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Running Title: SOIL MICROBES AND P IN AN AMAZONIAN RAIN FOREST

Short-Term Microbial Responses to Soluble Inorganic P Input in a Tropical Lowland Rain Forest in Amazonia

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ABSTRACT

In non-flooded lowland rain forests on low phosphorus (P) soils in parts of Amazonia, P cycling largely occurs by way of recycling from litter by arbuscular mycorrhizal (AM) fungal symbionts. Occasional high input of P into these ecosystems occurs during drought years with increased litterfall. As the length and frequency of drought events is projected to increase in the region, we carried out a single-dose nutrient addition experiment to test how this would impact P cycling. We applied 4 kg P ha^{-1}, which corresponds to twice the amount of litter-derived P in an average year. We hypothesised that (i) the added mineral P would be immobilised by soil microorganisms, leading to measurable increase in microbial biomass C and P, and that (ii) mycorrhizal colonization rates would be reduced.

The results did not support either of our hypotheses. The addition of P did not have an effect on AM root colonization, nor was P immobilised in the soil microbiota during the experimental period. The lack of a difference between control and treatment at our study site could be attributed to the relatively low one-off dose of P applied that did not change either the colonization rate of roots by AM fungi or the amount of soil available labile P. To obtain a mechanistic understanding of the availability, capture, and use of P by plant-symbiont associations in tropical rain forest ecosystems, further integrated studies of the soil-plant system combining longer-term nutrient manipulations, modeling, and experimental approaches are required.

Key Words: Amazon basin, arbuscular mycorrhizal fungi, nutrient addition, soil microbial biomass, tropical lowland evergreen rain forest

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INTRODUCTION

Mutualistic associations between plants and arbuscular mycorrhizal (AM) fungi are common in most tropical forests (Smith and Read, 2008) where they have important roles in nutrient cycling and productivity. The symbiosis helps explore the soil and thus enhances nutrient and water uptake by plant roots (Bolan, 1991; Finlay, 2004), thereby alleviating potential growth limitation. Mycorrhizal fungi represent a key component in the global carbon cycle (Nottingham *et al.*, 2013); by obtaining plant photosynthates, they contribute to heterotrophic soil respiration and release substantial quantities of CO_2 to the atmosphere (Nottingham *et al.*,

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2010). As the carbon (C) cycle is coupled with that of other elements such as phosphorous (P) and nitrogen (N), the functioning of AM fungal symbiosis directly affects both P and N cycles (Townsend *et al.*, 2011).

In the tropics, N is mostly limiting in montane forests (Corre *et al.*, 2010) while both N and P are in short supply in heath forests on podzolic 'white sand' soil (Mardegan *et al.*, 2009). Low available P in non-flooded lowland evergreen rain forest soils in Amazonia (locally known in Brazil as *terra firme*) results from the predominance of weathered soils, except for those mainly in western Amazonia, which receive inputs from ongoing erosion processes in the Andes (Quesada *et al.*, 2010).

Tropical forests stock and sequester large amounts of carbon, both above- and below-ground and they contribute substantially to the regulation of the global climate system (Field *et al.*, 1998). Net primary production in large parts of lowland Amazonia is considered to be related to, and appears to be limited by, the availability of soil P (Vitousek, 1984; Quesada *et al.*, 2012). It has been assumed that, in Amazonian *terra firme* forests, there is a 'tight' P cycling, which relies on the recycling from litter P by AM fungal symbionts (Herrera *et al.*, 1978; Aristizábal *et al.*, 2004); conversely, there appears to be a 'leaky' N cycling, often construed as N excess (Vitousek, 1984; Hedin *et al.*, 2009; Brookshire *et al.*, 2012). While the general pattern of AM fungal occurrence and diversity in tropical forests is largely unknown (Alexander and Selosse, 2009), it is widely assumed that the relative availability of C, N, and P determine the symbiotic function of AM fungi (Johnson, 2010). Some nutrient addition experiments have explored the nature and extent of nutrient limitations on tropical forest growth (Mirmanto *et al.*, 1999; Wright *et al.*, 2011) and on compositional and functional aspects of soil microorganisms (Tanner *et al.*, 1992; Fanin *et al.*, 2015; Buscardo *et al.*, 2018b) and AM fungi (Kivlin *et al.*, 2011; Liu *et al.*, 2013; Wurzburger and Wright, 2015). While it has been suggested that the levels of AM fungal root colonization can be reduced by the addition of P and N (Treseder and Vitousek, 2001; Pagano and Scott, 2010), the majority of these experiments, with the exception of some studies that looked at the medium- to long-term effects of litter manipulation on soil microbiota (Nemergut *et al.*, 2010; Sheldrake *et al.*, 2017), have used large and often unrealistic quantities of one or more nutrients, leading to stoichiometric imbalance of microbial resources (Fanin *et al.*, 2017). The input of P in lowland tropical rain forests can potentially increase during occasional severe droughts through a pulse of nutrients associated with an increased litterfall input (Mirmanto *et al.*, 1999; O'Connell *et al.*, 2018). This increase has two components: the higher quantity of litter that reaches the forest floor and its greater P concentration that results from less P resorption before leaf fall than in average climate years (Brando *et al.*, 2008; Cleveland *et al.*, 2010).

