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Abstract

1. Tropical rainforests are important components of global carbon and nutrient cycles. Many tropical rainforests grow on old and highly weathered soils depleted in phosphorus. While plants in such forests are usually considered phosphorus limited, it remains unclear if heterotrophic microbial communities are also limited by phosphorus or rather by carbon or energy. In my master thesis I therefore asked the question, if growth of soil microorganisms from tropical rainforests is limited by carbon or phosphorus.

2. I assessed the effects of carbon and phosphorus additions on microbial growth, respiration and microbial biomass increase in a laboratory incubation experiment, and compared them to a 4- year *in situ* nutrient addition experiment to account for direct and indirect effects of nutrient additions.

3. Microbial growth rates increased significantly only with additions of both carbon and phosphorus. This response to nutrient additions differed significantly to the response of microbial respiration and biomass. Moreover, microorganisms strongly accumulated phosphorus when amended, independently of a growth response. *In-situ* phosphorus additions alone led to an increase in microbial growth indicating an indirect effect of increased plant carbon inputs.

4. The significant increase in microbial growth with carbon and phosphorus additions hints towards a co- limitation of tropical soil microorganisms. This could be due to a profound adaption to a low energy environment as both organic carbon and phosphorus can be used as energy sources.

Zusammenfassung

1. Tropische Regenwälder sind von großer Bedeutung für die globalen Kohlenstoff- und Nährstoffzyklen. Viele tropische Regenwälder wachsen auf alten, stark verwitterten Böden, welche für Pflanzen kaum verfügbaren Phosphor besitzen. Während Pflanzen in solchen Wäldern oft als phosphorlimitiert angesehen werden, ist nicht bekannt ob Phosphor oder Kohlenstoff für Bodenmikroorganismen limitierend ist. In meiner Masterarbeit stelle ich daher die Frage, ob Mikroorganismen in stark verwitterten tropischen Böden eher durch Kohlenstoff oder Phosphor limitiert sind.

2. Ich evaluierte zu diesem Zwecke die Auswirkungen der Zugabe von organischem Kohlenstoff bzw. Phosphor auf das Wachstum von Bodenmikroorganismen eines tropischen Regenwaldes mit Hilfe einer Laborinkubation. Des weiteren verglich ich diese Resultate mit einem 4-jährigen Düngungsexperiment, um zwischen direkten und indirekten Effekten der Nährstoffzugaben zu unterscheiden.

3. Mikrobielle Wachstumsraten wurden nur durch die gemeinsame Zugabe von Kohlenstoff und Phosphor signifikant erhöht. Dieser Effekt der Nährstoffzugaben unterschied sich deutlich zu jenen auf mikrobielle Respiration und Biomasse. Des weiteren akkumulierten tropische Mikroorganismen hohe Mengen Phosphor, unabhängig davon, ob es zu höheren Wachstum führte. *In-situ* Phosphorzugaben führten zu einem erhöhten mikrobiellen Wachstum, welche in der Laborinkubation nicht gefunden wurden.

4. Der signifikante Anstieg in mikrobiellen Wachstum mit der gleichzeitigen Zugabe von Kohlenstoff und Phosphor deutet eine wahrscheinliche Ko-Limitierung der tropischen Bodenmikroorganismen hin. Dies könnte auf eine spezifische Adaptierung an die niedrige Verfügbarkeit von Energie hindeuten, da sowohl Kohlenstoff wie auch Phosphor als Energielieferant dienen können.

Part 1

General introduction

Tropical rainforests

Tropical rainforests are one of the major biome types in the world and are mainly found near the earth's geographic equator. They are characterized by a warm and humid climate and are commonly known to encompass a diverse fauna and flora. Recent estimates suggest that the pantropical forests inhabit between 40,000 to 53,000 tree species with the Neotropics and Indo-Pacific region alone contributing between 38,000 to 50,000 species[1]. These numbers are especially astounding when compared to the only 124 native tree species known in temperate Europe.

Tropical rainforests comprise the most productive regions on Earth[2] and constitute an important terrestrial carbon (C) pool[3]. Only covering about 8% of the earth's terrestrial surface, tropical rainforest store about 471 Pg C in soil organic C (SOC) up to 1 m and phytomass[3]. Hence, tropical rainforests are an essential part in the global biogeochemical cycles, particularly to the C- cycle, which has become a much discussed topic even outside of the scientific community. Moreover, while SOC is commonly dominating the C pool in most ecosystems, plant phytomass represents the largest pool of C in tropical rainforests[3]. Consequently, more than half of the global plant C is located in tropical rainforests[3].

Tropical rainforests are immensely important for the current climate regimes on the world. Multiple studies have shown that the hydrologic cycles residing in the tropics are largely dependent on evapotranspiration, a combination of evaporation from terrestrial land and water surfaces and transpiration of plant biomass[4]. These influences are not only measurable on regional scales, but can have far reaching teleconnections, as for example estimates suggest about 70% of the rainfall in the 3.2 million km^2 wide Rio de la Plata region is originating from evapotranspiration in Amazonia[5].

Tropical rainforest climate

Tropical rainforests are mostly found in South America, most notably Amazonia, Africa with the Congo Basin, and on the Malay Archipelago. The climate is characterized by high average temperatures and precipitation of 25.4 °C and 2178 mm, respectively[6], with considerable regional and continental differences[6].

Although not comparable to seasonality in higher latitudes, the tropical rainforest climate is experiencing slight seasonal variability in both temperature and precipitation[6] due to the movement of the Intertropical Convergence Zone (ITCZ). The ITCZ is a low-pressure zone formed through the high solar insulation at the equator heating up air and increasing evapotranspiration. The resulting moist and warm air is lighter than surrounding air masses due to thermal expansion and is therefore transported upwards. As it rises, air pressure declines leading to adiabatic cooling, which in turn lowers the air's water vapor capacity and results in strong cloud formation and rainfalls. The strong vertical movement of air leads furthermore to the formation of a low pressure zone in the lower atmosphere of the ITCZ creating also a horizontal movement of air from the North and South towards its center as a result of air pressure differences. The horizontal movement, also known as the Trade Winds, and the vertical movement of air masses are part of the Hadley Cells. The movement of the ITCZ mentioned above is brought forth by the Earth's axial tilt resulting in the ITCZ being approximately 23° North (Tropic of Cancer) in June and 23° South (Tropic of Capricorn) in December. As the ITCZ is characterized by high precipitation its North-South movement creates dry and wet seasons throughout the year, which also has a slight effect on the temperature regime.

The Amazon rainforest

One of the best studied tropical rainforests is the Amazon rainforest, or Amazonia, located in South America, which represents the largest connected rainforest on Earth comprising more than half of the current tropical rainforest area[7]. Most of its area is located in Brazil with smaller fractions present in Peru, Colombia, Ecuador, Bolivia, Venezuela, Guyana, Suriname and French Guiana.

Geological history

The geological history of Amazonia starts about 135 million years ago (Mya) with the separation of the South American Plate from the African Plate[8]. Through the growth of the Atlantic in the following million years, the South American continent further separated from the African continent, while not being connected to the North American continent until 3,5 Mya before today[9]. This long time of isolation to other continents gave arise to a very unique and diverse fauna and flora and Amazonia is therefore also often referred to be part of the Neotropics, in contrast to the Palaeotropics of Africa and the Malay-Archipelago.

Following the continental break the landscape was mainly under the influence of two Precambrian geological formations, the Guiana and the Brazilian Shield. At the time erosion of the Shields was the only introduction of sediments into the lowlands of Amazonia until around 65 Mya the Andes were being uplifted at the Western margin of the continent[9]. The emergence of the Andes would become the main driver of landscape evolution in Amazonia, as continuous crustal thickening of the Andes initially led to a flexure in the continental crust, inducing a depression area parallel to the mountain range about 30 Mya[10]. This depression area is postulated to have been around sea level and was either connected to the ocean or represented a large interior lake covering wide parts of today's Western Amazonia[10]. At the time Western Amazonia was divided from Eastern Amazonia by a forebulge and drainage systems most likely flew from the East to the West[10]. However, the continuous growth of the Andean mountain range increased cloud formation and precipitation on its eastern flanks as the Trade Winds, travelling from East to West, forced moist air to pass over it. This resulted in high rates of erosion and subsequent sediment transport into the depression area[9,10] and ultimately covered the forebulge in the continent's interior. The idea of how the subsequent landscape of Amazonia developed is strongly dependent on model parameters and therefore varies significantly[10]. Most likely drainage systems were still divided into (1) a small drainage system in Eastern Amazonia flowing into the Equatorial Atlantic with headwaters in the Guiana (North-East) and Brazilian Shields (South-East), and (2) a drainage system in Western Amazonia with its sources located in the Andes and the Shields discharging into the Caribbean Sea[10]. This would change with continuous sediment transport into Central Amazonia leading to the establishment of the Amazon river system around ~10 Mya[9,10]. The Amazon fluvially connected

Western and Eastern Amazonia for the first time resulting in the current landscape of Amazonia with the Andes being its Western boundary and the geologically old Guiana and Brazilian Shields in the North to North- East and South to South- East respectively (Fig. 1).

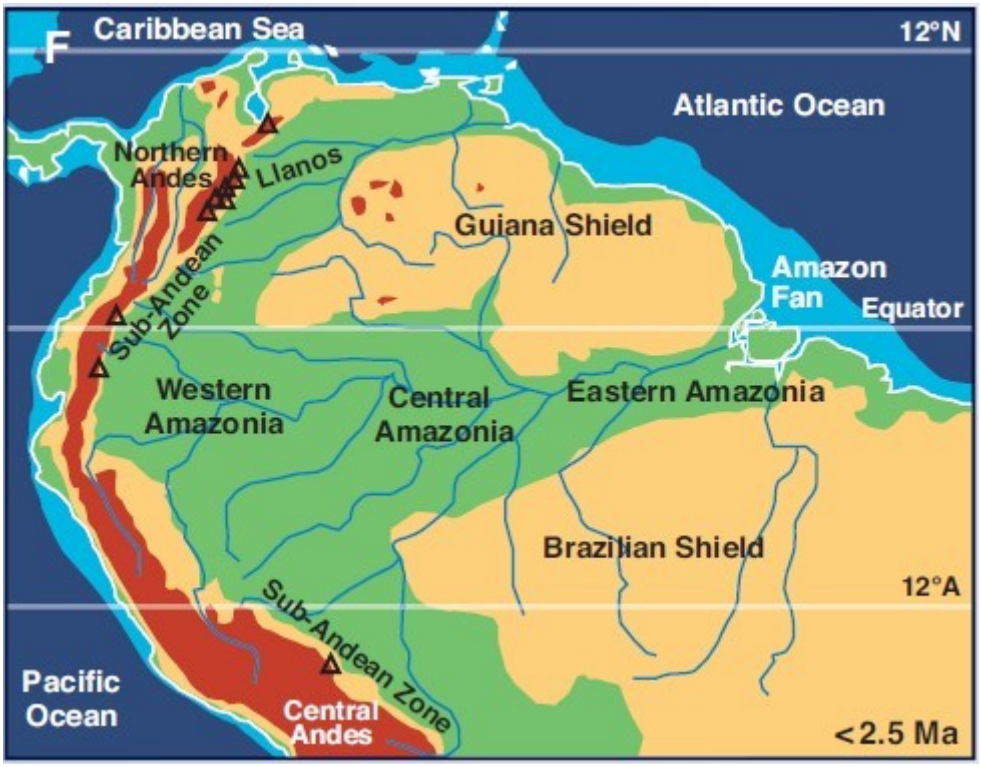

Fig. 1 Map of Amazonia in its current landscape and major geological formations: Andes, Guiana Shield and Brazilian Shield (from Hoorn et al., 2010[9])

White-, clear- and black-water river systems

Fueled by the high precipitation of the equatorial climate the Amazon and other rivers such as the Orinoco and the Rio Negro are constantly transforming the Amazonian landscape through their erosional power. The rivers of Amazonia are easily distinguishable by their water color and can therefore be classified into three river types: white-water-, black-water- and clear-water rivers. This first classification based on water color was later linked to physicochemical differences[11] and revealed white-water rivers to be comparatively rich in rock-derived nutrients such as calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P)[12], while black-water rivers showed low concentrations of rock-derived nutrients, but were darkly colored due to dissolved organic substances stemming from soils[13]. Clear-water rivers on the other hand transported low amounts of solutes in general and therefore appeared rather

colorless[13]. The differences in nutrient content among river types could further be linked to differences in the chemical properties of the soils they drained[13,14,15,16]. Amazonian rivers can therefore be used as proxies for rock-derived nutrient concentrations in soils. Interestingly, the distribution of river classes among Amazonia exhibits a pattern with white-water rivers predominantly having their headwaters in the Andes, while clear- and black-water rivers mainly originate on the Guiana and Brazilian Shield. Hence, soils on the Shields appear to be depleted, while soils in proximity to the Andean mountain range appear to be rich in rock- derived nutrients. The underlying reason for this uneven distribution of available nutrients is caused by the orogenic activity of the Andes counterbalancing an important soil formation process: soil weathering.

Soil weathering in tropical rainforests

Weathering generally sums up processes that lead to the breakdown, transformation and dissolution of rocks. Generally, when rocks (e.g., igneous rocks such as granite) weather, primary minerals (e.g., quartz, alkali feldspar, mica) will be set free and further transformed to secondary minerals (e.g., clay minerals like kaolinite or smectite). Weathering has a physical component that leads to the breakdown of solid rock due to changes in temperature (heat, freeze-thaw). However, chemical weathering plays a more important role in tropical soils. Chemical weathering can be induced through reactions between soil minerals and acids that form from reactive gases in the atmosphere, or by the exudation of organic acids by plants and microorganisms, the latter being classified as biological weathering. Chemical and biological weathering are important processes enabling plant and microbial uptake of essential elements like K, Mg, Ca or P from soil mineral. The introduction of organic acids by plants and microorganisms subsequently lowers the soil's pH adding to a characteristically low pH in strongly weathered soils.

While weathering is a process common to all soils it is particularly pronounced in tropical rainforests. The hot and humid climate exacerbates chemical and biological weathering by accelerating reaction rates[17] and increasing introduction of weathering reagents such as solubilized CO_2 into soil through high precipitation rates.

Another important reason for the highly weathered soils in Amazonia is the temporally stable climate during its formation. While glaciation events were common in higher

latitudes rendering the landscape free of vegetation, Amazonia was most likely always covered by vegetation fairly similar to present day[18]. Hence, biological weathering, in contrast to higher latitudes, continuously transformed tropical soils over long periods of time. The long and intensive weathering led to soils dominated by characteristic 1:1 clays such as kaolinite, together with iron (Fe), aluminum (Al) oxides and hydroxides[19,20]. 1:1 clays in comparison to the less weathered 1:2 clays exhibit a strongly reduced capacity for the binding of cations important for both plants and soil microbes including Mg, Ca, K and sodium (Na).

Moreover, availability of rock-derived nutrients are further reduced as climatic conditions allow for deep weathering disconnecting plants and soil microorganisms further from parent material.

On the role of phosphorus in tropical rainforests

While tropical soils are generally seen as deprived of nutrients due to their low capacity for nutrient retention, some nutrients are in far more limited supply to plants and soil microorganisms than others. Particularly the availability of nitrogen (N) and P, two elements most often viewed as limiting to plant growth, are differing considerably. The causes of these imbalances can be found in a fundamentally different availability of both nutrients during soil development (= pedogenesis), which was first discussed in detail in the study of Walker and Syers[21].

According to Walker and Syers, the availability of P is high in early stages of pedogenesis as P is a common constituent of parent materials on which soils develop. However, as pedogenesis continues weathering leads to a depletion in rock-derived P, which is either lost by leaching or taken up by plants and microorganisms. Hence, total available P in soils decreases, and mineral P, initially bound in parent material, is either lost or more and more immobilized in biomass as organic P. Moreover, weathering leads to the relative enrichment of Fe- and Al-oxides and hydroxides[19,20], which effectively bind P, making it unavailable for biological uptake[22]. Therefore, as P is supplied almost exclusively by the parent material, available P decreases with pedogenesis until a hypothetical steady state is reached[21] (Fig. 2).

N availability on the other hand contrasts the availability of P during pedogenesis. In addition to rock weathering, N is also introduced into soils by biological N_2 fixation and accumulates with progressive soil development[21,23,24].

Hence, young soils are often N limited as initial N availability is low, but P becomes limiting with increased amounts of weathering as losses of P during pedogenesis are inevitable[21].

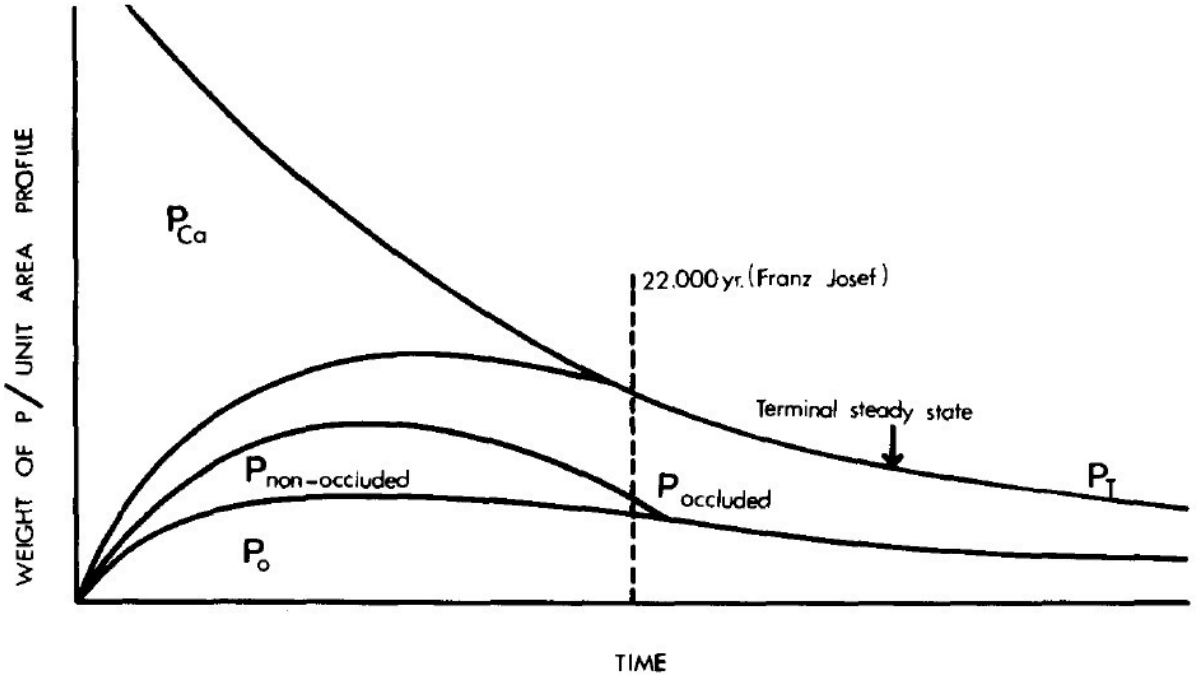

Fig. 2 Conceptualized distribution of soil phosphorus forms and amount over time (P_{Ca}= P bound in rocks and primary minerals; $P_{occluded}$= P strongly bound in soil matrix; $P_{non\text{-}occluded}$= P adsorbed to soil minerals; P_o= organic P; from Walker and Syers[21]).

