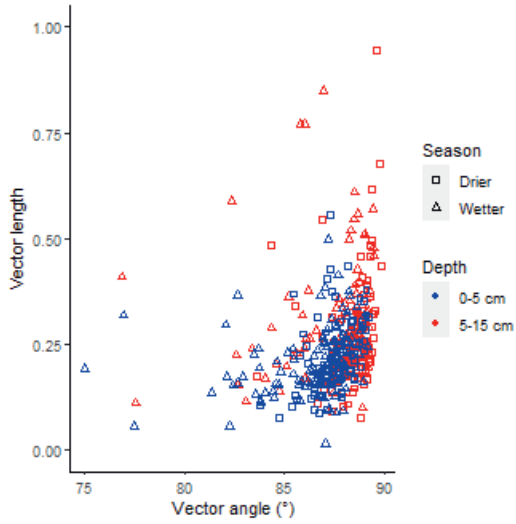
Corrected Figure 3.4: Relations between EE and extractable soil nutrients at 0-5 cm and 5-15 cm. Conditional R^2, F and p values for linear mixed effect models with sampling location as a random effect. a), b), and c) ß-glucosidase as related to eoC (natural logarithm), eN and Olsen P (natural logarithm); 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).



Corrected Figure 3.5: 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).



Corrected Figure S3.6: Relation between average enzyme activities and temperature and precipitation. Regression line only shown for significant relationships



Corrected Figure S3.7: Relation between enzyme vector length (unitless) and vector angle (°)

Corrected Table S3.1: Proportional enzyme activities as calculated according to Moorhead (2016). All means ± SE.

	Month	N	Proportional BG:AP	Proportional BG:NAG
0-5 cm	2016-02	15	0.0091 ± 0.0002	0.2377 ± 0.0063
	2016-03	18	0.0106 ± 0.0002	0.2131 ± 0.0048
	2016-04	18	0.0104 ± 0.0003	0.1924 ± 0.0032
	2016-05	18	0.0117 ± 0.0002	0.1967 ± 0.0039
	2016-06	18	0.0261 ± 0.0009	0.1958 ± 0.0037
	2016-07	18	0.0101 ± 0.0003	0.2322 ± 0.0066
	2016-08	18	0.0100 ± 0.0002	0.2099 ± 0.0047
	2016-09	18	0.0081 ± 0.0002	0.1932 ± 0.0035
	2016-10	18	0.0124 ± 0.0003	0.2315 ± 0.0051
	2016-11	18	0.0064 ± 0.0001	0.2475 ± 0.0042
	2017-01	17	0.0100 ± 0.0003	0.2498 ± 0.0057
5-15 cm	2016-02	15	0.0098 ± 0.0007	0.3263 ± 0.0112
	2016-03	18	0.0125 ± 0.0007	0.3056 ± 0.0079
	2016-04	18	0.0072 ± 0.0002	0.2615 ± 0.0040
	2016-05	18	0.0103 ± 0.0006	0.2674 ± 0.0085
	2016-06	18	0.0278 ± 0.0012	0.2524 ± 0.0062
	2016-07	18	0.0072 ± 0.0003	0.2480 ± 0.0044
	2016-08	18	0.0099 ± 0.0006	0.2870 ± 0.0063
	2016-09	18	0.0073 ± 0.0002	0.2426 ± 0.0050
	2016-10	17	0.0074 ± 0.0003	0.2104 ± 0.0056
	2016-11	18	0.0048 ± 0.0002	0.3919 ± 0.0106
	2017-01	18	0.0086 ± 0.0002	0.4068 ± 0.0063

Chapter 4:

Litter inputs and phosphatase activity affect the temporal variability of organic phosphorus in a tropical forest soil in the Central Amazon

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Abstract

Purpose

The tropical phosphorus cycle and its relation to soil phosphorus (P) availability are a major uncertainty in projections of forest productivity. In highly weathered soils with low P concentrations, plant and microbial communities depend on abiotic and biotic processes to acquire P. We explored the seasonality and relative importance of drivers controlling the fluctuation of common P pools via processes such as litter production and decomposition, and soil phosphatase activity.

Methods

We analyzed intra-annual variation of tropical soil phosphorus pools using a modified Hedley sequential fractionation scheme. In addition, we measured litterfall, the mobilization of P from litter and soil extracellular phosphatase enzyme activity and tested their relation to fluctuations in P fractions.

Results

Our results showed clear patterns of seasonal variability of soil P fractions during the year. We found that modeled P released during litter decomposition was positively related to change in organic P fractions, while net change in organic P fractions was negatively related to phosphatase activities in the top 5 cm.

Conclusion

We conclude that input of P by litter decomposition and potential soil extracellular phosphatase activity are the two main factors related to seasonal soil P fluctuations, and therefore the P economy in P impoverished soils. Organic soil P followed a clear seasonal pattern, indicating tight cycling of the nutrient, while reinforcing the importance of studying soil P as an integrated dynamic system in a tropical forest context.

Keywords: Amazon, Hedley fractionation, Lowland tropical forest, Phosphatase activity, Phosphorus cycle, Leaf litter

4.1 Introduction

The Amazon Basin contains about one third of the world's remaining tropical forests (Mayaux et al. 2005), playing an important role in the global carbon (C) cycle. Currently, the Amazon rainforest absorbs 0.5 GT C yr^{-1} (Pan et al. 2011), but recent evidence suggests that it is losing its C-sink capacity, potentially induced by rising temperatures and greater drought frequency or by reaching a new state of equilibria adapting to changing climate conditions (Brienen et al. 2015; Hubau et al. 2020; Gatti et al. 2021). One factor that could change the response of the forest to climate change is the CO_2-fertilization effect. This effect occurs when higher atmospheric CO_2 levels would allow an increase in photosynthesis rates, and could augment forest productivity (Zhu et al. 2016). However, the magnitude of this effect may depend on several factors, such as availability of light, water, and nutrients (Du et al. 2020). While in natural forest ecosystems in temperate regions nitrogen (N) is the most limiting nutrient for plant productivity (Vitousek 1982; Oren et al. 2001), tropical forest ecosystems are generally considered to be constrained by phosphorus (P) availability (Vitousek 1984; Townsend et al. 2011; Turner et al. 2018; Hofhansl et al. 2020) with feedbacks to the nitrogen cycle (Quesada et al. 2010; Nasto et al. 2014). Across the Amazonian basin, soil P has been shown to be positively related to forest productivity (Aragão et al. 2009; Quesada et al. 2012). The inclusion of P cycles in regional dynamic vegetation models suggest P limitation will be important in controlling forest productivity and the responses of tropical biomes to global change (Fleischer et al. 2019; Terrer et al. 2019). However, many key processes controlling P availability in tropical forests remain poorly understood.

Approximately 60% of Amazonian forests grow on geologically old and highly weathered soils, typically Ferralsols or Acrisols, with low nutrient concentrations (Quesada et al. 2011). This is in line with assumptions of the P pedogenetic model conceptualized by Walker and Syers (1976), where rock derived (mineral) P ends up in organic, occluded, and non-occluded (i.e., more available) pools at the start of pedogenesis, but after the parent material as a P source is depleted, P availability declines sharply. Total P declines during this soil aging process, due to losses - like leaching - from the system. Eventually most soil P will be either occluded or in organic pools - a phenomenon described as a "terminal steady state" (Walker and Syers 1976). In older, more weathered soils, a larger proportion of P can be found in organic forms and highlights the increased importance of biological activity.

While P is an essential element for plants and microbes, the directly plant-available fraction is usually a relatively small pool compared to the total P concentration (Tiessen 2008), and

chemical availability of P is determined by the solubility of ortho-P in a soil, which is mainly defined by sorption-desorption kinetics (Hinsinger 2001). A common method to characterize P accessibility in soils was developed by Hedley et al. (1982), to identify a series of P fractions based on their solubility that represent different levels of bioavailability for inorganic (P_i) and organic P fractions (P_o) (Tiessen and Moir 1993). The P_i fractions include water-soluble P, which should be directly available to plants, but also P bound to aluminum (Al) and iron (Fe), up to P bound to calcium (Ca) and primary P minerals and is therefore usually poorly accessible to plants (Cross and Schlesinger 1995). In soils with low or no Ca, this last fraction is likely to consist of recalcitrant organic matter or otherwise occluded P. In comparison to other anions, P_i has a relatively low mobility in soil (Johnson and Cole 1980). High kaolinite clay contents and high amounts of Al and Fe oxides amplified by low soil pH, common for tropical soils, facilitate sorption of P_i, further reducing its mobility (McGechan and Lewis 2002). The P_o fractions are more difficult to interpret than P_i fractions. They are on the same solubility continuum from water to Ca-bound, being directly derived from plant or microbial sources or from soil organic matter (SOM). Organic P forms are more complex (e.g. phospholipids, DNA, phosphate monoesters, glucophosphates, phytic acids) and have the potential to be an important contributor to P-bioavailability through chemical and biochemical plant acquisition strategies (Darch et al. 2016).

Plants have evolved various mechanisms to maintain a bioavailable pool of P; these mechanisms include an increased presence of root mats dominating the forest floor, fine roots, association with mycorrhizae for P uptake, root exudation of enzymes for mineralization of organic compounds, or exudation to change sorption or microbial activity through the “priming” effect (Herrera et al. 1978a; Stark and Jordan 1978; Hinsinger 2001; Buendía et al. 2014; Steidinger et al. 2015; Lugli et al. 2020). With declining plant available P and a relatively larger organic P pool (Turner et al. 2007), plant strategies for efficient recycling and uptake are increasingly relevant (Roberts et al. 2015). Plants might apply different strategies to compete for P (Steidinger et al. 2015; Nasto et al. 2017; Raven et al. 2018). From a plant perspective, those strategies can be categorized as either foraging or mining strategies (Richardson et al. 2011). Foraging strategies serve to explore more soil, while mining strategies are used to access forms of P by chemical alteration in the soil, for example through the excretion of enzymes (Hinsinger 2001; Lloyd et al. 2001). Although it is tough to distinguish the origins of soil phosphatase, root phosphatase appears to only account for a small part of total soil phosphatase activity, underlining the relative importance of microbial processes in the rhizosphere for P dynamics (Cabugao et al. 2021). While organic bound P is not directly available, phosphatases catalyzing the degradation of

organic molecules might rapidly change the available pool of P in the tropics (Wood et al. 2016; Turner et al. 2018) and thus can be used as a general proxy for demand of P (Vance et al. 2003).

With declining delivery rate of Pi from mineral sources, recycling of P and the dynamics in the organic P pools become increasingly important. One of the largest fluxes of organic matter in tropical forests is leaf litterfall (Hofhansl et al. 2012), with fluctuations and annual phenological cycles driven by changes in water availability and solar irradiation (Wu et al. 2016). Seasonality of rainfall is an important determinant of litterfall, though fluctuations may vary per region (Chave et al. 2010). Litterfall and its decomposition constitutes an important flux of organic material (and thus nutrients like P) to the soil (Luizao 1989), to maintain nutrient stocks and mineralize P bound in organic molecules and ultimately to plant uptake facilitating biomass production. However, there is an offset between litterfall peak production in the drier season and the mineralization of nutrients in Central Amazonia (Luizão and Schubart 1987). Typically, microorganisms decompose litter, showing a quick initial release of soluble nutrients at the onset of decomposition, which gradually reduces over time (Prescott and Vesterdal 2021). A large part of P released from litter is inorganic (Noack et al. 2012; Schreeg et al. 2017). Moreover, seasonal fluctuations in precipitation also affect decomposition dynamics, not only through the release of soluble compounds, but also by affecting soil moisture and the activity of the microbial community (Krishna and Mohan 2017). If nutrient pulses aboveground are synchronized with nutrient availabilities and plant strategies belowground, this might have implications for our understanding of the dynamic nutritional system that underlies the functioning of the forest (Janssen et al. 2021).

In this study, we aimed to evaluate the temporal dynamics of soil Hedley P fractions in a Central Amazonian forest with low soil P concentration. We suspected that different soil P fractions are not static but vary over time. We expected fluctuations to be most pronounced in the top 5 cm where the biological activity is highest, while in the soil below (5-15 cm) we would expect the same pattern but with a smaller amplitude. Moreover, we hypothesized that seasonal variation is driven by fluctuating inputs (litterfall), subsequential decomposition derived organic and inorganic P inputs to the soil, exchange between soil P fractions (catalyzed by phosphatase activities, among others), and outputs (i.e. plant uptake). We aimed to identify the relative importance of drivers controlling the fluctuation of different P pools, such as litterfall inputs, litter decomposition and phosphatase activity from either plant roots or microbes to degrade P_o compounds. We found that fluctuations in P_o are driven by (1) litterfall inputs, and on the other hand (2) degradation by phosphatase

activity, such that (3) both litterfall and enzyme activities follow a seasonal pattern, which reflects differences in biological activity and soil P-release, reflected in soil P_i fractions if not taken up by plants and microbes.

4.2 Methods

4.2.1 Site description

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 Lapola and Norby 2014; Pereira et al. 2019). Characteristic for the area are old-growth tropical forests locally known as "Terra Firme" forests, situated on plateaus with nutrient poor and clay-rich soils classified as Geric Ferralsols, with a pH of 3.94, in soils with 68% clay, 20% sand and 12% silt (Quesada et al. 2010). Average annual rainfall is about 2,400 mm, with a drier period from June to October, while the average temperature fluctuates from 25.8°C in April to 27.9°C in September (Araújo et al. 2002).

4.2.2 Soil sample collection

Soils were sampled from 18 sampling points. On 6 locations along a 400 m north-south transect (every 80 m), we sampled 3 points in the east-west direction, with a distance of 10 m between the 3 sampling points. The sampling scheme was adopted to consistently sample soils close to the AmazonFACE plots (for details, see Lapola and Norby 2014), without disturbing soil within the plots. Soils were sampled in February, May, August, and November 2016, 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. Soil aliquots were stored after weighing and oven drying (48 h at 65°C) until further analysis, while enzyme activity measurements were performed in fresh soil within 3 days of sampling. Moisture contents of fresh soil were calculated from the weight differences before and after drying (Fig. 4.1) to express all soil properties throughout this study on a dry soil basis. Soils were analyzed

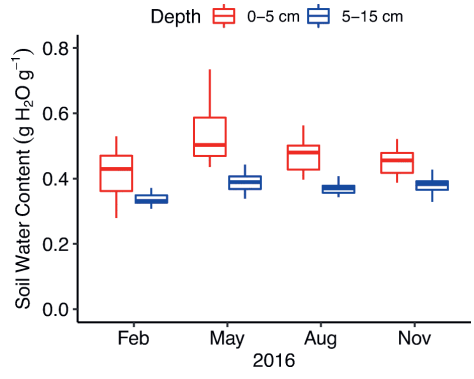

Figure 4.1: Soil water contents in g H_2O g^{-1} for each sampling date, at both sampling depths (n = 18)

individually and not bulked. All analyses were performed at the LTSP (Laboratório Temático de Solos e Plantas) laboratory at INPA (Instituto Nacional de Pesquisas da Amazônia) in Manaus, Brazil, nationally certified by Embrapa Soils (2016 Fertility Laboratory Quality Analysis Program, PAQLF, https://www.embrapa.br/en/solos/paqlf) and by the PIATV (Esalq/USP) inter-laboratorial program of vegetation tissue analysis (Grade A, http://piatv.com.br/).