The length of dry season and occurrence of drought periods is relevant in the context of projected climate change impacts. Across Amazonia, profound changes in rainfall quantity and patterns and temperature have been projected by recent model experiments, whether using dynamic vegetation models that couple atmospheric and soil processes, or those that consider land cover / land use change (deforestation; Marengo *et al.*, 2016). According to projected changes, drought frequency and severity could increase and may lead to changes in the characteristics of the ecosystems of the Amazon basin. This would necessarily involve changes in biogeochemical cycles.

To simulate the pulse of P associated with an increased litterfall input during a drought period, we undertook a P addition experiment in a *terra firme* forest stand in Amazonia during the dry season. A single-dose of P, corresponding to *ca*. twice the yearly amount of litter-derived P in *terra firme* forests (*ca*. 2 kg ha^{-1} $year^{-1}$; Buscardo *et al.*, 2016) was applied to 1 m x 1 m plots under a variety of tree species. This was comparable with the values expected in large and P-rich litter input during occasional drought years. To test the impact of P addition on soil microorganisms, we repeatedly measured in these plots soil microbial biomass C and P and AM fungal colonization to target the very short-term P effects in a 4-month period that was not coincident with the seasonal pulse-nutrient input.

We hypothesised that (i) the added mineral P would be immobilised by soil microorganisms, leading to measurable increase in microbial biomass C and P, and (ii) mycorrhizal colonization rates would be reduced by the pulse in mineral P available for plant uptake.

MATERIALS AND METHODS

Study area

The experiment was carried out in the Adolfo Ducke Forest Reserve (RFAD), located 26 km north-east of Manaus, Brazil. The Reserve covers about 100 km^2 of natural forest and it is characterized by a varied topography, with valleys and plateaux whose elevation ranges in between 40 and 140 m a.s.l. The climate is classified as Af Köppen's type (Kottek *et al.*, 2006), with a period with mild hydrologic deficit (<100 mm

month^{-1}) ranging from one to three months. The annual precipitation is on average 2100 mm while mean annual temperature is around 26 °C (precipitation and temperature obtained from RFAD meteorological station in 2014, the year of study, were 2760 mm and 24.1 ˚C; Fig. 1).

The soil in the well-drained plateau areas is classified as oxisol (Chauvel *et al.*, 1987) and it supports a tropical lowland evergreen rainforest with an emergent canopy that reaches 30-40 m, and an understory characterized by many palms (Ribeiro *et al.*, 1999). The annual litterfall has been estimated at 8 Mg ha^{-1} year^{-1} (Luizão, 1989) corresponding to an average input of P of *ca*. 3 kg P ha^{-1} year^{-1}, the Amazon basin-wide average being about 2 kg P ha^{-1} year^{-1} (Buscardo *et al.*, 2016).

Fig. 1

Fig. 1 Precipitation (unfilled bars) and temperature (broken line) in a lowland evergreen rain forest in the Adolfo Ducke Forest Reserve (ADFR), Manaus (Brazil), 2014. Source: meteorological station of ADFR. Black dots indicate soil sampling dates.

Field plots and experimental treatment

Ten trees were selected randomly as reference points along a 1600-m-long section of a plateau area, with a minimum distance between them of at least 40 m to avoid spatial autocorrelation of soil variables between plots (Buscardo *et al.*, 2018a). The random variety of target tree species served to represent the conditions that arise from high species richness that characterizes these ecosystems. At each point, two 1 m x 1 m plots (a control and a treatment) were established at a distance of 1.5 m from the reference tree, in a way so that the distance between the plots would be maximised. The treatment plots received an equivalent of 4 kg ha^{-1} year^{-1} of P in a single-dose in the form of $NH_4H_2PO_4$. The use of this compound resulted in concomitant addition of the equivalent of 2 kg ha^{-1} year^{-1} of N that could potentially have masked the effects of P. However, the amount of P received from litterfall in Amazonian *terra firme* forests on oxisols is about 2 kg ha^{-1} year^{-1}; in the meantime, litterfall returns, on average, 100 kg N ha^{-1} year^{-1} , not counting any potential atmospheric input and biological N_2-fixation (Buscardo *et al.*, 2016). In other words, the experimental treatment doubled the annual input of P and was < 2% of the annual input of N, the equivalent of about 7 days' worth of mean litterfall in a year, thus ecologically negligible. $NH_4H_2PO_4$ was added in 1 L of aqueous solution on 16 June 2014. Since 1 L of solution represented 0.5% of the previous 30-day average rainfall in the area, the control plots were not watered. To prevent leaching by rainfall of the applied P, fresh litter was carefully removed in a way so as not to disrupt too much fungal hyphae (Posada *et al.*, 2012), before the application of the fertilizer and replaced immediately afterwards.