Based on the conceptualized model by Walker and Syers[21], Amazonian soils are generally considered to be in a late or even final state of soil development[25], caused by long periods of intensive weathering, leaching and erosion, leading to low amounts of P remaining in soils[26]. Depletion of available P is so severe that most of the P inputs sustaining net primary production at the present are supplied by atmospheric deposition (mainly dust from the Sahara desert[27,28]) rather than weathering. Similarly other nutrients such as Ca, K, Na and Mg, that are derived mainly by parent material, are scarce in soils exhibiting a low nutrient holding capacity[26].

Examples for these highly weathered soils can be found on the Guiana and the Brazilian shield, geological formations uplifted at least 1,700 million years ago and subjected to multiple periods of weathering. Hence, rivers on the Shields are either black- or clear-water rivers, which are low in P and other rock-derived nutrients.

Contrary to soils on the highly weathered Shields, soils in Western Amazonia are comparatively rich in rock-derived nutrients[25,26], although still low when compared to higher latitudes. This is, as mentioned above, due to the orogenic activity of the Andes, which introduces new material into the surrounding areas via erosion[29], most importantly through the already mentioned white-water rivers. The input of new material counterbalances nutrient losses through weathering and subsequent leaching and erosion and sustains nutrient pools in soils of Western Amazonia. With declining influence of the Andes, the nutrient content of soil in Amazonia declines as seen on the Shields and parts of Central Amazonia.

Amazonian soil types (WRB)

Amazonian soils arise from a multitude of different factors like identity of the parent material, topography, microclimate and landforms[14,30] each affecting the development and nutrient content of soils. Differences in initial nutrient content of parent material or vulnerability to weathering for example can alter soil characteristics and have far reaching implications for soil organisms. Generalizing soils into soil classes grouped by soil development characteristics can be used to infer general nutrient availability, particularly of P and other rock-derived elements in soils.

Quesada et al identified soil types in Amazonia[25] using the World Reference Base for Soil Resources (WRB) an international soil identification system developed by the International Union of Soil Sciences (IUSS).

With a relative coverage of 31.6% Ferralsols are the most dominant soil type in Amazonia[25]. Ferralsols are characterized by very strong weathering, low pH and cation exchange capacity (CEC, metric describing plant and microbial available cations) and are usually dominated by kaolinite and Fe-oxides[19], which induce the often typical red color. Their occurrence is limited to old and stable regions[31] and they are therefore mostly found on the Guiana and Brazilian Shield. While low in nutrient and water holding capacity these soils are considerably deeper than other soils common to Amazonia and can therefore support a large plant biomass even through prolonged dry seasons[32]. Ferralsols are particulary deprived of plant and microbial available P as it is adsorbed effectively to the soil mineral fraction[22].

Second most common in Amazonia with about 28.9% are Acrisols[25], soils that similar to Ferralsols are strongly weathered, acidic soils with low CEC. Acrisols can also form

on younger parent material and are therefore also found in Western Amazonia. They are generally described to be more fertile than Ferralsols[25], however they are still strongly nutrient impoverished when compared to younger Amazonian soils[25,26]. Together Acrisols and Ferralsols cover most of Central and Eastern Amazonia (Fig. 3). Plinthosols cover about 8.9% of Amazonia and are found only in the middle of the South American rainforest[25] consisting mostly of its eponym Plinthite, an iron rich mixture of kaolinite and quartz[19,33]. The development of Plinthosols is induced due to frequent changes in groundwater levels and is therefore limited to the lowlands of the South American rainforest[30], mostly in Western and Central Amazonia[25].

Equally frequent as Plinthosols are Gleysols[25] which are similarly influenced by ground water levels, but are completely saturated for longer periods of time leading to reduction processes typical for these soils[33,34]. The resulting color patterns of the soil material is therefore also called gleyic. The necessity of water saturation in formation of these soils links their occurrence to lower topographic positions, but also to regions of high precipitation and in the proximity of rivers. They are mostly found next to the Andes and near rivers particularly the Amazon and are characterized by high CEC and silt and/or clay content[25].

Leptosols and Cambisols, both similarly frequent and covering about 5.5% of the Amazonian rainforest[25], are soils of low to moderate weathering[34].

Leptosols develop over weathering resistant rocks and are characterized by very shallow, coarse soils as erosion counterbalances soil formation, or soil has been removed[20]. Leptosols are low in CEC, but it should be noted that the low amount of exchangeable cations is due to low amounts of soil development, rather than low nutrient content of the parent material[25].

Cambisols are almost exclusively found in Western Amazonia and are characterized by high amounts of weatherable material and weak soil development[20,31] resulting in high CEC and increased P availability compared to Ferralsols and Acrisols[25,26].

The above mentioned soils cover almost 90% of the area in Amazonia with the remaining area made up by Arenosols (2.7%), Fluvisols (2.5%), Regosols (1.9%), Lixisols (1.9%), Podzols (1.9%), Alisols (0.3%), Histosols (0.2%) and Nitisols (less than 0.1%)[25]. Their distribution coincides largely with the geological age of parent material in Amazonia as soils on the Guiana and Brazilian Shield are predominantly highly developed Ferralsols and Acrisols, while Western Amazonia, particularly in close

proximity to the Andes, consists of more nutrient rich soils such as Cambisols, Gleysols and to some extent Plinthosols and Leptosols (see Fig. 3)[25,26]. Therefore, the availability of P and other rock-derived nutrients generally decreases from West to East.

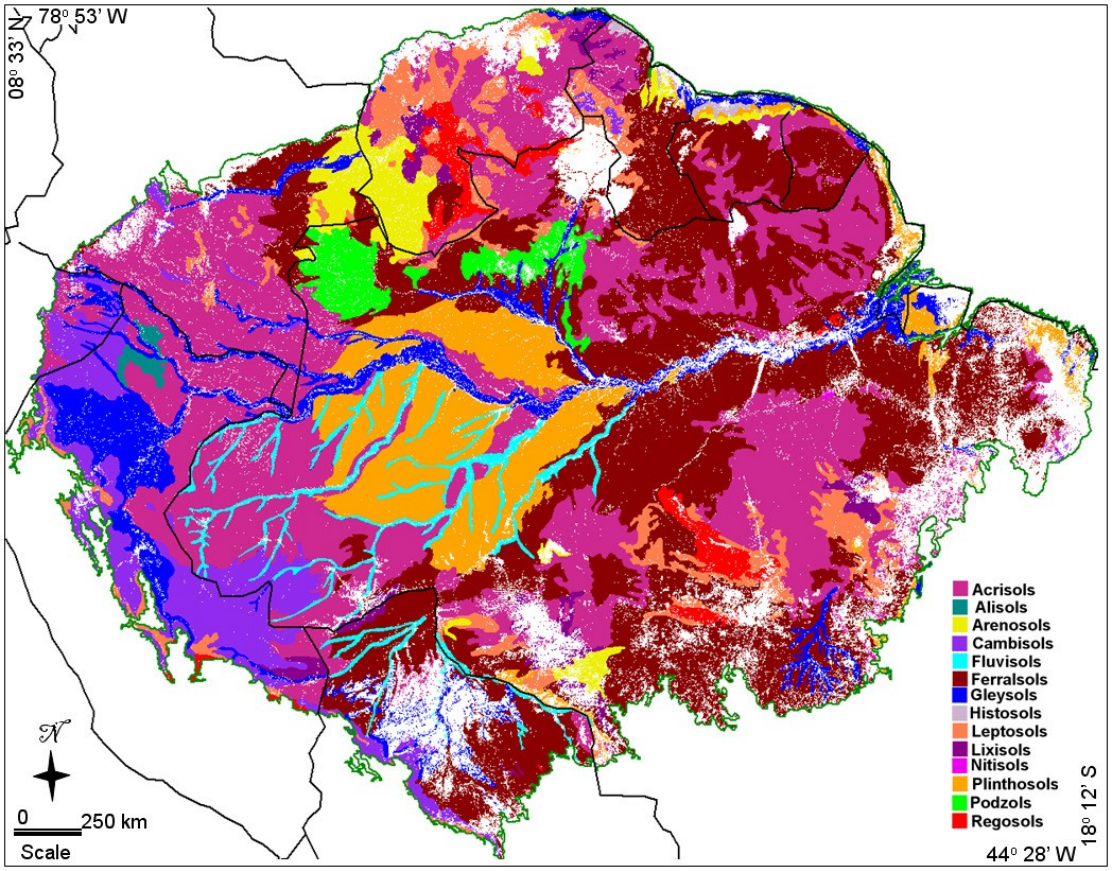

Fig. 3 Distribution of soil types among the Amazon Basin based on SOTERLAC-ISRIC soil database (version 2.0, 1:5 million scale) and the vegetation database of Saatchi et al. (2008) for South America (from Quesada et al., 2011[26])

Nutrient limitation concepts

The importance of nutrient availability for plants has been recognized by scientists for a long time and was first described by Justus von Liebig, with the definition of the law of the minimum in the 19th century[35]. Liebig hypothesized plants to be limited in growth by the scarcest element, while other elements would be available in excess. Consequently, addition of a limiting element would lead to increased plant growth until another element becomes limiting.

An alternative approach to the single nutrient limitation concept by Liebig are models that are based on the `Multiple Limitation Hypothesis´ developed by Gleeson and

Tilman[36], which proposes that organisms adapt their physiology to avoid excess energy and resource expenditure in acquiring non-limiting nutrients. Hence, the ideal organism would optimize its morphology and physiology to a state were all nutrients (C, N, P or trace elements) or other factors such as water or light are co-limiting to its growth.

In 1934 Redfield et al.[37] investigated the elemental ratios of C,N and P in sea water and associated single celled organisms and found a surprisingly constant ratio of C,N and P among marine epipelagic microplankton, today known as the “Redfield ratio” of 106 (C):16 (N):1 (P). This finding was particularly remarkable as the Redfield ratio was maintained irrespective of the C:N:P ratio in the surrounding sea water[38,39]. Hence, while certain elements were available for uptake and could theoretically be used for growth, microplankton rather remained in a state of elemental homeostasis. As marine phytoplankton can fix HCO_3^-, Redfield et al.[38] concluded that it was limited either by N or P, and that N was limiting, if the N:P-ratio of the surrounding sea water was below 16, while P was limiting at a ratio of above 16. These hypotheses were drawn from the fact that marine phytoplankton, in order to maintain its stoichiometric homeostasis, needed to take up 16 unit of N for each unit of P in accordance with the Redfield ratio. These findings showed for the first time the existence of homeostasis among organisms and allowed for the study of nutrient limitations by investigating the elemental stoichiometry of organisms and their resources.

While the discovery of the Redfield ratio was a significant advancement in understanding nutrient limitations and thus biogeochemical cycling in the ocean, it was initially not applied in terrestrial environments, because sea water is a fairly homogenous marine environment compared to soil. Moreover, soil microorganisms are almost exclusively heterotrophic in contrast to the autotrophic marine phytoplankton. However, studies revealed that even in the very heterogeneous soil environment microbial stoichiometry was constrained similar to marine microplankton[40]. Hence, elemental ratios can also be used to study nutrient limitations in plants and soil microorganisms and enable the application of ecological stoichiometry[41] in terrestrial environments.

Ecological stoichiometry (ES) and its application in soil ecosystems

ES uses elemental ratios (traditionally the elemental balance of C, N and P) of organisms and substrates to better understand controls of ecological processes[41].

One of the principles underlying ES is the inevitable difference in stoichiometric ratios among organisms and their environment, as a consequence of biological organization, in addition to an imbalance of the stoichiometric composition of consumers and their resources. In addition, microorganisms and most animals exhibit stoichiometric homeostasis, at least with regard to their C:nutrient ratios. Plants, on the other hands, are non-homeostatic organisms.

Consequently, ES can be used to explore relations between interacting organismal groups (e.g., predation, herbivory) or between organisms and their resources (e.g. photosynthesis, litter decomposition) and strives to elucidate the underlying ecosystem processes and their implications in biogeochemical cycling in ecosystems[41].

The application of ES has especially been useful in understanding soil biogeochemical cycling, a process that encompasses the introduction of material by plants in the form of above- and belowground litter to soil and the subsequent decomposition of soil microorganisms. Moreover, microbial decomposition leads to the formation of soil organic matter (SOM) as partially decomposed plant inputs or dead microbial biomass are protected from further decomposition by either chemical (e.g. organo-mineral interactions) or physical (e.g. occlusion in aggregates) interactions with soil particles[42–45].

Both litter and SOM are major sources of C for soil fungi and bacteria, but differ widely in their stoichiometry[46,47]. The underlying reason for this differences is their source of origin as litter more closely resembles the stoichiometry of plants with a generally high C:N:P ratio (global average 1183:19:1[47]), while SOM (C:N:P ratio global average 287:17:1[46]) is already transformed by soil microorganisms and thus converges towards the stoichiometric ratios of soil microbial biomass (global average 42:6:1[46]).

While individual microorganisms show little variation in their stoichiometric ratio[48], soil microbial communities cannot be seen as completely homeostatic, as stoichiometric variations do exist between microbial communities of, for example, forest to grassland soils. Still, there is ample evidence that microbial C:N:P stoichiometry is constrained even on a global scale[40].

Bacteria and fungi feeding on fresh litter will therefore face an excess of C and are

therefore rather limited by N or P. Soil microorganisms feeding on SOM however, are considered to be limited by C[49] even though their C:N and C:P ratio is lower than the respective ratios in SOM. This is caused due to losses of C through respiration as microorganisms have to satisfy energy demands for maintaining and building biomass[50]. Moreover, SOM is often physically and chemically protected from decomposition in the soil mineral matrix[51] further decreasing the availability of C (and other nutrients) in soil.

A consequence of stoichiometric imbalances between soil microorganisms and their resources is the excretion of elements that are in excess, a process termed mineralization. In litter, where C is present in excess to microbial demand, microorganisms will respire excess C in the litter as it cannot be used for growth due to limitations in other elements. Similarly, an excess of N and P, as it is the case in SOM, is mineralized by microorganisms to NH_{4+} and PO_{4-}, which can in turn be taken up by plants. This is well documented for N, but has not yet been clearly proven for P. This regulation of microbial elemental homeostasis is called microbial carbon or nutrient use efficiency. The microbial element use efficiency is defined as the fraction of an element used for growth (G) divided by the total uptake of the organic form of this element (U).

$$Element\ Use\ Efficiency = \frac{G}{U}$$

Operationally, for estimating microbial carbon use efficiency, the organic carbon uptake is calculated as the amount of carbon invested into growth plus the amount of carbon respired (R), while for microbial nitrogen use efficiency, growth is estimated as the amount of organic N taken up, minus the part that is mineralized back to the environment (M)[50,52,53].

$$Carbon\ Use\ Effiency = \frac{G}{U} = \frac{G}{G+R}$$

$$Nitrogen\ Use\ Efficiency\ = \frac{G}{U} = \frac{U-M}{U}$$

Consequently, for a given uptake rate, the use efficiency of an element is negatively related to its mineralization.

If we apply the formula stated above to the example of microorganisms feeding on litter

or SOM the microbial carbon use efficiency (CUE) is predicted to differ substantially between both groups of soil microorganisms, given that they behave homeostatically. As C:N and C:P ratios in SOM closely resemble microbial stoichiometry microbes feeding on SOM are thought to be C limited and thus would exhibit a maximum CUE. Microbes on plant litter (C:N and C:P ratios much higher than that of the microbes themselves) on the other hand would maximize the use of nitrogen and phosphorus, exhibiting maximum NUE but lower CUE[54].

This also links carbon and nutrient use to ecosystem carbon and nutrient fluxes, by what is known as the consumer driven nutrient recycling hypothesis (CNR)[55]. CNR links the stoichiometry of a consumer (e.g. soil microorganisms) to the stoichiometry of the consumed resource (e.g. litter, SOM) and describes the effects of this relationship on nutrient cycling. As mentioned above differences in stoichiometry between resource and consumer can lead to higher mineralization or release of non-limiting elements (lower use efficiency) and to lower mineralization or release of limiting nutrients (higher use efficiency). In other words, organisms are predicted to use limiting nutrients conservatively or recycle them, while non-limiting nutrients are predicted to be more likely mineralized or released back to the environment. The stoichiometry of mineralized particles can therefore change in dependence of the resource and the consumer and has further significant implications for nutrient cycling.

CNR is an important framework to improve the understanding of soil biogeochemical cycling by considering stoichiometric imbalances between soil microorganisms and their resource. Understanding the implications of these imbalances is important in defining limitations for common processes such as photosynthesis, decomposition and mineralization and predicting changes to these limitations brought forth by global change such as enhanced N deposition or elevated CO_2 levels.

Nutrient limitation in the tropics

As mentioned above, the Amazonian rainforest is to a large extent an ecosystem of very low P availability as soil development processes were continuous over hundreds of million years[21,25,26]. The low availability of P is particularly problematic for tropical primary production as plants are reliant on nutrient uptake via their root system from the soil. Studies who sought to identify nutrient limitations on plant growth thus found increasing evidence for P limitation in contrast to widespread evidence of N limitation

in temperate and boreal regions[27,56–59].

However, while plants in tropical ecosystems seem to be limited by P, much less is known about the limitations on soil microorganisms. Microorganisms are known to be the main drivers in biogeochemical cycles by decomposing and subsequently mineralizing inputs of plant material[60], thus making nutrients available again for plant uptake. The limitations on microbial growth are therefore of major importance in understanding biogeochemical cycling in tropical ecosystems with implications for primary production, but also higher trophic levels.

In contrast to plants, which are autotrophic and have access to an unlimited supply of C in the earth's atmosphere, soil microorganisms are mainly heterotrophic and are therefore reliant on organic C inputs. Studies have shown indications of a predominant C or energy limitation in soil microorganisms[49], which is in accordance with stoichiometric comparisons of bulk soil and microbial biomass[46]. However, the low availability of P in tropical soils led to a widespread belief of possible P limitation among microbial soil communities[61].

Thus, the main research question of my thesis is:

Is microbial growth of heterotrophic microorganisms in highly weathered tropical soils limited by carbon or phosphorus?

Nutrient addition experiments are best suited to identify nutrient limitations of microbial communities. Towards this end, nutrient additions can be either applied *in-situ* (in the field) or in laboratory incubations under controlled conditions. *In-situ* nutrient additions enable the study of microbial limitations under more natural conditions without disturbance of the soil matrix and additionally allows for interactions with plants (plant nutrient uptake and C exudation) and soil fauna (control of microbial community composition and nutrient availability). On the flipside, these interactions can theoretically cause indirect effects that stimulate or impede microbial growth. For example, a hypothetical positive stimulus on plant growth due to nutrient additions could indirectly effect soil microorganisms by increasing inputs of plant material into the soil. Disentangling direct and indirect effects is thus a complicated task.

Laboratory incubations on the other hand offer the possibility to control for a variety of physical and chemical parameters and allow for the exclusion of plants in the soil. Hence, nutrient additions into the soil under such controlled conditions can give direct

information on microbial nutrient limitations. However, inferring results from laboratory incubations to natural (field) conditions can be difficult as many biotic interactions present in nature are missing.

