Soil microbial CNP and respiration responses to organic matter and nutrient additions: evidence from a tropical soil incubation

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Abstract

Soil nutrient availability has a strong influence on the fate of soil carbon (C) during microbial decomposition, contributing to Earth's C balance. While nutrient availability itself can impact microbial physiology and C partitioning between biomass and respiration during soil organic matter decomposition, the availability of labile C inputs may mediate the response of microorganisms to nutrient additions. As soil organic matter is decomposed, microorganisms retain or release C, nitrogen (N) or phosphorus (P) to maintain a stoichiometric balance. Although the concept of a microbial stoichiometric homeostasis has previously been proposed, microbial biomass CNP ratios are not static, and this may have very relevant implications for microbial physiological activities. Here, we tested the hypothesis that N, P and potassium (K) nutrient additions impact C cycling in a tropical soil due to microbial stoichiometric constraints to growth and respiration, and that the availability of energy-rich

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labile organic matter in the soil (i.e. leaf litter) mediates the response to nutrient addition. We incubated tropical soil from French Guiana with a ^{13}C labeled leaf litter addition and with mineral nutrient additions of +K, +N, +NK, +PK and +NPK for 30 days. We found that litter additions led to a ten-fold increase in microbial respiration and a doubling of microbial biomass C, along with greater microbial N and P content. We found some evidence that P additions increased soil CO_2 fluxes. Additionally, we found microbial biomass CP and NP ratios varied more widely than CN in response to nutrient and organic matter additions, with important implications for the role of microorganisms in C cycling. The addition of litter did not prime soil organic matter decomposition, except in combination with +NK fertilization, indicating possible P-mining of soil organic matter in this P-poor tropical soil. Together, these results point toward an ultimate labile organic substrate limitation of soil microorganisms in this tropical soil, but also indicate a complex interaction between C, N, P and K availability. This highlights the difference between microbial C cycling responses to N, P, or K additions in the tropics and explains why coupled C, N and P cycling modeling efforts cannot rely on strict microbial stoichiometric homeostasis as an underlying assumption.

1. Introduction

Soil nutrient availability and stoichiometry have strong influences on soil carbon (C) cycling through their impact on the decomposition and formation of soil organic matter (Reed et al., 2011; Cotrufo et al., 2013; Poeplau et al., 2016). Different elemental C, nitrogen (N), phosphorus (P) stoichiometric ratios of plants (*ca*. C:N:P=3144:45:1; Cleveland and Liptzin (2007)), soil (*ca*. C:N:P=287:17:1; Xu et al. (2013)) and soil microorganisms (*ca*. C:N:P=42:6:1; Xu et al. (2013) or 60:7:1; Cleveland and Liptzin (2007)) involved in molecular transformations during decomposition are assumed to define the relationship between nutrients and C cycling (Sterner and Elser, 2002; Manzoni et al., 2012; Sinsabaugh et al., 2013; Zechmeister-Boltenstern et al., 2015). The maintenance of fixed ratios of elements in various organic substrates forms the basis of the Environmental Stoichiometry theory and provides a mechanistic understanding of biogeochemical transformations (Sterner and Elser, 2002; Spohn, 2016). Due to stoichiometric constraints, an increased availability of C and N in ecosystems due to global change should subsequently lead to increased demands for other macro-nutrients, such as P and potassium (K), thereby causing an imbalance between nutrient availability and nutrient demands in natural ecosystems (Peñuelas et al., 2012). Ecosystem nutrient and C enrichment from global change often corresponds with increased plant productivity and organic substrate inputs to the soil in the form of litter (LeBauer and Treseder, 2008; Gill and Finzi, 2016). Therefore, disentangling the direct responses of soil microbial activity to nutrient additions from the indirect responses via plant feedbacks *in situ* is not straightforward. In order to better predict how nutrient enrichment affects soil C cycling, more information is needed on the role of microbial C:N:P stoichiometric constraints to C cycling in direct response to nutrient enrichments as compared to addition of plant inputs.

Unlike temperate and northern ecosystems that are mainly N limited, ecosystems in the tropics are generally limited by low P availability due to the old age, strong weathering and high reactivity of Fe and Al oxide rich soils (Walker and Syers, 1976; Vitousek and Farrington, 1997; Turner and Wright, 2014; Grau et al., 2017). Relatively less is known about the role of K in C cycling, although evidence for possible K limitation of tropical systems has also begun to emerge (Doetterl et al., 2015; Sardans and Peñuelas, 2015). Given the essential role of nutrients in microbial functioning, human-induced changes in the

nutrient stoichiometry and subsequent exacerbation of nutrient limitations in tropical ecosystems can alter microbial physiological responses with potential consequences to C cycling. However, ecosystem stoichiometric theories based on more N-limited temperate ecosystems may not apply in the same way to P-limited tropical systems.

Microbial physiology is critical to ecosystem C cycling because microbial biomass and residues contribute significantly to the formation of persistent soil organic matter (SOM), while microbial respiration leads to immediate loss of C from the soil (Cotrufo et al., 2013; Kallenbach et al., 2016). Quantifying the partitioning of C from decomposing substrates into microbial biomass and respiration allows us to mechanistically link microbial activities with soil CO_2 fluxes and C sequestration at the ecosystem scale (Cotrufo et al., 2015; Soong et al., 2015; Campbell et al., 2016). Nitrogen additions tend to increase microbial C use efficiency due to the C and N co-limitation of microbial growth (Sinsabaugh et al., 2013). While N additions have been found to decrease microbial C respiration leading to an increase in relative C retention in biomass (Spohn et al., 2016), P additions tend to stimulate respiration activity relative to microbial biomass (Hartman and Richardson, 2013). Understanding the degree to which microbial respiration and growth are coupled with microbial N and P constraints would help to advance our understanding of how to integrate nutrients into models of C cycling (Reed et al., 2015).

Fresh organic matter inputs, such as leaf litter, provide a source of energy and nutrients for soil microorganisms. However, they can also prime the decomposition of SOM by providing an easily degradable energy source to microorganisms (Kuzyakov et al., 2000). Given a C-rich litter substrate, SOM decomposition can increase as microorganisms breakdown SOM to obtain N needed to maintain their stoichiometric constraints leading to priming of SOM decomposition (Moorhead and Sinsabaugh, 2006). However, similar P-mining effects in temperate ecosystems are not as common (Craine et al., 2007; Dijkstra et al., 2013; Poeplau et al., 2016). In tropical ecosystems reaching a terminal steady state, most soil P is in organic or mineral occluded forms, which specialized microorganisms can access through either enzymatic activity or acidification and complexing agents, respectively (Walker and Syers, 1976; Jones and Oburger, 2011). Understanding how P additions affect soil C decomposition through P-mining in response to labile C availability, would greatly improve our understanding of microbial P and C feedbacks in tropical ecosystems.