4.2.3 Soil P-fractionation

Phosphorus fractions were determined in oven dried soils, with an adaptation of the sequential extraction method developed by Hedley et al. (1982; described by Tiessen and Moir 1993; adapted by Quesada et al. 2010, Fig. 4.2). The extractant sequence was an anion exchange membrane (resin strip) in water, 0.5 M $NaHCO_3$ (bicarbonate fraction, pH 8.5), 0.1 M NaOH (hydroxide fraction) and 1 M HCl (hydrogen chloride fraction, Fig. 4.2), each of them shaking for 16 hours. All extracts were analyzed for inorganic Pi. In addition, the $NaHCO_3$ and NaOH extracts were digested with a sulfuric acid solution (H_2SO_4, 0.9 M) and analyzed for total P, which allowed the calculation of their respective organic (P_o) fraction. As an adaption to the Hedley et al./Tiessen and Moir (1993) method, the concentrated HCl-extraction step and digestion of the soil residue were not followed. Instead, another soil subsample was analyzed for total P by digestion with a concentrated sulfuric acid solution (H_2SO_4, 18 M), followed by H_2O_2 (Quesada et al. 2010). All seven extracts were analyzed for PO_4 concentrations photometrically (712 nm) using the Murphy-Riley method and are given

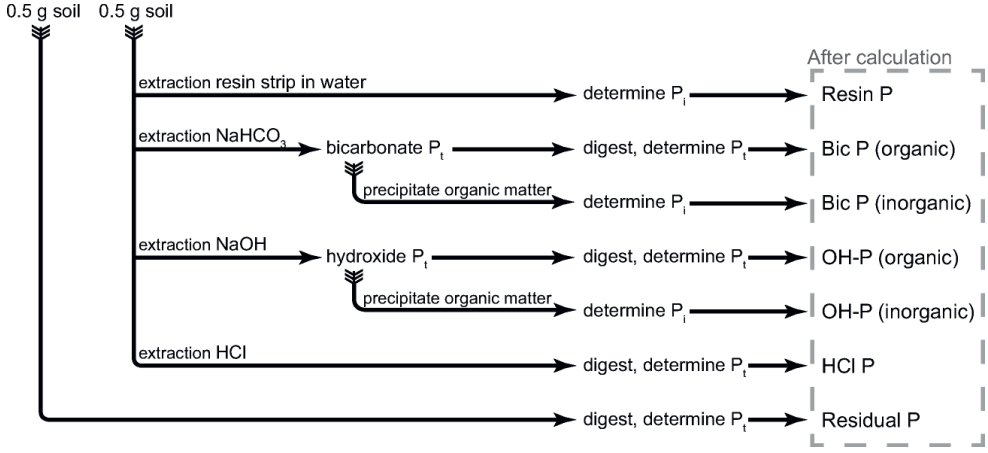

Figure 4.2: Schematic view of the Hedley fractionation method for sequential extraction of P, modified from Tiessen and Moir (1993). The organic P (Po) is the difference between the inorganic P (Pi) and the total P (Pt). The residual fraction is the difference between the total P from the entire sample and the sum of the extractable fractions. Details in Methods section 'Soil P-fractionation'.

in µg g^{-1} dry soil (Murphy and Riley 1962). Resulting fractions include four inorganic (P_i) fractions (in order of decreasing availability); the resin fraction, the bicarbonate fraction, the hydroxide fraction, and the hydrogen chloride fraction, along with the organic bicarbonate and hydroxide extractable fractions (P_o). The residual P fraction was obtained from subtracting the sum of the extractable inorganic and organic P fractions from the total P (Fig. 4.2). All analyses were accompanied by two method blanks (no soil) to account for contamination or background signal, and possible lab variation was accounted for by analyzing standards during each batch of photometric extract reading. The blanks from the standard curves (calibration blanks) were used to calculate the detection limit for each batch (defined as 3 × SD of the blanks) during analysis. Readings were discarded if they were under the detection limit, except for the method blanks that were subtracted from each sample value to account for background noise or contamination. Tests of the fractionation method resulted in an average coefficient of variation for individual soils analyzed in different batches of 0.11 for the resin fraction, 0.17 for the inorganic NaHCO3 fraction, 0.14 for the organic NaHCO3 fraction, 0.09 for the inorganic NaOH fraction, 0.13 for the organic NaOH fraction, and 0.20 for the HCl fraction.

4.2.4 Litterfall and P-input

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, weighed, and analyzed for total P (for total P subsamples were digested with nitric-perchloric acid and concentrations determined with the Murphy-Riley method as described above). Total litter P was scaled up to g m^{-2}, with the standard error showing the differences between individual litter traps. We aimed to estimate P release from litter over time accounting for a potential delay (time lag) between leaf litterfall, its decomposition, and subsequent P release into soil. To estimate P release from leaf litter over time, we used data from a litter decomposition experiment conducted at the same study site. The decomposition experiment measured remaining nutrients in litter, including P, which we used to fit a simple model following an exponential decline as Eq. (1) (adapted from Olson 1963), with *f* as the mass fraction of remaining P in leaf litter:

$$f(x) = e^{-bx} \quad \textit{(Equation 1)}$$

with b = 0.00178 as decomposition constant (R2 = 0.82, data from Martins et al. 2021) for the available 188 days of the decomposition data. We did not take into account further data

on litter stoichiometry or litter biomass, only the values for remaining P were used. To estimate P loss from litter from a certain litter subsample on a given day, the differential of Eq. 1, presented here as Eq. 2:

$$f'(x) = -b \times e^{-bx} \quad \textit{(Equation 2)}$$

was used. We combined this formula with litterfall data (i.e. leaf litter per day for each sampling interval) to account for seasonal variation in inputs, and summed the litter P-loss from litterfall (up to an arbitrarily chosen thousand days prior) to get an estimation of the litter P-input to soil (litter P-loss) on a given day from the following Eq. 3:

$$-\sum_{t=0}^{1000} r \times c_{t=0}(-b \times e^{-bt}) \quad \textit{(Equation 3)}$$

where t are the days prior to the day of interest (1000 days), c_t= 0 is the amount of litter at the start of each t and r is the initial concentration of litter P. Note that we use this formula with the same decomposition constant for the whole year, in both the wetter and the drier season. It is worth noting that the decomposition experiment took place across rainfall regimes as well, and that precipitation might have a limited impact on decomposition (Sanches et al. 2008b).

4.2.5 Potential extracellular soil acid phosphatase activity

We used a fluorescence method for analyzing potential extracellular acid phosphatase enzyme activities based on Marx et al. (2001) and calculations from German et al. (2011). Acid phosphatase was assayed in soil slurries of 0.5 g of fresh soil in 50 ml sodium acetate buffer (pH 5.5) and vortexed for 1 minute before pipetting (200 µl) in a black 96-well microplate. As a substrate, we used 4-methylumbelliferyl phosphate (M8168 Sigma), using Methylumbellliferyl as a standard (M1381 Sigma). In addition we measured substrate controls, sample controls and blanks to account for potential quenching effects. Microplates were incubated in the dark for 60 minutes (at 20°C) and fluorescence was measured using 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). Potential extracellular acid phosphatase activities were calculated following German et al. (2011) and are given in µmol g^{-1} day^{-1}, indicating potential activity of the enzyme at substrate saturation on a dry weight basis.

4.2.6 Statistical analysis

Data organization and calculations were performed with the “tidyverse” package (version 1.3.0, Wickham et al. 2019), graphs were made with the package “ggplot2” (version 3.3.2,

Wickham 2016). We calculated daily litter P loss based on field collections of litter and Eq. 3. Each soil P fraction was evaluated for differences between months and soil depths with linear models or linear mixed models using the lme function from the "nlme" package (version 3.1-148, Pinheiro et al. 2020) with the month and soil depth as fixed factors. We used sample location as a random effect and evaluated the best model fit according to the Akaike information criterion (AIC). For all fractions, models were allowed different variances per group combination (month and depth) using the VarIdent variance structure. Because the model fit was better with only depth included in VarIdent for the inorganic bicarbonate fraction, and month only for the HCl fraction, those models were fitted with only the mentioned grouping term in the variance structure. The models' residuals were checked for homogeneity and normality and variables were log-transformed if needed.

Since we hypothesized that P fractions changed over time, we calculated ΔP_o (the change in P_o fractions between two consecutive sampling dates for each sampling point). The same procedure as above was followed, with the ΔP_o as response variable, either phosphatase or litter P loss as the first fixed variable in separate models (since litter P-loss was not location-specific, while phosphatase was), the sample location was added as a random effect if this improved the model fit. We tested the influence of litter P inputs and phosphatase activity for each organic P fraction separated by soil depth. Again, resulting models were validated with visual checks of residuals on homogeneity and normality. All analyses were performed with R version 3.6.3 (R Core Team 2020).

Table 4.1: Average total P and soil P fractions (± SE) from the Hedley fractionation, in µg g^{-1}, with n = 69

Fraction		0-5 cm	5-15 cm
Total P		143.59 (± 0.61)	112.50 (± 0.43)
P_i	Resin	7.94 (± 0.05)	3.75 (± 0.03)
	$NaHCO_3$	4.85 (± 0.04)	1.61 (± 0.02)
	NaOH	14.31 (± 0.06)	9.89 (± 0.03)
	HCl	2.05 (± 0.02)	2.12 (± 0.02)
P_o	$NaHCO_3$	8.38 (± 0.08)	5.82 (± 0.04)
	NaOH	32.03 (± 0.28)	17.55 (± 0.13)
Residual P		78.43 (± 0.56)	72.61 (± 0.37)

Details on the extraction procedure in the Methods section 'Soil P-fractionation'

4.3 Results

4.3.1 Soil P-fractions and their dynamics

Total soil P was 143.6 µg g^{-1} for the top 5 cm (± SE 0.55), and 117.7 µg g^{-1} for 5-15 cm depth (± SE 0.73, Table 4.1). The P concentrations of all, except the HCl and residual fractions, were higher in the top 5 cm compared to 5-15 cm soil depth. The extractable inorganic fractions accounted for 29.1 µg g^{-1} (± SE 0.12, 20 % of total P) at 0-5 cm, and for 17.4 µg g^{-1} (± SE 0.07, 15% of total P) at 5-15 cm, the organic fractions accounted for 42.5 µg g^{-1} (± SE 0.34, 28% of P) and 23.3 µg g^{-1} (± SE 0.16, 19%) respectively. The residual P accounted for most of the total P, on average 78.4 (± SE 0.56, 51%) at 0-5 cm and 72.6 µg g^{-1} (± SE 0.37, 65%) at 5-15 cm.

Our results show that soil P fluctuated over the course of the year (Fig. 4.3) and differed between soil depths (0-5 cm and 5-15 cm) for most fractions (Table 4.2). Generally, the top 5 cm had higher P concentrations, reflected mainly in the extractable P fractions; the residual fraction did not show a significant effect of soil depth. The most labile fraction, resin P, was higher in the 0-5 cm (7.9, ± SE 0.05) as compared to the 5-15 cm layer (3.8, ± SE 0.03) and increased 38% from February to May, but decreased again until November (-15% between May and August, and -48% between August and November). The 5-15 cm resin fraction was significantly larger in February compared to the other months (60% higher than the average), while November had a significantly smaller resin fraction (51% below average). The inorganic bicarbonate P fraction showed some significant differences

Table 4.2: Analysis of variance F statistics, with p values in parentheses, for each fractions' responses to sampling time (Month), sampling depth (Layer) and their interaction as fixed factors

	Model terms	df	F (p)
Resin	Intercept	1, 113	819.4 (< 0.0001)
	Month	3, 113	45.0 (< 0.0001)
	Layer	1, 113	112.7 (< 0.0001)
	Month x Layer	3, 113	7.44 (0.0001)
$NaHCO_3$ inorganic	Intercept	1, 113	146.7 (< 0.0001)
	Month	3, 113	8.17 (0.0001)
	Layer	1, 113	156.5 (< 0.0001)
	Month x Layer	3, 113	12.9 (< 0.0001)
$NaHCO_3$ organic	Intercept	1, 113	1747 (< 0.0001)
	Month	3, 113	39.4 (< 0.0001)
	Layer	1, 113	19.7 (< 0.0001)
	Month x Layer	3, 113	ns
NaOH inorganic	Intercept	1, 112	1332 (< 0.0001)
	Month	3, 112	3.03 (0.0322)
	Layer	1, 112	131.5 (< 0.0001)
	Month x Layer	3, 112	6.50 (0.0004)
NaOH organic	Intercept	1, 113	1196 (< 0.0001)
	Month	3, 113	45.7 (< 0.0001)
	Layer	1, 113	106.6 (< 0.0001)
	Month x Layer	3, 113	ns
HCl	Intercept	1, 113	1851 (< 0.0001)
	Month	3, 113	6.92 (0.0001)
	Layer	1, 113	ns
	Month x Layer	3, 113	ns
Residual	Intercept	1, 109	1602 (< 0.0001)
	Month	3, 109	5.77 (0.0010)
	Layer	1, 109	ns
	Month x Layer	3, 109	ns

Includes sampling location as a random effect where this led to an improved model fit. Only significant values ($p < 0.05$) are shown

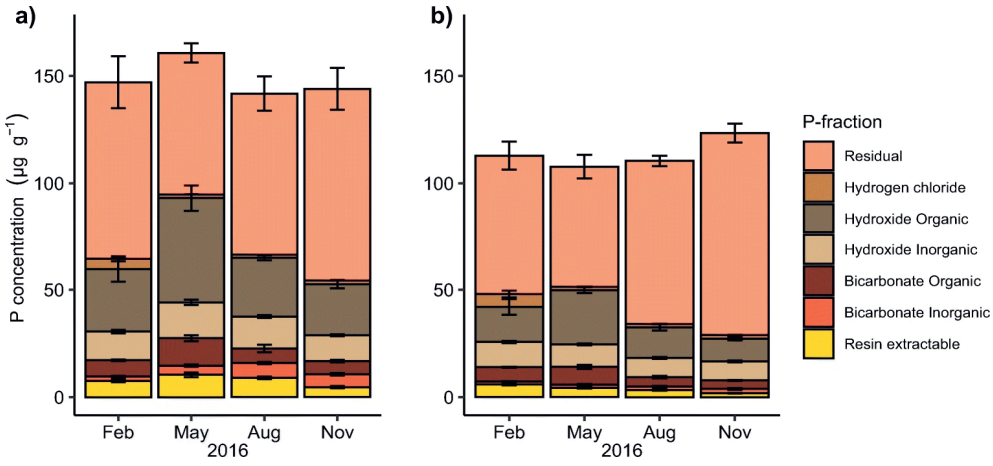

Figure 4.3: Cumulative Hedley soil P fractions determined in 4 campaigns at a) 0-5 cm depth, and b) at 5-15 cm soil depth. In their sequential extraction order (i.e. first extraction on the bottom, last extraction on top, stacked so the top of the bar represents total P). Concentration per dry soil, error bar represents standard error (n = 18)

between months in the topsoil, but those differences were not found at 5-15 cm. For the inorganic hydroxide fraction the November sampling proved significantly lower than the May and August samplings at both depths. The hydrogen chloride fraction had a more diverse pattern, with significantly higher values in February at both soil depths (Table 4.2).

In contrast to inorganic P fractions, the organic P fractions showed a stronger temporal fluctuation. While the F values for the inorganic fractions generally indicate a larger effect size for sampling depth, the organic fractions generally show a relatively stronger effect of the sampling month, and therefore show a stronger influence of seasonality. We found no direct trade-off between fractions, e.g., relatively smaller organic fractions did not lead to an increase in the inorganic fractions, but rather varied in roughly the same way across inorganic and organic forms.

The two organic soil P fractions showed their highest average values in May for both depths (Fig. 4.3). While other months had lower averages for the organic fractions, not all contrasts were significant. The changes in the organic fractions followed a pattern of a substantial increase in May, and a decline thereafter.

Since the organic fractions showed the clearest variation, we calculated the differences between consecutive sampling dates (Table 4.3). Between February and May, the organic P in the top 5 cm increased by a little over 27 µg g^{-1}, increasing the size of the organic fractions in 3 months with + 69% for both Po fractions. Between May and August, the organic

Table 4.4: Average change in the organic fractions of P (ΔPo) in µg g− 1, between sampling dates. Standard error between brackets

	Change between	Feb - May	May - Aug	Aug - Nov
		(n = 15)	(n = 18)	(n = 18)
0-5 cm	$NaHCO_3$ Organic	+ 5.44 (± 1.52)	− 6.02 (± 2.63)	− 0.64 (± 1.38)
	NaOH Organic	+ 21.78 (± 7.55)	− 21.69 (± 6.21)	− 3.56 (± 2.32)
5-15 cm	$NaHCO_3$ Organic	+ 2.13 (± 1.15)	− 3.83 (± 1.20)	− 0.51 (± 0.74)
	NaOH Organic	+ 8.71 (± 4.27)	− 11.05 (± 2.09)	− 3.63 (± 1.29)

fractions declined (-47% for bicarbonate Po, -44% for hydroxide P_o), with no increase in the inorganic pools (Fig. 4.3). The same pattern can be observed for the 5-15 cm depth, albeit in lower concentrations. November showed the lowest concentrations of P_o, indicating a continued depletion in the dry season.