As both *in-situ* and laboratory incubations can add valuable information in understanding the stoichiometric constraints microbial communities face in highly weathered tropical soils, I here compare the results of a 4-year *in-situ* nutrient manipulation experiment in a pristine tropical rainforest in Nouragues, French Guiana, with a laboratory incubation experiment. Soil samples for the laboratory incubation experiment were taken at the same site of the *in-situ* experiment, but outside the field fertilization plots. This guaranteed that soils in both experiments were comparable. The soils sampled were representative of the predominant highly weathered soils[25,26] in the Guiana Shield of Amazonia and were taken along a topographic gradient to account for the natural variation in P availability in lowland tropical forests. The experimental design and the integrative approach of comparing the results of an *in-situ* experiment and a laboratory incubation experiment allows to investigate both direct and possible indirect effects of nutrient limitation on the tropical microbial soil community.

The use of nutrient manipulation experiments, both *in-situ* and under laboratory conditions, is not *per se* a novel approach for investigating microbial nutrient limitations in highly weathered tropical soils. However, earlier studies investigating microbial nutrient limitations often focused on measurements of nutrient-induced changes in microbial respiration or biomass to infer limitations. Here, I argue that these are insufficient parameters for investigating microbial nutrient limitation as total respiration is comprised of respiration for enzyme production, maintenance and growth and can also be enhanced due to excess carbon supply, resulting in overflow respiration[50]. Microbial biomass on the other hand is known to be high during periods of little microbial activity, such as the winter season in temperate ecosystems, indicating that the increase might be due to a lack of turnover in the microbial community and therefore is also a poor metric in investigating microbial limitations. In this study I therefore measure gross growth rates to infer microbial nutrient limitations. I make use of the gold standard method for estimating growth, i.e., the incorporation of ^{18}O from labelled water into DNA, as only active microbial cells with sufficient energy and nutrient availability are able to replicate DNA.

My hypotheses are based on the assumption of a C limitation among heterotrophic microbial communities in tropical soil, due to their reliance on organic C inputs for growth.

I hypothesize that:

(I) Incubating soils with an organic carbon source leads to enhanced microbial growth, while phosphorus additions will not.

(II) *In-situ* phosphorus additions lead to an increase in microbial growth as the alleviation of P limitation on primary production increases plant carbon inputs into the soil.

Part 2

Manuscript

“What controls microbial growth in tropical soils? The role of carbon and phosphorus“

Introduction

Tropical rainforests are one of the major biome types on earth and encompass a diverse fauna and flora. They comprise the most productive ecosystems around the globe[2], storing about 471 Pg of carbon (C) in the upper 1 m in soils and in phytomass[3] and are thus important parts of the global carbon cycle. Tropical rainforest soils are among the oldest and deepest soils on Earth characterized by high amounts of weathering and a corresponding low availability of rock-derived nutrients, particularly P[21,25,26]. Moreover, enrichment of Fe- and Al-oxides further reduces the bioavailability of P due to strong organo-mineral interactions[22]. Nitrogen (N) on the other hand is usually enriched in tropical soils relative to P, most likely due to biological N_2- fixation[62]. Tropical rainforest ecosystems are therefore often considered rather limited by P, contrasting to a predominant N limitation of soils of temperate and boreal forests[27,56–59]. While there is evidence for P-limitation on plants in highly weathered tropical forests[59], the effects of low P availability on soil microorganisms is still poorly understood.

Soil microorganisms play an integral role in soil biogeochemical cycling, which is characterized by the input of fresh organic material through primary production by plants and its subsequent decomposition through extracellular enzymatic activity of heterotrophic soil microorganisms[60]. Decomposition not only benefits soil microbes by providing energy and nutrients for further growth, but makes essential nutrients available for plant uptake. Additionally, soil microorganisms play an important part in the formation of the largest global pool of organic C, soil organic matter (SOM)[42,43,51,63]. As SOM is very stable in soil it was previously believed to be composed of chemically recalcitrant organic molecules[64,65]. However, recent studies revealed that SOM is to a large extent originating from soil microbial biomass (e.g., from dead cells or part of cells), or from organic material transformed through microbial processes, which is partly stabilized in soil by chemical (e.g., organo-mineral interactions) or physical (e.g., occlusion in aggregates) mechanisms[42–45]. SOM is itself further decomposed by soil microorganisms and serves as the main C source in bulk soil. Soil biogeochemical cycling as a whole is hence largely dependent on the activity of soil microorganisms decomposing fresh plant material and SOM, providing essential nutrients to primary

producers, and to build-up SOM, a large, comparatively stable source of global C fixed in soil.

The crucial role of tropical rainforests in global ecology and the important role of soil microorganisms in soil biogeochemical cycles warrants for further studies on the extent and nature of microbial nutrient limitations in tropical ecosystems.

An important factor influencing microbial growth in soils are thought to be imbalances in elemental stoichiometric ratios among substrates such as litter (ca. C:N:P = 1183:19:1[47]), SOM (ca. C:N:P = 287:17:1[46]) and the microbial biomass itself (ca. C:N:P = 42:6:1[46])[41,50,52,66]. Soil microorganisms are generally considered to be constrained in their stoichiometry, following a rather strict stoichiometric homeostasis at least on a species level[40]. The stoichiometry of microbial communities can vary between ecosystems through differences in microbial community composition. Nevertheless, a comprehensive study by Liptzin et al.[40] found the stoichiometric ratio of soil microorganisms still to be homeostatic overall.

The application of ecological stoichiometry[41] in soils has a long tradition, and can be used to infer effects of resource stoichiometry on microbial decomposition and growth. Essentially, substrates such as litter and SOM differ in their stoichiometry from soil microorganisms, leading to some elements being available in excess, while others are lower in relative abundance to the requirements for building up new biomass[50]. Such differences in resource and soil microbial stoichiometry can also be interpreted as a limitation by certain elements based on the principles of Liebig's law of the minimum[35]. More precisely, this would mean that microbial growth is limited by the availability of the scarcest element given that microbes have to maintain a certain balance of elements. Alternatively to Liebig's law of the minimum, Gleeson and Tilman proposed the 'Multiple limitation theory'[36] stating that organisms adapt their physiology to avoid energy and resource expenditure in acquiring non-limiting nutrients. Hence, soil microorganisms may need to maximize their growth efficiency by being limited simultaneously by as many nutrients (C, N, P or trace elements) as possible.

A key concept in ES is the threshold element ratio (TER)[52,67,68], which describes the limitation of an organism or system in relation to its substrate. As soil microorganisms are homeostatic their element limitation is dependent on the stoichiometry of their substrate. Historically, considerations of TER focused on the macronutrient ratios of C:N and C:P. TER describes the elemental ratio where limitation of an ecosystem

process (here growth of soil microorganisms) changes from an energy or C limitation to a nutrient (N or P) limitation. For example, a higher C:P ratio in the substrate than in the microbial biomass would lead to a P limitation, while C would be limiting when substrate C:P was lower than in the microbial biomass.

As mentioned above SOM is the predominantly available substrate for soil microorganisms. While SOM is usually characterized by a slightly higher C:N and C:P ratio than the microbial biomass, soil microorganisms feeding on SOM are generally considered rather C limited[49], as a considerable amount of C is required for energy generation through respiration[50,52]. Yet, in contrast to higher latitude ecosystems the low P availability in tropical rainforests could be limiting soil microbial growth rather than C. Nutrient additions in tropical rainforests, both *in-situ* (in the field) and under laboratory conditions, revealed positive effects of P additions on heterotrophic microbial respiration and net microbial biomass changes[61,69–71], which would hint towards a general P-limitation of tropical rainforest ecosystems.

However, it can be argued that heterotrophic respiration and net biomass changes used as microbial parameters to describe effects of microbial nutrient limitation may not accurately describe microbial nutrient limitations[72]. Heterotrophic microbial respiration is in part dependent on resource stoichiometry and could increase because of overflow respiration[50]. Microbial biomass on the other hand is reported to be high in low activity ecosystems such as boreal forests or alpine grasslands[46]. An increase in net microbial biomass could thus occur due to reduced activity and slower turnover of the microbial community, rather than an alleviation of nutrient limitation. Another, potentially more accurate indicator of microbial nutrient limitation are gross microbial growth rates, that can be approached by determination of ^{18}O incorporation into DNA[73]. DNA synthesis requires water and by tracing ^{18}O-labelled water into DNA, newly synthesized biomass can be estimated. As only active microbial cells with sufficient supply of nutrients are growing and replicate DNA, estimation of gross microbial growth rates are a superior parameter in defining microbial nutrient limitations.

The main research question of this study is, if microbial growth of heterotrophic microorganisms in highly weathered tropical soils is limited by the access of microbes to carbon or by the low availability of phosphorus.

We have conducted microbial nutrient manipulation experiments, both *in-situ* and under laboratory conditions, to investigate microbial nutrient limitations. *In-situ* nutrient manipulation experiments offer the advantage of investigating the effects of nutrient additions under natural conditions, including indirect effects of nutrient- induced increases in primary production (increased plant nutrient uptake and carbon input). To disentangle these indirect from direct nutrient effects of nutrient addition on microbial growth, we also run a laboratory incubation experiment in which we were able to control for environmental parameters such as temperature and soil moisture, while impeding indirect effects via plants. Hence, variations in microbial growth in response to nutrient additions can be directly linked to the alleviation of nutrient constraints on soil microorganisms.

As both *in-situ* and laboratory incubation experiments can add valuable information in understanding stoichiometric constraints and nutrient limitations soil microorganisms face in highly weathered soils, we here compare the results of a 4-year *in-situ* nutrient manipulation experiment and a laboratory incubation experiment using soils of a pristine lowland tropical rainforest in Nouragues, French Guiana. The soils in this region are described as nutrient poor Acrisols and soil samples were therefore representative for the highly weathered soils predominant in tropical rainforests[74,75]. Samples were taken along a topographic gradient to account for the natural variation in soil physicochemical properties. The experimental design and the integrative approach of comparing the results of an *in-situ* and a laboratory incubation experiment allows to investigate both direct and possible indirect effects of nutrient additions on the tropical microbial soil community. We assumed that C was limiting growth of heterotrophic soil microorganisms in highly weathered tropical soils due to their reliance on plant C inputs.

More specifically, we hypothesized that:

(I) Incubating soils with a carbon source leads to enhanced microbial growth, while phosphorus additions will not.

(II) *In-situ* phosphorus additions lead to an increase in microbial growth as the alleviation of P limitation on primary production increases plant carbon inputs into the soil.

Materials and methods

Study site

Soils collected and analyzed in this study stem from a primary lowland rainforest in North-East Amazonia close to the research station Nouragues, French Guiana. Nouragues is located in inland French Guiana (5°15′ N, 52°55′ W) and is characterized by a perhumid climate with annual precipitation of 2.990 mm rainfall per year [76]. Annual mean temperature is near 26 °C[76] with low variability throughout the year. The site experiences seasonal rainfall variability due to the movement of the ITCZ and is therefore characterized by the occurrence of a wet season from December to July and a dry season from August to November with less than 100 mm rainfall per month[77].
The sampling area is characterized by an undulating topography with slopes ≤ 15° inclination and approximately 20 – 50 m elevational difference between valley bottoms and hill top plateaus. The geology in Nouragues is derived from the Bonidoro series, a Precambrian metamorphic formation and are comprised predominantly of Caraib gneiss[76]. Characteristic for this substrate is a particular low P content even among the generally nutrient- poor soils of Amazonia[74,75]. The soils that have developed on this bedrock are described as nutrient-poor Acrisols in the WRB taxonomy[78] (Ultisols in the USDA soil taxonomy). Soil texture varies with topography and ranges from sandy loam in lower topographies to silty clay on hill tops according to the USDA texture classification chart[79].

Experimental design: *In-situ* nutrient manipulation

To account as much as possible for the natural landscape and soil texture variation plots were established at three topographic regions: at the (1) bottom, i.e., in valleys, next to a creek intercepting hill-tops (2) at slopes, i.e., the section between the valley floor and the hill top, and at (3) the top of hill-tops or plateaus. For each topography four 50 x 50 m plots were established; one served as a control plot (no nutrient additions) while the three others received nitrogen (+N), phosphorus (+P) or a combination of both (+NP), respectively. Nutrient additions were applied by hand twice per year at a rate of 125 kg N ha^{-1} y^{-1} (+N) or 50 kg P ha^{-1} y^{-1} (+P), or both amounts together (+NP) starting in October 2016[80]. N additions were added as commercial urea

($(NH_2)_2CO$) and P was added as triple superphosphate ($Ca(H_2PO_4)_2$). Sampling was done in the inner 20 x 20 m area to avoid border effects. Distances between plots were 10-100 m. The experimental design is shown in more detail elsewhere[81].

Soil sampling strategy

Soil samples were taken at 0-5 cm soil depth during the wet season in June 2019. In each plot soil was sampled with a gouge auger (5 cm diameter) in each of the four corners and once in the middle leading to 60 soil samples (3 topographic categories, 4 plots per category, 5 samples per plot).

For the laboratory incubations additionally five samples at 0-10 cm soil depth were taken 5 m outside of the 50 x 50 m plots and pooled to one composite sample per plot for a total of twelve composite samples.

After sampling, each soil sample was homogenized and sieved to 2 mm and transported to the laboratory of Terrestrial Ecosystem Research (TER), CMESS, University of Vienna within a week after the soil was sampled in the field.

The samples taken from the *in-situ* nutrient manipulation experiment were analyzed for soil water holding capacity (SWHC), soil water content (SWC), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved inorganic, dissolved organic and total dissolved P (DIP, DOP and TDP), microbial C, N and P (C_{mic}, N_{mic}, P_{mic}) as well as microbial growth and respirations rates, as described below.

Experimental setup: Laboratory incubation

A laboratory incubation experiment was conducted to distinguish between direct and indirect (via increased plant C inputs) of P additions. For the laboratory incubation prior fertilization effects were excluded by using the composite soil samples, which were taken 5 m outside the fertilized plots in Nouragues, French Guiana. The laboratory incubation included four treatments: cellulose (C), phosphorus (P), cellulose and phosphorus (CP) and a control. Cellulose was chosen as a substrate for C as it is less labile than commonly used monosaccharides such as glucose and will be released slowly. Thus, while still serving as a C source cellulose is not metabolized shortly after addition and is hence not expected to trigger a priming effect as known for glucose. Moreover, cellulose more accurately simulates available C in bulk soil as the availability of large amounts of labile C is unlikely under natural conditions.

For each of the twelve composite soil samples four 150 ml volume incubation jars (mesocosms) were filled each with 25 g of fresh soil to a total of 48 mesocosms (12 composite soil samples, 4 treatments per composite sample). Mesocosms were closed with a lid that allowed for air exchange via a hole stuffed with cotton wool to reduce losses of water through air draft. Water content of the soil was adjusted to 60% of soil water holding capacity prior to addition of C to optimize conditions for microbial growth. Cellulose was added in a pre-incubation step three weeks before P. This pre-incubation step was chosen to ensure enough time for the decomposition and uptake of C from cellulose in order to accurately identify the effects of C addition alone or in combination with P.

Cellulose was added in an amount equaling 2.2% of total SOC of the respective sample. To elucidate effects of the carbon treatment alone a first harvest was done prior to P addition (n= 24 (Control); n=24 (C)). Afterwards P was added to P and CP mesocosms in the form of pH adjusted Sørensen buffer. The total amount of P added was 372 µg P applied by addition of 4 ml 3 mM Sørensen buffer. 4 ml of Milipore water was simultaneously added to mesocosms without P addition (Control and C). The addition of P to the mesocosms marked the beginning of the actual incubation. Mesocosms were incubated for seven days and were then harvested again.

The measurements at each of the two harvests included the determination of SWC, DOC, TDN, DIP, DOP, TDP, C_{mic}, N_{mic}, P_{mic} as well as the determination of $Growth_{mic}$ and $Resp_{mic}$. Additionally, heterotrophic soil respiration was measured four times directly from the mesocosms during the seven-day incubation. Measured respiration rates were subsequently used to calculate cumulative respiration.

Soil characterization

Soil water holding capacity

Soil water holding capacity (SWHC) was determined in a small amount (<15 g) of fresh soil, which was transferred into a funnel with an inserted pre- wetted ashless cellulose filter **paper** (110mm, Grade 40, Whatman) on top of a 50 ml tube (Greiner Bio-One). Milipore water was subsequently added to the point of maximum soil water saturation indicated by the dripping of water through the funnel into the 50 ml tube. Each funnel was then covered with parafilm (Parafilm M, Sigma Aldrich) and left for 24 h at room temperature to allow soil excess water to leach out of the soil samples. Approximately 5 g of soil was then weighed into aluminum dishes and subsequently dried at 105 °C for 24 h in a drying oven (UF260 plus, Memmert) to obtain dry mass. SWHC was determined gravimetrically by the mass of water (i.e. the difference in mass between of soil before and after drying) per mass of dry soil.

Soil water content

For the determination of the SWC roughly 5 g of soil was weighed and dried at 70 °C for 24 h in a drying oven (UF260 plus, Memmert) to obtain dry mass. SWC was determined gravimetrically by the mass of water (i.e., the difference in mass between the fresh weight of soil and the dry weight) per mass of dry soil.

Soil pH

Soil pH was determined in water by weighing 2 g of fresh soil into vials and subsequent addition of 10 ml Milipore water. pH was measured using a glass pH probe (E510, Metrohm).

Soil nutrient measurements and microbial biomass carbon, nitrogen and phosphorus

Total soil organic carbon, total soil nitrogen and phosphorus

Total soil carbon and nitrogen were determined by grinding oven dried soil (see section soil water content) with a ball mill (MM2000, Retsch). Grinded soil was weighed and analyzed for carbon and nitrogen content with an elemental analyzer (Carlo Erba 1110, CE Instruments) coupled to an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT). Due to low amounts of carbonate in tropical topsoils the measured soil carbon was approximated to soil organic carbon (SOC) and was hence not treated with an acidic agent prior to the measurement. The carbon isotope composition of the soils also did not indicate carbonates to be present.

Total soil phosphorus (TP) was determined by grinding oven dried soil (MM2000, Retsch). TP of the grinded soil was determined through acid digestion in an ultraWAVE digestor (Milestone) followed by ICP-MS (7500 ce model, Agilent).

Dissolved organic carbon, total dissolved nitrogen and microbial biomass carbon and nitrogen

DOC and TDN was determined by extracting fresh soil with 1 M KCl (weight to volume ratio 1:10); samples were shaken for 30 min on an orbital shaker (IKA, KS 501 digital) and filtered through ashless cellulose filter paper (110mm, Grade 40, Whatman). DOC (non-purgeable organic carbon) and TDN was determined on a TOC/TN analyzer (Shimadzu TOC-V CPH/CPN/TNM-1, Shimadzu). To determine microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) an aliquot of soil samples was fumigated with chloroform before extraction with 1 M KCl[82]. Measured DOC and TDN amounts from unfumigated samples were subtracted from those of fumigated samples and the result was corrected for a 45% efficiency of the fumigation. The resulting values equaled C_{mic} and N_{mic}.

Dissolved phosphorus and microbial biomass phosphorus

Estimations of dissolved P and microbial P were done using the Olsen-P method[83]. To this end fresh soil was extracted with 0.5 M $NaHCO_3$ (weight to volume ratio 1:10). Samples were subsequently treated as described for KCl extractions above. Dissolved inorganic P was determined in untreated $NaHCO_3$ extracts photometrically through the malachite green assay[82]. As only inorganic P (DIP) can be measured by the malachite green assay, a subsample of the $NaHCO_3$ extract was oxidized with sodium persulfate ($Na_2S_2O_{8)}$ using an autoclave to oxidize organic P (DOP) present in the sample to DIP, which was again quantified by the malachite green assay. Measured DIP values of the digested fraction correspond to total dissolved P (TDP), as it comprises both DIP and DOP. DOP was calculated by subtracting previously measured DIP values from the corresponding TDP sample.