In this study, we investigate how mineral nutrient additions themselves, or in combination with organic matter inputs, affect the decomposition of soil organic matter and leaf litter, and the partitioning of C into microbial biomass and CO_2 production. We also examine the microbial biomass C, N and P responses to mineral nutrient additions alone or in combination with a labile source of litter C. We hypothesized that microorganisms in this tropical forest mineral soil would respond most strongly to the addition of P, but that the presence of labile litter would also enhance microbial activity and nutrient uptake by providing a complex source of labile organic matter to the soil. This would help to explain the direct impact of N, P and K availability on microbially mediated soil C dynamics *versus* indirect effects via higher net primary productivity and organic matter inputs to the soil. To test these hypotheses, we incubated soil from the lowland tropical Amazon rainforest of French Guiana amended with either a labile, C-rich ^{13}C labeled leaf litter (i.e., an organic substrate containing C and nutrients), additions of mineral N, P and K, or their combinations. Over the course of a 30-day laboratory incubation we measured microbial C cycling by quantifying the fate of decomposing SOM and litter C into CO_2 fluxes and microbial biomass. We also measured the organic and mineral substrate addition effects on microbial C, N and P content at the end of the incubation. We chose a relatively short incubation to

120 focus on the initial stage of litter decomposition when most C-rich substrates are easily decomposed
121 (Cotrufo et al., 2015; Soong et al., 2015; Soong et al., 2016).

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123 **2. Materials and Methods**

124 *2.1 Soil*

125 The soils for our incubation came from an old-growth lowland Amazon rainforest at the Paracou
126 research station in French Guiana (5°15'N, 52°53'W; www.paracou.cirad.fr). We collected the soil from
127 the mineral topsoil (0-15 cm) within a 20 x 20 m area. This lowland tropical rainforest site receives 3041
128 mm of annual precipitation and has a mean annual temperature of 25.7 °C (Gourlet-Fleury et al., 2004).
129 Intra-annual temperatures range +/- 1.5 °C with minimum rainfall less than 100 mm $month^{-1}$ during the
130 dry season from August to November and maximum rainfall in the peak of the wet season of 500 mm
131 $month^{-1}$ (Gourlet-Fleury et al., 2004). The soil is classified as a nutrient-poor Acrisol, developed over a
132 Precambrian metamorphic formation called the Bonodoro series (Gourlet-Fleury et al., 2004). Soil
133 texture is sandy, with 79 % sand, 6 % clay, and 15 % silt and $pH_{(KCl)}$ is 3.99. We removed the litter layer
134 and collected five soil cores of mineral soil from the 0-15 cm depth at the four corners and center of the
135 20 m x 20 m sampling area. Soil was homogenized and sieved to 2 mm, dried at 40 °C and stored dry
136 until use. We determined % C and % N of the soil by dry combustion elemental analysis (Flash 2000
137 series CN analyzer, Thermo Scientific, Germany) and measured total P and K (Walinga et al., 1989) on a
138 continuous flow analyzer (SAN++, SKALAR, Breda, NL) after digestion with sulphuric acid, selenium and
139 salicylic acid. We used the Bray P method to approximate plant available P (Bray and Kurtz, 1945). We
140 measured the initial $^{13}C/^{12}C$ of the soil on an oven dried and ground subsample via elemental analysis
141 isotope ratio mass spectrometry (IRMS) using a PDZ Europa ANC-GSL elemental analyzer coupled with a
142 Sercon 20-20 IRMS with SysCon electronics (SerCon, Cheshire, UK).

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144 *2.2 Soil incubations and nutrient additions*

145 In the laboratory, we re-wetted the air-dried soils to 60% of field capacity and pre-incubated
146 them at 21 °C for four days prior to the start of the incubation. Field capacity was determined by first
147 oven drying three 50 g sub-samples of the starting soil, saturating them with water, letting them drain
148 for one hour, then determining water retention gravimetrically. We determined soil moisture after pre-
149 incubation gravimetrically by oven drying three 10 g aliquots of soil at 70°C for 72 hours. Approximately
150 40 g of dry mass equivalent soil was used in each incubation unit. The experiment tested for the effects
151 of two main treatments (mineral nutrient and litter additions) and their interactions, 1) Mineral nutrient
152 additions with six levels: +K, +N, +NK, +PK, +NPK and a Control, and 2) Litter additions in the form of leaf
153 litter with two levels: addition of 0.5 g of *Andropogon gerardii* ^{13}C labeled litter mixed into the soil (Soil
154 & Litter treatment) and Control (Soil Only treatment). We also examined the effects of the interaction
155 between the mineral nutrient and litter additions, by applying the six nutrient addition treatments to
156 both the soils with and without litter additions. Therefore, the incubation experiment consisted of four
157 replicates per each treatment and combination, plus four soil-free blank jars to correct the CO_2 flux
158 measurements, for a total of 52 incubation units.

Mineral N was added at a rate of 367 g N/ kg soil and mineral P additions were added at a rate of 195 g P/ kg soil. This is equivalent to approximately two times the annual natural N input from litter at the site (6.5 g N m^{-2} y^{-1}), and fifty times the natural P input at the field site (0.14 g P m^{-2} y^{-1}), and is equivalent to previous fertilization experiments at this site (Barantal et al., 2012; Fanin et al., 2014). While these nutrient addition rates are somewhat greater than natural inputs they help to stimulate existing mechanisms and therefore better identify them. Though this methodology may push the microorganisms into a situation not faced in the field, this is a classical approach used to better understand natural processes (Benton et al., 2007). Mineral nutrient treatments were added in 1 ml solutions containing 0.0263 g of NH_4NO_3 for the +N treatment, 0.0159 g KNO_3 and 0.0199 g NH_4NO_3 for the +NK treatment, 0.0214 g of KH_2PO_4 for the +PK treatment, 0.0117 g KCl for the +K treatment, and 0.0214 g KH_2PO_4 and 0.0262 g NH_4NO_3 for the +NPK treatment. One ml of deionized water was added to the Control treatment. We used KH_2PO_4 as our P source because it is soluble, C-free and had no effect on soil pH, therefore, we did not have a P-only nutrient addition. In an attempt to isolate the P-only nutrient affects, and assuming additive responses of nutrient combinations, we added +K (as KCl) in an equivalent amount as is contained in the KH_2PO_4 and KNO_3 additions in order to help differentiate the +K from +PK effects. Thus, mineral K was added at a rate of 246 g K/ kg soil.