4.3.2 Litterfall and litter decomposition

The total leaf litterfall amounted to 5377 kg ha^{-1} y^{-1} (± SE 49, or an average of 1.47 ± SE 0.01 g m^2 day^{-1}) (Fig. 4.4a), the annual amount of P in that litter was 0.71 kg ha^{-1} y^{-1} (± SE 0.01,

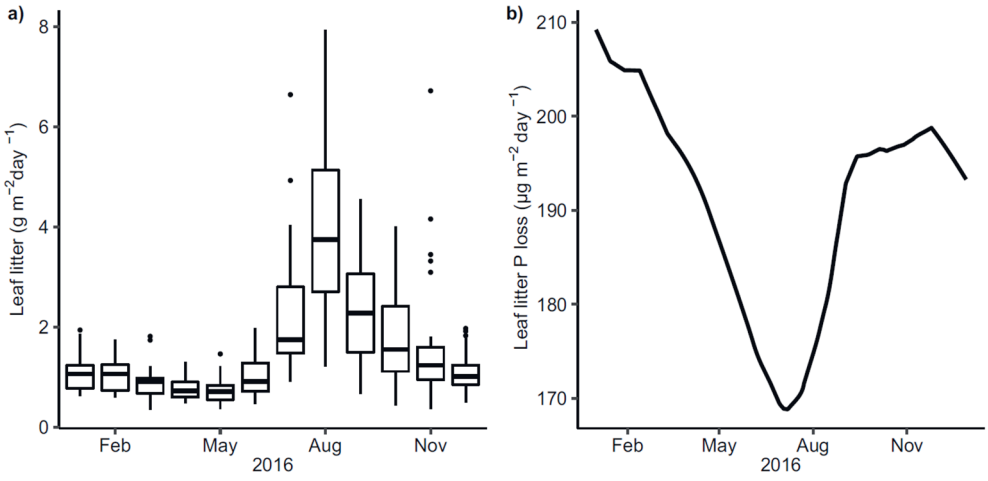

Figure 4.4: a) Leaf litter collected at the AmazonFACE study site in 2016, and b) the modeled P-loss from that litter according to Eq. (3), used in this study as soil P-input from litter. Note that the litter data used to calculate this litter P loss is not entirely shown in a) (i.e. pre-2016 data was also used to get soil P-input). Details in Methods section 'Litterfall and P-input'

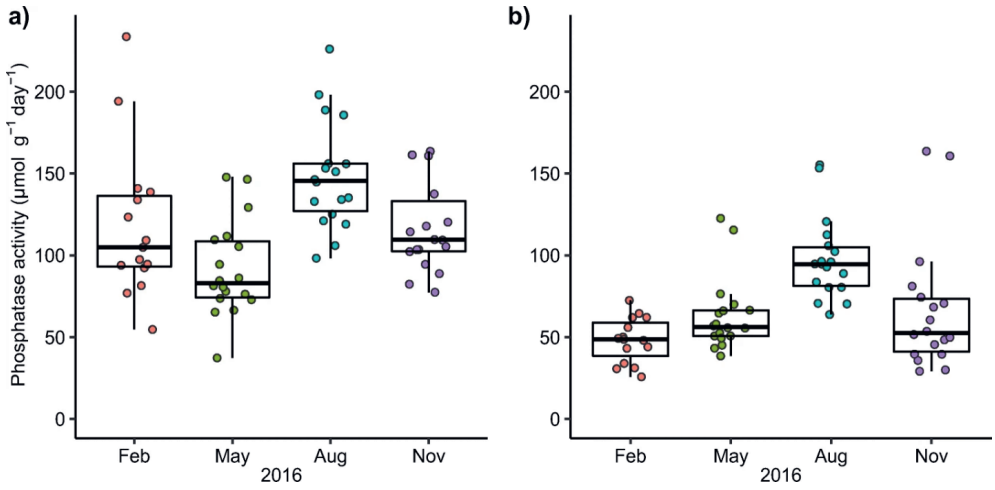

Figure 4.5: Potential activities of soil extracellular acid phosphatase (n = 18 per timepoint) at a) 0-5 cm depth, and b) at 5-15 cm depth

or an average of 194.8 ± SE 1.8 µg m^2 day^{-1}). Litterfall showed a clear seasonal pattern, with a peak at the end of the drier part of the year (August). According to the applied decomposition model, the estimated litter P-loss in 2016 averaged 190.9 (± SE 0.03) µg m^2 day^{-1} (including decomposition of leaf litter produced from previous years), accounting for the time between litterfall and P mobilization from litter to soil (Fig. 4.4b). The cumulative modeled P input over the whole year amounted to 0.69 kg ha^{-1}; giving a modeled average daily input of P to the soil of 196 µg m^2 day^{-1} (± SE 0.06) between February and May, 174 µg m^2 day^{-1} (± SE 0.04) between May and August and 193 µg m^2 day^{-1} (± SE 0.06) between August and November. Overall, the half time for litter P was 379 days according to the model, and because decomposition follows an exponential pattern most of this loss took place at the start of decomposition (i.e., with each daily litter input).

4.3.3 Extracellular acid phosphatase activities

Potential phosphatase activity at 0-5 cm soil depth amounted to 119.0 µmol g^{-1} day^{-1} (± SE 0.6) on average, at 5-15 cm the average was 69.9 µmol g^{-1} day^{-1} (± SE 0.5). Phosphatase activity ranged from 91.5 µmol g^{-1} day^{-1} (± SE 1.6) in May, to 148.8 µmol g^{-1} day^{-1} (± SE 1.8) in August (in the topsoil), while the 5-15 cm depth showed a similar pattern with a lower average of 48.2 µmol g^{-1} day^{-1} (± SE 0.9) in February, to a high 97.9 µmol g^{-1} day^{-1} (± SE 1.4) in August (Fig. 4.5).

4.3.4 Combining litter, enzyme, and organic P dynamics

Phosphatase activity was related to the changes in organic P (ΔP_o) (Fig. 4.6 a, b). In the 0-5 cm depth a negative relationship was observed between the phosphatase and the change in organic bicarbonate P ($F(1, 49) = 11.86$, $p < 0.01$), and a significant negative relationship between phosphatase and the changes in the organic hydroxide fractions ($F(1,46) = 16.76$, $p < 0.001$), while at the 5-15 cm depth the regression results were not significant, despite following similar pattern as in the top 5 cm.

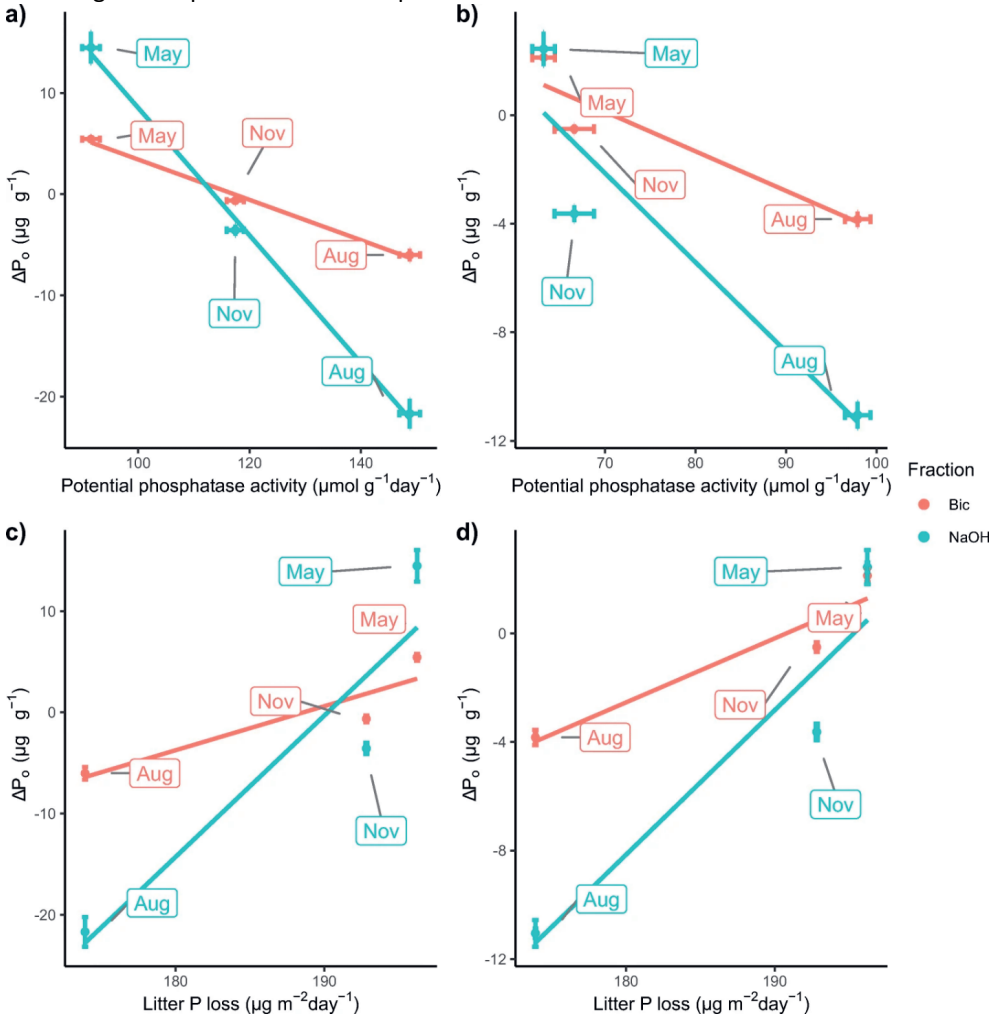

Figure 4.6: Relation between average changes in the organic fractions of soil P (ΔP_o) and assumed drivers of those changes. a) relation between average phosphatase and ΔP_o at 0-5 cm depth, b) at 5-15 cm depth, c) the relation of modeled litter inputs (average between sampling dates, Fig. 4.4) with average ΔP_o at 0-5 cm, and d) at the 5-15 cm depth. This graphical representation of the found effects does not depict the applied (mixed) models referred to in the text, since here only the averages of the tested relations are shown. Error bars show the standard error

We evaluated the average P loss from litter, i.e. P input to soil, between sampling dates, and its effect on the organic fraction changes (ΔP_o) (Fig. 4.6 c, d) as was done with potential phosphatase activity above. The effect of P loss from litter on the change in the organic P fractions was significant, this time for all organic fractions. In the topsoil, bicarbonate ΔP_o showed a slightly weaker relation to the litter input ($F(1, 49) = 13$, $p < 0.01$) than in the 5-15 cm depth ($F(1, 49) = 14.27$, $p < 0.01$) while for hydroxide ΔP_o this relation was slightly stronger at 0-5 cm ($F(1, 49) = 16.16$, $p < 0.01$) compared to the lower depth ($F(1,49) = 16.57$, $p < 0.01$). The phosphatase showed a negative relationship with the change in organic fractions, whereas litter inputs showed a positive relation with the change in organic fractions; despite some variation in strength of the response, phosphatase decreased the size of the organic fractions, while litter P inputs increased the size of the organic fractions.

4.4 Discussion

4.4.1 Overview

In this study we aimed to disentangle the controls over seasonal dynamics of soil P cycling in a tropical forest, by studying P inputs by leaf litter, changes of several fractions of soil P through time, as well as phosphatase activity catalyzing the turnover of organic P. Given that the soils at our study site are very old, we would expect that P inputs drive soil P cycling. In line with our hypothesis that P availability varies seasonally we found a clear seasonality of soil P fractions. Furthermore, we found that soil phosphatase activities were significantly negatively related to net changes in organic P fractions at the 0-5 cm depth (with higher phosphatase activity causing a decline in P_o pools, or a lower phosphatase activity causing an increase in P_o pools, in particular in the top 5 cm of soil.), but not significant at 5-15 cm. The decomposition model, relating the observed fluctuations in litterfall to changes in the soil organic P fractions, confirmed our hypothesis that input of P from litter decomposition and associated activities of microbial and plant derived phosphatases were controlling soil (organic) P fluctuation in central Amazonian terra-firme forests.

4.4.2 Soil P pools and turnover

Our results suggest that the organic P fraction was the most variable component of the local soil P pool intra-annually, arguably driving soil P_i availability though mineralization into plant accessible pools. However, we could not detect a clear seasonal pattern for P_i pools. Our site showed typical P concentrations reported for Ferralsols. Quesada et al. (2010)reported that two thirds of the studied soils across the Amazon contain below 100 mg kg^{-1} total extractable P. When comparing our results with other natural ecosystems, the total P concentration and total extractable P at our study site were low (Cross and Schlesinger 1995; Johnson et al. 2003; Turner and Engelbrecht 2011; Yang and Post 2011), especially considering the 5-15 cm soil depth, which could arguably be more suitable for comparison since this depth is more representative of mineral soil P stocks. The top layer played a more active role in biological (re)cycling and showed larger seasonal fluctuations, and P decreased rapidly from the top 5 cm down to 5-15 cm. As Johnson et al. (2003) and others have argued, the controls over labile P might be less dependent on soil weathering status (and thus total P) than often assumed; other mechanisms, including sorption-desorption dynamics, redox state, and mineralization, are likely to play a large role.

According to Helfenstein et al. (2020), turnover times for the extractable inorganic fractions of the Hedley fractionation procedure are minutes to hours for the resin and bicarbonate

fractions, while hydroxide fractions have a turnover of days to months, and only hydrochloric acid fractions have longer residence times. The P_i pools may vary in their bioavailability and since the turnover times of the more available fractions may be fast (Helfenstein et al. 2020), it is likely that we may have missed some of the variation between sampling intervals. In our study, labile P_i fractions showed little variation and were not related to either litter inputs or phosphatase activity. This could be due to an adverse effect on P_i fractions caused by sample pre-treatment (oven drying at 65∘C), which could have affected solubility and inflating the fraction sizes of especially the most labile fractions (see also Ajiboye et al. 2004). However, across sampling dates soil water content was not highly variable (Fig. 4.1) and did not significantly affect labile P_i fractions. The less available (slow turnover) fractions are probably better represented considering the products of mineralization of P_o do not stay in the soil solution long enough for a net increase of available P_i pools to be detectable at our sampling frequency.

In a tropical ecosystem, organic P fractions are crucial as buffer for the shorter-term P-availability, while the actual variability of the (inorganic) ortho-P fractions might be hard to measure in an observational study, especially when P-demand and thus turnover is high. In tropical soils, organic forms of tropical soil P can constitute about a quarter of total P according to Turner and Engelbrecht (2011) although there is substantial variation across the Amazon (Quesada et al. 2010). Approximately two thirds of the organic P fraction can be bound in the microbial biomass (Turner et al. 2015). While in our study the proportion of organic P compared to total P is similar overall, we show substantial variation over the year. The relative peak of the organic P in May, together with the low concentration of organic P in November (about a factor 2 difference) have large implications suggesting that the organic fractions are the prime regulator of more available forms; if the regulator "stock" of P_o varies, the cycled P_i is likely to be impacted by the same magnitude at shorter time spans. Although the importance of organic P has been well described and is of central importance to the Walker and Syers model (1976), its annual variation is known to a lesser extent.

4.4.3 The effect of soil phosphatase and litter decomposition

High phosphatase activity indicates a high P demand (Allison et al. 2011) but absolute values of soil phosphatase activities at our study site were low compared to global averages (Margalef et al. 2017) which suggests that P limitation is not as high as in other (tropical) forests. However, this meta-analysis was done with colorimetric assays, and fluorescent assays generally show lower values (Nannipieri et al. 2011). Our results are slightly higher

than other fluorescent enzyme assays performed in tropical forest soils (e.g. Turner and Wright 2014; Nottingham et al. 2016). Even so, absolute activities might not be the best indicator of nutrient limitation (Moorhead et al. 2016), and should be used with caution. Interestingly, phosphatase activity was not related to the size of the inorganic P fractions but rather to the organic fractions. As mentioned before, the soil drying process might have affected the solubility of the more labile fractions, limiting their accuracy. Even so, this result might indicate that the (labile) inorganic P fractions are rapidly taken up by plants and microbes. Moreover it suggests that plant roots and the microbial community are able to access organic P-pools by releasing enzymes, but also that the organic P pool, including P stored in microbial biomass, can act a buffer stabilizing the P-supply throughout the year.

The production of phosphatase is demand driven, rather than supply driven (Kitayama 2013), but there are alternative hypotheses including a supply-driven philosophy (Turner 2008), as well as indications that P cycling in tropical forests may be affected by climate, and especially precipitation (Huang et al. 2011; Wood et al. 2016). In our study, it might very well be that the in-situ mineralization rates were generally not limited by climate at any time during the year (as is suggested by our relatively constant soil moisture throughout the year, Fig. 4.1), but by enzyme or substrate availability. If phosphatase activity peaks when the soil organic P fractions are relatively small, P demand might be driving investments in phosphatase since the substrate has largely been transformed. On the other hand, the higher investment in phosphatases could be synchronized with higher inputs of substrate from litter, and therefore variation in enzyme production could be supply- rather than demand-driven. It is also worth emphasizing that the potential enzyme activities were rather high throughout, which suggests that the in situ mineralization rates were more dependent on P_o supply than on P_i demand. However, due to the nature of nutrient re-cycling between vegetation and soil it is challenging to distinguish cause and effect. What we can conclude, however, is that the phosphatase, whether demand-driven or supply-driven in its activity, is correlated with changes in the organic P fractions and thus affects P-availability.

Leaf litterfall increased during the drier season, as has been described for other studies conducted in the region (Luizao 1989; Wu et al. 2016). Without a doubt, the phosphorus return via litter inputs was crucial to sustain cycling of nutrients within in the forest. Observations underlining the relation between litter inputs and soil organic P are found for both tropical and temperate forests (Beck and Sanchez 1994; Tiessen et al. 1994; Chen et al. 2003). Litter manipulation and fertilization experiments in Panama found that three

years of litter addition induced substantial increases, while litter removal decreased organic P pools (Vincent et al. 2010), whereas after six years this effect decreased (Sheldrake et al. 2017) possibly due to the changes in P_o turnover in the manipulated plots (Sayer et al. 2020). Especially under the litter removal treatment, the decrease in Po pool size seems to signal its importance, but on such a timespan some additional sources of P from deeper soil or from more recalcitrant fractions could be responsible for maintaining the nutrient cycle (Sheldrake et al. 2017; Sayer et al. 2020). Under P addition, a fertilization experiment in the same area resulted in higher microbial P, as well as significant changes to other microbial nutrients, indicating the links of the P cycle to other nutrients and reenforcing the hypothesis of P-limitation from a microbial point of view (Turner and Wright 2014).