For the determination of microbial P forms a second set of samples was fumigated with chloroform[82]. Samples were again extracted with 0.5 M $NaHCO_3$, and P concentrations were determined photometrically[82]. Calculation of microbial P was done by subtracting measured DIP values of the unfumigated samples from the corresponding fumigated samples. Values were corrected for the 45% extraction efficiency of the fumigation.

Due to frequently higher values of inorganic P to total P in both fumigated and unfumigated samples it was decided to use only data on inorganic P for both soil and microbial P analysis. Hence, inorganic microbial P will be hereafter referred to as P_{mic}.

Analysis of microbial physiology

Cumulative respiration (Laboratory incubation)

Four separate respiration rate measurements have been done over the span of the seven-day laboratory incubation in order to calculate cumulative respiration. Determination of respiration rates was done by removing the cotton wool in the lid of the vessel and replacing it with a butyl rubber septum, to prevent air exchange between air in the mesocosm and the surrounding environment. A 10 ml gas sample was then taken at the beginning of the experiment (T0) and measured with an Infrared Gas Analyzer (IRGA, EGM-4, PP Systems). Air was replaced with 10 ml of synthetic air containing 200 ppm CO_2. A second measurement (T1) was taken after 2 hours of incubating at room temperature and respiration rates were calculated as the ΔCO_2 between T0 and T1.

Cumulative respiration was calculated by averaging the determined heterotrophic respiration rates of two subsequent measurements (e.g., the average of the first and the second measurement, second and third measurement etc.), multiplying it by the time passed between them and adding the calculated respiration between each of the measurements together.

Microbial growth and respiration rates

Microbial growth ($Growth_{mic}$) and respiration rates ($Resp_{mic}$) were determined by weighing 400 µg of fresh soil into vials applicable for freezing and placing them open into 27 ml serum bottles, which were closed with airtight caps with a butyl rubber septum, to allow gas sampling. Samples were adjusted to approximately 60% SWHC and 20 at% ^{18}O by the addition of ^{18}O labelled water. A gas sample (5 ml) was taken immediately after closing the incubation vials (T0) and the CO_2 concentration was measured on an IRGA (EGM-4, PP Systems). The sampled gas volume was replaced with synthetic air with 200 ppm CO_2. The samples were then incubated at room temperature for 24 h. At the end of the 24 h another gas sample (T1) was taken and measured again with the IRGA. Respiration rates were calculated as the ΔCO_2 between T0 and T1. T0 values were corrected for the addition of standardized 200 ppm air. After the second gas sample was taken the incubation vial was opened, the cryovial

containing the soil was closed, immediately frozen in liquid N_2 and then stored at -80 °C.

$Growth_{mic}$ was determined by measuring ^{18}O incorporation into DNA as well as total amount of DNA present in the soil sample and was standardized per g dry soil[84]. ^{18}O incorporation into DNA was determined by first extracting and purifying DNA using a DNA extraction kit (Fast DNA™ SPIN Kit for Soil by MP BIO). DNA concentrations were subsequently determined by the usage of the Quant-iT™ PicoGreen™ dsDNA Assay Kit and dsDNA Reagent (Thermo Fisher). ^{18}O enrichment of DNA was determined using a High Temperature Conversion Elemental Analyzer (TC-EA, Thermo Fischer) coupled via a Conflo III open split system (Thermo Fisher) to an Isotope Ratio Mass spectrometer (IRMS, Delta V Advantage, Thermo Fisher).

In order to address microbial limitations we further calculated mass specific microbial respiration rates (Specific $Resp_{mic}$) and mass specific microbial growth rates (Specific $Growth_{mic}$) by standardizing respiration and growth rates to the measured C_{mic}. This allowed to determine microbial growth rates relative to the microbial biomass stock and is hence better suited to study microbial nutrient limitations.

Some of the measured and calculated respiration and microbial growth values in the second harvest of the laboratory incubation had to be removed from further statistical analysis as ^{18}O was erroneously added in dilution with DEPC treated water (Water, sterile, ready-to-use, nuclease-free, autoclaved, DEPC-treated water, Carl Roth) containing comparatively large amounts of ethanol, which in dilution, led to considerable increases in respiration and decreases in microbial growth compared to samples where pure ^{18}O water was added. Seven samples were therefore excluded from statistical analysis.

CUE

Carbon use efficiency was calculated following the formula after Manzoni et al., 2012[50] and Sinsabaugh et al., 2013[52]:

$$CUE = \frac{Growth_{mic}}{Growth_{mic} + Resp_{mic}}$$

Statistical analysis

Linear mixed effect models were used to test the effect of added nutrients and topography and their interactions. Presence or absence of a nutrient and topography were used as fixed factors and plot identity as random factor, leading to the general model (presence/absence of nutrient * topography). All models were run in the R package nlme[85]. Tests were run separately for the *in-situ* nutrient manipulation experiment and the laboratory incubation experiment. Model residuals were tested for normality and variance homogeneity using standard functions of R (R version 3.6.3, 2020) and variables were log transformed, if conditions were not met. If normality and variance homogeneity could not be achieved by transformation alone, the linear mixed effect model was adjusted with by a weight function (varIdent - R package nlme) that accounted for the variation among topographies or treatment. Multiple comparisons were performed using Tukey post hoc tests, but were excluded from the results as the correction yielded only few significant differences for the full factorial design.

Results

In-situ nutrient manipulation experiment

Topography-specific differences in soil nutrient availability, microbial biomass and microbial physiology

Soil nutrients

Dissolved organic carbon did not differ significantly among topographies with top and slope controls averaging 366 (± 55) µg C g^{-1} and 361 (± 18) µg C g^{-1} respectively and bottom controls having the lowest DOC averaging 311 (± 52) µg C g^{-1} (Table 1). The amount of total dissolved nitrogen (TDN) was also only slightly different among topographies, similarly to DOC. Highest TDN was measured in top controls with 35.5 (± 8.2) µg N g^{-1}, followed by bottom controls with 30.2 (± 1.4) µg N g^{-1} and was lowest in slope controls with 26.8 (± 1.8) µg N g^{-1} (Table 1). Dissolved inorganic phosphorus (DIP) was highest in bottom controls, which averaged 1.40 (± 0.20) µg P g^{-1}, while top and slope controls were slightly lower with 1.14 (± 0.13) µg P g^{-1} and 1.01 (± 0.03) µg P g^{-1} respectively (Table 1). Overall, topography had no effect on either DOC, TDN or DIP (Table 2).

Microbial biomass carbon, nitrogen, phosphorus and their stoichiometry

Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) varied substantially among topographies and were highest in top controls with 667 (± 192) µg C g^{-1} and 189 (± 28) µg N g^{-1}, followed by slope controls with 574 (± 67) µg C g^{-1} and 157 (± 10) µg N g^{-1} (Table 1). We found lowest C_{mic} and N_{mic} in bottom controls with 398 (± 112) µg C g^{-1} and 133 (± 16) µg N g^{-1} (Table 1).

Contrary to C_{mic} and N_{mic} microbial biomass phosphorus (P_{mic}) was similar for top and bottom controls, which averaged 6.5 (± 0.9) and 6.2 (± 0.9) µg P g^{-1} respectively. Slope controls were considerably lower in P_{mic} than the other topographies with 4.6 (± 0.7) µg P g^{-1} (Table 1).

As a result of a similar patterns in C_{mic} and N_{mic} the microbial C:N ratio among topographies did not vary significantly between top (3.2 (± 0.6):1 C:N), slope (3.6 (± 0.3):1 C:N) and bottom (2.8 (± 0.5):1 C:N) controls (Table 1).

However, we observed a considerable variation in both microbial C:P and N:P ratios among topographies. Slope plots had both the highest microbial C:P (146 (± 82):1) and N:P ratio (39 (± 20):1) followed by intermediate ratios in top plots (C:P= 108 (± 33):1; N:P= 31 (± 4.9):1) and considerable lower C:P (68 (± 18):1) and N:P (23 (± (8.3):1) ratios in bottom controls (Table 1).

In summary, topography significantly affected C_{mic}, N_{mic}, P_{mic},microbial C:P and N:P, but had no significant effect on microbial C:N (Table 2).

Microbial physiology

Topography was an important factor in explaining the variation of microbial respiration rates ($Resp_{mic}$) (Table 2). Top controls had higher $Resp_{mic}$ (1.6 ± 0.12 µg C g^{-1} h^{-1}) than slope (1.2 (± 0.05) µg C g^{-1} h^{-1}) and bottom controls (1.0 (± 0.08) µg C g^{-1} h^{-1}) (Table 1). When standardized to microbial biomass, the resulting mass specific respiration rates (Specific $Resp_{mic}$) in both top and bottom controls were similar with 4.1 (± 1.6) and 3.8 (± 1.7) µg C mg^{-1} C_{mic} h^{-1} respectively, while slope controls with a Specific $Resp_{mic}$ of 2.2 (± 0.4) µg C mg^{-1} C_{mic} h^{-1} were considerably lower (Table 1).

Microbial growth rates ($Growth_{mic}$) were almost identical in top (0.48 (± 0.14) µg C g^{-1} h^{-1}) and slope controls (0.49 (± 0.07) µg C g^{-1} h^{-1}), but less than half of the other topographies in bottom controls (0.22 (± 0.07) µg C g^{-1} h^{-1}) (Table 1). When $Growth_{mic}$ was standardized to microbial biomass, the resulting mass specific growth rates (Specific $Growth_{mic}$) rates were highest in slope (0.85 (± 0.03) µg C mg^{-1} C_{mic} h^{-1}), intermediate in top (0.76 (± 0.07) µg C mg^{-1} C_{mic} h^{-1}) and again lowest in bottom controls (0.55 (± 0.03) µg C mg^{-1} C_{mic} h^{-1}) (Table 1).

Microbial carbon use efficiency (CUE) varied to a minor extent among topographies and was highest in slope controls at 0.29 (± 0.02), followed by top controls at 0.22 (± 0.05) and lowest in bottom controls at 0.18 (± 0.04) (Table 1).

Overall, topography was significantly affecting respiration and growth rates of the microbial communities, as well as the resulting CUE (Table 2).

Effects of *in-situ* nutrient additions on soil nutrient availability, microbial biomass and microbial physiology

Soil nutrients

N additions had no significant effect on DOC in any of the three topographies, while P additions had a significant negative effect on DOC ($F_{1,44}$= 5.9, P= 0.019, Table 2).
We observed significant effects of both N ($F_{1,44}$= 19.21, P< 0.001) and P additions ($F_{1,44}$= 23.33, P< 0.001) on TDN (Table 2). Responses to both nutrient additions were contrasting each other as N additions increased TDN by 38%, while P additions decreased TDN by 30% (Table 1).
P additions had a strong positive effect on DIP ($F_{1,44}$= 148, P< 0.001) leading to a 7-fold increase in P amended plots compared to plots without P additions (Table 1, Table 2). Additions of N had a significantly negative effect on DIP overall ($F_{1,44}$= 8.07, P= 0.007), which was largely driven by a strong negative interaction with simultaneous additions of P ($F_{2,44}$= 7.79, P= 0.008, Table 2). Consequently, simultaneous additions of N and P were leading to less pronounced differences (3.7-fold increase) compared to non-P amended plots than in plots were P was added alone (11-fold increase) (Table 1).

Microbial biomass carbon, nitrogen, phosphorus and their stoichiometry

The addition of N had no significant effect on C_{mic}, N_{mic} or P_{mic} in either of the three topographies (Fig. 4-6, Table 2).
The response of C_{mic} and N_{mic} to P additions was dependent on topography; in top plots P additions increased C_{mic} and N_{mic} by 54% and 53%, respectively, but showed no effect in slope plots, and decreased C_{mic} and N_{mic} by 36% and 13% in bottom plots compared to plots without P additions (C_{mic}; $F_{2,44}$= 4.05, P= 0.024; Fig. 4, Table 2) (N_{mic}; $F_{2,44}$= 7.22, P= 0.002; Fig. 5, Table 2).
There was a consistent increase of P_{mic} with P additions in all topographies, which led to an 8-fold increase compared to plots without P additions ($F_{1,12}$= 309, P< 0.001; Fig. 6, Table 2).
The degree of response of P_{mic} to P additions varied between topographies ($F_{2,44}$= 3.50, P= 0.039; Fig. 6, Table 2) as the increase was even more pronounced in bottom plots were P additions led to a 13-fold increase in P_{mic} (Fig. 6).

There was no significant interaction between N and P additions for C_{mic}, N_{mic} or P_{mic} (Table 2).

Consistent with the effect on C_{mic}, N_{mic} and P_{mic}, N additions had no effect on microbial C:N, C:P or N:P ratios (Table 1, Table 2).

P additions had no effect on microbial C:N, however, they led to 8-times lower microbial C:P ($F_{1,44}$= 119, P< 0.001) and 7-times lower microbial N:P ratios ($F_{1,44}$= 234, P< 0.001) across all topographies (Table 1, Table 2).

The decrease of microbial C:P and N:P ratios after P additions showed differences among topographies for both microbial C:P ($F_{2,44}$= 5.97, P= 0.005) and N:P ($F_{2,44}$= 7.64, P= 0.001) ratios and was lowest in top plots with 3.4-times lower microbial C:P and 3.6-lower microbial N:P ratios compared to plots without P amendment (Table 1, Table 2). Microbial C:P ratios decreased the most in bottom plots with 12-times lower microbial C:P and 10-times lower microbial N:P ratios compared to plots without P additions (Table 1). We observed no significant interaction effects between N and P additions for microbial C:N or C:P ratios. However, a significant interaction effect between N and P additions ($F_{1,44}$= 6.01, P= 0.018) led to slightly higher N:P ratios in the NP treatment compared to the P-only treatment (Table 1, Table 2).

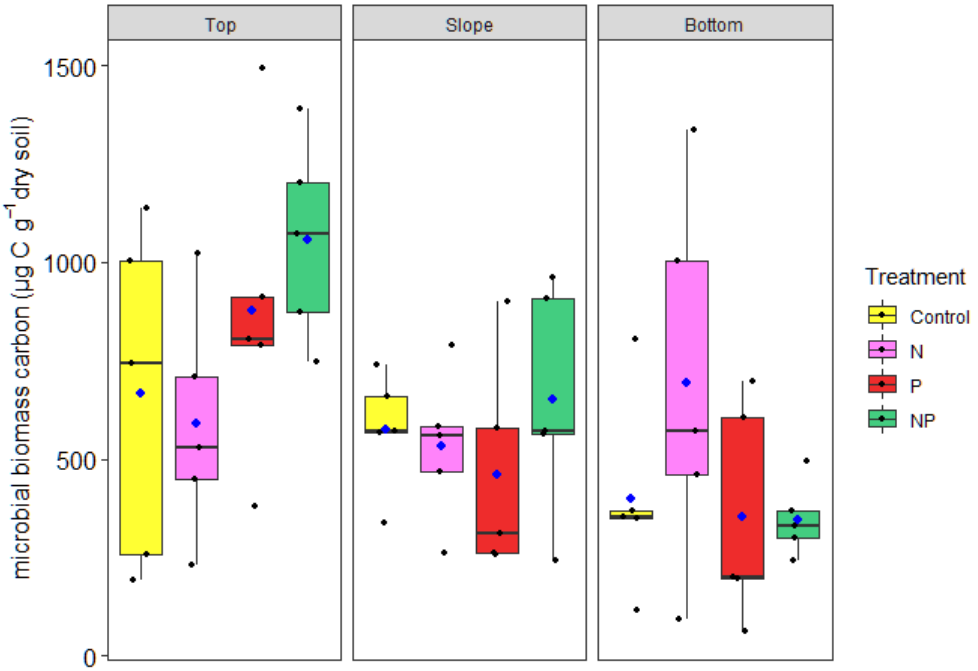

Fig. 4 Microbial biomass C (µg C per g dry soil) for three topographies (top, slope, bottom) and treatment (N, nitrogen addition; P, phosphorus addition; NP, combined nitrogen and phosphorus addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 5 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 2.

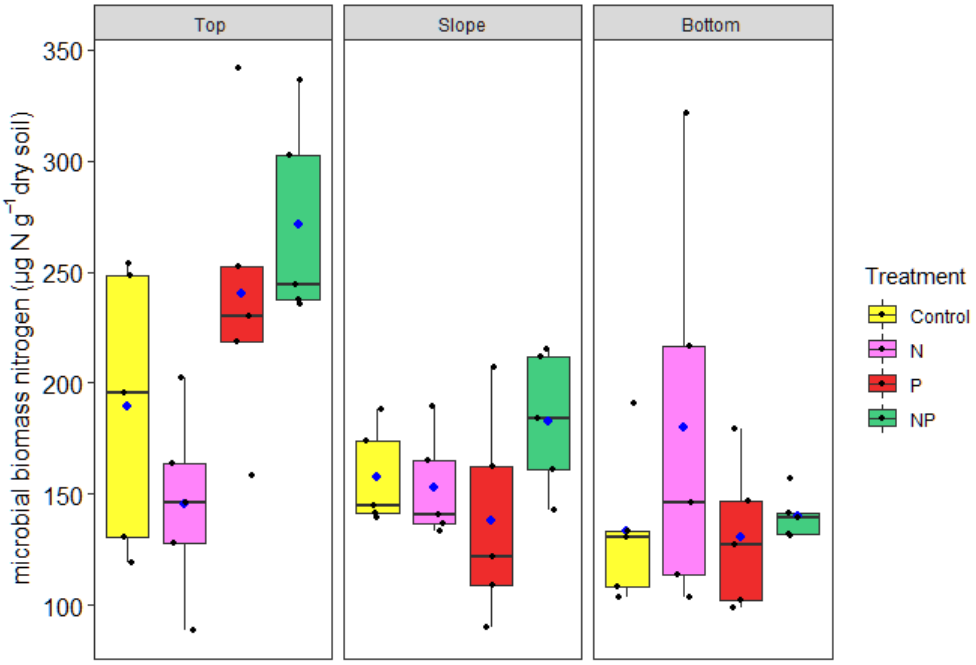

Fig. 5 Microbial biomass N (µg N per g dry soil) for three topographies (top, slope, bottom) and treatment (N, nitrogen addition; P, phosphorus addition; NP, combined nitrogen and phosphorus addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 5 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 2.