A labile *Andropogon gerardii* Kaw grass leaf litter uniformly labeled with ^{13}C was used as a source of organic substrate addition. ^{13}C enriched *A. gerardii* was grown from seedling to maturity and harvested as leaf litter in an continuous isotope labeling chamber (Soong et al., 2014b). The aboveground biomass was harvested at senescence, air dried and cut into approximately 1 cm lengths. The *A. gerardii* litter represents a complex, C-rich and labile source of organic substrate addition to the soil, and it has been frequently used in previous studies as a model substrate to examine decomposition dynamics (Soong et al., 2014a; Cotrufo et al., 2015; Soong and Cotrufo, 2015; Campbell et al., 2016; Haddix et al., 2016; Soong et al., 2016). This litter was therefore used as a labile yet complex organic substrate to contrast with the inorganic, mineral nutrient additions. In this way, the direct effect of mineral nutrient additions themselves on microbial activity and stoichiometry could be discriminated from indirect effects via the stimulation of net primary productivity accompanied by more organic matter inputs to the soil. The dried leaf litter material contained 29% cellulose, 4% lignin, and had a 4.46 atom % ^{13}C isotopic signature (McKee et al., 2016). We measured litter C and N content by dry combustion elemental analysis (Flash 2000 series CN analyzer, Thermo Scientific, Germany) and P and K content after digestion on a continuous flow analyzer (SAN++, SKALAR, NL).

The mineral fertilizer addition accounted for 35 % of the initial soil N, 187 % of the initial soil P, and 31 % of the initial soil K. The litter addition represented a 1.25 % addition of mass relative to the soil and a source of organic nutrient additions. This accounted for a 62 % addition of C, a 21 % addition of N, a 26 % addition of P and a 37 % addition of K with respect to the respective nutrient concentrations in to the soil (Table 1). We incubated the soil incubation units in plastic cups inside Schott Duran glass bottles in a climate-controlled incubator. We added a small amount of water (25 ml) to the bottom of each glass bottle to maintain high humidity and prevent soil drying. Gravimetric soil moisture stayed constant throughout the incubation. All samples were incubated for 30 days at 21 °C.

2.4 CO_2 fluxes

Soil CO_2 flux and C-isotopic composition were determined on days 1, 2, 3, 5, 7, 10, 15, 21 and 30 of the incubation. Each day, the incubation bottles were closed and both initial and final concentrations of ^{12}C- and ^{13}C- CO_2 were measured using a Picarro G2131-i cavity ring-down spectrometer (Picarro inc., Santa Clara, CA, USA). The analyzer was calibrated for high enrichments and we utilized a specially designed discrete sampling system (Dickinson et al., 2017). To measure CO_2 fluxes, the bottles were closed for 24 hours for the first three time points, in order to capture the initial high CO_2 fluxes from the litter, and for 2 hours for the rest of the time points. The CO_2 concentration was measured at the start and end of each closure period and flux rate was calculated as the accumulation of CO_2 over the given time period. After each measurement, we ventilated the bottles with CO_2-free air until the concentration of CO_2 was close to ambient. Between measurements the bottles were loosely covered with parafilm to prevent drying but allow for some gas exchange. All flux calculations were corrected for the exact volume of each bottle minus the volume of the sample. The concentration of CO_2 never exceeded 3% during any of the incubation periods. Flux rates were integrated between sampling dates to estimate total cumulative CO_2 respiration. We used the initial soil and litter isotopic values as end members in a two-end member isotope-mixing model to calculate the amount of soil-derived or litter-derived C contributing to the measured CO_2 fluxes from the Soil & Litter units (see section 2.6 for details).

2.5 Microbial biomass and chemical analyses

After 30 days we removed the samples from each incubation jar, weighed them, homogenized them and subsampled them for chemical analysis. We sieved the soils to 2 mm and picked the remaining litter pieces out of the soil before any soil analyses. One aliquot of soil was oven dried at 70 °C for 72 hours to measure gravimetric soil water content. The remaining soil was sub-sampled into 5 g aliquots and either extracted with an 0.25 M HCl and 0.03 M NH_4F solution for available P (Bray and Kurtz, 1945), or with 0.5 M K_2SO_4 for extractable C and N (Brookes et al., 1985), or fumigated with $CHCl_3$ for 72 hours and similarly extracted for available P or extractable C and N. The HCl and NH_4F solution was chosen for the P extraction because F^- promotes P desorption in these Al^{3+} rich soils, and promotes P desorption. We filtered all extracts over pre-wetted Whatman #40 ash-less filters. We extracted the soils and began fumigations on fresh soils within 48 hours after the last CO_2 flux measurement on day 30 of the incubation. The difference between C, N and P contents of the fumigated and non-fumigated soils was used as a measurement of microbial biomass C, N and P, respectively (modified from Brookes et al. (1985) due to different solution for P extractions). This difference between fumigated and non-fumigated samples was not corrected for extraction efficiencies, and thus should be considered as a proxy for microbial biomass values in this soil.

Extractable organic C concentrations and isotopic signatures were measured using wet oxidation (heated persulfate) total organic C analysis (IO Analytical Aurora 1030W, College Station, TX, USA), coupled via a custom-made cryofocusing device with an isotope ratio mass spectrometer (Thermo Finnigan Delta V Advantage; USA) (Geeraert et al., 2016). We measured the concentration of N and P of the soil extracts after digestion on Skalar San++ continuous flow analyzer (Skalar Analytical B.V., Breda, The Netherlands).