The decomposition model used for our study has a mediating effect on peaks observed in the litterfall and conceptualizes a delay between litterfall and litter soil P input. Even though this model serves well for our time scale, on shorter timescales decomposition (i.e. litter P loss) can be argued to be more complex and dynamic than our (simple) model; mainly rainfall and soil moisture have controls over the shorter-term dynamics, and there are several transformation pathways that could add an additional layer of complexity to the decomposition process (Prescott and Vesterdal 2021). Moreover, litter P-loss in inorganic forms which constitute the majority of soil P inputs (Noack et al. 2012; Schreeg et al. 2017) might be taken up quickly by plants and microbes alike. The uptake of P_i by plants and the returns of P via root litter are beyond the scope of this study, and our P_o pool includes microbial derived P. The resulting P input to the soil from decomposition might not be as smooth as the model predicts, but it shows that a simple model for decomposition lines up well with soil P if integrated over time, despite the limitation of having only 4 sampling timepoints.

4.4.4 The dynamic nature of the P-cycle

Our study underlines the dynamic nature of the plant-soil system regarding P availability in tropical forest ecosystems. Research in another lowland forest in the Amazon showed the importance of available bicarbonate and hydroxide fractions in P cycling as opposed to more recalcitrant fractions (McGroddy et al. 2008), but the fractions had not been differentiated between inorganic and organic forms. Studies in drier forests or sites with a more pronounced dry season did differentiate between organic and inorganic P. Turner et al. (2015) studied the impact of fertilization on organic soil P fractions in a Panamanian forest (about three times higher total P compared to our site), and found that large parts of the seasonal variation and the fertilization effects were explained by the microbial biomass P,

suggesting a relative stable extracellular P_o pool and a more seasonal microbial one. Mirabello and colleagues (2013) found a decline in the organic bicarbonate fraction for the dry season in the same study region, which might indicate increased mineralization during that time and thus would support our findings of increased phosphatase activity in drier months. The hydroxide extractable organic P fraction showed the opposite pattern however. Studies in drier tropical forests also indicate the importance of precipitation in the P-cycle, both in terms of litter dynamics (Valdespino et al. 2009), and sorption of P (Campo et al. 1998), while Waring et al. (2021) compared different forests and found that soil development stage was the major driver of the soil P balance.

Overall, our results suggest that soil organic P pools in highly weathered tropical soils are more dynamic than previously reported. In low-P soils organic P inputs are the main source of the nutrient and the biological cycling appears to be highly relevant. Future studies should focus on microbial community dynamics to ultimately identify processes driving P-cycles in tropical forests. Turner et al. (2013) highlighted the crucial role of microbial biomass P in the retention and cycling of P during ecosystem development, which indicates that microbial community dynamics might be paramount to understanding organic soil P dynamics in addition to the factors accounted for in this study. Mycorrhizal interactions with different soil fractions and partitioning thereof is adding another layer of complexity to the interaction of P with the ecosystem (Liu et al. 2018). Although ecosystem models are increasingly recognizing the importance of P in tropical systems (Fleischer et al. 2019), and starting to implement P cycle dynamics and processes, mechanistic understanding of P in ecosystem processes is far from complete (Vitousek et al. 2010; Wright et al. 2018). Our results show substantial variability in the soil P pools during the year, contributing a dynamic representation of the P cycle and the seasonal pattern of its different components (i.e., soil P-pools) and its drivers.

This dynamic view of the soil P cycle also indicates a high efficiency of P cycling in tropical forests, of which the deeper implications point toward a sustained limitation by P on forest functioning under global change scenarios (Fleischer et al. 2019). Plants might increase the amount of bioavailable P in the system by accessing relatively occluded forms of P, by mining in deeper soil layers, or by exudation of organic acids to liberate currently unavailable P (Jin et al. 2015). While increased CO_2 might stimulate investments below ground (Hoosbeek 2016) - i.e., increased root growth, root exudation including phosphatases - the current dependence on mineralizing P from organic compounds limits the amount of P that could be easily liberated to sustain an acceleration or intensification

of the P-cycle, especially if soils are almost at Walker and Syers' (1976) terminal steady state. If global changes affect the current seasonality, the tight cycling that is reported here could be affected - leading to a less effective cycling of the nutrient.

4.5 Conclusions

Our study considering seasonal variation of soil P and its drivers shows how Central Amazonian soil P fractions may fluctuate inter-annually, in response to litter inputs to the soil and phosphatase through root and microbial demand. Our study indicates that litter P inputs are correlated with the soil organic P pool, while potential biochemical mineralization through soil enzymes showed a negative relation to those organic fractions. Albeit the fact that a tight cycling of P in tropical forest ecosystems indicates that this nutrient is in short supply, the specific limiting steps are still up for debate, and the observed diametric relationship between different factors within the plant-microbe-soil system further highlights the relevance of studying the P-balance as an integrated dynamic system.

Chapter 5:

Main Findings and Discussion

5.1 Research context and focal points of this thesis

Tropical forests, which are among the most diverse and abundant ecosystems on Earth, play a significant role in regulating the planet's climate. Due to their vast extent the influence of these forests extends far beyond tropical regions. Their role in global carbon and water cycles is of paramount importance due to their ability to absorb carbon dioxide, a critical factor in climate regulation. In the Amazon basin, these forests thrive on highly weathered soils.

However, a challenge arises as in those soils the primary source of phosphorus (P), a vital nutrient for forests, is nearly depleted. Many of these regions have relatively low soil P concentrations, limiting biological processes in this energy-rich environment. Phosphorus is essential for all forms of life as it is a key component of compounds such as nucleic acids and phospholipids found in cells. Despite the low P concentrations in the highly weathered soils, tropical forests remain highly productive year after year. This paradox raises intriguing questions about the biochemical mechanisms that enable forest productivity in a P-impoverished environment.

Tropical forests, despite their year-round warm and humid conditions, exhibit clear seasonal variations. An observant eye can discern seasonality in several ways. The drier season is characterized by increased litterfall, leading to litter accumulation that persists until the wetter months. My thesis explores what happens next, focusing on seasonality in soil nutrients, examining the relative abundance of these nutrients throughout the year and how this is reflected in soil nutrient demand.

Since tropical forests are often considered to be in a steady state (Bruijnzeel 1991; Chambers et al. 2013) – sometimes even called "terminal" because of the low P concentrations (Walker and Syers 1976) – many studies on tropical soils overlook potential seasonal dynamics of soil nutrients. This oversight is probably largely due to a lack of strong seasonal temperature differentiation. However, (seasonal) variation in moisture could have large implications to soil biogeochemistry (Dietterich et al. 2022). Complementing existing data about seasonality of aboveground processes such as litterfall (Zhang et al. 2014), this thesis details the seasonality of tropical soil C and nutrient cycles in a Central Amazon tropical forest. Seasonal differentiation between seasons can be highly consequential if the length or intensity of seasons is increased in a changing climate, as is projected in the latest IPCC report (Douville et al. 2021). Such changes could have significant implications for the

nutrient economy and overall functioning of forests. In this thesis, I unravel how nutrients follow a seasonal pattern and how those dynamics are driven, so we can have a nuanced view of potential risks to this part of the forest nutrient cycle. Special attention is given to the phosphorus (P) cycle, considering the low-P nature of these soils. Ultimately, we want to find answers to what these elemental cycles reveal about nutrient availability when seasonal patterns are altered. Through climate change, those alterations can almost be considered inevitable.

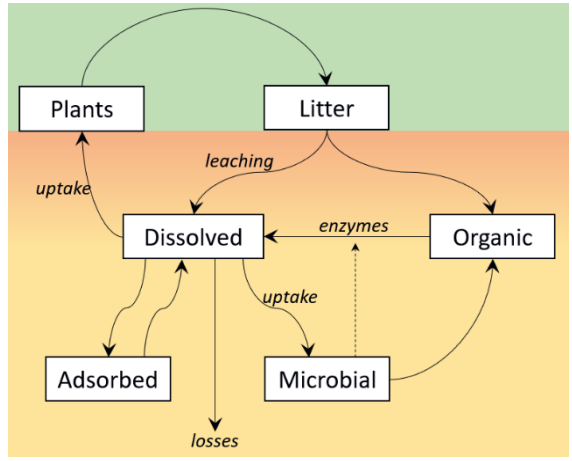

Figure 5.1: Schematic display of pools and processes in the soil. My thesis highlights the differences and relations between C, N and P cycles and their seasonality in tropical soils.

I investigated seasonal variation of tropical soil biogeochemistry, focusing on carbon (C), nitrogen (N) and phosphorus (P). I provided an analysis of the (stoichiometric) variation in total, available, and microbial soil C, N, and P pools (Chapter 2). Additionally, I examined their relationship to extracellular enzyme activities which serve as a proxy for nutrient demand (Chapter 3). In Chapter 4, I explored soil P pools with differing plant availability. In this concluding chapter, I will summarize the key findings from the experimental chapters, discuss broader implications and limitations of this thesis, provide recommendations for further study, and finally provide overall conclusions.

5.1.1 Carbon and nutrient stoichiometry of tropical soils and microbes

To enhance our understanding of the seasonal dynamics of C and nutrient pools in tropical soils, I designed and conducted a detailed study of the intra-annual variation of total, extractable, and microbial C, N, and P pools in Chapter 2 (Fig. 5.1). Additional to the intra-annual differences in C and nutrient concentrations of different fractions (total, extractable and microbial), I also explored their soil nutrient stoichiometry, the relative balance of these elements within those fractions. Stoichiometry can provide insight into the interactions and transformations that occur within the soil ecosystem. This can shed light on the dynamics controlling soil organic C (SOC) formation, stabilization, and sequestration, since they are influenced by intra-annual organic matter inputs and microbial decomposer activity.

I hypothesized an increase in extractable soil C and nutrient fractions during the wet season, as decomposed litter inputs are leached and incorporated into the soil (Hypothesis 2.1). Through this increased resource availability, I anticipated an increase in the microbial C, N and P fractions (Hypothesis 2.2). However, despite these changes I expected that, through microbial homeostasis, the stoichiometry of the microbial biomass would be relatively constant between months. Furthermore, I hypothesized that the upper soil layer (0-5 cm) would exhibit the most pronounced seasonality and fluctuations since this layer is more exposed to the effects of seasonal litter inputs (Hypothesis 2.3).

		Feb (Wet)	May (Wet, transition)	Aug (Dry)	Nov (Dry)
Extractable C	0-5 cm	+	++	-	-
	5-15 cm				
Extractable N	0-5 cm	-		++	+
	5-15 cm	-	-	+	++
Extractable P	0-5 cm	+	+		-
	5-15 cm	+	+	-	-
Microbial C	0-5 cm				
	5-15 cm		+	-	-
Microbial N	0-5 cm	+	+	+	-
	5-15 cm	+	+	+	-
Microbial P	0-5 cm	+	-	-	-
	5-15 cm	+			-
Total C	0-5 cm		+	+	-
	5-15 cm		+	+	-
Total N	0-5 cm	+	+	+	-
	5-15 cm	+			-
Total P	0-5 cm		+		-
	5-15 cm				

Figure 5.2: Observed temporal dynamics regarding soil nutrient pools from Chapter 2. The symbols indicate where peaks and minima occur during the season, empty cells indicate no significant difference with those peaks. The colors indicate aligned observations at both soil depths; green for an increase at both soil depths, red for a decrease at both depths. For further detail, see Chapter 2, Table S1.

Chapter 2 confirmed the hypothesis that extractable C and P concentrations increase during the wet season, while N peaked in the dry season (Fig. 5.2). In the wet season, higher substrate leaching from accumulated plant litter and easier substrate diffusion led to an increase in C and P availability. The extractable N showed a different pattern than C and P; I postulate this was because drier conditions have a distinct effect on the N cycle (Hinko-Najera Umana and Wanek 2010; Homyak et al. 2017). This indicates a seasonal change in litter and soil organic matter decomposition and nutrient mineralization dynamics by the microbial community.

My study also revealed a surprisingly high degree of variation in the stoichiometry of microbial C, N and P. I observed a decrease in microbial P relative to microbial C and N during both the wet and the dry seasons. Contrary to my hypothesis of a relatively constant

microbial stoichiometry due to expected stoichiometric homeostasis of the microbial community, the reported variation suggests influences from factors that I did not directly test, such as changing community structure and microbial storage of P. Microbial storage of nutrients is not just linked to the abundance of nutrients, but can even occur in times of scarcity, following environmental and physiological signals (Mason-Jones et al. 2022) Although the dynamics of nutrient storage cannot be discarded as a factor of influence, the more evident explanation is that this variation is driven by shifts in soil microbial and fungal community composition, as has been found in studies investigating drought effects (e.g., Buscardo et al. 2021). In my study, seasonal shifts in microbial stoichiometry reflect substantial and frequent changes in nutritional balance within a single year. I conclude these changes are likely attributable, at least in part, to variations in community composition.

Despite large differences dominating in the soil nutrient stoichiometry between sampling months, my results did not indicate a unidirectional seasonal effect. However, I presented evidence suggesting that the composition of the microbial community undergoes shifts in response to seasonal changes. I observed seasonal variation in the pools of total C, N, and P, and argued that this could be due to three factors; a) leaf litter and root litter inputs (Yavitt and Wright 2001; Green et al. 2005; since root mortality shows seasonality, e.g., Cordeiro et al. 2020), b) the influence of the preceding El Nino year, which may have resulted in higher litter inputs (Hilker et al. 2014; Hofhansl et al. 2014; Oliveira de Morais et al. 2021), and c) rapid turnover of C, N and P pools, potentially accompanied by shifts in microbial community composition (Buscardo et al. 2018). The high variation in SOM pools shows that biogeochemical nutrient cycles are highly dynamic in tropical soils. Second, total C and N pools followed a more pronounced seasonal pattern than total P; this could be because of the large occluded pool (as further detailed in Chapter 4), and through the fundamentally different nature of the nutrient cycles (Vitousek 1984; Filippelli 2019).

With seasonally driven changes in total, available, and microbial C, N, and P pools in tropical soils, we confirm that the soil system in tropical forests is a dynamic one (Fig. 5.1). This is exemplified by the contrasting dynamics of the C and P pools in comparison to the availability of N (Fig. 5.2). Moreover, the observed changes in microbial stoichiometry throughout the year indicate changes in the microbial community structure. Further understanding these shifts in nutrient availability and microbial biomass brings us to the next chapter, where we dive into the dynamics of microbial activity and nutrient demand.

5.1.2 Shifts in nutrient demand: what soil enzymes tell us

In Chapter 3, I conducted a comprehensive analysis of the interplay between precipitation, litterfall, extracellular enzyme activities (EEA) as a proxy of nutrient demand of microbial communities, and nutrient availability in the context of seasonality. I calculated enzyme activity vectors to illustrate shifts in relative nutrient demand and related those changes with fluctuations in nutrient availability and climate. I expected that the seasonal variation in enzyme activity would be predominantly driven by new litter inputs to the soil. Given the relatively constant temperature and soil moisture in the study area, I hypothesized that leaf litter inputs would be the primary driver of soil EEA, rather than factors such as soil moisture, precipitation, or temperature (Hypothesis 3.1). Furthermore, I expected that reduced nutrient availability would lead to increased microbial investments in EEA (Hypothesis 3.2), with a particular emphasis on increased phosphatase activity due to the low P status of the soils (Hypothesis 3.3).

My results (Fig. 5.3, 5.4) corroborated the first hypothesis, demonstrating significant (positive) relationships between leaf litterfall and demand driven enzyme activities for C and P acquisition. This indicates synchronization between substrate availability and enzymatic investments. Precipitation exhibited weaker or insignificant negative relations with EEA (Table 3.1). On the other hand, temperature had consistent positive relations with acid phosphatase (AP) activity. I postulated that the elevated enzyme activities could be attributed to stimulation of microbial enzyme production in the dry season. This stimulation of production could occur through two mechanisms: the immobilization of enzymes during periods of decreased soil moisture, and higher return of investment through increased substrate availability. The immobilization of enzymes might sound contradictory as a driver for their increased activity, while immobilized enzymes are likely measured in an enzyme

		Feb (Wet)	May (Wet, transition)	Aug (Dry)	Nov (Dry)
C-enzyme	0-5 cm				
	5-15 cm	-		+	-
N-enzyme	0-5 cm			+	-
	5-15 cm	-	+	+	--
P-enzyme	0-5 cm	+	-	++	+
	5-15 cm	-	-	+	-

Figure 5.3: Observed temporal dynamics of enzyme activities from Chapter 3, in selected months according to the data from other chapters. The symbols indicate where peaks and minima occur during the season, empty cells indicate no significant difference with those peaks. The colors indicate aligned observations at both soil depths; green for an increase at both soil depths, red for a decrease at both depths. For further detail, see Fig. 3.3 and Fig. S3.5; BG activity is shown here as "C-enzyme", NAG-activity as "N-enzyme" and AP activity as "P-enzyme".

assay they might not provide any mineralization in-situ; this lack of in situ reaction could cause enzyme production to continue despite limited effectivity, thereby increasing demand-driven enzyme production. In contrast, if enzymes are secreted in a substrate-rich environment, they might be able to mineralize nutrients easily if microbes adjust their enzyme production to profit from the supply of substrate, forming a supply-driven relationship between nutrient and enzyme (see also Box 1.2 in the Introduction).