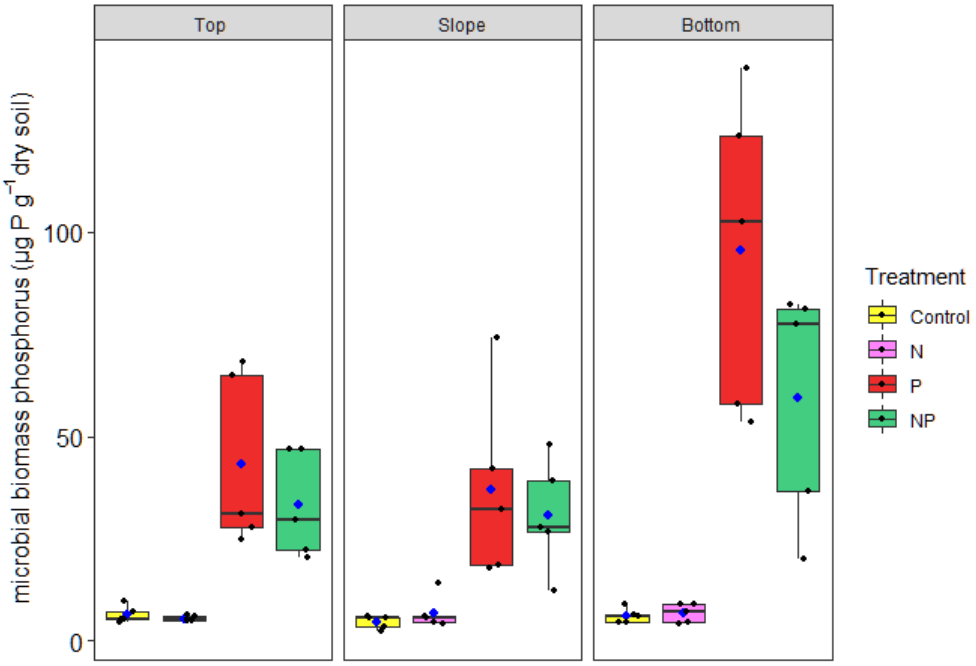

Fig. 6 Microbial biomass P (µg P per g dry soil) for three topographies (top, slope, bottom) and treatment (N, nitrogen addition; P, phosphorus addition; NP, combined nitrogen and phosphorus addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 5 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 2.

Table 1 Soil and microbial parameters of an in-situ nutrient manipulation experiment in a lowland tropical forest in Nouragues, French Guiana. Values represent means ± 1 standard error (n=5). SOC, SN and SP, are soil organic carbon, nitrogen and phosphorus; DOC, TDN and DIP, are dissolved organic carbon, total dissolved nitrogen and dissolved inorganic phosphorus; Mic. C:N, C:P and N:P are the ratios of microbial carbon to nitrogen, carbon to phosphorus and nitrogen to phosphorus; $Resp_{mic}$ stands for microbial respiration rates; $Growth_{mic}$ stands for microbial growth rates; CUE is the microbial carbon use efficiency).

	Top				Slope				Bottom			
	Control	**N**	**P**	**NP**	**Control**	**N**	**P**	**NP**	**Control**	**N**	**P**	**NP**
SOC (%)	5.17 ± 0.41	7.3 ± 1.22	5.02 ± 0.21	5.57 ± 0.3	4.36 ± 0.25	4.54 ± 0.3	4.7 ± 0.71	3.74 ± 0.5	3.83 ± 0.11	4.63 ± 1.12	3.17 ± 0.47	3.83 ± 1.02
SN (%)	0.36 ± 0.02	0.42 ± 0.06	0.36 ± 0.01	0.40 ± 0.02	0.29 ± 0.02	0.33 ± 0.03	0.27 ± 0.04	0.29 ± 0.03	0.26 ± 0.01	0.33 ± 0.07	0.22 ± 0.03	0.37 ± 0.05
SP (‰)	22.4 ± 0.7	30.2 ± 1.14	59.4 ± 1.27	66.0 ± 2.12	10.4 ± 0.39	14.6 ± 0.7	22.9 ± 1.79	23.3 ± 3.3	7.04 ± 0.6	9.58 ± 1.73	19.6 ± 5.7	21.9 ± 1.4
DOC ($\mu g\ C\ g^{-1}$)	366 ± 55	404 ± 49	343 ± 35	277 ± 33	361 ± 18	381 ± 35	268 ± 19	353 ± 21	311 ± 23	288 ± 22	264 ± 25	332 ± 32
TDN ($\mu g\ N\ g^{-1}$)	35 ± 8.2	50 ± 11.1	22 ± 3.4	25 ± 4.2	27 ± 1.8	38 ± 2.5	14 ± 2.1	30 ± 3.8	30 ± 1.4	31 ± 2.7	22 ± 1.9	35 ± 3.5
DIP ($\mu g\ P\ g^{1}$)	1.14 ± 0.13	1.22 ± 0.18	14.4 ± 3.06	6.63 ± 1.01	1.01 ± 0.03	1.17 ± 0.08	8.75 ± 2.00	4.94 ± 1.59	1.40 ± 0.20	1.09 ± 0.11	19.9 ± 6.08	4.82 ± 1.27
Mic. C:N	3.2 ± 1.3	4.0 ± 1.3	3.5 ± 0.7	3.9 ± 0.5	3.6 ± 0.7	3.4 ± 0.9	3.1 ± 0.8	3.4 ± 1.1	2.8 ± 1.1	3.6 ± 1.6	2.4 ± 1.5	2.5 ± 0.7
Mic. C:P	108 ± 73	108 ± 52	26 ± 17	37 ± 17	146 ± 82	97 ± 46	13 ± 5	22 ± 7	68 ± 40	95 ± 47	4.6± 5.1	8.9 ± 8.9
Mic. N:P	31 ± 11	27 ± 8	6.9 ± 3.7	9.2 ± 3.8	39 ± 20	27 ± 11	4.4 ± 1.5	4.0 ± 3.1	23 ± 8	26 ± 5	1.6 ± 1.0	3.2 ± 2.3
$Resp_{mic}$ ($\mu g\ C\ g^{-1}\ h^{-1}$)	1.62 ± 0.12	1.64 ± 0.18	1.62 ± 0.16	1.19 ± 0.06	1.19 ± 0.05	1.18 ± 0.15	1.18 ± 0.10	1.10 ± 0.05	0.98 ± 0.05	1.05 ± 0.18	1.18 ± 0.08	1.35 ± 0.07
$Growth_{mic'}$ ($\mu g\ C\ g^{-1}\ h^{-1}$)	0.48 ± 0.14	0.60 ± 0.22	0.79 ± 0.15	0.72 ± 0.12	0.49 ± 0.07	0.70 ± 0.11	0.44 ± 0.12	0.61 ± 0.11	0.22 ± 0.07	0.49 ± 0.17	0.25 ± 0.08	0.23 ± 0.03
CUE	0.22 ± 0.05	0.24 ± 0.05	0.32 ± 0.05	0.30 ± 0.03	0.29 ± 0.03	0.37 ± 0.02	0.25 ± 0.05	0.35 ± 0.05	0.18 ± 0.04	0.28 ± 0.06	0.16 ± 0.04	0.15 ± 0.02

Table 2 Statistical summary of the effects of topography, N addition, P addition and the interaction between topography and N addition (Topo x N), topography and P addition (Topo x P) and N and P addition (N x P) for the in-situ nutrient manipulation experiment. Depicted are F-values and p-values from a linear mixed effect model p-values < 0.05 are highlighted in bold).

	Topography	N addition	P addition	Topo x N	Topo x P	N x P
DOC	$2.63_{2,44}$; 0.084	$1.18_{1,44}$; 0.283	**$5.9_{1,44}$; 0.019**	$1.05_{2,44}$; 0.36	$1.42_{2,44}$; 0.253	$0.21_{1,44}$; 0.648
TDN	$1.65_{2,44}$; 0.203	**$19.2_{1,44}$; <0.001**	**$23.3_{1,44}$; <0.001**	$1.88_{2,44}$; 0.164	$3.05_{2,44}$; 0.058	$1.67_{1,44}$; 0.203
DIP	$1.49_{2,44}$; 0.238	**$8.07_{1,44}$; 0.007**	**$149_{1,44}$; <0.001**	$0.85_{2,44}$; 0.433	$0.94_{2,44}$; 0.4	**$7.79_{1,44}$; 0.008**
Mic. C	**$7.11_{2,44}$; 0.002**	$1.34_{1,44}$; 0.253	$0.38_{1,44}$; 0.538	$0.13_{2,44}$; 0.876	**$4.05_{2,44}$; 0.024**	$0.17_{1,44}$; 0.686
Mic. N	**$10.8_{2,44}$; <0.001**	$1.29_{1,44}$; 0.262	$3.82_{1,44}$; 0.057	$0.72_{2,44}$; 0.494	**$7.22_{2,44}$; 0.002**	$1.38_{1,44}$; 0.246
Mic. P	**$6.88_{2,44}$; 0.003**	$0.83_{1,44}$; 0.368	**$309_{1,44}$; <0.001**	$0.93_{2,44}$; 0.403	**$3.5_{2,44}$; 0.039**	$3.12_{1,44}$; 0.084
Mic. C:N	$3.12_{2,44}$; 0.054	$1.65_{1,44}$; 0.205	$1.16_{1,44}$; 0.287	$0.35_{2,44}$; 0.709	$0.81_{2,44}$; 0.45	$0.16_{1,44}$; 0.69
Mic. C:P	**$13.4_{2,44}$; <0.001**	$2.92_{1,44}$; 0.095	**$119_{1,44}$; <0.001**	$0.83_{2,44}$; 0.442	**$5.97_{2,44}$; <0.005**	$2.86_{1,44}$; 0.1
Mic. N:P	**$13.2_{2,44}$; <0.001**	$2.32_{1,44}$; 0.135	**$234_{1,44}$; <0.001**	$0.81_{2,44}$; 0.453	**$7.64_{2,44}$; 0.001**	**$6.01_{1,44}$; 0.018**
$Resp_{mic}$	**$22.2_{2,44}$; <0.001**	$0.09_{1,44}$; 0.763	$0.8_{1,44}$; 0.376	$0.61_{2,44}$; 0.546	$2.05_{2,44}$; 0.141	$0.01_{1,44}$; 0.959
Specific $Resp_{mic}$	$2.28_{2,44}$; 0.114	$1.67_{1,44}$; 0.204	$0.04_{1,44}$; 0.849	$0.07_{2,44}$; 0.929	**$4.94_{2,44}$; 0.01**	$0.35_{1,44}$; 0.555
$Growth_{mic'}$	**$8.99_{2,44}$; <0.001**	$2.61_{1,44}$; 0.114	$0.01_{1,44}$; 0.905	$0.47_{2,44}$; 0.628	$2.25_{2,44}$; 0.117	$1.48_{1,44}$; 0.231
Specific $Growth_{mic}$	**$30_{2,44}$; <0.001**	$1.43_{1,44}$; 0.238	$0.01_{1,44}$; 0.998	**$3.67_{2,44}$; 0.03**	**$3.8_{2,44}$; 0.029**	**$17.9_{1,44}$; <0.001**
CUE	**$9.59_{2,44}$; <0.001**	$3.41_{1,44}$; 0.072	$0.04_{1,44}$; 0.836	$1.07_{2,44}$; 0.353	**$4.16_{2,44}$; 0.02**	$1.05_{1,44}$; 0.312

Microbial physiology

Neither $Resp_{mic}$ nor Specific $Resp_{mic}$ did significantly change with N additions in any of the three topographies (Table 2). P additions had no significant effect on $Resp_{mic}$, however we observed a significant topography dependent effect on Specific $Resp_{mic}$ ($F_{2,44}$= 4.94, P= 0.012, Table 2). P additions decreased Specific $Resp_{mic}$ by 47% in top plots, while slope and bottom plots were increased by 16% and 64% respectively (Fig. 7).

$Growth_{mic}$ was not significantly affected by N or P additions in either of the three topographies (Table 2). We also observed no interactive effects between topography, N and P additions for $Growth_{mic}$ (Table 2).

The responses of Specific $Growth_{mic}$ to N and P additions were dependent on topography (N; $F_{2,44}$= 3.67, P= 0.03) (P; $F_{2,32}$= 3.83, P= 0.029) and additionally had a significant negative interactive effect ($F_{1,44}$= 17.9, P< 0.001), which led to divergent responses among topographies to both sole and combined additions of N and P (Fig. 8, Table 2). In top plots the addition of either N or P alone, led to an increase of 22% in Specific $Growth_{mic}$, while the combined addition of both nutrients decreased Specific $Growth_{mic}$ by 11% when compared to the controls (Fig. 8).

In slope plots additions of N increased Specific $Growth_{mic}$ by 55%, while the P-only treatment and the NP treatment were similar to the controls (Fig. 8).

In bottom plots sole additions of P led to an increase of Specific $Growth_{mic}$ by 37% when compared to the controls, while the N-only and NP treatment increased by 17% and 19% respectively (Fig. 8).

Microbial CUE was not significantly affected by N-additions, however it should be noted that additions of N alone led to a 57% increase in bottom plots (Table 1, Table 2). The response of microbial CUE to P additions was dependent on topography ($F_{2,44}$= 4.16, P= 0.02) and increased CUE in top plots by 38%, while decreasing CUE in bottom plots by 32% (Table 1, Table 2).

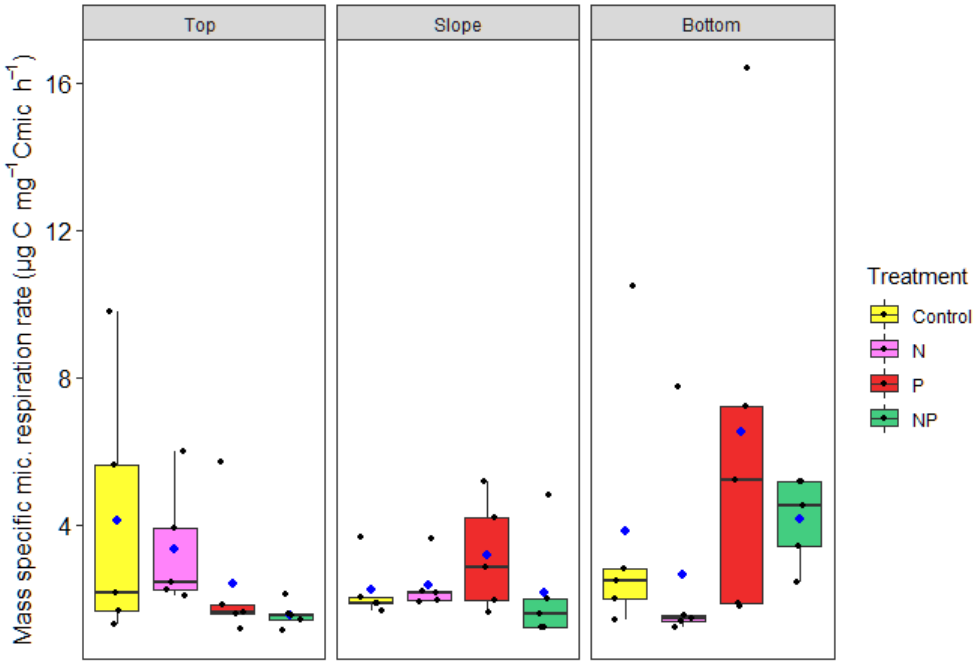

Fig. 7 Mass specific microbial respiration rates (µg C mg^{-1} C_{mic} h^{-1}) for three topographies (top, slope, bottom) and treatment (N, nitrogen addition; P, phosphorus addition; NP, combined nitrogen and phosphorus addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 5 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 2.

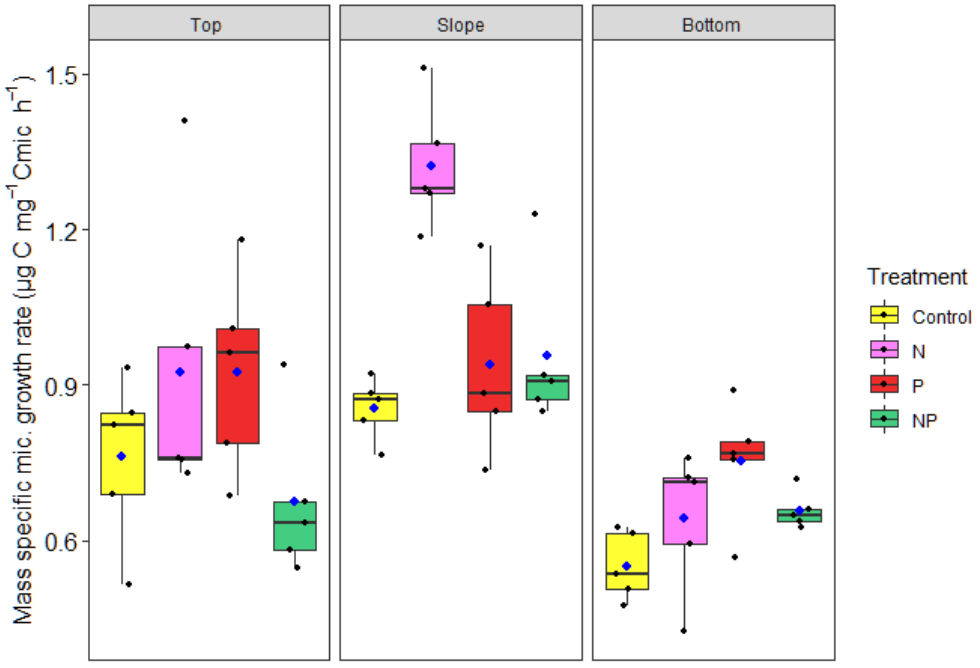

Fig. 8 Mass specific microbial growth rates (µg C mg^{-1} C_{mic} h^{-1}) for three topographies (top, slope, bottom) and treatment (N, nitrogen addition; P, phosphorus addition; NP, combined nitrogen and phosphorus addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 5 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 2.

Laboratory incubation experiment

Topography- specific differences in soil nutrient availability, microbial biomass and microbial physiology

Soil nutrients

The origin of soils (i.e., the topography) significantly affected DOC, TDN and DIP in the laboratory incubation experiment (Table 4). DOC among mesocosms varied considerably with topography and was highest in top (182 (± 28) µg C g^{-1}), intermediate in slope (132 (± 8) µg C g^{-1}) and lowest in bottom mesocosms (103 (± 13) µg C g^{-1}) (Table 3).

Highest TDN values were observed in top mesocosms (104 (± 9) µg N g^{-1}), followed by bottom mesocosms (66 (± 11) µg N g^{-1}) (Table 3). TDN was lowest in slope mesocosms (55 (± 4) µg N g^{-1}), which is a 47% decrease compared to top mesocosms (Table 3).

DIP was, in contrast to DOC and TDN, highest in bottom mesocosms (0.59 (± 0.17) µg P g^{-1}), closely followed by top mesocosms (0.54 (± 0.04) µg P g^{-1}) (Table 3). While DIP only marginally differed between bottom and top, it was considerably lower in slope mesocosms (0.36 (± 0.06) µg P g^{-1}) (Table 3).

Microbial biomass carbon, nitrogen, phosphorus and their stoichiometry

Overall, topography significantly affected C_{mic} and P_{mic}, but had no effect on N_{mic} (Table 4). C_{mic} was highly variable between topographies and was by far the highest in top mesocosms with an average of 733 (±71) µg C g^{-1}. Both slope and bottom mesocosms were considerably lower in C_{mic} with 439 (± 36) µg C g^{-1} and 475 (± 55) µg C g^{-1} (Table 3). N_{mic} was varying slightly, but not significantly with topography, with highest observed values in top mesocosms averaging 99 (± 22) µg N g^{-1} and similar values in slope and bottom mesocosms at 68 (± 6) µg N g^{-1} and 67 (± 5) µg N g^{-1} respectively (Table 3).

P_{mic} varied differently among topographies than the other microbial biomass parameters and was highest in bottom mesocosms with an average of 10.5 (± 1.9) µg P g^{-1} (Table 3). This was markedly higher than P_{mic} in top and slope mesocosms, which averaged 6.8 (± 0.5) µg P g^{-1} and 5.1 (± 0.6) µg P g^{-1} respectively (Table 3).