Soil sampling

The period of the experiment spanned the transition from the rainy to the dry season until the transition from the dry to the wet season. This period was chosen so that the pulse of mineral P addition would not coincide with the natural peak of nutrient pulse, after peak litterfall, at the beginning of the rainy season. This way we expected to observe the effects of the P addition without confounding background effects. Soil samples were taken at transition period between the rainy and dry season (pre-fertilization, day 01), at the beginning of the dry season (day 20), at the peak of the dry season (day 88), and at the transition period between dry and wet season (day 119). Each plot was subdivided into 15 subplots and three of them were randomly sampled with a volumetric ring of 5 cm in diameter and 5 cm in depth at each sampling (during the period of the experiment each subplot was sampled only once); the samples were bulked in a soil composite sample per plot. Soil samples were stored in plastic bags and taken to the laboratory within the same day and processed.

Soil physical and chemical analyses

A physico-chemical characterization of the soil at each reference tree was carried out. Particle size analysis was made with 10 g of air dried soil, following the pipette method (Gee and Bauder, 1986). Exchangeable cations were determined by extraction from 5 g of air-dried soil, using the silver-thiourea method (Pleysier and Juo, 1980), followed by atomic absorption spectrophotometry (AASpectrometer Thermo Scientific iCE3000 Series). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined in lanthanum solution while sodium (Na^{+}) and potassium (K^{+}) dilution were made in cesium chlorite solution. For the

quantification of aluminum (Al^+), the method of EMBRAPA (2009) was followed – soil extraction was made using 5 g of dried air soil in 1 M KCl solution and Al^+ was then titrated with 0.025 M NaOH solution.

For the determination of soil density (D), soil samples (0-5 cm) were collected with a volumetric ring outside of, but close to the edge of the plots, and dried in an oven at 105 °C for 48 h (or until constant weight). Soil density was determined by the formula: D (g cm^{-3}) = mass of dry soil / ring volume. For the determination of soil volumetric water content (θ) a fresh sub-sample of 5 g of soil was brought to a temperature of 105 °C for 24 h immediately after collection. Volumetric water content (cm^3 water cm^{-3}) was calculated using the apparent soil density and the weight difference between fresh and oven dried soil. Soil pH was measured with a pH meter (mPA210 - MS Tecnopon) after shaking 10 g of air-dried soil for 1 h in 25 ml of water.

Total soil carbon (C_{total}) and nitrogen (N_{total}) were determined for each plot with a Vario Max CN Analyser (Elementar Analysensysteme GmbH) by using 1 g of milled soil per sample. Due to the insufficient amounts of samples, this analysis could not be made for two of the samples collected on day 119.

Root colonization by arbuscular mycorrhizal (AM) fungi

The rate of colonization of roots by AM fungi was determined using the modified method of Kormanik and McGraw (1982). We selected about 50 segments of fine roots of 1 cm per soil sample. The roots were washed with water to remove soil and organic matter particles and then they were heated for 1 h in a 10% KOH solution to remove cytoplasmic contents. The roots were subsequently cleared in 10% H_2O_2 for 30 minutes, followed by a period of 5 minutes of acidification in a 3.5 % HCl solution. Between treatments the roots were washed with abundant distilled water. Subsequently, they were coloured with a heated solution of trypan blue in lactoglycerol for 1 h. The rate of colonization was determined by using a stereoscopic microscope (40-x). Segments of roots were classified according to the presence or absence of fungal structures. The rate of colonization (defined as the percent of root fragments with AM colonization present) was calculated as the proportion of colonized root segments in relation to the total number roots segments per sample.

Soil microbial biomass carbon

Soil microbial biomass carbon (C_{mb}) was determined using the chloroform fumigation method (Brookes *et al.*, 1985; Vance *et al.*, 1987) and quantified as the difference between the concentration values of C of a sample of 25 g of soil extracted in 1M K_2SO_4, and another one following fumigation with alcohol-free chloroform and incubation for 24 h at room temperature.

Soil P fractions

The determination of soil P fractions was made using the method proposed by Hedley *et al.* (1982) and modified by Tiessen and Moir (1993). For our experiment, we extracted the bicarbonate extractable fraction form the labile soil P pool ($P_{ilabile}$), readily available for plant uptake. The determination of the $P_{ilabile}$ was made spectrophotometrically (1240 Shimadzu, Kyoto, Japan), following the method of Murphy and Riley (1962). For determining P total (P_{total}), an aliquot from the bicarbonate extract was digested with ammonium persulphate and P determined spectrophotometrically. The organic fraction was calculated as the difference between P_{total} and $P_{ilabile}$. The P immobilized in soil microbial biomass (organic P, P_{mb}) was made by calculating the difference between the P_{total} and $P_{ilabile}$ recovered with and without fumigation. At day 88, the calculation for determination of organic P resulted in negative data for two samples; these data were excluded from the statistical analyses.

Statistical analyses

Treatment effects (P addition) on the analyzed parameters were tested with a mixed effect linear model in the R environment (R Development Core Team, 2016), using the following model:

model<-lme(Variable~Day+Treatment, random=~1|Plot).

The temporal dynamics were analyzed by paired t-test, using the difference between the values of the variable of interest in fertilized and control plots. Prior to statistical analyses in order to meet normality and homoscedasticity assumptions, data on mycorrhizal colonization rate, soil C and N, C:N ratio were transformed by using arcsin = $\sqrt{}$ (datum/ 100), and P data by sin $\log_{10}$.