	Litterfall	C-availability	N-availability	P-availability
C-enzyme	*Both*		*0-5*	
N-enzyme	*Both*		*0-5*	
P-enzyme	*Both*	*Both*	*Both*	*5-15*

Figure 5.4: Observed relations between litter and nutrients and acquisition as expressed by enzyme activity from Chapter 3. The green color indicates positive relations, the red color indicates negative relations. For more details, see Fig. 3.3 and Fig. 3.4 (Chapter 3).

However, my analysis also showed that most total soil N and P contents had weak relations with enzyme activities. For the available nutrient fraction, only P availability demonstrated the hypothesized "demand-driven" relationship with its corresponding enzyme, acid phosphatase (Fig. 5.4). Surprisingly, N availability exhibited positive correlations with most enzyme activities, suggesting that most enzymes are to some extent dependent on a supply of available N. The activity of phosphatase, an enzyme responsible for P acquisition, remained relatively high throughout the study. This indicates a persistent and high demand for this nutrient and verifies my hypothesis of increased microbial demand due to P limitation in this tropical ecosystem. Nevertheless, my results suggest that microbial nutrient demand in these ecosystems is not solely driven by P availability but also influenced by N availability. These findings have important implications for our understanding of microbial nutrient turnover in tropical forests and the role of soil biogeochemistry in regulating microbial processes.

As previously established by Weintraub et al. (2013), my study confirms the pivotal role substrate availability plays in regulating enzyme activities. This corroborates the notion that litter inputs can be considered a major driver for microbial investments in enzymes. My research further suggests a link between N availability and enzymatic expression; the positive correlation we observed between extractable N and enzyme activities implies that this nutrient could be an important nutrient in facilitating enzyme production. Conversely, the negative correlation between phosphatase and available P suggests that microbial

demand for P increases when its availability is low. To evaluate the relative demand for nutrients, I used enzymatic vector analysis (Moorhead et al. 2016). The observed patterns of nutrient demand reveal a chronic high demand for P throughout the study period, although this demand was reduced prior to the dry season when there was a shift toward greater N acquisition (Fig. 3.5 c, d). This increased demand for N prior to the dry season is possibly related to the increased production of enzymes during the dry season, or, when considering the findings of Chapter 2, changes to the microbial community composition. The demand for P reached its peak at the end of the dry season. The findings also suggest that nutrient availability (particularly N) is a stronger driver of enzymatic activities in the top 5 cm of soil (Fig. 5.4). However, at depths between 5-15 cm, variations in enzymatic activities are slightly better accounted for by substrate inputs (Fig. 3.3). This could be an artifact of high variation in the top layer, but could also point to relatively high organic matter (substrate) contents in combination with higher leaching of available forms of nutrients in the top 5 cm, inducing a demand driven rather than a substrate driven enzyme dynamic.

5.1.3 Organic P fractions maintain the bioavailable P pool,

In Chapter 4, I conducted an analysis of the drivers of seasonality in the phosphorus cycle. Phosphorus concentrations in tropical forests are generally relatively low, since these soils consist of geologically old material that has been highly weathered. Since the primary source for P is through soils, this limitation is crucial in understanding tropical forest functioning (Fig. 1.1). Using a widely used P-fractionation method (Hedley et al., 1982; adapted by Quesada et al., 2010; described by Tiessen & Moir, 1993) I obtained soil P pools of varying bioavailability. I analyzed the same soil samples for phosphatase activity and applied a simple litter decomposition model to assess if I could correlate changes in soil P pools with litter inputs and transformations in the soil (Fig. 5.5).

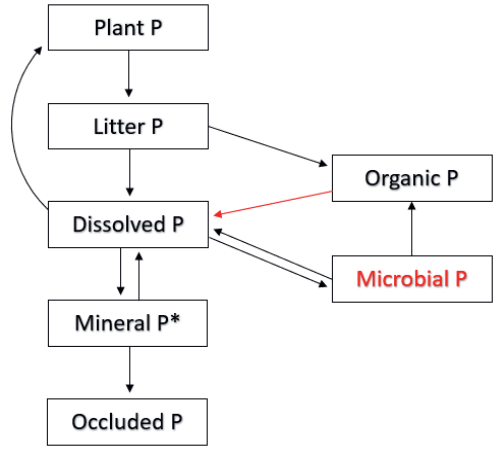

Figure 5.5: Simplified soil P-model, with some of the different pools. The red arrow indicates enzymatic mineralization step from organic P sources. The data for microbial P (red) was not included in Chapter 4.

I hypothesized (Hypothesis 4.1) that fluctuations in phosphorus (P) levels would occur more visible in the top 5 cm of soil, where biological activity is highest. However, the overall patterns found would be reflected in the soil below (5-15 cm). Additionally, I hypothesized (Hypothesis 4.2) that seasonal variation is driven by fluctuating inputs such as litterfall, and (Hypothesis 4.3) subsequent decomposition derived organic and inorganic P inputs to the soil which is catalyzed by phosphatase. The missing part in this balance would be outputs (i.e., plant uptake). Overall, the aim was to identify the relative importance of drivers controlling the fluctuation of different P pools, such as litterfall inputs, litter decomposition, and phosphatase activity from either plant roots or microbes to degrade organic P compounds.

We observed distinct seasonal trends in soil P fractions (Fig 5.6). We also found a significant negative correlation between soil phosphatase activities and net changes in organic P fractions in the top 5 cm of soil. This confirms that an increase in phosphatase activity results in a reduction in organic P pools, especially in the uppermost 5 cm of soil. This correlation was not significant in the 5-15 cm soil layer. A decomposition model, which links the observed fluctuations in litterfall to changes in soil organic P fractions, supported our hypothesis. It confirmed that the input of P from litter decomposition, along with the associated activities of microbial and plant-derived phosphatases, are key factors controlling the fluctuation of soil organic P.

I concluded that the seasonal dynamics and dependency of organic P on incorporation of litter and subsequent mineralization by phosphatase (AP) in the soil indicate a tight cycle, while providing valuable insights into the timing of P fluxes and transformations. Given that vegetation and microbes demand P and this nutrient is

		Feb (Wet)	May (Wet, transition)	Aug (Dry)	Nov (Dry)
Resin	*0-5 cm*	-	+	+/-	--
	5-15 cm	+	-	-	--
NaHCO3 inorganic	*0-5 cm*	--	-	+	+/-
	5-15 cm				
NaHCO3 organic	*0-5 cm*	+	++	-	+/-
	5-15 cm	+	+	-	-
NaOH inorganic	*0-5 cm*	-	+		-
	5-15 cm	+		-	-
NaOH organic	*0-5 cm*		+	-	-
	5-15 cm	+	+	-	-
HCl	*0-5 cm*				
	5-15 cm				
Residual	*0-5 cm*				
	5-15 cm		-		+

Figure 5.6: Observed dynamics of Hedley phosphorus fractions from Chapter 4, in selected months. The symbols indicate where peaks and minima occur during the season, empty cells indicate no significant difference with those peaks. The colors indicate aligned observations at both soil depths; green for an increase at both soil depths, red for a decrease at both depths. For further detail, see Fig. 4.3.

generally considered to limit various processes, these findings could have significant implications in scenarios of global change such as a prolonged dry season. Although in Chapter 2 I showed that the total content of C and N showed more variation than total P, Chapter 4 gives a more nuanced view, with a large part of the total P pool of the soil being present as the "residual" or non-reactive pool. Thus, more available pools (e.g., Al- and Fe-bound inorganic fractions, Resin fraction, see Cross & Schlesinger, 1995) and organic pools of P are considered to be the reactive P-pool. Resin, bicarbonate, and hydroxide P fractions are likely to have a faster turnover than the applied sampling interval (Helfenstein et al. 2020), once again underlining the importance of the organic fraction in the system when considering changes over a year. The large variation and relationships of the organic fractions with the phosphatase activity and P inputs of the decomposing litter suggest that these pools are paramount to maintaining a bioavailable P pool in tropical forest ecosystems.

5.1.4 How seasonal interactions shape nutrient dynamics

Nutrient cycles, which are fundamental ecological processes, can be highly complex. In the previous paragraphs I highlighted my main findings. The nutrient economy differs significantly between the drier and wetter periods of the year (Fig. 5.7), and changes to the

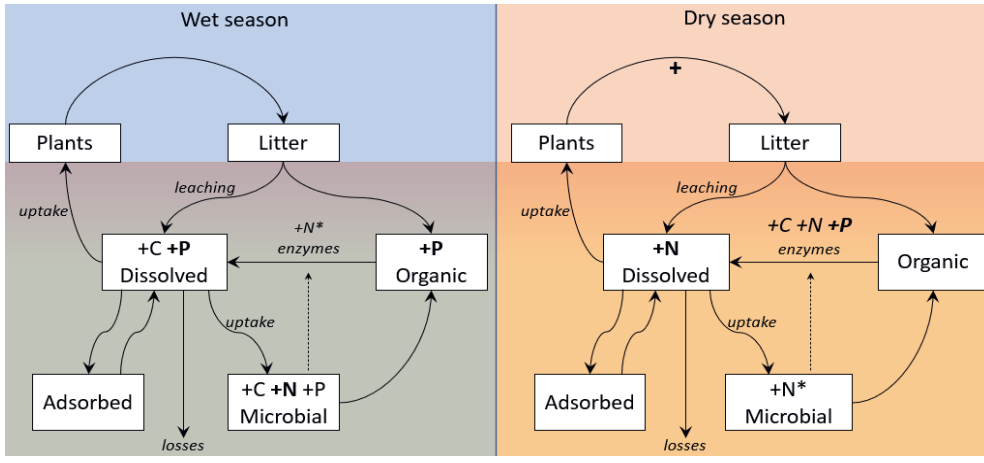

Figure 5.7: Graphical summary of the main observations from the previous chapters, indicating relative increases in nutrient pools and fluxes between either the wet (left) or dry (right) season. In Chapter 2, I showed the seasonal dynamics of the extractable (shown here as dissolved), microbial and total (not shown) C, N and P pools. In Chapter 3, I focused on the enzymatic activities of C, N and P-targeting enzymes. In Chapter 4, I further refined the distinction between different P-pools, and related these to enzymatic turnover. (*) The microbial N pool is still relatively large at the first part of the dry season, while the enzyme activity related to N- mineralization increases at the end of the wet season.

precipitation regime or weather patterns could challenge this dynamic balance. Studies have shown an increase in the intensity and duration of dry season droughts in the Amazon Forest in response to climate change (Chadwick et al. 2016; Zemp et al. 2017; Barkhordarian et al. 2019). In Chapter 2, I postulated longer dry seasons could exacerbate existing nutrient limitations. In tropical forests, plant productivity is generally considered to be limited by P, given the relatively low P concentrations in tropical forest soils (Cunha et al. 2022). For microbes, C is generally considered to be the main limitation (Soong et al. 2020). However, in Chapter 3, I highlight a vital role for N in enzyme dynamics, which play a crucial role in C and nutrient cycles. This emphasizes the importance of considering the interplay between different nutrient cycles. Given the relative scarcity of available P tropical forest soils, the "recycling" of this nutrient is paramount, and in Chapter 4 I highlighted the central role of mineralization of organic P.

The findings presented in this thesis underscore the pronounced seasonal variation across almost all measured variables. This highlights the critical need for further exploration of the dynamics and drivers of nutrient cycling in tropical ecosystems, with a particular emphasis on the influence of seasonal changes on these processes. As I progress to the next chapter, I will delve into the limitations and implications of this research (5.2). I will scrutinize the academic challenges encountered during this study and discuss the recommendations, suggesting ways forward on the basis of my findings, in the broader scientific context of nutrient cycling in tropical ecosystems (5.3). This will provide a comprehensive perspective on the significance of my research and its contribution to the field.

5.2 Implications and limitations

5.2.1 Seasonality in C, N and P cycles: inputs and turnover rates

My results indicate large intra-annual shifts in the nutritional status of a tropical soil. Building on previous collaborative work in the same study area, we can gain valuable insights into various aspects of tropical forest ecology. Many pools and fluxes are relevant to the functioning of the tropical soil system, with many organisms providing inputs, or taking up nutrients from the soil. Given my findings, it is evident through the dynamic nature of tropical soil pools and fluxes, that the nutritional balance is not only influenced by the chemical properties of the soil, but also shaped by the biological interactions within the ecosystem, particularly the role of plants, their root systems, and the microbial biomass.

Through roots, plants take up water and nutrients, and they can be an input to the soil as plant litter (Fig. 5.5). It has been found that the productivity and mortality of roots is positively related to seasonal precipitation (Cordeiro et al. 2020). Plants can develop fine adsorptive roots with low tissue density which secrete enzymes for efficient nutrient uptake, although multiple root strategies are needed to maintain plant productivity (Lugli et al. 2020). Through these strategies, roots stimulate nutrient release from decomposing litter (Martins et al. 2021). In a nutrient addition experiment with similar forest composition and soil as in this thesis, it has been shown that roots respond rapidly to nutrient additions (Lugli et al. 2021). This suggests a high degree of adaptability in root systems to changing environmental conditions. Rapid responses of roots to changing moisture levels and biogeochemical conditions (Wurzburger and Wright 2015; Lugli et al. 2021; Cusack et al. 2021) – particularly in relation to limited nutrients such as P – highlight the intricate interplay between plants and soil. This underscores the necessity to consider not only soil chemistry, since in a changing climate the biotic feedbacks and interactions can to a large extent define the nutrient economy.

Another consideration is the resolution of the measurements in relation to the speed of the interactions and reactions. My results on the seasonality of the soil C:N:P did not provide us with an unambiguous pattern of how the biogeochemical changes take place while considering combined nutrient cycles. Especially the dynamics of nutrient fractions that are readily available in the ecosystem are likely influenced by processes that occur at a faster pace than the frequency at which I collected samples. Logistical factors and local capacity limitations for sampling and analysis of the available facilities and resources taken into account, our research was performed at the maximum feasible sampling frequency.

However, a rapid pace of change means that the conditions could have altered back and forth between two subsequent sampling campaigns, making it challenging to accurately track and get insight in these dynamics (this is further discussed in section 5.3.6 of this chapter). Essentially, the nutrient fractions are part of a complex, fast-moving system, and capturing a snapshot of this system at any given moment might not fully reflect the ongoing processes. As another example, in Chapter 2 I reported higher SOC concentrations in the wet season and lower concentrations in the dry season, while litterfall showed the opposite pattern. The significant temporal variation in the C pools in the top 15 cm of soil suggests C might be cycling rather quickly in this layer. This implies that C dynamics in the topsoil are sensitive to changes, such as variations in litter inputs and/or shifts in the biogeochemical balance that affect the quality of SOC. Residence times of C in tropical ecosystems are high on average, partially as a result of the relatively large (total) soil SOC pools (Jobbágy and Jackson 2000). However, residence times of soil carbon increase with depth, and most respired CO_2 comes from recent sources (Trumbore, 2000). This corroborates that the turnover rate of soil SOC at relatively shallow depth is relatively high, as it responds quickly to changes in environmental conditions such as moisture and temperature. These high turnover rates also imply a vulnerability. Specifically, it implies soil SOC at shallow depth might be more susceptible to loss when disturbances occur, such as droughts, fires, or land use changes. Such disturbances could compromise the role of the ecosystem as a C sink in the long term, especially if they occur more frequently or intensely than the soil community can adapt to.

In light of these observations and considerations, it becomes clear how important it is to monitor and understand how soil C dynamics are affected by various types of disturbances and how resilient soil functioning is to recover from such events. This understanding is particularly important when considering future precipitation and soil moisture decreases in the Amazon region in response to global change (Douville et al. 2021). Under drier conditions, soil C emissions could increase rapidly - provided that soil moisture is at levels that still allow for microbial activity - while available inorganic P decreases (O'Connell et al. 2018). It could be particularly important to consider the influence of N availability in this context, as I have shown that the activity of soil enzymes seems dependent on N availability.

The most solid relations between the N cycle and C and P cycles were found while analyzing the enzyme data; extractable N was related to the expression of most of the soil enzymes studied. This could be related to the N-cost of enzyme production; since enzymes are proteins, microbes need N to produce them (Friedel and Scheller 2002). The observed

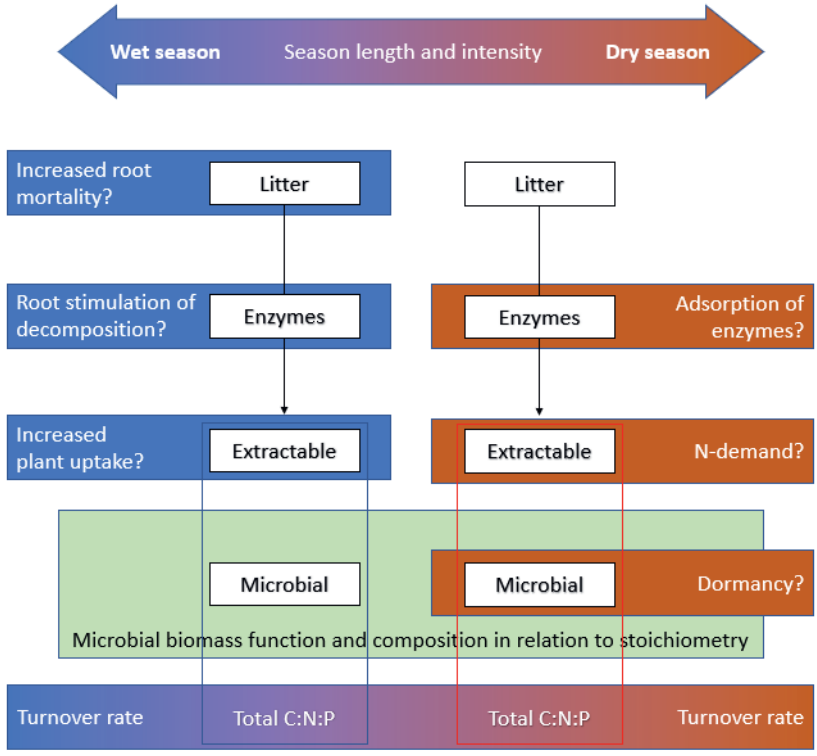

Figure 5.8: Some of the uncertainties highlighted in this chapter. The wet (blue) and the dry (orange) season each have their own challenges.

dependency of enzyme production on nitrogen availability as I explored in Chapter 3, could mean nitrogen availability has a key role in the fate of organic phosphorus, by facilitating enzymes that break down organic P into forms that are readily available for uptake by plants (Chapter 4). This underscores the need to further deepen our understanding of the interplay between different nutrient cycles in tropical soils.