Topography also significantly affected microbial C:P and N:P (Table 4). Although C_{mic} and N_{mic} varied to great extent between topographies, the microbial C:N ratio was not significantly different in top (8.9 (± 2.3):1 C:N), slope (6.6 (± 0.6):1 C:N) and bottom mesocosms (7 (± 0.4):1 C:N) (Table 3, Table 4). This is in contrast to microbial C:P and N:P ratios, which varied significantly among topographies with lowest ratios in bottom mesocosms averaging 48:1 (± 6.9):1 C:P and 7 (± 2.2):1 N:P, while top and slope mesocosms were closely matched in their microbial C:P and N:P ratios with top averaging 109 (± 3.8):1 C:P and 14.1 (± 2.5):1 N:P and slope mesocosms averaging 91 (± 12):1 C:P and 13.7 (± 1.6):1 N:P (Table 3, Table 4).

Microbial physiology

Overall, we found a significant effect of soil topography on microbial respiration (Table 4). $Resp_{mic}$ was similar in top and bottom mesocosms with 1.15 (± 0.02) and 1.12 (± 0.13) µg C g^{-1} h^{-1} respectively, but was 40% less in slope mesocosms with an average of 0.68 (± 0.06) µg C g^{-1} h^{-1} (Table 3). When standardized to microbial biomass, the resulting Specific $Resp_{mic}$ were highest in bottom mesocosms with 2.4 (± 0.1) µg C mg^{-1} C_{mic} h^{-1}, while being similar in top and slope mesocosms with 1.64 (± 0.23) µg C mg^{-1} C_{mic} h^{-1} and 1.50 (± 0.10) µg C mg^{-1} C_{mic} h^{-1} respectively (Table 3).
Topography had no significant overall effect on $Growth_{mic}$ (Table 4), albeit C invested into $Growth_{mic}$ varied with topography and was highest in top mesocosms with an average rate of 0.97 (± 0.42) µg C g^{-1} h^{-1}, while slope and bottom mesocosms were not significantly different from each other with rates of 0.59 (± 0.07) and 0.62 (± 0.2) µg C g^{-1} h^{-1} respectively (Table 3). Similarly, when growth rates were standardized to microbial biomass, the resulting Specific $Growth_{mic}$ differed (non-significant) among topographies with top, slope and bottom growth rates being 1.26 (± 0.42), 1.32 (± 0.17) and 1.28 (± 0.33) µg C mg^{-1} C_{mic} h^{-1} (Table 3, Table 4).
CUE was also not significantly affected by topography (Table 4) and was highest in slope mesocosms with 0.46 (± 0.02). CUE in top mesocosms were intermediate among topographies with 0.42 (± 0.01) and consequently lowest CUE was observed in bottom mesocosms with 0.34 (± 0.05).

Pre-incubation – the impacts of labile C additions in tropical forest soils

Three weeks after the addition of C in the form of cellulose we found a small positive effect on microbial respiration ($F_{1,30}$= 7.6, P< 0.001), mainly in top mesocosms ($F_{2,30}$= 4.91, P= 0.014) (Table S1, Fig S2). No significant effect was found when respiration rates were standardized to Specific $Resp_{mic}$ (Table S1). C additions also had a small positive effect on P_{mic} in slope mesocosms ($F_{1,33}$= 6.88, P= 0.013, Table S1, Fig S1). No other significant effects of C- additions were found.

Incubation of highly weathered tropical soils with labile sources of C and P – Identifying nutrient controls on microbial growth

Soil nutrients

Neither DOC nor TDN changed significantly in response to the addition of C and P in any of the three topographies (Table 3, Table 4).

Contrasting to other soil nutrients DIP was, similarly to results reported in the *in-situ* nutrient manipulation experiment (Table 1), strongly increased by P additions ($F_{1,27}$= 356, P< 0.001) in all topographies (Table 3, Table 4).

Microbial biomass carbon, nitrogen, phosphorus and their stoichiometry

Additions of C did not affect C_{mic}, N_{mic} or P_{mic} in either of the three topographies (Fig. 6-8, Table 4). P additions had a significant negative effect ($F_{1,27}$= 6.17, P= 0.02, Fig. 9) on C_{mic}, which was mainly driven through a 10% decrease in C_{mic} in P-amended top mesocosms compared to mesocosms without P additions, but had no effect on N_{mic} (Fig. 9-10, Table 4).

The addition of P increased P_{mic} 2-fold compared to mesocosms without P-additions ($F_{1,27}$= 248, P< 0.001, Fig. 11, Table 4). Slope mesocosms were more strongly affected by P additions with an increase of 133%, while top and bottom mesocosms increased by 76% and 73% respectively ($F_{2,27}$= 4.57, P= 0.02, Fig. 11, Table 4).

C additions had no effect on microbial C:N, C:P or N:P ratios, and the addition of P did not affect microbial C:N (Table 4). In contrast, both microbial C:P ($F_{1,27}$= 277.27, P< 0.001, Table 4) and N:P ($F_{1,27}$= 141.54, P< 0.001, Table 4) ratios were significantly decreased by P additions in all three topographies.

This decrease was strongest in slope mesocosms with a 60% decreased microbial C:P and 57% decreased microbial N:P ratio, while top mesocosms decreased 49% and 45% and bottom mesocosms decreased 44% and 42% in microbial C:P and N:P ratios respectively (C:P; $F_{2,27}$= 18.30, P< 0.001) (N:P; $F_{2,27}$= 10.62, P< 0.001) (Table 3, Table 4).

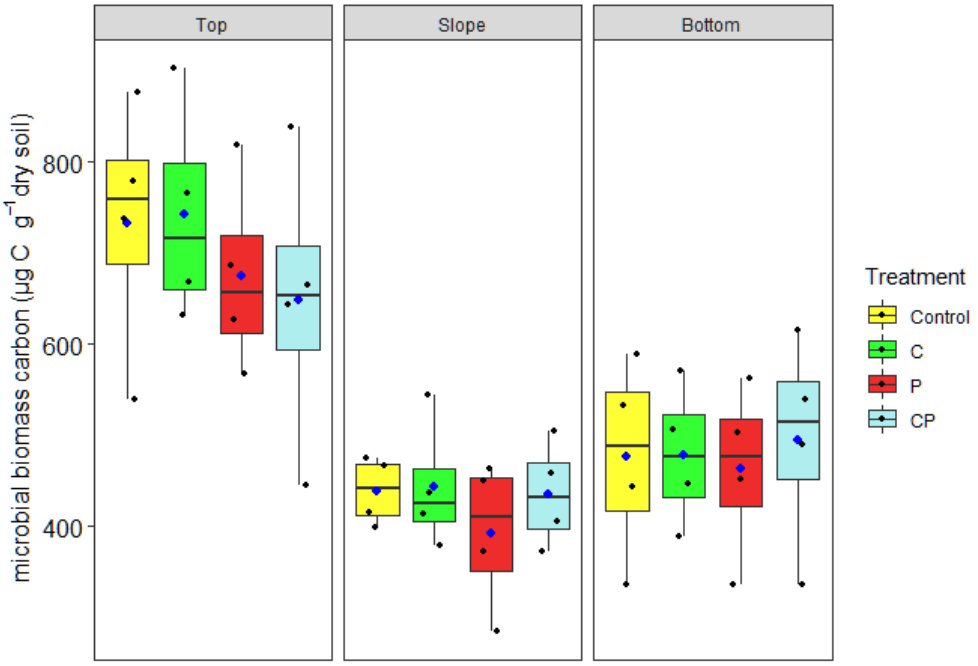

Fig. 9 Microbial biomass C (µg C per g dry soil) for three topographies (top, slope, bottom) and treatment (C, carbon addition; P, phosphorus addition; CP, combined carbon and phosphorus addition; Control, no additions) of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 4 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 4.

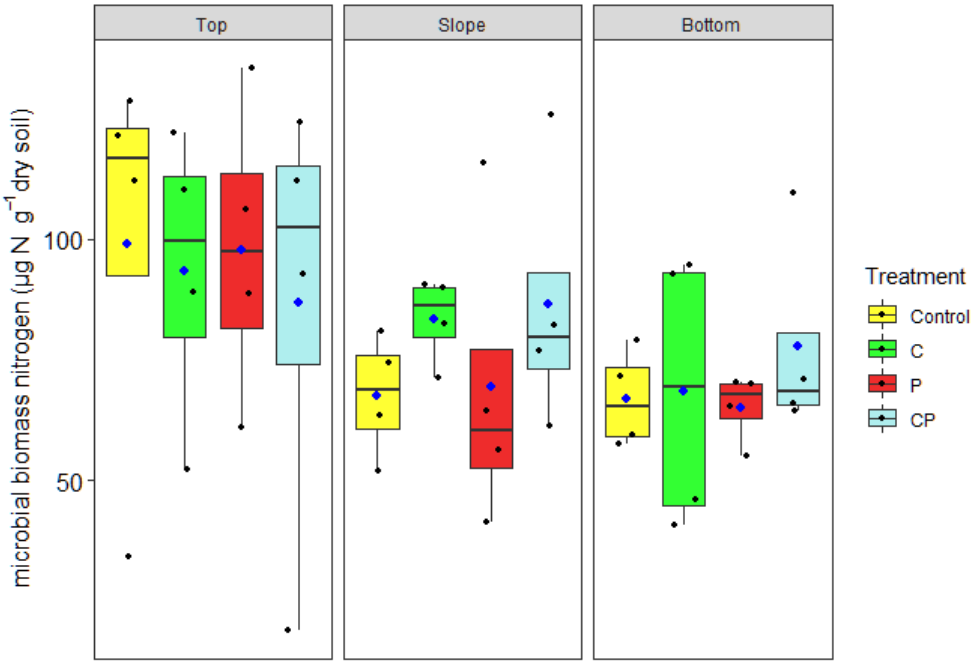

Fig. 10 Microbial biomass N (µg N per g dry soil) for three topographies (top, slope, bottom) and treatment (C, carbon addition; P, phosphorus addition; CP, combined carbon and phosphorus addition; Control, no additions) of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 4 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 4.

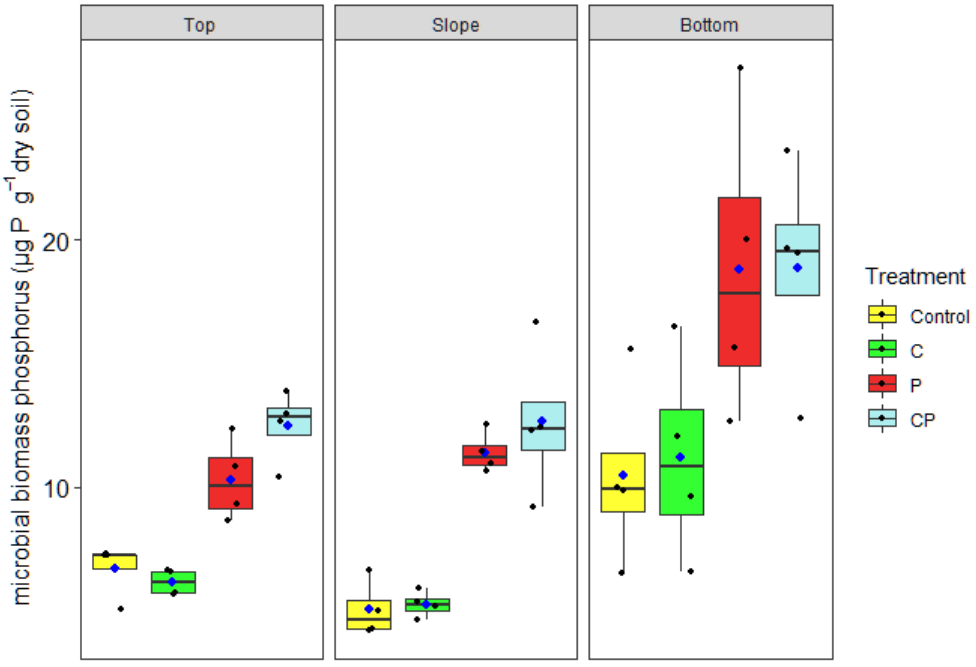

Fig. 11 Microbial biomass P (µg P per g dry soil) for three topographies (top, slope, bottom) and treatment (C, carbon addition; P, phosphorus addition; CP, combined carbon and phosphorus addition; Control, no additions) of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 4 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 4.

Table 3 Soil and microbial parameters of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Values represent means ± 1 standard error (n=4). SOC and SN, are soil organic carbon and soil nitrogen; DOC, TDN and DIP, are dissolved organic carbon, total dissolved nitrogen and dissolved inorganic phosphorus; Mic. C:N, C:P and N:P are the ratios of microbial carbon to nitrogen, carbon to phosphorus and nitrogen to phosphorus; $Resp_{mic}$ stands for microbial respiration rates; $Growth_{mic}$ stands for microbial growth rates; CUE is the microbial carbon use efficiency).

	Top				Slope				Bottom			
	Control	**C**	**P**	**CP**	**Control**	**C**	**P**	**CP**	**Control**	**C**	**P**	**CP**
SOC (%)	4.68 ± 0.93	5.11 ± 0.86	4.94 ± 0.55	5.61 ± 0.54	2.92 ± 0.8	2.69 ± 0.5	4.58 ± 0.42	3.78 ± 0.21	2.69 ± 0.48	1.78 ± 0.67	2.64 ± 0.73	2.42 ± 0.39
SN (%)	0.32 ± 0.05	0.35 ± 0.04	0.35 ± 0.04	0.39 ± 0.01	0.20 ± 0.05	0.19 ± 0.04	0.34 ± 0.04	0.27 ± 0.02	0.19 ± 0.03	0.12 ± 0.05	0.18 ± 0.04	0.16 ± 0.02
DOC ($\mu g\ C\ g^{-1}$)	182 ± 28	192 ± 23	186 ± 24	198 ± 19	132 ± 8	135 ± 11	135 ± 10	136 ± 10	103 ± 7	113 ± 11	109 ± 8	107 ± 8
TDN ($\mu g\ N^{-1}$)	104 ± 9	106 ± 13	102 ± 8	105 ± 12	55 ± 4	51 ± 3	56 ± 4	57 ± 3	66 ± 11	67 ± 10	66 ± 10	63 ± 11
DIP ($\mu g\ P^{-1}$)	0.54 ± 0.04	0.57 ± 0.04	1.27 ± 0.12	1.25 ± 0.17	0.36 ± 0.03	0.36 ± 0.02	1.36 ± 0.13	1.06 ± 0.12	0.59 ± 0.17	0.51 ± 0.09	1.99 ± 0.33	2.18 ± 0.45
Mic. C:N	8.9 ± 2.3	8.5 ± 1.3	7.2 ± 0.7	10.8 ± 4.2	6.6 ± 0.6	5.3 ± 0.4	5.3 ± -0.4	6.2 ± 0.8	7.0 ± 0.4	7.7 ± 1.3	7.1 ± 0.6	6.6 ± 1.1
Mic. C:P	109 ± 4	120 ± 6	66 ± 3	51 ± 4	91 ± 12	84 ± 6	34 ± 3	35 ± 3	48 ± 7	46 ± 7	26± 4	27 ± 2
Mic. N:P	14.1 ± 2.5	15.1 ± 2.3	9.4 ± 1.0	6.7 ± 1.7	13.7 ± 1.6	15.9 ± 0.9	6.0 ± 1.1	6.8 ± 0.3	7.0 ± 1.1	6.7 ± 1.5	3.7 ± 0.4	4.3 ± 0.7
$Resp_{mic}$ ($\mu g\ C\ g^{-1}\ h^{-1}$)	1.15 ± 0.02	1.59 ± 0.21	1.24 ± 0.03	1.50 ± 0.24	0.68 ± 0.06	0.66 ± 0.06	0.69 ± 0.02	0.74 ± 0.04	1.11 ± 0.13	1.01 ± 0.16	0.93 ± 0.13	1.37 ± 0.24
$Growth_{mic'}$ ($\mu g\ C\ g^{-1}\ h^{-1}$)	0.97 ± 0.43	1.02 ± 0.43	0.91 ± 0.14	0.81 ± 0.21	0.59 ± 0.07	0.58 ± 0.09	0.58 ± 0.10	0.69 ± 0.11	0.62 ± 0.20	0.59 ± 0.15	0.64 ± 0.15	0.89 ± 0.19
CUE	0.42 ± 0.10	0.37 ± 0.05	0.42 ± 0.03	0.35 ± 0.04	0.46 ± 0.02	0.46 ± 0.03	0.45 ± 0.04	0.47 ± 0.03	0.34 ± 0.05	0.37 ± 0.05	0.40 ± 0.03	0.38 ± 0.02

Table 4 Statistical summary of the effects of topography, C addition, P addition and the interaction between topography and C addition (Topo x C), topography and P addition (Topo x P) and C and P addition (C x P) for the laboratory incubation experiment. Depicted are F-values and p-values from a linear mixed effect model, p-values< 0.05 are highlighted in bold).