RESULTS

Soil analyses

Soils were clayey in texture (73 ± 1 % mean ± SE, clay; 16 ± 1 %, silt; 11 ± 0.5 % sand) and acidic (pH, 4 ± 0.05; see Table SI for full information). Water content and pH varied over the duration of the experiment, indicating seasonality effects (Fig. 2; Table SII), but there were no significant differences between control and treatment plots either at the beginning of the experiment (i.e. before P application; day 01) or following nutrient addition. The values of θ decreased gradually between day 1 (transition between rainy and dry season, 0.22 cm^3 water cm^{-3} soil) and day 88 (peak of the dry season, 0.19 cm^3 water cm^{-3} soil), to increase again at the transition between dry and rainy season (day 119, 0.2 cm^3 water cm^{-3} soil; Fig. 2). The values of pH were higher at the beginning and at the end of the experimental period (pH, 4.1) while significant lower values were detected at the beginning (day 20, 3.5) and during the peak of the dry season (day 88, 3.7; Table SII).

Fig. 2

Fig. 2 Soil chemical and microbial composition of the top 5 cm soil analysed in response to phosphorous (P) addition in a tropical lowland rain forest in Amazonia (n = 10); day 01, pre-treatment; day 20, beginning of dry season; day 88, peak of the dry season; day 119, resumption of rains. Values are mean (± standard error). θ, volumetric water content; AM root colonization, colonization rate by arbuscular mycorrhizal fungi; P_{mb}, microbial biomass P; C_{mb}, microbial biomass carbon (C); $P_{ilabile}$ plus P_{mb}, soil available P following fumigation; blue, control; red, P addition treatment.

Total soil carbon and nitrogen

Total soil C (C_{total}, 4.62 %, control; 5.03 %, treatment), total soil N (N_{total}, 0.35 % *vs*. 0.37 %) and C:N_{total} ratio (13.2 *vs*. 13.7) did not differ significantly between fertilized and control plots (Fig. 2; Table SIII). However, these elements showed significant seasonal variations along the experimental period (Table SII). Values of C_{total} were significantly higher on day 88 when compared with day 01 and 20 and decreased again on day 119. A similar trend was observed for N_{total} with the highest values at the peak of the dry season and for C:N_{total} ratio that showed the highest values on days 88 and 119.

Soil microbial biomass carbon, available inorganic phosphorus and microbial phosphorus

Soil microbial biomass carbon (C_{mb}) values did not differ significantly between control and fertilized plots (Fig. 2; Table SIII). The last day of collection (day 119), corresponding to the transition period between dry and rainy season, was characterized by the highest values both in fertilized and control plots (342 *vs*. 433 μg g^{-1}). The sum of available inorganic phosphorus ($P_{ilabile}$) and microbial phosphorus (P_{mb}) did not differ significantly between control and fertilized plots at any sampling occasion (Fig. 2; Table SIII). No significant temporal changes were detected in $P_{ilabile}$ plus P_{mb} values along the study period (Fig. 2; Table SII). The P_{mb} fraction did not change significantly between control and fertilized plots (6.13 ± 0.90 *vs*. 5.58 ± 0.62 μg g^{-1}) and along the experimental period between different sampling dates. The C:P_{mb} ratio was also not significantly affected by fertilization (77 ± 12 *vs*. 100 ± 16) and did not follow a temporal pattern (Fig. 2; Table SII).

Root colonization by arbuscular mycorrhizal (AM) fungi

Root colonization by AM fungi was on average 32.8 % in the control and 28.5 % in the P-treated plots, with no significant differences between them at any occasion (Fig. 2; Table SIII). However, significant differences were detected in the colonization rates in the control plots between day 01 and 20, with lower colonization rate values at the beginning of the dry season, and between days 20 and 88 with an increase of the colonization rate during the peak of the dry season (day 88; Fig. 2; Table SIII). The highest root colonization rate was recorded for both control and fertilized plots on day 88.

DISCUSSION

Doubling the input of annual P that reaches the forest floor of an Amazonian *terra firme* forest through litterfall (4 kg ha^{-1} $year^{-1}$) had no effect on either on soil labile P, on microbial C and P biomass, or on the

rate of root colonization by arbuscular mycorrhizal (AM) fungi during the 4-month period that followed the P addition.

Contrary to our first hypothesis (H1), microbial biomass (both C and P) did not increase after the application of P. Soil microbiota in tropical rain forests appears to be limited by P (Cleveland *et al.*, 2002) and generally responds positively by increasing its biomass after P application (Turner and Wright, 2014; Fanin *et al.*, 2015; Zhu *et al.*, 2015), even if, in some cases, the effects are transient (Liu *et al.*, 2013) or no changes are detected due to P saturation (Chen *et al.*, 2014). However, most of the cited fertilization experiments have used high doses of P (50-150 kg P ha^{-1} $year^{-1}$), making it difficult to make comparisons with the results of the current experiment, in which a more realistic, but much lower dose of inorganic P was applied. A litter manipulation experiment conducted in tropical lowland forest in Costa Rica showed that doubling the amount of litter during a 2-year period could increase microbial biomass, both C and N (Nemergut *et al.*, 2010), while there was no P effect in a similar experiment after a 9 years in a *terra firme* forest in Panama (Sheldrake *et al.*, 2017). However, in these cases, it has to be considered that the extra amount of added litter represents a much more complex C and nutrient input than the mere addition of inorganic P. Recent fertilization experiments have suggested that microbial communities in tropical forests could be co-limited by C and P (Barantal *et al.*, 2012; Fanin *et al.*, 2012) and may respond with greater biomass and activity to the increased availability of a labile C source (Camenzind *et al.*, 2018).