The fractionation method of soil P pools I analyzed in Chapter 4 is widely used to assess pools of different availability in soils, by extracting them with increasingly aggressive extractants. Since this method is widely used, this allows for comparison with different studies, such as the study performed by Helfenstein et al. (2020) that experimentally established reference residence times of different P pools. However, the pools are operationally defined, which hinders a more mechanistic view of what soil processes are involved in the transformations of P. This thesis focused on the seasonal transformations of nutrients. As such, sorption-desorption dynamics, redox and chemical interactions with Iron (Fe) and Aluminum (Al) (hydr)oxides, the residence times of the different nutrient forms, and the reactivity of different mineral surfaces with other ions were not studied. Since these

dynamics could hold important clues over the fate of inorganic P in highly weathered, low P soils (e.g., Mendez et al. 2022), the integration of these pedologic transformations with biological P transformations would greatly enhance our overall understanding of the P cycle. Moreover, the chemical speciation of organic P could also add to the complexity of the studied processes, as has been demonstrated in another study by Helfenstein et al. (2018). This would be especially illuminating to our process based understanding if performed in a design where the combination with, for example, enzymatic transformations of those species can be examined, as I further expand upon in section 5.3.2 and 5.3.3.

Although soil SOC plays a crucial role in providing microbes with energy (Soong et al. 2020) and SOC is of great interest as a carbon sink in the global climate system (Lal 2004), this thesis has limitations in identifying robust drivers of variation in most soil C and N pools. This limitation underscores the complexity of nutrient dynamics in ecosystems and the challenges faced in studying them. It highlights the need for more frequent sampling or alternative methods that can better capture these rapid changes. Despite these challenges, the findings still provide valuable insights into nutrient availability and its role in ecosystem functioning. However, since enzymes are the rate limiting step of microbial nutrient and C transformations (Sinsabaugh et al. 2008), we can say something about how the nutritional demand in the forest changes with season by studying the relative activities of soil enzymes. Since the findings suggest that microbial activity may be limited under wetter conditions, or alternatively, that enzyme production may be stimulated during the drier season, we should wonder what mechanisms are behind this. One of the possible causes could be that enzymes are adsorbed to OM or minerals, as we discuss below.

5.2.2 Uncertainties in the drivers of enzyme activity

Soil microbial nutrient demand, measured here as potential extracellular enzyme activities, indicated different patterns of demand between nutrients. Often expressed through changes in heterotrophic respiration, soil microbial activity has been shown to follow a seasonal pattern (Cusack et al. 2019). This seasonality, combined with observed seasonal shifts in microbial community composition (Buscardo et al. 2018), could hold important clues for C and nutrient cycling under a changing climate. Indeed, the enzymes related to C and P mineralization showed increasing activity during the dry season, correlated with litterfall (Fig. 3.3). The microbial biomass size showed a different pattern (e.g. Fig. 2.2); apparently the extracted biomass size and its stoichiometry are not directly linked to the activity of specific enzymes, which would imply that enzymatic activity is driven by other processes. These include processes such as adsorption of enzymes to OM and mineral

surfaces (Steinweg et al. 2012), but also the state of the microbial biomass. Microbes can be dormant for extended periods if limited by C (Joergensen and Wichern 2018), and produce most phosphatases when the C supply is sufficient (Bilyera et al. 2021). This suggests that the size of the microbial biomass is of limited explanatory value as compared to enzyme activity, or alternatively, heterotrophic soil respiration.

Microbial activity is crucial for nutrient cycling in soil, but roots and mycorrhizae also contribute to P mineralization by secreting enzymes (German et al. 2011). This adds a layer of complexity to the interpretation of enzyme dynamics, and can be problematic especially if enzymes are interpreted as signaling microbial demand only. Evidence on the relative contribution of root-produced phosphatase is unconclusive; some indicate it only constitutes a small contribution to total soil phosphatase activity (Cabugao et al. 2021), while others maintain that the relative contribution of roots to phosphatase activity is more substantial in litter decomposition (Martins et al. 2021). Roots and microbes might very well respond differently to climatic drivers. If we assume for argument's sake that the observed variation in phosphatase activity was indeed caused by root exudation of said enzymes, this could mean that the synchronization of litterfall and enzymes is driven by increased plant nutrient demand during the dry season. If in the dry season the production of root enzymes increases as part of a plant strategy to obtain phosphorus during e.g., new leaf production, this would mean plants have the capacity to quickly and directly modify soil nutrient cycling. On the other hand, if we attribute the primary variation in enzyme activity to the microbial community, this could imply two scenarios. First, the introduction of fresh litter might stimulate microbial activity, including enzyme synthesis. Second, the soil moisture regime, which also affects leaf senescence and therefore affects the amount of litter reaching the soil, could facilitate increased enzyme activities.

Since my study did not distinguish between the origin of the enzymes (microbial origin or root exudation, see also section 5.3.3), it is hard to make generalized statements. It is clear however that roots play a role in the cycling of nutrients through different mechanisms, including through the direct exudation of phosphatases to mineralize P, emphasizing the complexity of controls over nutrient cycling. If the clear increase in phosphatase activity during dry months can be partly attributed to changes in root exudation dynamics, it would raise intriguing new questions about tropical nutrient ecology. These questions could include the evolution of plant functioning and development, the interactions and controls of belowground nutrient cycling, and the competitive advantages that plants might have developed over time. These advantages might be to either outcompete other plants in

phosphorus acquisition or to increase phosphorus use efficiency, for example by adjusting P allocation (i.e. resorption). In a highly diverse tropical forest, evolutionary strategies to obtain P, through root exudation of enzymes, priming of the microbial biomass, or through the timing of deployment of those acquisition strategies, might be crucial to understand the response of the forest to disturbances such as changes in precipitation or other forms of seasonality.

In this context, it could also be important to critically examine microbial functioning and structure in relation to these root interactions and competition. Since different species have different acquisition strategies for nutrients, symbiotic relations such as mycorrhizal interactions or the priming of the microbial biomass are definitely worth investigating further in a tropical context. The intricate relationships that plants establish with soil via their root systems further emphasize the critical need to integrate above and below ground cycles, as well as their seasonal variations.

5.2.3 Enzyme vectors: underestimated N-demand?

The vector analysis in Chapter 3 followed the methodology established by Moorhead et al. (2016). However, I opted to exclude leucine aminopeptidase (LAP) activity from the calculations. Despite adhering to established laboratory protocols, I had reservations about the assay results for LAP. Noise around zero activity (including values below zero) after calculations persisted even across lab replicates. The uncertainty stemmed from either degraded reagents (or otherwise not reacting properly) or very low LAP activity. Enzymatic assays inherently come with limitations (e.g. Wallenstein and Weintraub 2008). Notably, the lack of a positive control for calibration in our methodology introduced uncertainty about the results. Consequently, I made the decision to exclude the LAP-activity.

Early on in the discussion by Moorhead et al (2016), it is highlighted that the “relative contribution of LAP to the sum of NAG + LAP is low at pH < 7”. Subsequently, they discuss how this resulted in high proportional activities of BG/(BG+LAP+NAG) when NAG was omitted. Although Moorhead et al. recommend to therefore include both LAP and NAG, they explicitly indicate that the contribution of LAP is low, and cite Sinsabaugh et al (2008). It is not uncommon for LAP activity to be low, especially at low pH, since the pH optimum for LAP is at about 8.0 (Sinsabaugh et al. 2008). Given that the soils studied in this thesis are acidic, I assumed that excluding LAP activity would not significantly alter the quantitative assessment of EE-vectors. In combination with the observed high AP activities (consistently exceeding the other enzyme activities, which resulted in AP-dominated vectors), inclusion of LAP in the vector calculation would be unlikely to change the conclusions in Chapter 3.

If activity of LAP is indeed significant, and the seasonal dynamics of LAP are different from those of NAG, this might have implications to the interpretation of our data. While both NAG and LAP contribute to N (and C) mineralization, they act on different substrates. NAG primarily reflects microbial biomass turnover, whereas LAP is linked to protein mineralization. In this light, an asynchronous pattern is plausible if the enzyme activities are, for example, supply-driven: litter input dynamics may provide LAP with a different substrate-availability pattern than the microbial biomass provides for NAG. If LAP activity would be negatively correlated with NAG activity, the observed dynamics of the vector angles over the months might have shown a different pattern. However, since we were not able to reliably test LAP-activity, this remains speculative. Although conclusions based solely on vector analysis may raise questions, we have used the vector analysis in combination with a broader assessment of drivers of enzyme activities and microbial demand.

5.2.4 Supply-demand dynamics of enzymes and their relation to nutrient cycling

According to exoenzymatic theory, enzyme activities are either supply driven or demand driven (See Box 1.2). By using the analytical framework suggested by Moorhead et al. (2016) the enzyme activity is considered to be driven by demand (i.e. enzyme production increases under C or nutrient limitation) rather than supply (increases in enzyme activity when substrate is available), and the proportional activities can be calculated into vectors which indicate relative C-, N- and P-limitation. The enzyme activities I reported indicate a turnover potential which is larger than the nutrient stocks (Table 5.1). Litter at the site contains very little P, about 0.13 mg kg^{-1}, arguably through resorption before leaves are shed. Especially when considering the turnover of phosphorus, the data strongly suggest both demand driven enzyme production (i.e. nutrient demand drives enzyme production) and substrate limited mineralization of organic P pools.

In this thesis, the uppermost soil layers are examined. This study design was adopted because I assumed seasonality to be most visible in those layers. In the forest, the uppermost mineral soil is characterized by relatively large root stocks (Cordeiro et al. 2020) while fine roots grow upwards into the litter layer positioned on top of the mineral soil surface (Stark and Jordan 1978). This leads to mineralization and uptake of ortho-P directly from litter without touching the mineral soil and the chance of reaction with Al- and Fe-oxides (Herrera et al. 1978b; Stark and Jordan 1978; Martins et al. 2021). This adds to the evidence that plant and microbial acquisition of nutrients is highly influenced by the limited availability of substrate.

5.2.5 The challenge of quantifying pools and fluxes

Although it remains a challenge to quantify the in-situ rate of turnover, Chapter 4 integrates several observations and gives an indication of how different P-pools are connected. I suggested the modeled P-loss obtained from a litter decomposition experiment provides a basis for understanding possible litter inputs that increase the organic P fractions in the soil, while the activity of AP decreases the size of the organic fractions. The cycling of nutrients in litter maintains forest production, even in forests with moderate soil P concentrations (Sayer et al. 2024). Litter return to the soil could be a good indicator of turnover rates, yet in our study only the leaf litter fraction was measured. The coarse wood and root litter inputs could also play a substantial role in soil C and nutrient turnover (Cordeiro et al. 2020; Wu et al. 2023), yet for our site these effects remain to be quantified. Even so, it is improbable this would fully explain the magnitude of observed changes in soil P pools. The microbial biomass could also be of influence: if turnover rates of the microbial pool are high and microbial necromass accumulates this could stabilize substantial amounts of SOM (Miltner et al. 2012).

Table 5.1: Average stocks of several soil C, N and P pools, in comparison with litter input and potential enzyme activities (litter data from Martins et al. 2021 and bulk density data from Hoosbeek et al. 2023)

	unit	Litter (year^{-1})	0-5 cm	5-15 cm
Total C	kg ha^{-1}	2 573.4 ± 10.2	20 907 ± 1 056	21 817 ± 1 170
Extractable C	kg ha^{-1}		340.5 ± 23.3	709.4 ± 57.1
Microbial C	kg ha^{-1}		268.7 ± 15.9	516.4 ± 43.0
C-enzyme (BG)	kg C ha^{-1}year^{-1}		1611.6 ± 4.6	2132.3 ± 11.3
Total N	kg ha^{-1}	80.7 ± 1.1	1 056.3 ± 45.5	1 629.4 ± 61.2
Extractable N	kg ha^{-1}		34.3 ± 1.2	60.6 ± 1.9
Microbial N	kg ha^{-1}		26.43 ± 2.35	36.03 ± 3.16
N-enzyme (NAG)	kg N ha^{-1}year^{-1}		8045.9 ± 33.2	6873.6 ± 33.4
Total P	kg ha^{-1}	0.70 ± 0.11	45.5 ± 0	91.8 ± 0
Extractable P	kg ha^{-1}		0.74 ± 0.06	0.84 ± 0.06
Microbial P	kg ha^{-1}		0.81 ± 0.09	0.80 ± 0.11
P-enzyme (AP)	kg P ha^{-1}year^{-1}		403 738 ± 956	608 319 ± 1659

The challenge remains that the observed monthly averages for total C and total N show variation that is substantially larger than can be explained by the inputs of litter (Table 5.1). The peak in May for C (0-5 cm, Fig 2.2) is about a third larger than the lowest average in November: this would mean that some 7000 kg of C is lost (per hectare) during the dry season in the top 5 cm. Although the inputs of leaf litter could replenish max. 2600 kg, considering some 70% of litterfall in tropical forests is leaf litterfall (Shen et al. 2019), we can assume that another 1100 kg is added through other forms of aboveground litter. This would mean about 3300 kg C ha^{-1} is unaccounted for by aboveground inputs. It is not unrealistic this amount is provided by plant litter below ground, as these orders of magnitude have been reported for Amazonian forests (Aragão et al. 2009). Additionally, the effects of root litter inputs can be larger than the effect of above ground litter inputs (Liu et al. 2019). This quick calculation, however, does not take into account any soil below the 0-5 cm depth. Considering the layer below had less intra-annual variation and we did not consider contributions from e.g. microbial necromass, this illustrates the challenge of scaling up and quantifying seasonality in the turnover. A larger problem in this calculation is the relative loss of C when litter progresses to the soil: while litter has a C:N of 35, the top 5 cm had an C:N of 19, and at 5-15 cm depth this dropped to 15.5. Lower C:N ratios with increasing soil depth are common since C is respired during microbial turnover.

For the quantification of the P-balance, the organic P fractions indicate higher differences between months than can be explained by the inputs of litter-P. However, the total amount of P does not show large variation, which implies the transformations from inorganic to organic P (and vice versa) could largely explain these dynamics. Given the high activity of the microbial biomass and the large differences in microbial P between months (Table S2.1), it seems likely that changes in the organic P fractions can be largely attributed to the transformations of soil pools to the microbial biomass, and subsequent stabilization as necromass. The size of the microbial biomass in this thesis was not adjusted for extraction efficiency, since an extraction efficiency has to be determined specifically for each soil. This limitation does not affect the goal of this thesis to study the dynamics of the microbial biomass size, but it does indicate that the reported differences between microbial biomass size might underestimate the absolute differences between observations. The dynamics between P-pools within the same layer are thus an important next step for increasing our understanding of the P-cycle in tropical forests (see also sections 5.3.2 and 5.3.4).

5.2.6 Models and process-based insights in relation to my findings

In this thesis I investigated the links between litterfall, stoichiometry of soil nutrient pools (total, extractable and microbial), enzymatic activity, and organic P cycling in soils. In simple terms, process-based insights, translated into conceptual models and generalized statements, can be building blocks for more complex models. However, as we have indicated above, some limitations exist. This thesis gives insight in how the bioavailable pool of inorganic P is maintained through the mineralization of organic P forms. My results are limited in the sense that I did not incorporate seasonal differences in decomposition rate (as for example observed by Wieder and Wright 1995) in our simple decomposition model (Fig. 4.4b). The inclusion of such detail to the decomposition rate might bring more complexity to the interaction between nutrients and climate, which could result in a more accurate representation of the process.

Moreover, the influence of roots can be detailed and included in the representation of litter decomposition dynamics. Due to the interplay between climate, root productivity, and the stimulating effect of roots on decomposition (Cordeiro et al. 2020; Martins et al. 2021) they are of paramount importance to the processes taking place in the soil. Climate affects the rate of organic matter breakdown, while root productivity is related to the amount of organic matter available for decomposition. Moreover, roots can stimulate decomposition by secreting enzymes and other organic compounds, and influencing the soil microbial community (see also section 5.3.3). Incorporating these factors into decomposition models could lead to more accurate predictions of nutrient cycling and tropical ecosystem functioning. My thesis shows that there are distinct peaks in enzyme activity, possibly related to root exudations either directly (for AP) or indirectly (mycorrhizal symbiosis, and priming of the microbial biomass through the secretion of available C), and in soil nutrient budgets, according to season.