2.6 Data analysis

For statistical analysis of CO_2 fluxes, we used a repeated measures mixed effects model with mineral nutrient addition, litter addition and their interaction as fixed factors and individual sample as a random factor in order to account for the repeated sampling in the analysis of CO_2 fluxes. For the cumulative CO_2 fluxes used in the biomass-to-respiration ratio and priming calculations, and for all microbial biomass data, we used a generalized linear model with mineral nutrient addition, litter addition, and their interaction as fixed effects. We then analyzed the mineral nutrient effect in Soil Only or Soil & Litter treatments separately using a Tukey *post hoc* analysis to make pairwise comparisons between mineral nutrient addition effects within the mineral only and organic plus mineral nutrient treatments. Normality and homogeneity of variance was checked for each analysis and an log transformation was used when necessary to fit the assumptions of the parametric models. Statistically significant differences were defined by p-values <0.05. All statistics were done using the nlme (Pinheiro et al., 2017) and multcomp (Hothorn et al., 2008) packages in RStudio version 0.99.892.

In the Soil & Litter treatments, both litter and SOM decomposition contributed to microbial biomass and CO_2 fluxes. Therefore, we used the isotopic mixing model to quantify the contribution of litter-derived C and soil-derived C to CO_2 flux and microbial biomass.

(1) $$f_A = \frac{atom\%\ ^{13}C_x - atom\%\ ^{13}C_B}{atom\%\ ^{13}C_A - atom\%\ ^{13}C_B}$$

Where f_A is the proportion of soil-derived C (A), and $atom\%\ ^{13}C_A$ is the percent ^{13}C of starting soil, $atom\%\ ^{13}C_B$ is the percent ^{13}C of the added residue (B), and $atom\%\ ^{13}C_x$ is the percent ^{13}C measured from the "Soil & Litter" sample. The f_A-value was then multiplied by the total CO_2 flux, or total microbial biomass, in order to calculate the amount of soil-derived C in the CO_2 flux or microbial biomass C. The amount of litter-derived C was calculated as the total CO_2 or microbial biomass pool minus the soil-derived contribution.

The microbial biomass-to-respiration ratio was used as an indication of C partitioning during decomposition. This ratio tells us proportionally how much C from litter or SOM decomposition is retained in the microbial biomass at the end of the incubation, versus how much is lost as CO_2 flux to the atmosphere. It was calculated separately for litter- and soil-derived C, based on isotopic partitioning using equation 1. Therefore, the amount of litter C in microbial biomass was the final amount of litter-derived C in the microbial biomass after 30-days, while the amount of soil-derived C in microbial biomass at the end of the incubation was calculated as the difference between the final amount of soil-derived C in microbial biomass minus the mean microbial biomass in the pre-incubated, starting soil. This measure of microbial biomass gives us a net microbial biomass C production value, including both growth and turnover, over the 30-day incubation. CO_2 fluxes were integrated between sampling points to calculate the total litter- or soil-derived CO_2 respiration over the 30-day incubation. We used a 0.45 correction factor to account for the efficiency of the extraction for the microbial biomass C assimilation value in the biomass-to-respiration ratio (Jenkinson and Powlson, 1976). Although this correction factor is not specific to this soil, it is likely that the chloroform fumigation extraction method does not quantify all microbial biomass C so a 0.45 correction factor helps to put our microbial biomass C measures on a more comparable scale with respiration C values in the biomass-to-respiration ratio.

The effect of the litter addition and nutrient treatments on priming of SOM was calculated as the difference between the cumulative CO_2 flux of soil-derived cumulative CO_2 fluxes per g of soil from the Soil & Litter treatment and the Soil Only treatment.

3. Results

3.1 SOM respiration as affected by mineral and organic nutrient additions

The litter addition increased the overall measured CO_2 flux by an order of magnitude, while nutrient addition and the interaction between nutrient addition and litter addition did not have a significant effect on CO_2 fluxes in the Soil & Litter treatment (Figure 1b, Table 2). Mineral nutrient additions significantly affected the total soil CO_2 flux only in the Soil Only treatment in the absence of organic substrate amendment (Table 2). In the Soil Only treatments, the +NPK mineral nutrient addition had significantly greater soil CO_2 fluxes than the Control, +K, +N and +NK treatments ($p<0.05$; Figure 1a). When we partitioned the total CO_2 flux from the Soil & Litter treatment into soil-derived and litter-derived CO_2 flux, there was no significant effect of mineral nutrient additions on either soil-derived or litter-derived CO_2 fluxes. Litter-derived C constituted approximately 90% of the total CO_2 flux in the Soil & Litter treatments. This disproportionately large contribution of litter *versus* soil to the CO_2 flux resulted in somewhat large uncertainty in the soil-derived CO_2 fluxes in the Soil & Litter treatment, as seen in the large error bars in Figure 1.

The combined litter addition and mineral +NK treatment led to a greater loss of soil C to CO_2 flux ($t_{17}=2.85$; $p=0.0110$), and the soil C losses were significantly greater than in the correspondent Soil Only +NK treatment (Figure 1a). This indicates priming of SOM decomposition by the combined litter and +NK nutrient treatment interaction. The litter addition did not lead to a significant increase in soil-derived CO_2 flux, or priming, in any of the other nutrient treatments (Figure 1a).

3.2 Microbial biomass carbon

The litter addition led to a nearly two-fold increase in total microbial biomass in the Soil & Litter treatments as compared to the Soil Only treatment (Table 2; Figure 2). This priming of SOM by the litter addition in the +NK treatment was accompanied by an increase in soil-derived microbial biomass C at the end of the 30-day incubation in the same treatment (Figure 2). Within the Soil Only treatment, mineral nutrient additions had a statistically significant impact on microbial biomass C (Table 2). In a pairwise comparison the +NPK treatment had significantly lower biomass C than the Control ($p=0.0022$) and the Control had the highest mean microbial biomass C of the Soil Only mineral nutrient treatments (Figure 2). Within the Soil & Litter incubations the +NK treatment had significantly greater total microbial biomass than the +K, +N, and +NPK treatment ($p<0.05$). The +PK treatment also had high total microbial biomass, which was only significantly different from the +N treatment ($p=0.001$). Overall, the interaction between the litter addition and mineral nutrient treatments also had a statistically significant impact on total microbial biomass C (Figure 2; Table 2).