Interestingly, the average value of the C_{mb} on the last day of collection in our experiment, at the transition between dry and rainy seasons, was higher than in any of the other sampling occasions, with a more visible trend in the fertilized plots. Generally, the values of C_{mb} tend to be smaller in evergreen tropical rain forests during the dry season, when compared with those in the rainy season (Turner and Wright, 2014). The increase in precipitation frequency in concomitance with our last soil sampling could therefore explain the observed increase in C_{mb}, limited during the dry season by water availability. However, other concomitant factors may have influenced the increase of C_{mb} at the transition between dry and rainy season. Higher values of total soil C and N were observed in soil at the peak of the dry season when compared with the other sampling dates. This could be explained by the fact that, during the driest period of the year, litter accumulates on the forest floor; the start of the rainy season promotes the movement of readily decomposable dissolved organic matter from the litter layer (Cleveland *et al.*, 2006) that becomes available to the soil microbiota (Nottingham *et al.*, 2012). In the present study, the experimental period was chosen to avoid an overlap between the pulse of mineral P addition and the natural peak of nutrient pulse and to be able therefore to observe the effects of the P addition without confounding background effects. Our results indicate however that soil microbes could potentially benefit from an extra P supply exactly after the litterfall peak, at the beginning of the rainy season, when microbial activities tend to increase.

The colonization rate by AM fungi that we observed at our study site (on average 31 %) is comparable to that estimated by Treseder and Cross (2006) for tropical forests (*ca*. 36 %). Contrary to our second hypothesis (H2), the amount of P applied to our experimental plots did not change the rate of root colonization by AM fungi. A study conducted in four Neotropical rain forests over large geographic separation distances and with differing soil fertility and above-ground biomass, has shown that while both root biomass and length were negatively correlated with soil N and P, AM hyphal length, which together with the rate of fungal colonization of roots represents another way to measure the intensity of AM symbiosis, was not related to nutrients, root properties or above-ground biomass (Powers *et al.*, 2005). Similarly, a recent study conducted in a lowland tropical forest in Panama to investigate the consequences of long-term litter addition on the AM fungal communities has shown no significant effect of litter manipulation on the proportion of root length colonized by AM hyphae, arbuscules or vesicles or on extra-radical AM fungal biomass (Sheldrake *et al.*, 2017). Previous experiments on P nutrient addition conducted in Neotropical forests have used large quantities of inorganic P. The results from these experiments were conflicting; some have shown no significant alteration (100 kg ha^{-1} $year^{-1}$ for 7-9 years; Treseder and Allen, 2002); others a decrease (100 kg ha^{-1} $year^{-1}$ for 2 years; Treseder and Vitousek, 2001); and yet others an increase in the rate of AM fungal colonization (50 kg ha^{-1} $year^{-1}$ for 14 years; Wurzburger and Wright, 2015) by hyphae and other structures. However, Treseder and Allen (2002) have reported differences in AM hyphal biomass between soil with previous low P concentration (then fertilized for 9 years) and soils with initial higher levels of P (then fertilized for 7 years). Phosphorous addition increased the hyphal biomass in the first case, while an opposite effect was observed in the second case. While the authors suggested that these results could be related, to a certain extent, to soil fertility prior to P addition, they also pointed out that the role played by mycorrhizal fungi in forest ecosystems goes beyond being a structure that facilitates plant nutrient absorption. Results obtained from other experiments, conducted in controlled conditions, have shown that P addition was not the main factor regulating the mycorrhizal symbiosis (Gamage *et al.*, 2004; Patreze and

Cordeiro, 2004), indicating that carbohydrates transferred from the host tree may be more important form AM fungi than soil P. An additional consideration is that added P may have resulted in root growth that outpaced AM colonization, which may have led to relatively lower observed rates of colonization (D. Janos, pers. comm.). On the other hand, the observed differences in AM colonization rates over time, as soil moisture reduced, might indicate a slowing of root growth and thus, higher apparent colonization rates. Alternatively, the lack of a difference between control and treatment at our study site could be attributed to the one-off relatively low dose of P applied that did not change either the colonization rate of roots by AM fungi or the amount of soil available labile P.