This thesis offers a dynamic perspective on the interactions between Carbon (C), Nitrogen (N), and Phosphorus (P) cycles. The differentiation of nutrient contents across seasons and the enzyme activities' reliance on nitrogen availability are notable features of this plant-soil system. Such dependencies are increasingly incorporated in ecosystem models, as outlined in Box 5.1, to predict the fate and future of forests through mathematical representations of ecological processes. These models are thus becoming more comprehensive and insights they provide can generate valuable hypotheses for experimentalists – especially in the context of large scale ecosystem manipulation experiments such as AmazonFACE. Therefore, it has been argued that model and experiment integration could be beneficial in

such experiments (Hofhansl et al. 2016). For example, by incorporating the P cycle in a range of ecosystem models, each with different process representations of P, not only called attention to the apparent P limitation of tropical ecosystems, but also identified several uncertainties in the underlying processes (Fleischer et al. 2019). When these key processes are included in ecosystem models, they could potentially decrease the uncertainty in predicting how tropical forests might respond to increased CO_2 concentrations. Among those key processes, the pathways of biochemical P mineralization (through phosphatase) and the controls of N availability on P acquisition are specifically addressed in this thesis (Fleischer et al. 2019), as is the control of the water cycle and water availability over the P cycle. Interestingly, Goll et al. (2018) argue, on the basis of their model including the P-cycle, that tropical forest susceptibility to droughts decreases on low-P soils, since P-limitation decreases water uptake.

Each chapter of this thesis contains elements that are highly relevant to modeling the processes in the tropical forest, to ultimately get insight in the fate of the forest under for example higher CO_2 concentrations or changed precipitation patterns. The novelty of Chapter 2 may not lie in the parts that are directly applicable to models, but the dynamic observation of the microbial biomass (Fig. 2.4) could be expanded upon in a similar way as the conceptual model of Moorhead (Moorhead et al. 2013) provides insight into the enzymatic expression of nutrient demand dynamics, by using enzyme vectors based on the relative activities of C, N and P-targeting enzymes (See also Chapter 3). My proposed conceptual model (Fig. 2.5) shows the microbial biomass size as a fraction of the total nutrient content, from which vectors can also be calculated as indication of the relative size of the microbial biomass, and integrating the stoichiometries of total soil C, N and P pools with the microbial stoichiometry. Chapter 3 provides potential soil enzyme activities, thereby indicating the turnover potential of organic nutrient pools in tropical soils at different times of the year, moreover, it postulates a special role for the availability of N as a driver to this enzymatic turnover potential. Chapter 4, in particular, is relevant to future modeling efforts aimed at predicting forest functioning and its dependence on the P cycle under varying conditions, such as drought or elevated CO_2 scenarios. By demonstrating that enzyme activities are responsible for the mineralization of P from organic compounds, the goal was to enhance the process-based understanding of the P-cycle. Furthermore, the correlation between phosphatase activity and litter inputs to the change in the organic P pools provides a tangible relationship that can be incorporated into a wide variety of models.

Box 5.1 **Climate models**

Climate models are essential tools for predicting future climate. They simulate large-scale patterns under diverse scenarios, incorporating the latest scientific understanding of the elements and interactions that play a role in our climate and its changes. One of the major challenges is predicting the extent to which increases in greenhouse gases, such as CO_2, will affect climate. Ecosystem models play a pivotal role in these computations, particularly in the context of nutrient cycling. These models simulate the flow and transformation of nutrients within an ecosystem, capturing the complex interplay between biotic and abiotic components. Nutrient availability, for instance, plays a critical role in ecosystem processes, which in turn have significant implications to climate feedbacks. Describing those patterns involves complex calculations with many interactions and feedbacks. To improve the accuracy of the results, it is crucial to study interactions and feedbacks in detail. Ecosystem processes regulate climate, but might be limited by nutrient availability. By gaining a deeper understanding of nutrient limitations to those processes, we create more accurate and reliable climate models for our predictions of future climate feedbacks.

The main ecosystem processes that ecosystem models aim to capture are the dynamics C and nutrients in ecosystems in various situations. A core process is the forests' productivity as defined by the assimilation of C in biomass, and the trajectory and release (emissions) of C to the atmosphere as organisms use it. As models grow more comprehensive, they include the fixation and emission of C and N from those plants, and the usage of nutrients, including P.

This thesis focuses on a tropical soil system, specifically the dynamics of C and the macro-nutrients N and P. We observe how organic matter inputs containing C, N and P progress through the soil system in different seasons. This increases our understanding of soil biochemistry and how microbes and enzymes affect the transformations organic matter undergoes under different conditions within a year. By doing so, we can identify discrepancies between model representations and observations about the soils system nutrient pools and fluxes. Describing these pools and fluxes throughout the seasons in a tropical forest could challenge modelers to improve their representations, ultimately enhancing our understanding about the future of tropical ecosystems in a changing climate.

5.2.7 Seasonality, scale, and the representativeness of this thesis

Every ecosystem on Earth exhibits some form of seasonality, and the Amazon forest is no exception. In fact, significant variations can be observed across different regions within the basin (Zubieta et al. 2019). This thesis presents findings from soils that are representative of approximately 60% of the Amazon basin, based on soil type and age (Lapola & Norby, 2014; Quesada et al., 2011; Quesada et al., 2012). Climate and forest structure might differ between regions with this soil type within the Amazon, as they do within other (neo)tropical forests (Hofhansl et al. 2020). Still, if edaphic conditions are similar, most of my results and conclusions should hold up, but I also reenforce the notion that seasonal variation and sampling moment in a year can cause significant differences even when sampled at the same site. Therefore, while my results indicate patterns and interactions that are likely to be valid for large parts of the Amazon, comparative studies between regions or sites should be interpreted with caution; results might be substantially influenced by seasonality-related nutritional shifts. In other words, it is important to note that seasonal variation can lead to significant differences in soil chemistry, even when samples are taken from the same site. The timing of sampling within a year should always be seen in the context of those temporal patterns, which might differ in timing and magnitude between sites (also see section 5.3.1).

Understanding temporal patterns in ecological systems is essential for unraveling underlying processes and predicting ecosystem responses to change. Seasonal patterns can be differentiated from long-term, intra-annual variation as exemplified by the seasonal dynamics I established related to litter dynamics, extracellular enzymes (EE), and microbial biomass. However, they are to be treated with some caution, and should be solidified by expanding on the topics of this thesis in future work. Confirming the observed temporal patterns, while addressing the quantification of turnover of C, N and P pools in tropical soils (as has been discussed in 5.2.5), is a feasible yet challenging effort that requires a combination of methods (See 5.3.4).

This thesis provides one of the first datasets exploring the temporal variation of soil nutrient dynamics influenced by enzymes in the Amazon Forest. Despite the broad representativeness of the edaphic conditions and the smallest feasible measurement interval we could manage, there is always room for further refinement and detail. The conclusions drawn in chapters 2 and 4 are based on data from four sample dates within a year, demanding some caution when generalizing the results. Many processes could be too fast to properly have been captured with the used measurement interval (see also 5.3.6). Moreover, as mentioned in the previous chapters, some of the observed patterns might

have had some unidentified effects from an El Niño year that preceded 2016 (Van Schaik et al. 2018). However, the observed precipitation in the year of the sampling campaigns was within the range of a typical year (see Chapter 2, Fig. 2.1, or Araújo et al., 2002).

5.2.8 The influence of plants: from soil processes to ecosystem scale

In this thesis I looked at tropical soil biogeochemistry from an ecological perspective, in the context of which I identified shifts in soil nutrient pools and their stoichiometry, which I argue are the result of seasonal changes to the microbial community composition, changes in nutrient demand, and litter inputs. From a soil chemical perspective, plants are the drivers of most of the inputs to the soil, and play a crucial direct role belowground as well, through nutrient acquisition and through interactions with microbes. These P-acquisition strategies include the foraging by fine roots, phosphatase exudation, organic acid exudation and arbuscular mycorrhizal symbiosis (Reichert et al., 2022). Tropical forests are very diverse, and several strategies for the acquisition of P can be distinguished in plant roots (Lugli et al., 2020). Some plants might have advantages over others when it comes to either their use or acquisition of nutrients, and before it can be assessed what community shifts would mean belowground, we would need to identify what functional plant groups currently play what role. Phosphorus (P) is an essential element for many energy-dependent processes in plants and microbes, such as growth, functioning or reproduction. The seasonal availability of P in the soil can have cascading effects on organisms. One area of interest is how periods of relative P shortage affect plant growth and which species have a competitive advantage in this situation. Some plants may even have the ability to store P (Rosell et al. 2023), making them more resilient to seasonal P shortages. By identifying which organisms have these advantages we can begin to understand the mechanisms behind it. Equally for microbes; increasingly we can identify functional groups and their traits. Like plants, microbes may be affected by P limitation and have the ability to store P (Mason-Jones et al. 2022).

The findings presented here should be seen as a stepping stone towards a more nuanced understanding of the Amazon basin's complex ecosystem. In the last sections, I have discussed the limitations of the methods and the data used in this study, and the challenges in interpreting the results. Furthermore, I have highlighted the importance of monitoring and understanding the soil carbon and nutrient dynamics and how they are affected by disturbances, especially in the context of global change and the role of the Amazon region as a carbon sink. The findings of this thesis have implications for ecosystem models that predict the fate and future of forests under changing climate conditions, and the need to

incorporate the factors that affect the decomposition and nutrient cycling processes. In the next section, I will provide recommendations for future research.

5.3 Recommendations for further research

The seasonal dynamics of tropical soil biogeochemistry are underrepresented in scientific literature. My results indicate that microbial functioning and soil nutritional status exhibit strong and often distinct temporal patterns. Future research efforts should focus on refining these results to better understand the biotic and abiotic drivers that influence these processes throughout the year. The temporal resolutions used in this study provide insight into the significant changes in biogeochemistry. However, the lack of direct or statistically significant relations between several of the analyzed pools suggests that there's a wealth of intricate interactions yet to be explored at a higher resolution. Especially when performing research at relatively remote field sites in a tropical forest, where locally available facilities are limited. As with all projects, there is a limit to the amount of work one can do, and to the capacity of the laboratory facilities to process samples. Although I am proud to present the results of this work in this thesis, I can conceptualize a range additional research directions to unravel these complex interactions and enhance our understanding of biogeochemical processes. Below are some of my main recommendations to guide future research efforts.

5.3.1 Routinely reporting sampling dates

The main recommendation from this thesis is to consistently and systematically provide sampling dates and seasons when reporting information about (tropical) soils, particularly if the data involves soil nutrients and nutrient cycles. Considering the dynamic nature of the soil nutrient pools, this information is vital as it can greatly influence tropical soil biogeochemistry - and consequently, forest functioning. Many published articles have not, or not sufficiently, reported this information. Considering seasonal effects is essential in many contexts, especially if multiple study sites are involved or results are compared between sites. Ideally, the (effect of) sampling season/date should not only be considered during the reporting phase, but should be included as a central consideration during the experimental design phase. This will reduce unexplained variation resulting from seasonality, and can thereby potentially strengthen the robustness of datasets.

5.3.2 Further speciation of nutrient pools

Further studies building and expanding upon the data and findings from my thesis could enhance our understanding of the dynamics and reveal more about the underlying processes by further refinement of the C, N, and P pools. Although I did not include any chemical speciation for C and N beyond the distinction between total, extractable and

microbial pools, this would be a logical next step in understanding their variability. Questions such as what forms change and how they are bound in the clay rich soil could be explored. SOM stoichiometry might be an important driver for processes, but here we lack further specification of what SOM forms are dominant in what season. Similarly for soil P, we identified the organic pool as an important pool in maintaining soil P bioavailability. However, we still need to determine which organic forms are dominant, and which are most important to the bioavailability of P.

Refining soil C pools involves distinguishing between various components such as sugar, starch, carbohydrates, lignin, and organic acids. These components significantly influence the bioavailability and turnover time of the soil organic carbon fraction. Techniques like nuclear magnetic resonance (NMR) spectroscopy (solution and solid state) and X-ray absorption near-edge structure (XANES) have been utilized for SOM speciation (Doolette and Smernik 2011; Wang and Zhong 2016; Luo et al. 2017; Liu et al. 2017). Moreover, pyrolysis- gas chromatography/mass spectrometry (py-GC/MS) is increasingly used to this end, creating insight in the whole molecular composition of organic matter (Schellekens et al. 2017; Picó and Barceló 2020). Additionally, isotopic methods using the relative abundance of 14C/15N/18O can provide insights into the age and turnover of existing pools, their interactions, and can also serve as markers for assessing the turnover of new additions to the system. More specifically, isotopic techniques have been employed to disentangle transformation rates, with 15N used as a tracer element (Weintraub et al. 2016). For organic P, solution 31P NMR spectroscopy can be used for speciation of chemical forms (Turner et al. 2014). As referenced previously, isotopic kinetic data and sequential extraction methods have been combined before to estimate residence times of Hedley P pools (Helfenstein et al. 2020). In this way, combining different extraction methods can be applied to further differentiate between chemical forms, residence times and nutrient transformations from organic P pools (see also Kruse et al. 2015).

Since we observed large stoichiometric shifts in the microbial community, I concluded that it was likely that large shifts in community structure take place seasonally. Explicitly linking microbial nutrient content and microbial community structure should be a research priority, considering the central role the microbial biomass plays in nutrient cycling. Describing what communities are dominant during what precipitation regime could form the first step in predicting the implications of changes to precipitation. Moreover, the effects of elevated CO_2 could include more C allocation belowground, which could have implications for the microbial community as well (See also section 3.4). Similarly, the link between the microbial

community composition and microbial functioning – specifically traits such as enzyme production (see section 3.3) – should be elaborated upon. Molecular methods on DNA extractions can provide information on fungal and bacterial groups (Buscardo et al. 2018; Buscardo et al. 2021), as can group-specific fatty acid methyl ester analyses (Fanin et al. 2013) either one of which could be a logical step to expand on the results and conclusions of Chapter 2. If certain functional groups are more abundant under specific precipitation conditions, it would be interesting to explore the implications for the community's functioning. As an example, this could affect the production of phosphatase enzymes or the resilience to drought.

5.3.3 Improving insight in the drivers of enzyme activity

The dynamics of soil enzymes are widely studied, yet our understanding on their controls can be refined, especially at short temporal intervals. Since we have shown the activity of the enzymes studied in this thesis are related to N-availability in tropical soils, but also show the influence of seasonal litter inputs and the effects of enzymatic activities on organic P-pools, I here give some directions for future research efforts in the unraveling of the drivers of these catalysts of organic matter turnover.

First, the enzymes described in this thesis are used as a proxy for microbial demand, yet we have a limited understanding of what microbial groups are producing what enzyme. Although low P-availability is generally considered to have an adverse effect on microbial growth (Waring et al. 2014), increased N availability might stimulate fungal groups in the microbial community through which enzymatic activity is more efficient (Bonner et al. 2018). Some work has been done on the effects of season and drought on tropical soil fungi and enzymes, showing an increase in dark septate fungi and a decrease in fungal pathogens, and a shift towards N-demand (Buscardo et al. 2021), suggesting the forest is shifting towards more N-limitation as drought becomes more pervasive. I would therefore recommend to explicitly study the connection between microbial functional groups and the enzymes that mineralize N and P under different stress situations. Since drought, and the interplay with an increased plant nutrient demand from increased atmospheric CO_2 concentrations might well shift microbial processes through changing community composition, it is crucial to integrate findings about the community structure and the enzymatic implications of such shifts.

Yet microbes are not the only organisms that play a role in the activities of enzymes in soil. Plant roots, as mentioned previously, can also play a role in the secretion of phosphatase (Cabugao et al. 2017; Martins et al. 2021). Through methods such as isotopic labeling and

root exclusion techniques, clarifying the relative contribution of plant roots in the activity of phosphatase enzymes is a feasible research objective. This would also provide a stepping stone towards an exploration of the evolutionary ecological implications of changes in biochemistry as mentioned in section 5.2.6 of this chapter.

In the next section I provide some recommendations about the integration of field and lab-studies, which might be crucial in improving our process based insight. Specifically for the activity of enzymes, I would recommend to clarify the in-situ substrate turnover catalyzed by enzymes. In general, enzyme activity is analyzed as a potential activity. To correlate this potential to actual turnover, and describing what conditions have to be met for an enzyme to fulfill its potential would constitute a big advancement in our insight in nutrient cycling in tropical soils. Different moisture levels, sorption/desorption dynamics and the complexity of substrate all play a role in the enzymatic breakdown of organic matter, which I would recommend to explore by integrating field observations and lab incubations as described below.

5.3.4 Field tests and laboratory incubations: solidifying interactions by eliminating other variables

Sampling tropical soils from a remote field site can prove to be a challenge in itself. Transporting samples to the lab and analyzing them in a short timespan is another challenge. Both of those challenges have to be met to get the kind of data and relations I found in this thesis. Some of those relations can be validated by performing incubation experiments, or be improved by performing measurements in the field. I mainly recommend incubation studies to get a more process-based understanding of the reactions taking place in the soil, focusing on the effect of enzymes, simulated root exudates and temperature/moisture sensitivity of reactions.