Using the ^{13}C isotopic signature to differentiate litter-derived from soil-derived microbial biomass C within the Soil & Litter treatments, we found a significant interaction between litter addition

and mineral nutrient addition in their effect on soil-derived microbial biomass C (Table 2; Figure 2 white bars). Within the Soil & Litter treatment, mineral nutrient addition had a significant effect on soil-derived microbial biomass (Table 2) with the +NK treatment having more soil-derived microbial biomass C than the Control, +K, +N and +NPK treatments ($p<0.05$). The +PK treatment also tended to have larger soil-derived microbial biomass C on average, but it was not statistically significantly different from the other treatments (Figure 2). The nutrient effect on litter-derived microbial biomass was the same, with the +NK treatment having larger litter-derived microbial biomass than the Control, +K, +N and +NPK treatments ($p<0.05$) and the +PK treatment not being significantly different from any other treatment (Figure 2). Soil and litter-derived C contributed equal amounts to the microbial biomass at the end of the 30-day incubation across all Nutrient treatments (Figure 2). This shows that after 30 days, litter derived C made up approximately half of the microbial biomass C in the Soil & Litter treatment.

3.3 Microbial carbon partitioning

The biomass-to-respiration ratio of the soil-derived C was significantly greater in the Soil & Litter treatments than in the Soil Only treatments (Table 2; Figure 3). Within the Soil Only treatment alone the mineral nutrient addition had a significant effect on the biomass-to-respiration ratio of the soil-derived C (Table 2), where the +NPK mineral nutrient addition had a significantly lower biomass-to-respiration ratio than the control, +K and +N treatments ($p<0.05$). There was no significant effect of mineral nutrient addition on the partitioning of soil-derived C within the Soil & Litter treatment, although again the +NPK treatment had on average the lowest biomass-to-respiration ratio (Figure 3; Table 2). The biomass-to-respiration ratio of the litter-derived C was an order of magnitude lower than that of the soil (Figure 3) and nutrient additions did not have a significant effect on the microbial partitioning of the litter C.

3.4 Microbial biomass nitrogen and phosphorus

Nutrient additions did not have a significant effect on microbial biomass N in the overall model. However, post-hoc pairwise comparisons of nutrient treatments within the Soil Only treatment revealed that the +N treatment had a significantly larger microbial biomass N than the Control and +K treatments (Figure 4a). This led to a decreased microbial biomass C:N ratio in the Soil Only +N treatment, however the C:N ratio was not significantly different from any of the other treatments (Figure 4c). There was no overall or pairwise nutrient effect on microbial biomass C:N (Figure 4c). The litter addition alone significantly increased microbial biomass N compared to the Soil Only treatment (Table 2). However, due to the consistently greater increase in microbial biomass C with the litter addition, microbial C:N was significantly greater in the Soil & Litter treatment than the Soil Only treatment (Figure 4c; Table 2).

In contrast to the N fertilizations, there were significant effects of mineral nutrient additions, litter addition and their interaction on microbial biomass P in the overall model (Figure 4b, Table 2). Within the Soil Only treatment, microbial biomass P in the +PK treatment was significantly larger than all of the other nutrient treatments. Within the Soil & Litter treatment, microbial biomass P in both the +PK and the +NPK treatments were significantly larger than all of the other non-P added treatments. Within the Soil & Litter treatment, both the +NPK and +PK treatments had low C:P ratios, while only the +PK

treatment had a lower C:P ratio in the Soil Only treatment (Figure 4d) and there was only a significant effect of the Nutrient treatment on C:P ratios in the Soil Only treatment (Table 2). We do not suspect that the litter addition or any of the nutrient treatments affected the fraction of P added that was adsorbed to soil minerals because there was no difference in the post-fumigation, extractable P between any of the P added treatments nor was there a litter x nutrient interaction effect.

4. Discussion

4.1 Nutrient effects on soil organic matter decomposition

Our results provide evidence for both organic matter and mineral nutrient effects on SOM decomposition in this tropical soil. Microbial respiration and biomass responded to the addition of a labile litter substrate, indicating a clear response to labile organic matter, which was mediated by nutrient availability. Rapid decomposition of leaf litter under hot and humid conditions in tropical soils can lead to a strong C limitation as seen in the low C content of this top-soil (Table 1). Moreover, it is possible that the labile litter addition effect masked any potential nutrient addition effects in the Soil & Litter treatment on SOM or litter decomposition. However, in the absence of the litter addition, the addition of N, P and K in combination significantly increased the mineralization of soil C to CO_2 as compared to the control. No other single or dual nutrient addition had any significant effect on soil respiration, although the +PK treatment slightly increased CO_2 flux, indicating a tri-NPK limitation of mineral soil respiration.

Although we did not have a P-only nutrient addition, we found evidence to support the hypothesis that P stimulates soil CO_2 fluxes. In the Soil Only treatment, both nutrient treatments containing P had the highest cumulative CO_2 production and were not significantly different from one another. The +NPK treatment had the largest CO_2 flux, and was significantly greater than the Control, +N, +K and +NK treatments. The +PK treatment had significantly greater CO_2 flux than the +K treatment. If we assume additive effects of nutrients on microbial activity, we would then deduce that P is the nutrient responsible for the increased CO_2 production in the +PK and +NPK nutrient treatments. Without a true +P treatment, however, this remains an interpretation.

Soil microorganisms in tropical mineral soils may be both C and NPK co-limited. Previous studies on the same tropical French Guianese soils have demonstrated the impact of N and P additions on litter decomposition rates and microbial communities (Barantal et al., 2012; Fanin et al., 2014; Fanin et al., 2016). However, our results clearly demonstrate the overwhelming importance of fresh litter inputs on soil respiration and microbial biomass. Tropical forest leaf litter varies widely in composition (Hattenschwiler et al., 2008). While the *A. gerardii* litter used in our study had a slightly greater P content than most tropical forest leaf litter, we used it here to highlight the difference in mineral nutrient effects *versus* organic matter and mineral nutrient effects on microbial activity in this mineral tropical top soil. While Fanin et al. (2014) found that P fertilization alone affected microbial community structure and cellulose paper decomposition, they also found strong synergistic effects of C and N additions along with P fertilization. Similarly, in our tropical soil incubation the addition of labile organic matter led to a large increase in microbial activity with no significant nutrient addition effects, while nutrient additions alone did stimulate microbial activity.