We can offer some possible explanations in relation to the fate of the applied P. Duke *et al.* (1994) have suggested that soil nutrient pulses may cause fine root proliferation and that this could represent a more important mechanism adopted by plants for the capture of nutrients. Fine root proliferation could have represented an alternative response mechanism to the application of inorganic P at our study site. While it is known that litter can be colonized by mycorrhizal fungi that directly transfer P to plants (Herrera *et al.*, 1978), rapid pulses of mineral nutrients can promote the fast growth of roots at the expense of mycorrhizal symbiosis (Duke *et al.*, 1994). This fact could help understanding why we did not observe alteration in mycorrhizal association over the experiment. However, since fine root growth was not assessed in the present study, and the mycorrhizal colonization peak at day 88 suggests slow root growth over the dry season as confirmed by previous research in the area and in other lowland neotropical rain forests (Luizão *et al.*, 1992; Malhi *et al.*, 2014; Buscardo *et al*. 2016; Buscardo *et al.*, 2018a), further investigation should be carried on to test this possibility.

Most P in tropical forests resides in either organic forms (i.e. organic matter, microbial biomass, stable organic P), or in geochemically protected forms (i.e. P that is 'sorbed' onto mineral surfaces or protected within mineral matrices), the so called occluded P-fraction, which is relatively unavailable for biological uptake (Reed *et al.*, 2011). Sayer *et al.* (2012) have reported that the addition of nutrients, including P, in the form of litter facilitates the absorption of greater proportion of nutrients by plants compared with P applied in an inorganic form. This fact has been related to the rapid cycling of organic matter and colonization of the litter by fine roots, suggesting that the majority of the P used by plants is derived from internal cycling rather than from mineral P. On adding inorganic P, it enters the labile P pool, however temporarily. Using the extractable (resin plus bicarbonate) P values as a guide, it appears that our P addition did not increase the available fraction of inorganic P. As there were no increases in microbial P either, the added inorganic may have been absorbed on soil material (entered the stable inorganic P pool), or was taken up by plant roots; rapid adsorption by the predominantly Al and Fe oxides (along with kaolinite clay minerals) is likely (e.g., Barber, 1984).

There are many open questions that remain with regard to P and plant-soil interactions in tropical forest ecosystems. For example, some recent studies may call into question the 'tight' litter P recycling for the P cycle. The results of an experimental litter removal have shown that litter removal did not reduce plant productivity and leaf litter P during the first 6 experimental years, indicating that trees were able to access P from alternative sources to maintain productivity (Sayer *et al.*, 2012). A previous study conducted at the same study site had shown that while the overall soil inorganic P pool remained unchanged 3 years after litter removal, the stable organic P pool in the upper 2 cm was reduced by 23 % (Vincent *et al.*, 2010). These studies, while interesting and by far more realistic that high-dose agricultural fertilizer addition experiments, fail to propose an underlying hypothesis that could explain a natural phenomenon that would produce long-term doubling of litter production in tropical forest ecosystems. On the other hand, periodic drought-induced peaks do occur and can substantially impact nutrient cycling in the short term (O'Connell *et al.*, 2018). The results of our short duration experimental study, conducted 4 years after the last drought year in Amazonia, did not indicate comparable impacts. We agree with Mori *et al.* (2018) that we are yet to understand some fundamental biological and soil chemical processes and their interactions on P dynamics of soil microbiota in tropical forests before we can realistically tackle P limitation *per se* – for example, by being able to test P effects by establishing *a priori* ecologically significant expected differences.

CONCLUSIONS

The addition of P in our study did not appear to have caused significant changes in the levels of organic and inorganic forms of labile P in the soil surface layer. The lack of effects of P on the colonization of roots by AM fungi suggests that inorganic P is not the main factor that regulates the intensity of AM symbiosis. Similarly, the fact that soil microbial biomass was not altered following P addition suggests that P, applied in relatively small doses, did not stimulate the soil microbiota during the study experimental period. We suggest

that further careful experimental evidence is needed to disentangle the role of biological processes and soil chemical properties in the dynamics of P in tropical forests.

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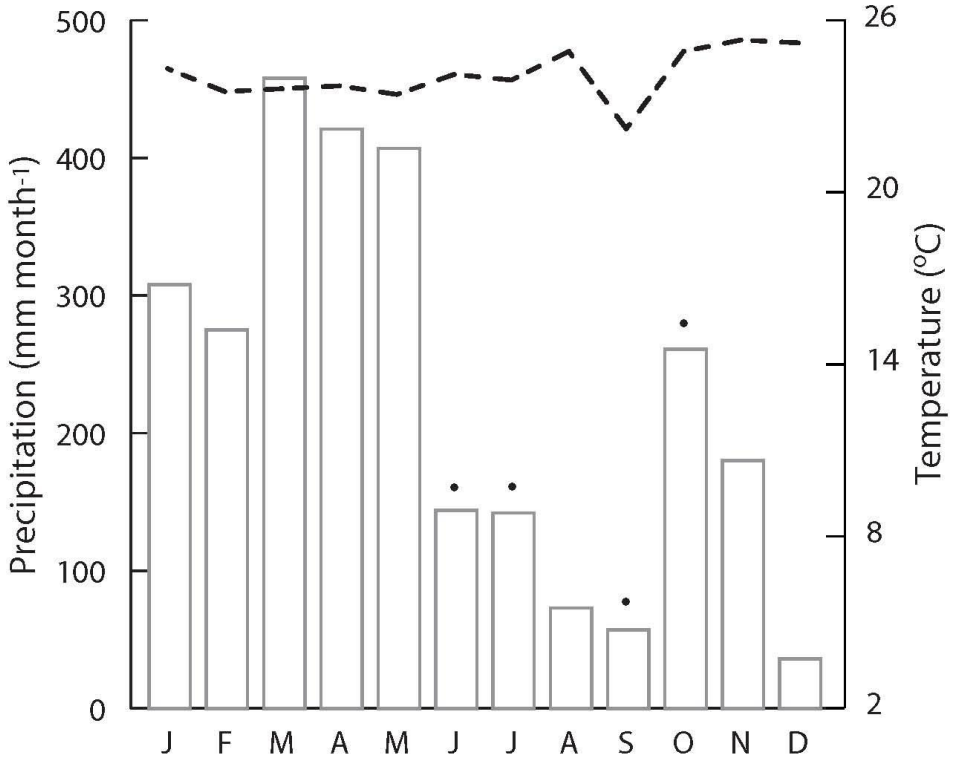
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Precipitation (mm month-1)
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Figure 1