Specifically from our study, Chapter 2 presented the dynamics of the different C and nutrient pools. The extractable fractions showed a contrasting pattern for N as compared with C and P. A detailed study on N dynamics may be performed, in which samples should be analyzed as quick as possible to prevent adverse effects from sample storage. Additionally, we saw changes in the microbial biomass stoichiometry that we attributed to the changes in microbial composition. In an incubation experiment, I would recommend to experimentally test what stressor or stimulant (moisture, nutrients, temperature, etc.) would provoke such shifts in stoichiometry, and on what timescale.

Following from the third chapter, the enzymatic interactions and effects should be refined by confirming the dependence of enzyme production on N availability, by applying different amounts of substrate or available N, and analyzing the effect on potential enzyme activities. Equally for the availability of other nutrients, what enzymes, for example, are repressed when available nutrients are chronically added to a tropical forest soil? Is microbial demand indeed driven by nutrient limitation, and what are the effects of increased or decreased organic-P substrate to the activity of phosphatase on different timescales?

With the results of the Chapter 4 in mind, I would recommend to specifically test transformations of P between the different Hedley fractions and how this could have been provoked by different conditions, such as changes in moisture, as well as changes in P-availability induced by roots (Hinsinger 2001). Specifically, I would recommend to investigate for the AmazonFACE site how P availability changes when adding organic acids or labile C (imitating root exudates) to the tropical forest soil at different concentrations and in different seasons. There are various examples of those types of experiments (e.g. Huang et al., 2021), yet in the context of increasing CO_2 concentrations in the tropical forest, this question remains relevant. Labile C inputs have been shown to stimulate the production of phosphatase by microbial composers in the litter layer (Martins et al. 2021), yet in the mineral soil, sorption-desorption dynamics might alter the effect of these exudates. Studying these simulated effects of exudation in the mineral soil would give results on a relative short term, while investigating the in-situ effects of elevated atmospheric CO_2 concentrations on the soil nutritional balance might be more time consuming. Furthermore, considering the insights from Chapter 4, it would be beneficial to apply different quantities of phosphatase to incubated soil. By studying the subsequent transformations of organic compounds, one could estimate the relationship between mineralization rate and phosphatase activity. This approach would provide valuable insights into the microbial cost of phosphorus acquisition.

These are only some directions of how incubation studies could solidify the conclusions from the previous chapters. Ideally, these experiments would be integrated with some of the methodologies suggested above. As an example, the composition of the microbial biomass could be linked to microbial stoichiometry (as described in section 5.3.2). A study like this could be performed in combination with a range of different soil nutritional or moisture conditions through addition of different substrates or maintaining the soil in a specific moisture range. Moreover, the enzymatic transformations of organic fractions can be studied with additions of specific organic nutrient forms, to increase insight in the drivers

of enzymatic turnover of organic matter, and the role of substrate type. Ultimately, insight in the interactions between drivers and transformations in tropical soil pools should be focused on creating a comprehensive understanding of nutrient pathways in the soil, with special attention to the P cycle.

Incubations to test specific drivers, such as temperature, moisture levels, pH or substrate availability, should preferably be performed alongside field studies on the in-situ reactions of the soil biochemistry. This dualistic approach ensures that results and insights are realistic, applicable and accurate, differentiating between controlled and natural conditions. By integrating data from incubation studies with in-situ experiments, we can maintain benchmarks that are closely aligned with field observations while also accounting for the effects of specific drivers, such as nutrient availability. This combination allows for a more comprehensive understanding of the soil biochemical system, as it captures detailed insights from controlled conditions as well as the complexity of real-world conditions, such as the interplay between multiple environmental factors in a soil ecosystem.

5.3.5 Atmospheric CO_2 increase and the intensification of nutrient cycles

The implications of responses of tropical forests to elevated CO_2 concentrations reach far beyond the gas exchange dynamics of the forest. Among the questions that are relevant to the soil chemistry in this context are how forest functioning might differ in water and nutrient dynamics under elevated CO_2. The possible CO_2-enrichment related increases to the efficiency of water use (Hatfield and Dold 2019) are relevant to the cycling of nutrients, as we have also observed in the rainfall seasonality in this thesis. Although some have hypothesized that low P availability increases the capacity of forests to deal with droughts (Goll et al. 2018), this remains to be confirmed in in-situ experimentation. Such findings underline the value of FACE experimentation (Box 1.3) in a tropical forest.

When studying seasonality in tropical forests in this context, it is crucial to carefully consider the nuances and details in the study design phase. Factors such as the species or functional types of plants can have significant implications for nutrient cycling (Prieto-Rubio et al. 2023). To fully understand the competition between plants below ground, comprehensive and detailed studies should be undertaken to investigate which traits provide an advantage in obtaining crucial resources such as phosphorus and water. If elevated atmospheric CO_2 concentrations increase the demand for P, a range of P acquisition strategies (Reichert et al. 2022) could be relevant. I would argue that especially the pathways that include phosphatase are relevant for thorough investigation. Since this enzyme is not only secreted by microbes, but also by roots, increases in its activity are indicative of a broader limitation.

Studies have already established that higher diversity has positive effects on the phosphatase activity and thus the availability of P (Chen et al. 2022a). Moreover, root-phosphatase might directly stimulate decomposition. For instance, in Martins et al. (2021) we suggest that roots may play a crucial role in shaping soil nutrient cycling through their influence on decomposition. In the context of elevated CO_2, these competitive differences also have implications to the capture and processing of carbon.

5.3.6 Timescales of turnover and change

In this thesis, I presented data on the seasonality of soil biochemistry, but how can these results be put into context when considering the timescales of changes to the biosphere? As indicated in the introduction, soil development takes place over geological timescales and any studied soil has to be viewed within that context. I studied highly weathered tropical soils representative of large tropical areas. They are of particular interest because of their mineralogical and nutritional makeup. The mineralogy of these fine textured soils is characterized by kaolinite, Fe- and Al-hydroxides and quartz, causing relatively large specific surface areas (Hoosbeek et al., 2023; Quesada et al., 2010). In terms of C-storage, those soils are storing a substantial amount of C (Quesada et al., 2020), while they have low P availability leading to P-limitation of tropical forest (Cunha et al. 2022). Their development over geological timescales was in concert with the development of the ecosystems and large-scale hydrology in the area (Herrera et al. 1978a; Quesada et al. 2010; Osman 2013). Changes to either the land use or shifts in hydrological cycles could very well mean a fundamental change in the biochemical functioning of those soils, and could lead to the loss of C, and by extent to the loss of other nutrients.

Of course, these shifts in climate or land use provoke changes in soil biochemistry. However, gradual changes in seasonality or exacerbation of existing nutrient limitations by for example the CO_2 fertilization effect would also have deep implications in the way tropical forests develop. Fleischer at al. (2019) showed that including P cycles in ecosystem models dampens the effect of CO_2 fertilization on the forest, which could have far reaching consequences for tropical forests as a carbon sink. The future of the Amazon forest as a carbon sink is of great interest, and there is evidence this sink is decreasing (Brienen et al. 2015). Gradual changes to climate, such as decreases in precipitation, increases of average dry season length, or other alterations to the hydrological cycle induced by climate change could undermine the capacity of the forest to maintain the biochemical cycles synchronized between ecosystem components. The forest and the life therein have evolved over long timescales, and the seasonality in nutrient cycles suggest that some form of synchronization

of nutrient cycles is advantageous to organisms. If climatic changes outpace the capacity of the forest to naturally adapt to the new balance, the function of the forest as a C sink might be affected. Moreover, if a cycle like the P-cycle becomes disturbed by environmental or land use changes, the loss of nutrients might force the ecosystem to deal with even lower concentrations of available P, and this would affect the composition of the forest on the long run. Therefore, long term systematic studies have to be undertaken to envision the possible futures of tropical forests.

On shorter timescales, the development of models that capture the plethora of processes involved in the cycling of C, N and P is the main way to gain insight into the future of C and nutrient cycles. In order to improve model representations, the following should receive extra consideration: a) Generally considering the implications of the effects of seasonality and moisture on biological processes and nutrient turnover rates, and specifically extrapolating and validating the seasonal effects on biogeochemistry as observed for microbial biomass, enzyme activity, and nutrient availability in this thesis, b) carefully considering how occurring or existing "disturbances", such as seasonality or droughts caused by the El Niño–Southern Oscillation, affect soil nutrient cycles and their biotic drivers, and c) contextualizing results from individual studies on soil biochemistry and stoichiometry in a broad ecological context, in order to extract patterns relevant to the long term nutrient ecology of forests, such as changes in plant nutrient uptake strategies, shifts in microbial functioning, or enzyme mediated nutrient mineralization. In this way, short term studies can better generate results that can be incorporated into long term predictions and models.

In Chapter 2, I established seasonal variation of nutrient stoichiometry of extractable, microbial and total soil pools, and in Chapter 4 I detailed the changes in different pools of soil P. Both of these chapters consider data collected every three months, while in Chapter 3 I established dynamic differences in microbial demand by studying enzyme activities on a monthly basis. The resolutions on which these data were collected can be refined, and further research should carefully address the rate at which the biochemical changes occur, and at which rate for example changes in enzymatic activity affect in situ turnover of soil C and nutrient pools. Decomposition experiments already established relations between mass loss of substrate and the activity of enzymes (Martins et al. 2021), yet controls over and quantification of turnover rates in the soil matrix remain uncertain. If drought immobilizes enzymes and affects the turnover of SOM, incubation experiments may further disentangle the complex relations between biochemistry and turnover.

5.4 Conclusions

The accurate prediction of the future of tropical forests in response to climate change hinges on detailed insight into the combined cycles of carbon (C), nitrogen (N), and phosphorus (P). These elements are essential for plant and microbial growth and their availability in the soil can greatly impact the health and productivity of a forest ecosystem. One of the key questions is how soil biogeochemistry will adapt to a scenario where increased CO_2 could modify plant nutrient demands and the return of these nutrients to the soil. Our goal was to gather as much detail as possible on the current status quo. In this study, I aimed to unravel the seasonality of these nutrient cycles.

We observed significant seasonal changes in soil biogeochemistry. We demonstrated how nutrient concentrations changed and hypothesized how C, N, and P were interconnected through various soil processes. We concluded that the microbial biomass shows strong signs of P-limitation. This was also reflected in the enzyme activities, particularly through relatively high phosphatase activities. We showed that organic P is a crucial pool for maintaining a bioavailable P-pool; replenished through litter P-loss and mineralized by enzyme activity. In the context of elevated CO_2 concentrations, we hypothesize that the P-cycle is already very tight. Inorganic, non-occluded P pools only account for a small part of total P, suggesting a likely intensification of the organic cycling of P under increasing nutrient demand due to elevated atmospheric CO_2 concentrations.

In this final chapter, I synthesized the findings and discussed the limitations of the thesis. I emphasized the importance of considering the interplay between different nutrient cycles, and the effects of climate change on soil biogeochemical processes. With a rise in atmospheric CO_2, soil biogeochemistry, particularly soil P, could become a significant limitation to potential increases in forest productivity. The phosphorus cycle is already very tight, yet this may intensify even further under increasing CO_2 and subsequent nutrient demand. On longer timescales, this will shift the nutritional balance and have profound implications for species composition above and below ground.

Tropical forests play a crucial role in climate regulation by absorbing atmospheric CO_2 and affecting global carbon and water cycles. Their functioning is highly relevant in a changing climate. At the core of their functioning are the processes that transform carbon and nutrients into the complex web of life. When viewed from a distance, from the perspective of nutrient ecology, these forests face rapid changes which present a range of challenges to their functioning. This thesis underscores the importance of understanding the complex

interactions between climate and nutrient cycling in tropical forests. By deepening our understanding of these processes, we can better predict how these ecosystems will respond to future changes in climate and land use.

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English summary

Soils are fundamental to the functioning of tropical forests. Soils are not merely the physical basis a forest can grow on, they are also the provider of nutrients and moisture that sustain the plants in the forest. The nutritional composition of soils and the plants that grow on them have a geological and biological context, in which the interplay between parent material and tropical climatic conditions affect soil nutrient availability. The nutrient that is considered to be limiting tropical forest processes, phosphorus, is generally low in the older tropical soils, the result of phosphorus depletion over the years.

Soil nutrient availability is crucial for plant and microbial functioning. Largely due to the dependency of flora on nutrients, their cycling in the context of plant productivity has been studied for many decades if not centuries. Although we have gone to great lengths to describe the relation between nutrient availability and (plant) production, the influence of freshly deposited plant litter (production) on soil nutrient availability is known to a lesser extent. Since a detailed understanding of the interconnected cycles of carbon (C), nitrogen (N), and phosphorus (P) is required to accurately predict the future of tropical forests in response to climate change, it is crucial we understand this interplay between seasonality, plants, and microbes in the soil.

The geological and ecological context sets the scene for one of the major questions, the answer of which will determine the fate of many forests; how will plants adapt to changes in climate and CO_2 concentration? Will an elevated CO_2 concentration mean that plants can produce and grow more, or will there be nutrient limitations to this CO_2 fertilized growth? How will soil biogeochemistry change in scenarios where increased CO_2 could alter plant nutrient requirements or the seasonal cycling of these nutrients in the soil?

In this thesis, my objective is to explore the patterns of seasonality in major soil nutrient cycles in a tropical ecosystem, with special attention to the P-cycle. The seasonality in soil nutrients can provide important insights into how the forest will respond to changes in seasonality or precipitation. Drier and wetter seasons in the year can provide us with some insight in how the pools of C, N and P vary according to changes in their environment. This provides a basis for models predicting how future nutrient cycles will be altered and how the ecosystem might respond to shifts in seasonality or changes to rainfall patterns.

Ultimately, those nutrient cycles are central to the functioning of tropical forest, the ecosystem that has a crucial role in regulating global climate.

In **Chapter 1,** the topic of nutrient cycling and seasonality is introduced by detailing the geological context that has led to widespread P-limitation in tropical forests. Many of the old and highly weathered soils of the tropics have low phosphorus (P) availability, which limits the growth and productivity of tropical plants. The combined cycles of C, N and P in tropical forests are influenced by seasonal variations in rainfall and temperature, as well as by global change factors such as elevated CO_2 and land use change. The role of microbes in determining nutrient availability in soils is highlighted, as they mediate the decomposition and mineralization of organic matter and the release and immobilization of nutrients. A general description of the study site and methodologies is provided, and central questions for the subsequent chapters are introduced. The main aim of the thesis is to investigate how soil C, N and P dynamics are affected by seasonality in a central-eastern Amazon forest.

In **Chapter 2,** we investigated how soil C, N and P pools fluctuate in a year. We show how in the dry season, the relative importance of available (easily extractable) C and available P decrease, while available N increases. We explore how microbial biomass pools and stoichiometry vary across seasons and soil depths. Microbial biomass P was found to be low in both wet and dry season, yet had a relative increase during the transition period. These variations suggest potential shifts in microbial community composition due to changes in soil nutrient availability which can be influenced by climate or land use changes.

In **Chapter 3,** we demonstrate that soil EE activity is synchronized with precipitation-driven substrate inputs and seems dependent on the availability of N. We further indicate high investments in P acquisition, with an increase in microbial N demand 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 resources suggest dynamic changes in microbial activity, aligning with climate seasonality and resource limitations in the central-eastern Amazon forests.

In **Chapter 4,** I conclude that input of P by litter decomposition and potential soil extracellular phosphatase activity are the two main factors related to seasonal soil P fluctuations, and therefore the P economy in P impoverished soils. Organic soil P exhibits a clear seasonal pattern, indicating a tight nutrient cycle. This underscores the importance of studying soil P as an integrated dynamic system within the context of a tropical forest.

In **Chapter 5,** the importance of considering the interplay between different nutrient cycles is emphasized, as is the effect of climate change on soil biogeochemical processes. With a rise in atmospheric CO_2, soil biogeochemistry, particularly soil P, could pose significant limitations to increases in forest productivity, or alternatively, the already tight soil P cycle may intensify under increasing CO_2 and subsequent alterations to nutrient demand. This could shift the nutritional balance and have profound implications for forest functioning and composition above and below ground. Tropical forests, crucial in climate regulation and carbon and water cycles, face rapid changes. These changes underscore the importance of understanding the interactions between climate and nutrient cycling. By enhancing our understanding of these processes in a tropical soil, we can better anticipate how tropical ecosystems will respond to changes in climate and seasonality.

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- Environmental research in context (2017)
- Research in context activity: 'Support for the presentation of the AmazonFACE-project to stakeholders and funders, Washington DC' (2017)

Other PhD and Advanced MSc Courses

- AmazonFACE – Amazonia and climate change field course. Instituto Nacional de Pesquisas da Amazônia (INPA) (2015)
- School of Advanced Science on nitrogen cycling, environmental sustainability and climate change, University of São Paulo (2016)

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- Forest management, INPA, Brazil (2018)
- Global Change and the Amazon, INPA, Brazil (2018)
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