Partitioning the decomposition of soil organic C to microbial biomass *versus* CO_2 flux is critical to understanding soil C sequestration during decomposition of organic substrates. Respiration is the main loss pathway of C from the soil, while microbial biomass contributes to the formation of persistent SOM (Mambelli et al., 2011; Kallenbach et al., 2016). The microbial biomass-to-respiration ratio was significantly lower with the +NPK addition due to both greater soil-derived CO_2 fluxes and smaller C retention in microbial biomass. This shift in C partitioning, along with the low microbial biomass P content in the Soil Only +NPK treatment, indicate increased turnover of microbial biomass. Previous soil incubations have found P additions to increase microbial biomass specific respiration, or qCO_2 (Hartman and Richardson, 2013), yet here we found that the tri-nutrient effect in the +NPK treatment decreased the net biomass-to-respiration ratio, while the +PK and +NK treatment did not. This could be due to the differential P demands by various microorganisms at different developmental stages or a shift in the microbial community (Elser et al., 2003). Here, it appears that all three N, P and K nutrients were needed for this outcome, pointing again toward the need for a greater understanding of the interactive effects of nutrients on microbial functioning and carbon cycling to inform coupled C-nutrient cycling models (Huang et al., 2018; Wang et al., In Review).

Along with litter addition, the +NK treatment primed SOM decomposition via a larger and more active microbial community as seen in the larger microbial biomass and greater CO_2 flux as compared to the Soil Only +NK treatment. This stimulation of SOM decomposition uniquely occurred in the +NK and litter addition treatment indicating a C, N, K stimulation of SOM decomposition. It is not entirely clear from our dataset why this treatment alone resulted in a positive SOM priming effect. One explanation could be a shift to a larger and more active microbial community. Another explanation could be enhanced SOM decomposition to acquire organic P when C, N and K were provided in excess. Microorganisms can obtain organic P via enzymatic activity during SOM decomposition and inorganic P through acidification and complexing agents. Therefore, the stimulation of SOM decomposition to obtain limited P resources is only one potential P access pathway. Mining of SOM for P via enzymatic activity could be more likely in P limited tropical soils than in more N limited temperate ecosystems where P is more abundant (Craine et al., 2007; Dijkstra et al., 2013). In these highly weathered, Eastern Amazonian soils it is likely that almost all of the soil P is divided between organic and occluded forms (Walker and Syers, 1976). Thus, when provided with C-rich labile organic matter and +NK nutrients, the enhanced decomposition of SOM measured here indicates a potential mining of SOM for organic P. If ecosystem C, N and K enrichment is likely to stimulate plant primary productivity (Reich et al., 2006), our results suggest that increased organic matter inputs to the soil in combination with N and K enrichment could possibly cause enhanced decomposition of SOM in tropical soils due to priming.

The approximately ten-fold greater biomass-to-respiration ratio of soil-derived C compared to litter C reflects the difference in microbial metabolism of organic matter of contrasting quality. Isotopic partitioning of the CO_2 flux allowed us to see that there was no significant mineral nutrient effect on litter-derived CO_2 fluxes or on microbial partitioning between biomass and respiration. While the +NK treatment did lead to larger microbial biomass, slightly greater CO_2 fluxes from the same treatment did not lead to significant change in C partitioning. The nutrient content of the decomposing litter itself, which was being rapidly decomposed due to its high lability, may have masked any effects of the mineral nutrient additions indicating a strong C limitation to microbial activity in this tropical soil. Due to the high C-to-nutrient ratio of leaf litter compared to microbial biomass, much more C is lost as CO_2 rather than retained in biomass during litter decomposition than during SOM decomposition, which is

stoichiometrically more similar to microbial biomass (Mooshammer et al., 2014). Furthermore, the co-metabolic cost of oxidative degradation of more recalcitrant SOM compounds is energetically less favorable than the decomposition of more labile carbohydrates and hemicellulose that are abundant in *A. gerardii* litter (Blagodatskaya and Kuzyakov, 2008; Klotzbucher et al., 2011; McKee et al., 2016). This leads to a more rapid turnover of fresh litter inputs than SOM during decomposition, suggesting that both fresh litter and nutrient availability co-limit SOM formation and C losses during decomposition.

4.2 Microbial biomass stoichiometry

Across all our treatments, CN ratios of microbial biomass communities were constrained between 3.5 and 8 while microbial CP ratios varied between 4 and 28 (Figure 4). This was in spite of the fact that the mass of the N additions was nearly twice as large as the P additions (367 g N/ kg soil and of 195 g P/ kg soil). This demonstrates the much greater potential variability in microbial biomass P content and CP ratios than N content CN ratios in this tropical soil. Although we cannot say whether this resulted from a shift in the microbial community or a direct flexibility in cellular P content (Fanin et al., 2013; Kaiser et al., 2014), we can conclude that the same community within a given treatment combination often had a greater divergence in C:P than C:N ratios from the Control (Figure 4). While the addition of NH_4NO_3 and KH_2PO_4 could have acidifying properties, the soil used here had a pH of 3.99 and was not likely further acidified. The variability of microbial biomass P content relative to C and N may be a key aspect in the complex role of microorganisms in soil C cycling, particularly in P limited tropical biomes. The production of organic acids and phosphatase enzymes by bacteria, fungi and actinomycetes could allow bacteria and fungi to immobilize P even in the absence of organic matter inputs (Jones and Oburger, 2011). Tropical foliar P content is also responsive to P fertilization, while foliar N content is not responsive to N fertilization (Elser et al., 2003; Mayor et al., 2014; Wang et al., 2017). The potential implications of this are that both plant and microbial variability in cellular P content could be adaptive to seasonal or temporal changes in P availability. However, the immobilization of available P by microorganisms, particularly in the presence of available C, could also mean that fast growing microorganisms could possibly compete with plants for available P particularly when litter inputs or root exudation is high. The lack of a microbial P response in the Soil Only, +NPK treatment along with its greater CO_2 flux and lower microbial biomass points toward high microbial turnover in this treatment rather than P accumulation. This could account for the lack of a microbial biomass P response in this treatment if high turnover inhibits the accumulation of microbial biomass P.

One possible mechanism for the difference in N and P limitation effects on SOM priming could be the de-coupling of microbial P uptake from SOM decomposition and microbial growth (Dijkstra et al., 2013). A large portion of P is occluded in minerals while most soil C and N is in organic matter (Gerard, 2016). Carbon and N are both required to build and maintain microbial cell walls and enzymes, which both have rather constrained stoichiometry, explaining why microbial biomass C:N ratios show little variation. Phosphorous, in contrast, controls the rate of metabolic processes but may be less intrinsically linked to microbial biomass (Elser et al., 2003). Thus, while microbial respiration rates may be responsive to P additions, microbial growth is more responsive to C and N additions (Hartman and Richardson, 2013). Moreover, the ability to store P in non-organic forms could allow microorganisms to thrive in tropical ecosystems where plant inputs are low in P (Jones and Oburger, 2011). A de-coupling of

483 microbial P utilization from microbial biomass C and N indicates that microbial stoichiometric theories
484 based on strict C and N coupling may not apply to C:P or N:P ratios at the community level.