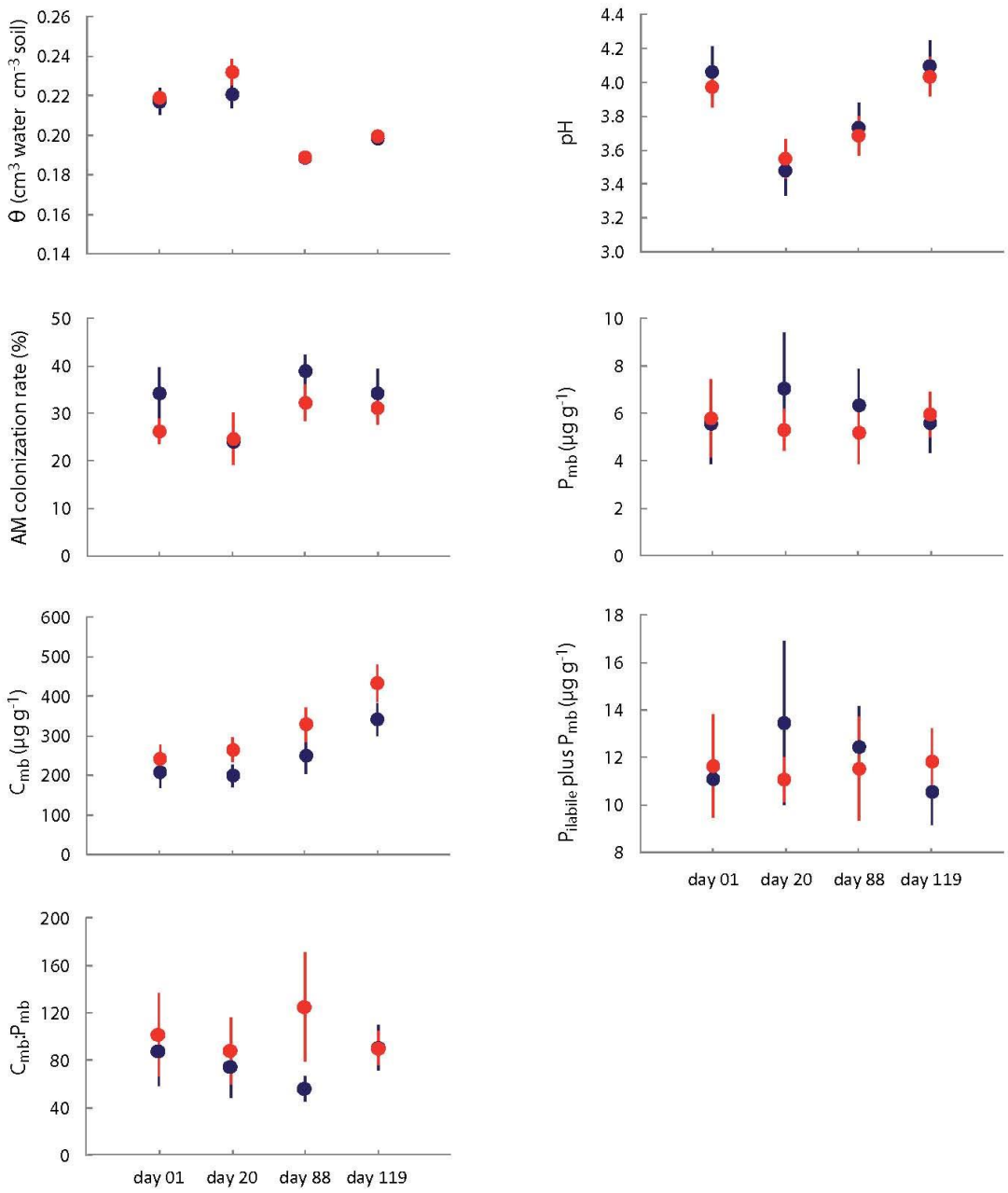

Figure 2

Supplementary material

Short-term microbial responses to soluble inorganic P input in a tropical lowland rain forest in Amazonia

Table SI

Soil chemical properties (0-5 cm) in a lowland rain forest in Amazonia prior to phosphorous application (n = 10). Al^{2+}plus H^{+}, exchangeable acidity; SB, sum of bases.