	Topography	C addition	P addition	Topo x C	Topo x P	C x P
DOC	**$7.72_{2,9}$; 0.011**	$3.41_{1,27}$; 0.076	$0.76_{1,27}$; 0.392	$0.86_{2,27}$; 0.432	$0.18_{2,27}$; 0.839	$1.03_{1,27}$; 0.318
TDN	**$7.13_{2,9}$; 0.014**	$0.78_{1,27}$; 0.385	$0.06_{1,27}$; 0.815	$0.43_{2,27}$; 0.657	**$3.39_{2,27}$; 0.049**	$0.92_{1,27}$; 0.347
DIP	**$6.60_{2,9}$; 0.017**	$0.9_{1,27}$; 0.351	**$356_{1,27}$; <0.001**	$0.75_{2,27}$; 0.48	**$7.4_{2,27}$; 0.003**	$1.77_{1,27}$; 0.194
Mic. C	**$7.94_{2,9}$; 0.01**	$1.07_{1,27}$; 0.31	**$6.17_{1,27}$; 0.02**	$0.88_{2,27}$; 0.43	$1.73_{2,27}$; 0.196	$0.27_{1,27}$; 0.605
Mic. N	$1.21_{2,9}$; 0.343	$0.90_{1,27}$; 0.352	$0.02_{1,27}$; 0.887	$1.78_{2,27}$; 0.187	$0.19_{2,27}$; 0.83	$0.05_{1,27}$; 0.828
Mic. P	**$6.65_{2,9}$; 0.017**	$2.38_{1,27}$; 0.135	**$249_{1,27}$; <0.001**	$0.11_{2,27}$; 0.9	**$4.57_{2,27}$; 0.02**	$1.58_{1,27}$; 0.22
Mic. C:N	$2.26_{2,9}$; 0.161	$0.15_{1,27}$; 0.702	$0.63_{1,27}$; 0.434	$1.56_{2,27}$; 0.228	$0.04_{2,27}$; 0.961	$0.12_{1,27}$; 0.734
Mic. C:P	**$36_{2,9}$; <0.001**	$0.41_{1,27}$; 0.526	**$277_{1,27}$; <0.001**	$0.04_{2,27}$; 0.958	**$18.3_{2,27}$; <0.001**	$1.07_{1,27}$; 0.309
Mic. N:P	**$7.11_{2,9}$; 0.014**	$0.29_{1,27}$; 0.592	**$141_{1,27}$; <0.001**	$1.89_{2,27}$; 0.171	**$10.6_{2,27}$; 0.004**	$1.98_{1,27}$; 0.171
$Resp_{mic}$	**$8.45_{2,8}$; 0.01**	**$12.88_{1,21}$; 0.002**	$1.59_{1,21}$; 0.221	$2_{2,21}$; 0.16	$0.37_{2,21}$; 0.697	**$6.55_{1,21}$; 0.018**
Specific $Resp_{mic}$	**$13.1_{2,8}$; 0.003**	**$9.82_{1,21}$; 0.005**	**$5.89_{1,21}$; 0.024**	$2.07_{2,21}$; 0.151	$0.13_{2,21}$; 0.879	**$9.07_{1,21}$; 0.007**
$Growth_{mic'}$	$0.95_{2,8}$; 0.428	$2.97_{1,21}$; 0.1	**$3.27_{1,21}$; 0.085**	$0.3_{2,21}$; 0.745	$1.45_{2,21}$; 0.257	**$4.54_{1,21}$; 0.045**
Specific $Growth_{mic'}$	$0.1_{2,8}$; 0.904	$1.6_{1,21}$; 0.219	**$12.9_{1,21}$; 0.002**	$0.44_{2,21}$; 0.648	$0.8_{2,21}$; 0.463	**$8.44_{1,21}$; 0.009**
CUE	$2.31_{2,8}$; 0.161	$0.57_{1,21}$; 0.458	$0.38_{1,21}$; 0.544	$1.46_{2,21}$; 0.255	$0.72_{2,21}$; 0.5	$0.19_{1,21}$; 0.665
Cumlative Resp	**$8.71_{2,8}$; 0.008**	**$6.24_{1,21}$; 0.019**	**$34.9_{1,21}$; <0.001**	**$4.88_{2,21}$; 0.016**	$0.85_{2,21}$; 0.437	$0.14_{1,21}$; 0.708

Microbial physiology

C additions had a significant effect on $Resp_{mic}$ overall ($F_{1,21}$= 12.9, P= 0.002), while addition of P alone did not significantly change $Resp_{mic}$ in either of the three topographies (Table 4). However, C addition alone increased $Resp_{mic}$ only in top mesocosms by 38%, whereas $Resp_{mic}$ in slope mesocosms did not change and bottom mescosms decreased by 10% (Table 3). Therefore, the significant C effect most likely was driven by the positive interactive effect of C and P additions ($F_{2;21}$= 6.55, P= 0.018), increasing $Resp_{mic}$ by 30%, 9% and 23% in top slope and bottom CP mescosms compared to the respective controls (Table 3, Table 4).

The additions of both C ($F_{1,21}$= 9.82, P= 0.005) and P ($F_{1,21}$= 5.89, P= 0.024) had significant effects on Specific $Resp_{mic}$ in the overall model, although this was driven by a strong interactive effect between both nutrients ($F_{1,21}$= 9.07, P= 0.007) (Table 4). This led to an increase in Specific $Resp_{mic}$ in top, slope and bottom mesocosms by 42%, 17% and 15% respectively, when nutrients were added in combination (Fig. 12). In contrast C addition alone only increased Specific $Resp_{mic}$ in top mesocosms by 26%, while showing no effect in slope and decreasing Specific $Resp_{mic}$ in bottom mesocosms by 12% (Fig. 12). Similarly, P additions alone elicited different responses among topographies increasing Specific $Resp_{mic}$ by 11% and 7% in top and slope mesocosms, and decreasing it by 26% in bottom mesocosms (Fig. 12).

$Growth_{mic}$ was not stimulated by the addition of only C or P (Table 3, Table 4). However, we found a significant interactive effect of C and P on $Growth_{mic}$ ($F_{1,21}$= 4.54, P= 0.045, Table 4). P additions had a significant effect on Specific $Growth_{mic}$ ($F_{1,21}$= 12.89, P= 0.0017) according to the overall model (Table 4). However, this effect is driven due to a strong interactive effect with C- additions on Specific $Growth_{mic}$ ($F_{1,21}$= 8.43, P= 0.009) as little to no effect of P addition was observed in the P-only mesocosms (Fig. 13, Table 4).

Although no significant interaction between nutrient additions and topography was detected, responses of $Growth_{mic}$ and Specific $Growth_{mic}$ were varying between topographies. $Growth_{mic}$ in slope and bottom mesocosms increased with additions of C and P by 16% and 42% respectively, while decreasing by 16% in top mesocosms (Table 3). Similarly, Specific $Growth_{mic}$ was increased by 21% in slope mescosoms and 34% in bottom mesocosms. Top mesocosms were again responding differently and showed no changes in Specific $Growth_{mic}$ between the control and the CP treatment (Fig. 13).

CUE was not significantly affected by C or P additions in any of the three topographies (Table 3, Table 4).

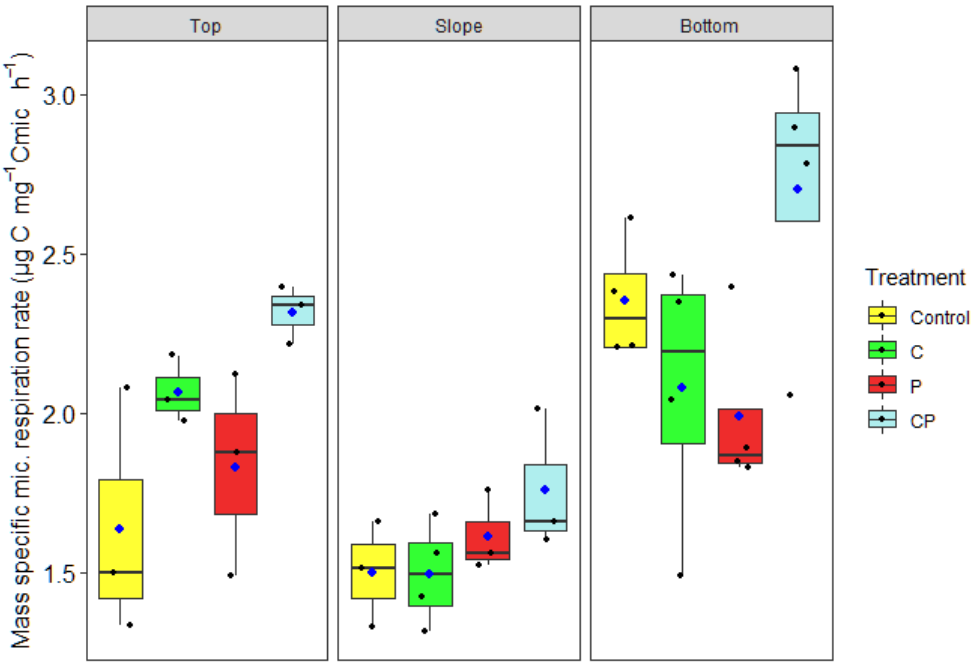

Fig. 12 Mass specific microbial respiration rates (µg C mg^{-1} C_{mic} h^{-1}) for three topographies (top, slope, bottom) and treatment (C, carbon addition; P, phosphorus addition; CP, combined carbon and phosphorus addition; Control, no additions) of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 4 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 4.

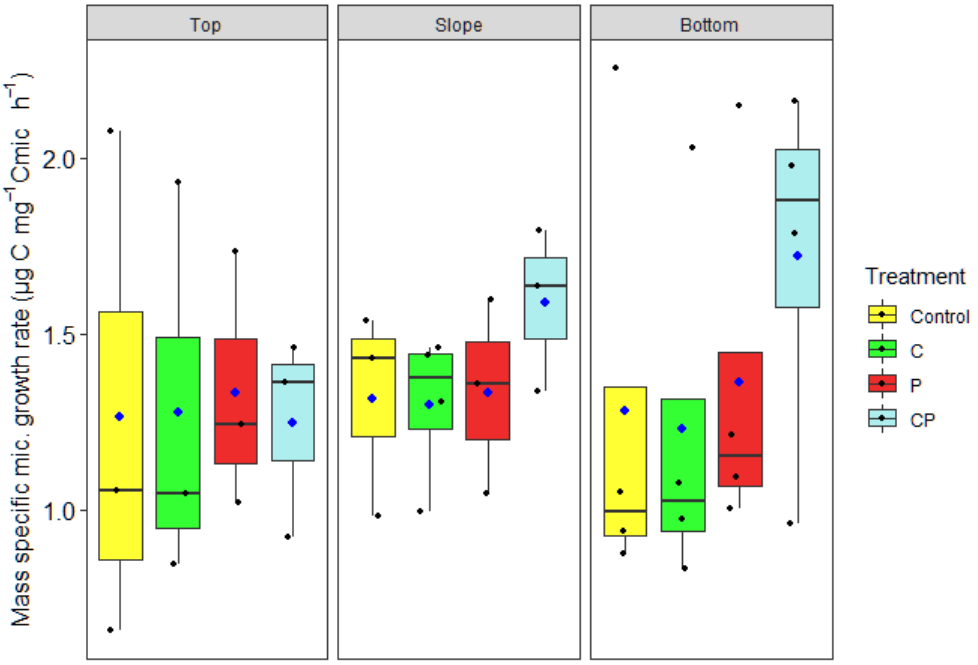

Fig. 13 Mass specific microbial growth rates (µg C mg^{-1} C_{mic} h^{-1}) for three topographies (top, slope, bottom) and treatment (C, carbon addition; P, phosphorus addition; CP, combined carbon and phosphorus addition; Control, no additions) of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 4 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 4.

Cumulative Respiration

Overall, the cumulative respiration measured over the duration of the whole laboratory incubation experiment was positively stimulated by both C ($F_{1,27}$ = 6.24, P= 0.019) and P additions ($F_{1,27}$= 34.90, P< 0.001) (Table 4). While P additions alone led to an increase in cumulative respiration in all three topographies, additions of C alone increased cumulative respiration only in top (+16%) and bottom (+15%) mesocosms with slope mesocosms showing no response (Fig. 14). Additionally, despite no significant interactive effect between C and P additions the CP mesocosms had the highest measured cumulative respiration in each topography (Fig. 14).

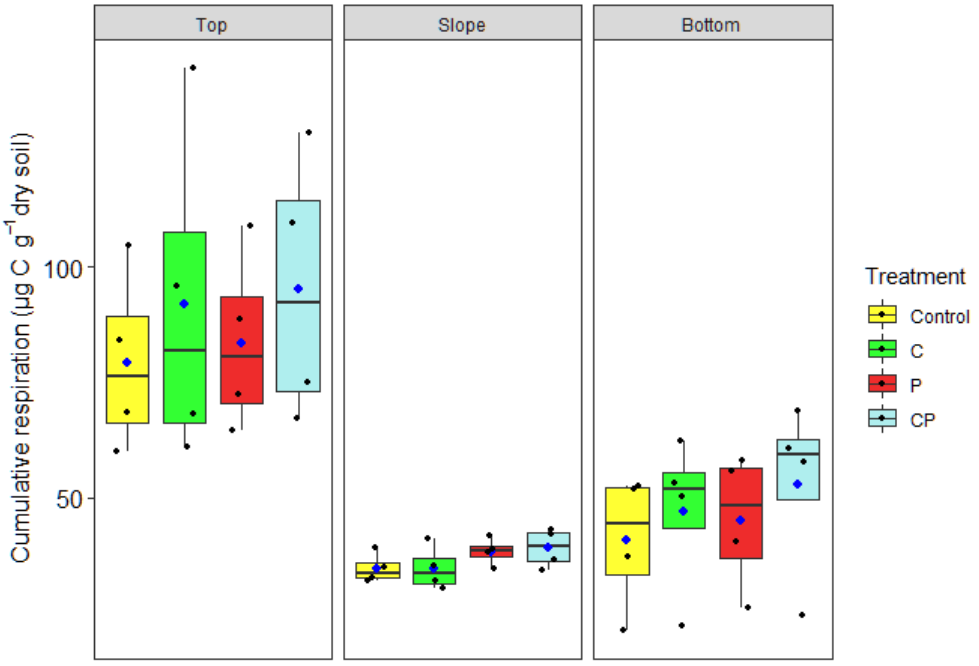

Fig. 14 Cumulative respiration (µg C g^{-1} dry soil) for three topographies (top, slope, bottom) and treatment (C, carbon addition; P, phosphorus addition; CP, combined carbon and phosphorus addition; Control, no additions) of a laboratory incubation experiment with soils from a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 4 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table 4.

Discussion

Nutrient limitations of tropical soil microorganisms

This study aimed at elucidating possible nutrient limitations of microbial communities in highly weathered tropical soils. We initially hypothesized that C would limit microbial growth, as the heterotrophic soil microbiome depends on organic C[49] and because the C:nutrient ratios in such soils are low. To this end we investigated the effects of nutrient additions on mass specific microbial growth rates to exclude the variation of gross microbial growth rates due to differences in microbial biomass. We expected the addition of an organic C source to increase mass specific microbial growth rates in our laboratory incubation experiment. In contrast, we expected the addition of P under controlled laboratory conditions to not affect mass specific microbial growth rates.

The results of our laboratory incubation showed no significant effects of sole C additions on mass specific microbial growth rates in either of the two harvests (Fig. 13, Table 4) contradicting our initial hypothesis of an overall C limitation. The lack of response to C additions alone is particularly evident in the results of our first harvest, in that after three weeks of incubation with cellulose we did not find any differences in mass specific microbial growth rates between the controls and the C-amended incubations (Table S1). However, P additions alone also had no significant effect on microbial growth rates, in line with our initial hypothesis (Fig. 13, Table 4). Although neither sole addition of C or P alleviated microbial growth limitations in our study, combined additions of C and P stimulated mass specific microbial growth rates significantly, at least in soils from the slope and bottom topography (Fig. 13, Table 4).

The response of mass specific microbial growth rates to additions of C or P in this study contradict the idea of single nutrient limitation of microbial growth and showcase a more likely co- limitation of at least C and P. Hence, growth limitations on tropical soil microorganisms do not follow the law of the minimum proposed by Liebig, but rather are more accurately described by the multiple resource limitation hypothesis developed by Tilman and Gleeson[36].

Earlier incubation studies on similar tropical French Guianese soils, both *in-situ* and under laboratory conditions, already found evidence for a limitation of microorganisms by multiple resources[69–71]. Fanin et al.[70] measured net microbial biomass changes in response to additions of C, N and P and found a synergistic effect of P with both C and N. Soong et al.[71] incubated French Guianese soils with litter and mineral nutrients in a laboratory incubation experiment and found an overwhelming influence of litter addition on respiration. However, in the absence of litter addition the combined additions of N, P and K significantly increased decomposition of SOM. The authors therefore deduced a possible co- limitation of soil microorganisms by both C and mineral nutrients.

Although earlier studies found hints towards multiple resource limitation of tropical soil microorganisms the conclusions were based on microbial biomass and respiration measurements. Changes in these parameters in response to nutrient additions can serve as indicators for microbial limitations. However, they cannot directly be linked to the actual alleviation of nutrient limitation[72].

The results presented in this study showcase the decoupled response of mass specific microbial growth rates vs. microbial respiration or changes in microbial biomass. We observed effects of sole additions of C and P on mass specific microbial respiration rates (Fig. 12, Table 4), which was not the case for mass specific microbial growth rates (Fig. 13, Table 4). These differences are particularly pronounced in soil mesocosms from top positions were nutrient additions had no effect on microbial growth (Fig. 13), but additions of each, C and P, increased mass specific microbial respiration by 26% and 12% respectively, and by even 41% when both elements were added simultaneously (Fig. 12). Moreover, this was also evident when heterotrophic respiration was calculated by unit of soil mass, where a stimulation of both sole and combined addition of nutrients was observed (Fig. 14, Table 4). Hence, microbial respiration, both per unit soil mass and microbial biomass, exhibits a decoupled response to nutrient additions from microbial growth. To understand this asynchronous response we have to first consider that microbial respiration consists of respiration for growth ($Resp_G$), maintenance ($Resp_M$), enzyme production ($Resp_E$) and overflow respiration ($Resp_O$)[50,52]. As we observed no increase in mass specific microbial growth rates enhanced microbial respiration rates after nutrient additions cannot be caused by higher $Resp_G$.

Moreover, while $Resp_O$ could occur after additions of C in the absence of nutrients, the additions of P is unlikely to lead to overflow respiration. Increases in mass specific microbial respiration to single nutrient additions are therefore likely caused by an increase in maintenance ($Resp_M$) and/or enzyme production ($Resp_E$), indicating an increase in microbial activity that is not accompanied by growth. Moreover, while mass specific microbial growth was increased with combined additions of C and P we observed no changes in microbial biomass C (Fig. 9, Table 4). This is most likely due to a concomitant increase in microbial biomass turnover rates, which may offset the increase in microbial growth and result in no net change in biomass.

Here, we could confirm the existence of a previously postulated growth limitation by multiple resources by measuring mass specific microbial growth rates. The existence of a multiple resource limitation has previously been proposed for plants[86]. The cause for a possibly prevalent multiple resource limitations among organisms is thought to be found in the fact that in natural (nutrient limiting) environments, co-limitation offers a competitive advantage for organisms as energy expenditure into the uptake of non-limiting nutrients is minimized. The occurrence of a nutrient co-limitation of microorganisms in soil can therefore be interpreted as a logical consequence of an energy limitation and a profound adaptation of tropical soil microorganisms to low C and P availability. Furthermore, as other minerals like K, Ca, Mg and some trace minerals are similarly in low abundance in highly weathered tropical soils[25,26], microorganisms are possibly co-limited by other elements than C and P as well. The identity and number of limiting nutrients could furthermore be dependent on factors such as topography, microclimate and soil physical or chemical characteristics, such as clay content or pH.