485 The wide range of microbial C:P and N:P ratios that we found here contrasts with the more
486 constrained C:N ratios of microbial biomass in response to N fertilization in both temperate and tropical
487 ecosystems (Hartman and Richardson, 2013; Turner and Wright, 2014; Zechmeister-Boltenstern et al.,
488 2015). This direct measurement of flexibility in microbial P content relative to C and N demonstrates the
489 direct responsiveness of microbial C:P and N:P ratios to P addition and explains the relatively weak
490 correlations between P and N or C globally (Cleveland and Liptzin, 2007; Xu et al., 2013). Our results
491 support those of Fanin et al. (2013) who also measured soil microbial stoichiometric variability and
492 found that microbial C:N:P stoichiometry mirrored litter C:N:P stoichiometry in low-P tropical soils.
493 These two studies provide direct evidence for the responsiveness of microbial C:P and N:P stoichiometry
494 to both organic matter inputs and mineral nutrient availability, and call for caution in applying strict
495 C:N:P stoichiometric constraints to estimates of microbial C cycling responses to nutrient availability
496 (Sinsabaugh et al., 2013). Evidence for some degree of soil microbial C:N:P homeostasis has been found
497 in two large global datasets (Cleveland and Liptzin, 2007; Xu et al., 2013) and has provided the
498 foundation for a body of work applying strict microbial stoichiometric constraints to theoretical
499 relationships between nutrients and C cycling during decomposition (Manzoni et al., 2012; Anders et al.,
500 2013; Sinsabaugh et al., 2013; Mooshammer et al., 2014). However, the capacity for microbes to vary
501 their C:N:P stoichiometry in response to mineral and organic nutrient additions, and the impacts of
502 combined nutrient additions on microbial respiration and biomass can help to inform coupled C:N:P
503 modeling efforts (Wang et al., 2017; Huang et al., 2018; Wang et al., In Review). Our results particularly
504 highlight the need for closer examination of microbial physiological and functional responses to P in P-
505 limited tropical ecosystems, which are important drivers of the global C cycle.

506

507 *4.3 Conclusions*

508 The combination of ^{13}C labeled leaf litter additions and N, P, K mineral nutrient additions have
509 allowed us to examine the individual and interacting effects of mineral nutrient and organic matter
510 additions on soil organic C dynamics, including priming, as driven by microbial biomass C and nutrient
511 stoichiometry in a tropical forest soil. Our results reveal the unique and interacting effects of N, P and K
512 on SOM decomposition, both with and without fresh organic matter inputs. Large increases of microbial
513 biomass and CO_2 respiration in response to litter addition indicate a clear labile organic matter limitation
514 in these soils. However, microbial biomass-to-respiration partitioning of soil C was lowest in the +NPK
515 treatment while CO_2 flux was highest, which points to enhanced microbial biomass turnover. In contrast,
516 litter addition and +NK fertilization stimulated both biomass production and soil CO_2 efflux, priming SOM
517 decomposition. Microbial biomass C:N:P stoichiometry responses to mineral fertilizer and litter
518 additions reveal the tighter constraints of microbial C:N ratios in response to N additions as compared to
519 C:P ratios in response to P additions. This demonstrates a potentially strong competitive ability of soil
520 microorganisms to immobilize available P in the soil independent of low organic matter constraints.
521 These results have important implications on our understanding of how soil microorganisms respond to
522 altered environmental stoichiometry and how microbial nutrient and C cycling mechanisms can be
523 incorporated into models of ecosystem functioning.

524

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533 Table 1. Composition of the soil, litter and mineral nutrients used in the incubation units on a mass
534 basis. NA= Not applicable because this was not measured.

	Soil	Litter	Mineral nutrients
Mass (g)	40	0.5	1
Organic C (g)	0.376	0.23315	0
Total N (g)	0.0266	0.0056	0.0092
Total K (g)	0.0201	0.0073	0.0062
Total P (g)	0.0026	0.00067	0.0049
Bray Available P (ppm)	2.74	NA	NA
Atom $\%^{13}C$	1.07	4.46	NA

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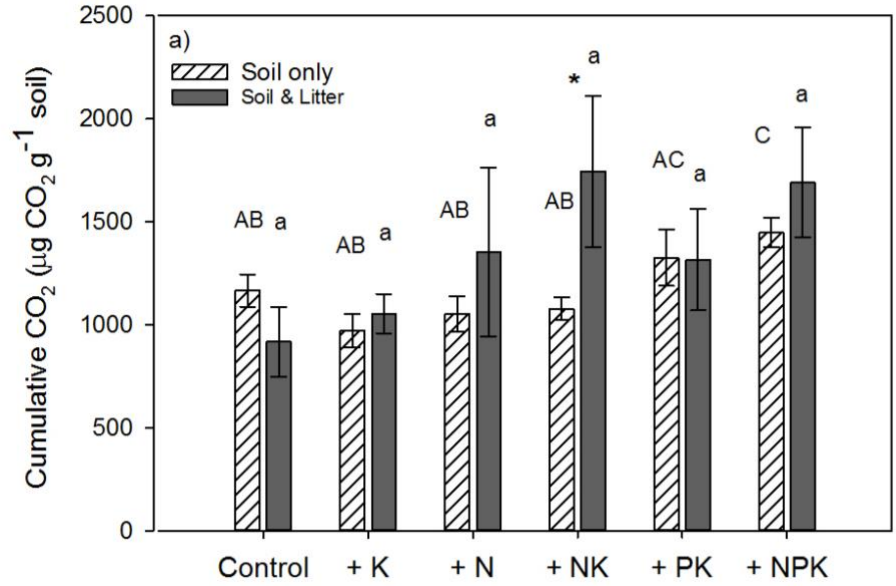
536

Table 2. Statistical summary of litter and nutrient addition treatment effects on CO_2 flux and microbial biomass carbon, nitrogen and phosphorus content. Statistically significant treatment effects with p-values <0.05 are in bold.