Plot	pH	Ca^{2+} cmolc kg^{-1}	Mg^{2+} cmolc kg^{-1}	K^{+} cmolc kg^{-1}	Na^{+} cmolc kg^{-1}	SB cmolc kg^{-1}	$Al^{2+}+H^{+}$ cmolc kg^{-1}	$P_{ilabile}$ mg kg^{-1}
mean	4.018	0.223	0.272	0.787	0.109	1.391	2.29	5.69

SE	0.045	0.019	0.023	0.053	0.009	0.083	0.101	0.661

Table SII

P-values and relative degrees of freedom (df) obtained by carrying out a paired *t*-test on soil chemical and microbial composition from a lowland rain forest in Amazonia between different sampling dates, for both control and phosphorus- treated plots; day 01, pre-treatment; day 20, beginning of dry season; day 88, peak of the dry season; day 119, resumption of rains; θ, volumetric water content; AM root colonization, root colonization rate by arbuscular mycorrhizal fungi; C_{mb}, microbial biomass carbon; $P_{ilabile}$ plus P_{mb}, soil available phosphorus following fumigation; P_{mb}, microbial biomass phosphorus; C_{total}, total soil carbon content; N_{total}, total soil nitrogen content.

Comparing days	01→20		01→88		01→119		20→88		20→119		88→119	
	p-value	df	p-value	df	p-value	df	p-value	df	p-value	df	p-value	df
Control plots												
Θ (cm^3water cm^{-3}soil)	0.629	9	**0.002**	9	**0.013**	9	**0.001**	9	**0.003**	9	**0.000**	9
pH	**0.000**	9	**0.000**	9	0.660	9	**0.002**	9	**0.000**	9	**0.001**	9
AM root colonization	**0.035**	9	0.485	9	0.974	9	**0.034**	9	0.111	9	0.377	9
C_{mb} (μg g^{-1})	0.850	9	0.476	9	**0.053**	9	0.463	9	**0.030**	9	0.131	9
$P_{ilabile}$ plus P_{mb} (μg g^{-1})	0.930	9	0.494	9	0.830	9	0.617	9	0.917	9	0.524	9
P_{mb} (μg g^{-1})	0.795	9	0.278	9	0.641	9	0.595	9	0.874	9	0.766	9
C:P_{mb}	0.778	9	0.772	9	0.587	9	0.883	9	0.417	9	0.412	9
C_{total} (%)	0.434	9	**0.011**	9	0.113	8	**0.003**	9	0.068	8	0.440	8
N_{total} (%)	0.690	9	**0.013**	9	0.296	8	**0.017**	9	0.231	8	0.244	8
C:N_{total}	0.194	9	**0.047**	**9**	0.200	8	**0.002**	9	**0.024**	8	0.992	8
Fertilized plots												
Θ (cm^3water cm^{-3}soil)	0.089	9	**0.000**	9	**0.000**	9	**0.000**	9	**0.000**	9	**0.000**	9
pH	**0.002**	9	**0.002**	9	0.419	9	**0.032**	9	**0.001**	9	**0.004**	9
AM root colonization	0.523	9	0.332	9	0.133	9	0.240	9	0.235	9	0.854	9
C_{mb} (μg g^{-1})	0.540	9	0.107	9	**0.011**	9	0.212	9	**0.006**	9	0.081	9
$P_{ilabile}$ plus P_{mb} (μg g^{-1})	0.752	9	0.990	9	0.577	9	0.674	9	0.707	9	0.550	9
P_{mb} (μg g^{-1})	0.786	9	0.899	7	0.359	9	0.902	7	0.276	9	0.378	7
C:P_{mb}	0.975	9	0.957	7	0.405	9	0.879	7	0.335	9	0.853	7
C_{total} (%)	0.847	9	**0.007**	9	**0.060**	8	**0.011**	9	**0.014**	8	0.892	8
N_{total} (%)	**0.043**	9	**0.002**	9	0.118	8	**0.066**	9	0.565	8	0.496	8
C:N_{total}	**0.061**	9	0.535	9	0.378	8	**0.001**	9	**0.006**	8	0.201	8

Table SIII

Results obtained using a mixed-effect linear model for the effects of phosphorus fertilization on soil physico-chemical and microbial variables. *P*-values > 0.05 indicated no difference between treatment and control. θ, volumetric water content; C_{total}, total soil carbon content; N_{total}, total soil nitrogen content; AM root colonization, colonization rate by arbuscular mycorrhizal fungi; C_{mb}, microbial biomass carbon; $P_{ilabile}$ plus P_{mb}, soil available phosphorus following fumigation; P_{mb}, microbial biomass phosphorus.

	df	t	p-value
θ	18	0.727	0.476
pH	18	0.497	0.625
C_{total}	18	1.121	0.276
N_{total}	18	-1.842	0.081
C:N_{total}	18	0.222	0.826
AM root colonization	18	0.458	0.652
C_{mb}	18	-1.105	0.283
$P_{ilabile}$ + P_{mb}	18	-1.055	0.305
P_{mb}	18	-0.809	0.429

$C:P_{mb}$	18	-1.039	0.312