Microbial adaptations to a low energy-environment

The results of our laboratory incubation do not suggest a sole P-limitation based on the measurements of mass specific microbial growth rates (Fig. 13). However, we could observe a strong immobilization in the microbial biomass (i.e., increase in P_{mic}) with the addition of readily available P-sources (Fig. 11, Table 4). This immobilization was not accompanied by an increase in microbial C (Fig. 9,

Table 4), showing that the microbial community did not exhibit net growth. This was leading to a change in microbial stoichiometry. Both microbial C:P and N:P ratios drastically decreased within six days after P-amendments in our laboratory incubation experiment, while C:N ratios remained stable across treatments (Table 3, Table 4). This accumulation of P was not only a short term response to sudden increases in P availability, as even lower microbial C:P and N:P ratios were found in the *in-situ* nutrient manipulation experiment after four years of P additions (Table 1). Hence, accumulation of P seems to be a consistent strategy of microorganisms in tropical soils and may be important to cope with the environmental conditions prevalent in highly weathered tropical soils. The efficient immobilization of P in microbial cells raises the question why soil microorganisms bother to take up and store P without using it for their growth. To answer this question we must first consider the form in which P is stored in microbial cells. Our results show that the vast majority of microbial P is inorganic, as organic P was consistently low and sometimes not even detectable in our measurements (data not shown).We know from previous studies, particularly by Kornberg et al.[87], that the most common inorganic P-compound found in microbial cells are inorganic polyphosphates (PP_i), which, even though not confirmed by molecular analysis, probably make up most if not all of the microbial inorganic P found in our study. PP_i are polymers which consist of phosphates linked together by a highly energetic anhydride bond and are found in bacteria, archaea as well as fungi such as arbuscular mycorrhizae[88] and yeast[87]. The size of PP_i can vary from tens to many hundreds of phosphate units and they have been reported to account for up to 20% of dry mass in yeast[87]. Moreover, PP_i are hypothesized to be one of the oldest molecule classes and are ubiquitously found in nature[89–91]. While the large increase in inorganic P is likely due to an accumulation of PP_i in microbial cells it is not entirely clear what benefits it brings. The purpose of PP_i is not well documented in the literature and only few studies were conducted on the possible functions of these molecules. However, PP_i has been reported to serve as an alternative energy source to ATP and has been shown to phosphorylate adenosine di-phosphate to ATP and other biomolecules like glucose and even some proteins in bacteria[92]. It is also thought to be important in metabolic pathways linked to stress tolerance; for example, bacteria such as *E. coli* mutants unable to produce

PP_i showed a higher vulnerability to stresses such as nutrient deficiency and heat[93]. Reported interactions between PP_i and proteins could be an indicator of regulatory functions on gene expressions leading to these observed effects on stress tolerance. Another more obvious function of PP_i is as reservoir for phosphorus. To store P as PP_i is osmotically advantageous over free phosphorus and can therefore be used to accumulate P in times of high availability. In addition, storage of PP_i does not require carbon. In tropical soil microorganisms the high accumulation of PP_i is likely an adaptation to a low energy environment as both P and C are comparably low in highly weathered tropical soils. While initially not used for growth PP_i could serve as an alternative, carbon-free energy storage compound, optimizing C use towards growth. Particularly arbuscular mycorrhizal fungi, known to accumulate PP_i rapidly when P is available[88], could be storing P rather than transferring it to host plants. Plants in temperate regions are known to downregulate C transferred to their fungi symbiont when inorganic P is available in sufficient quantities[94]. Arbuscular mycorrhizal fungi could in return stop the transfer of P to plants as they gain no C in return and rather store P in the form of PP_i. C and P thus exhibit similarities in their role as an energy source, which needs to be taken into consideration when trying to disentangle the effect of microorganisms to single and multiple nutrient additions. In our incubation study only supplying both C and P to mesocosms soil microorganisms yielded a significant increase in growth, while sole addition of either element did not suffice in alleviating growth limitations. Thus, tropical soil microorganism seem to be limited by both C and energy (the latter can be alleviated by P), but most likely not by P for the buildup of biomass.

The not so uniform lowland tropical forests: The influence of topography

While we found an overall positive effect of C and P additions on microbial growth rates our results demonstrate that responses to nutrient limitations depended on topography.

In this study we included a topographical gradient in order to account for the natural variation of soil parameters in lowland tropical rainforests particularly in P-availability. Prior studies at our site in French Guiana have shown that clay-rich top soils, while rich in total C, N and P, were impoverished in available P in comparison

to sandier bottom soils[79]. This is likely the effect of an increased amount of Fe- and Al-oxides in top soils leading to an increase in P bound to minerals[22]. The implication of this gradient in available P is a more likely P-limitation in clay-rich top soils than in sandier bottom soils. However, we observed no significant effect on mass specific microbial growth rates of either C or P addition in top mesocosms (Fig. 13, Table 4).

One possible explanation for this lack of a response among top mesocosms could be the existence of other limiting elements. Minerals like K, Ca, Mg, S, molybdenum (Mo) and other trace elements are similarly in low abundance in highly weathered tropical soils[25,26], as they are also replenished mainly from parent material. While only few studies evaluated the effect of these elements on tropical soil microorganisms some long-term studies do exist. For example, Barron et al.[95] conducted a long-term experiment in Panama to identify the influence of specific micronutrients and found a significant positive effect of molybdenum (Mo), a key component of the enzyme nitrogenase, on N_2- fixing microorganisms. Other studies found positive effects of combined P and zinc (Zn) addition on decomposition rates[96], a positive effect of K- addition on cellulose decomposition[97] or a positive effect on microbial respiration with sulfur (S) and K- addition[98].

As these elements are important for microbial physiology (e.g., K as an important intracellular osmoregulator or Zn as an important co-factor in a variety of enzymes[99]) they very well might be limiting or co- limiting growth of the overall microbial community in tropical soils. The French Guianese top soils used in this study are therefore possibly (co-) limited by such nutrients rather than by C or P alone.

An alternative explanation for the lack of a response in top soils could be a stronger immobilization of C and P through Fe- and Al-oxides leading to a decreased availability of energy-rich compounds in the soil. If tropical soil microorganisms are limited solely by carbon and energy, the reduced availability of these elements in top soils could have left microorganisms beyond the threshold needed for further growth. While this is speculative as we did not determine soil texture of the individual soils used in this study, we did observe less increase of P_{mic} and available (dissolved) P in the soil of top mesocosms than in slope and bottom mesocosms one week after P was added (Fig. 11, Table 3). The discrepancy in

measureable P_{mic} and DIP between top soil and the other topographies becomes even more apparent when considering that the amount of P added per g dry soil was highest in top soils. Moreover, C could have been similarly to P bound to Fe- or Al-oxides in top soils, which would decrease available energy sources compared to slope and bottom soils. Thus, in contrast to slope or bottom soils, where energy limitation was alleviated through nutrient additions, top soils were still energy limited and additions of C and P increased microbial activity, but not microbial growth.

Effects of four years of nutrient addition on tropical soil microorganisms in an *in-situ* experimental design

Through our laboratory incubation experiment we could observe a significant effect of C and P additions on microbial growth rates. While this points to a co- limitation by C and P, or a C and energy limitation of microbial communities in tropical soils, it is also important to consider that *in-situ* soil microorganisms depend closely on plants as they are the main source of C inputs. Hence, positive effects of nutrient additions on plants could indirectly benefit soil communities via increased inputs of C through litter and root depositions. Multiple studies reported beneficial effects of P additions on tropical primary production leading to the assumption of a widespread P limitation on plant growth in the tropics[27,56–59]. We therefore hypothesized that P additions in our *in-situ* experiment would increase primary production, which would subsequently increase microbial growth.

Results of mass specific microbial growth rates in our *in-situ* experiment showed a general increase with P additions alone, which was masked by a negative interaction effect with N additions (Fig. 8, Table 2). This effect of P additions was not observed in our laboratory incubation and may therefore be attributed to an increase of plant C inputs into the soils. Additions of P have been reported to increase litter fall in tropical rainforests[100], which may lead to a stronger influx of C into the soil, and could possibly also increase root turnover. The greater supply of both C and P would again alleviate carbon and energy limitations on microbial growth, explaining the different response in our laboratory incubation and *in-situ* experiment to P additions. This is of course a very simplistic view as plant adaptations to increased P supply are known to be notoriously complex. For

example, studies have shown a decreased association of plants with arbuscular mycorrhizal fungi in response to mineral P additions in a tropical rainforest in Panama[101] indicating that plants reduced C allocated into the soil as root depositions. However, Lugli et al. found no effect of P additions on arbuscular mycorrhiza colonization[59]. Plant C- allocation into the soil might be further affected by plant investments into fine root biomass. This response has been shown to be highly variable as previous nutrient addition experiments found P additions both to increase[59] and decrease[102] root biomass or show no effect at all[103].

Hence, as we did not measure effects of nutrient additions on litter production and root deposition the possible cause of increased microbial growth due to P additions in our *in-situ* experiment is speculative.

Moreover, N additions surprisingly had a strong effect on mass specific microbial growth rates, particularly in slope plots (Fig. 8, Table 2). While we observed no significant effect of N overall, we argue that this is again masked by the negative interactive effect of N and P additions as N additions alone let to moderate increases of top and bottom plots and even increased mass specific microbial growth rates by 55% in slope plots. This increase was unexpected as tropical rainforests are generally assumed to be relatively rich in N compared to P, and show signs of N losses[104–106]. The underlying reason for the apparent effect of N additions alone is difficult to interpret as we did not include N additions in our laboratory incubation experiment and are thus unable to disentangle direct and indirect effects of nutrient additions. However, we argue for a more likely indirect effect via increased plant inputs into bulk soil, as N is generally highly enriched in tropical rainforest soils and would therefore not limit microbial growth.

Summary and Conclusion:

The increase in mass specific microbial growth rates in the laboratory incubation experiment by the addition of both C and P suggests either a multiple resource limitation or a carbon and energy limitation of tropical soil microorganisms. P can serve as an alternative energy source to organic C in the form of polyphosphates (PP_i). Thus, the strong accumulation of P reported in this study might be the consequence of a profound energy limitation on microbial growth, which was alleviated in the laboratory incubation when both C and P were supplied. However, energy has to be invested first to build-up PP_i from added P_i, thus energy gains from P_i uptake are not possible as they are from organic carbon uptake. However, PP_i can be used to store energy for growth.

Multiple resource limitation is a possible explanation for the significant increases in microbial growth rates with combined additions of C and P. On the other hand, our results also demonstrate that microbial communities are able to store P beyond their immediate need, even in soils that are depleted in available P and even in the presence of strongly P-limited plant communities. P is strongly depleted in highly weathered tropical soils and thus could have led to a strong selection for fast P uptake among soil microorganisms when made available. This strategy seems to be applied by soil microorganisms even when P is not able to be used for growth as we observed drastic decreases of C:P and N:P ratios in P-amended mesocosms, which were not accompanied by growth. However, as mentioned before P is very likely stored as PP_i in microbial cells and thus the formation of PP_i from P_i would need an investment of energy (organic C) by microorganisms. The initial investment of converting available P_i into PP_i could make sense when other elements than C would be (co-) limiting or if the environmental conditions would be unfavorable for growth. In this scenario, conserving energy in the form PP_i for later use could offer significant advantages.

Topography had a strong effect on the responses to nutrient additions overall. We did not observe any change in mass specific microbial growth rates in clay- rich top soils in our laboratory incubation experiment. This may indicate a strong limitation of microbial growth by nutrients other than C and P, or by environmental factors that we did not analyze. Intensive weathering in tropical soils does not only lead to

the depletion of P, but has also been reported to affect the availability of many micronutrients essential to soil microorganisms such as K, Ca, Mg, S, and Mo. Additionally, the high amount of Fe- and Al-oxides present in top soils could have led to a strong immobilization of C and P further reducing their availability for soil microorganisms. The strong effect of topography on mass specific microbial growth indicates the importance of considering differences in physicochemical variations among tropical soils.

By comparing the results of our laboratory incubation with results gained from the *in-situ* nutrient manipulation experiment we aimed to distinguish between direct effects of nutrient additions and indirect effects resulting from plant-microbe interactions. Additions of P alone increased mass specific microbial growth rates in top, slope and bottom soils by 22%, 10% and 37% respectively. The positive effect of P additions was not observed in our laboratory incubation and can thus be interpreted as an indirect effect by plants. However, we cannot directly link the increase in microbial growth to increased plant C inputs, as neither litter fall nor root exudation were measured during the experiment. An alternative explanation for the differential effect of P amendments in the laboratory and field is the different time scale of observation that ranged from days/weeks (in the laboratory) to years (in the field).

Finally, the results presented in this study demonstrate that the response to nutrient additions can vary greatly when different approaches are used to evaluate microbial limitations, such as microbial growth rates, net changes in microbial biomass, or microbial respiration. Thus, a clear definition and a conceptual framework are needed when working on microbial nutrient limitations.
A recent meta- study carried out by Camenzind et al.[61] gathered information on microbial responses to nutrient additions of N and P in tropical soils. Most study sites were located in the Neotropics and microbial parameters included microbial respiration and biomass measurements as well as rates of decomposition, free-living N fixation, N mineralization, net nitrification, soil methane uptake and P immobilization. While the meta- study revealed overall no effect of N additions on the aforementioned microbial parameters, P additions stimulated a positive effect

particularly in microbial respiration and biomass. This positive effect could be rooted in the alleviation of nutrient limitation on microbial growth, however, as demonstrated in our results, could also result in a misinterpretation of microbial nutrient limitations.
Hence, commonly used parameters such as microbial respiration and biomass should be interpreted with caution, as already discussed by Mori et al.[72].

The highly differentiated responses of microbial biomass, respiration and growth suggest a complex regulation of microbial physiology in highly weathered tropical rainforest soils. This demands for a holistic approach integrating microbial biomass, respiration and growth data to correctly assess microbial limitations. Only focusing on either one of these parameters is not sufficient in eliciting microbial nutrient limitations.

Supplement

Table S1 Statistical summary of the effects of topography, C- addition and the interaction between topography and C- addition (Topo x C) for the laboratory incubation experiment before phosphorus was added (pre- incubation). Depicted are F-values and p-values from a linear mixed effect model, p- values < 0.05 are highlighted in bold).

	Topography	**C- addition**	**Topo x C**
DOC	$0.71_{2,9}$; 0.518	$1.74_{1,33}$; 0.196	$2.28_{2,33}$; 0.118
TDN	**$12_{2,9}$; 0.003**	$1.22_{1,33}$; 0.277	$0.03_{2,33}$; 0.97
DIP	**$16.1_{2,9}$; <0.001**	$0.46_{1,33}$; 0.502	$1_{2,33}$; 0.379
Mic. C	$3.29_{2,9}$; 0.084	$1.05_{1,33}$; 0.314	$0.01_{2,33}$; 0.991
Mic. N	$0.94_{2,9}$; 0.427	$0.18_{1,33}$; 0.671	$0.12_{2,33}$; 0.886
Mic. P	$3.72_{2,9}$; 0.067	**$6.88_{1,33}$; 0.013**	$1.05_{2,33}$; 0.362
Mic. C:N	$0.06_{2,9}$; 0.944	$1.22_{1,33}$; 0.278	$0.21_{2,33}$; 0.814
Mic. C:P	**$9.14_{2,9}$; 0.007**	$0.66_{1,33}$; 0.423	$2.8_{2,33}$; 0.075
Mic. N:P	**$7.08_{2,9}$; 0.014**	$2.63_{1,33}$; 0.114	$0.10_{2,33}$; 0.901
$Resp_{mic}$	**$25.32_{2,9}$; <0.001**	**$7.6_{1,30}$; 0.01**	**$4.91_{2,30}$; 0.014**
Specific $Resp_{mic}$	**$4.49_{2,9}$; 0.044**	$2.330_{,30}$; 0.138	$2.01_{2,30}$; 0.152
$Growth_{mic'}$	$1.88_{2,9}$; 0.208	$0.230_{,30}$; 0.642	$0.17_{2,30}$; 0.848
Specific $Growth_{mic'}$	$2.05_{2,9}$; 0.185	$0.16_{1,30}$; 0.69	$0.18_{2,30}$; 0.838
CUE	$3.48_{2,9}$; 0.076	$2.23_{1,30}$; 0.146	$0.95_{2,30}$; 0.399

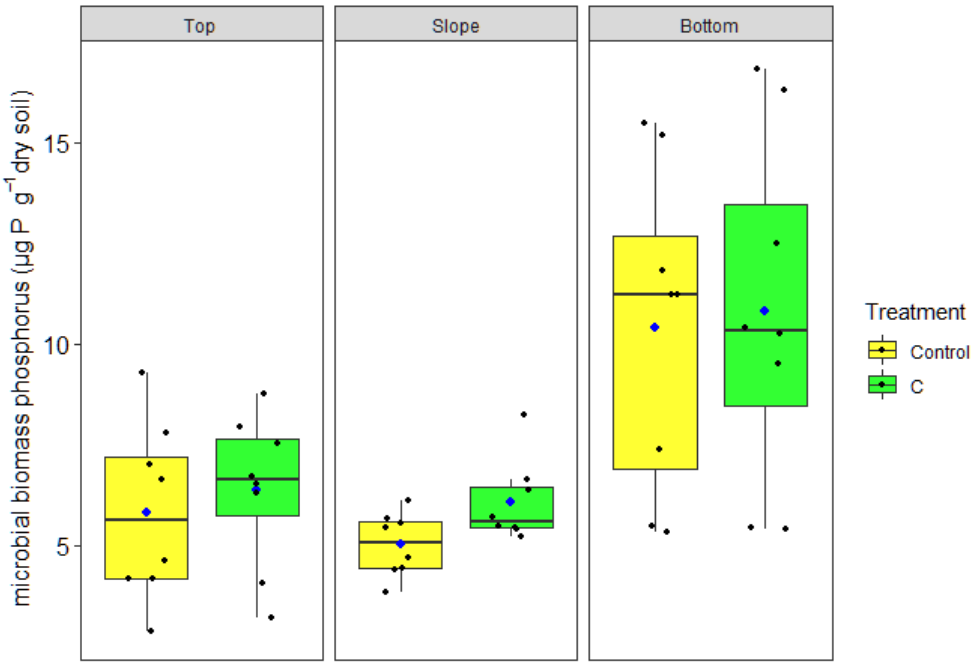

Fig. S1 Microbial biomass phosphorus (µg P g dry soil) for three topographies (top, slope, bottom) and treatment (C, carbon addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 8 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table S1.

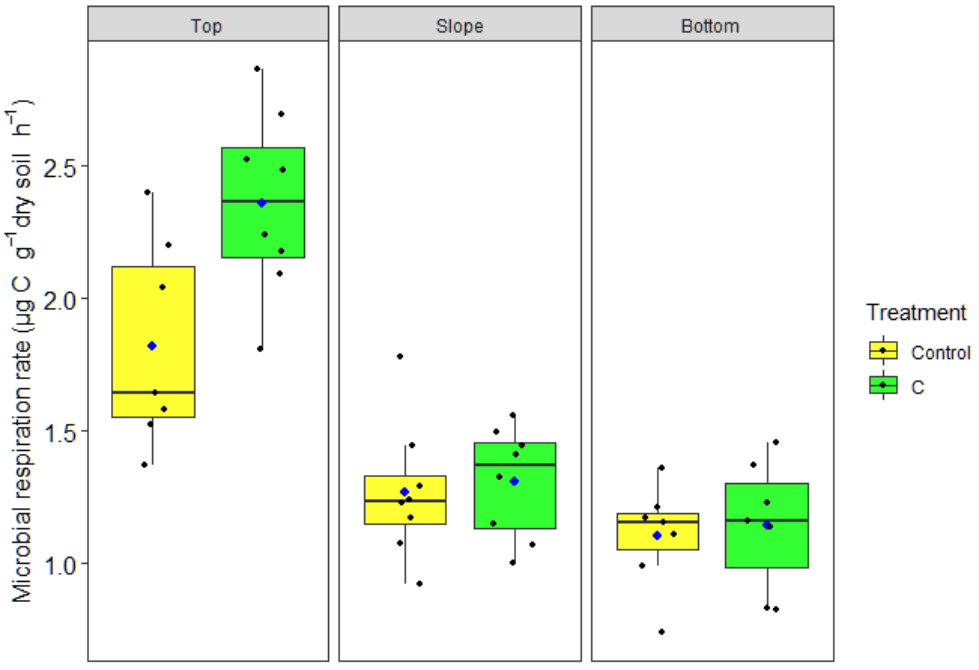

Fig. S2 Microbial respiration rates (µg C g dry soil h^{-1}) for three topographies (top, slope, bottom) and treatment (C, carbon addition; Control, no additions) of a lowland tropical forest in Nouragues, French Guiana. Boxes represent values from the first to the third quartile of 8 replicates. Anchors represent the range from lowest to highest value. The horizontal lines in each box plot represent the median and blue dots represent the mean. For statistics, see Table S1.

Part 3

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