	Litter addition	Nutrient	Litter x Nutrient	Nutrient (Soil only)	Nutrient (Litter added)
Total CO_2	**$387_{(1,35)}$; <0.0001**	$0.586_{(5,35)}$; 0.710	$0.650_{(5,35)}$; 0.6634	**$9.68_{(5,17)}$; 0.0002**	$0.707_{(5,17)}$; 0.626
Soil derived CO_2	$2.13_{(1,35)}$; 0.154	$2.24_{(5,35)}$; 0.072	$1.17_{(5,35)}$; 0.346	**$9.68_{(5,17)}$; 0.0002**	$2.10_{(5,17)}$; 0.116
Litter derived CO_2	NA	NA	NA	NA	$0.601_{(5,17)}$; 0.700
Total microbial biomass C	**$54.2_{(1,35)}$; <0.0001**	**$4.60_{(5,35)}$; 0.0025**	**$3.84_{(5,35)}$; 0.0071**	**$3.01_{(5,17)}$; 0.0399**	**$5.18_{((5,17)}$; 0.0046**
Soil derived microbial biomass C	**$8.87_{(1,35)}$; 0.0056**	**$3.46_{(5,35)}$; 0.0134**	**$3.25_{(5,35)}$; 0.0179**	**$3.01_{(5,17)}$; 0.0399**	**$3.57_{(5,17)}$; 0.0272**
Litter derived microbial biomass C	NA	NA	NA	NA	**$3.34_{(5,17)}$; 0.0341**
Microbial biomass N	**$6.95_{(1,35)}$; 0.0124**	$1.14_{(5,35)}$; 0.359	$0.816_{(5,35)}$; 0.547	$2.54_{(5,17)}$; 0.068	$0.660_{(5,17)}$; 0.658
Microbial biomass P	**$30.1_{(1,35)}$; <0.0001**	**$24.1_{(5,35)}$; <0.0001**	**$4.09_{(5,35)}$; 0.005**	**$14.5_{(5,17)}$; <0.0001**	**$14.7_{(5,17)}$; <0.0001**
Microbial biomass C:N	**$6.62_{(1,35)}$; 0.015**	$1.97_{(5,35)}$; 0.112	$0.2114_{(5,35)}$; 0.955	$0.959_{(5,17);}$ 0.4712	$0.565_{(5,17);}$ 0.725
Microbial biomass C:P	$0.493_{(1,30)}$; 0.488	$2.41_{(5,30)}$; 0.060	$1.72_{(5,30)}$; 0.161	$1.44_{(5,15)}$; 0.2661	**$4.14_{(5,14)}$; 0.016**
Soil Biomass:Respiration partitioning	**$5.72_{(1,35)}$; 0.022**	$1.60_{(5,35)}$; 0.186	$0.443_{(5,35)}$; 0.815	**$4.16_{(5,17)}$; 0.012**	$1.17_{(5,17)}$; 0.361
Litter Biomass:Respiration partitioning	NA	NA	NA	NA	$2.26_{(5,17)}$; 0.096

Figure 1



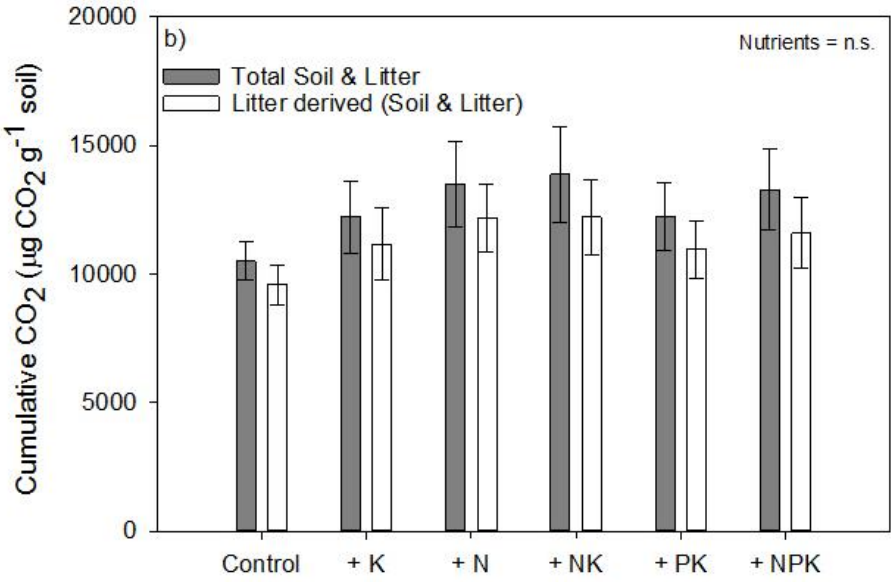
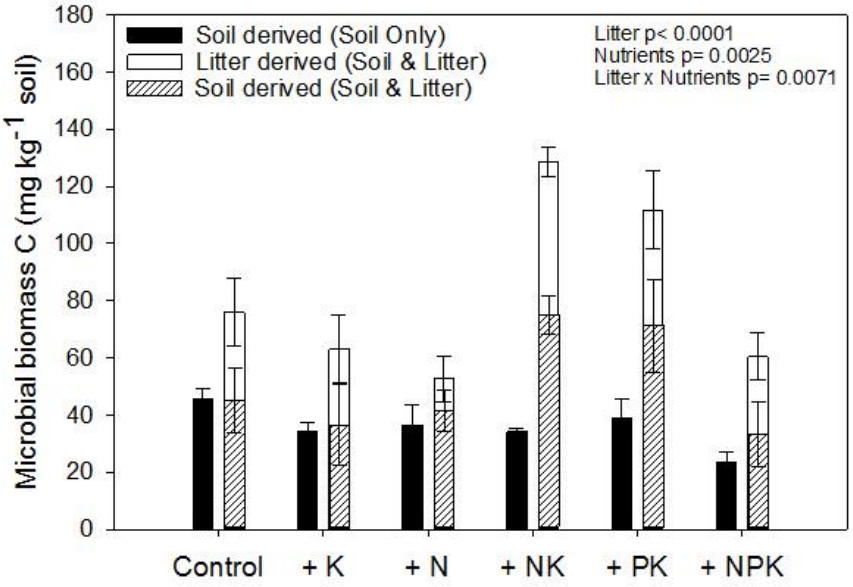


Figure 1. a) Cumulative soil derived CO_2 over 30 days in the Soil Only (striped bars) and Soil & Litter (grey bars) treatments, and b) Cumulative CO_2 from the soil and litter in the Soil & Litter treatments (grey bars) and litter derived only CO_2 (white bars) over 30 days. Bars are means of four replicates with standard error bars. Capital letters indicate significant differences between nutrient treatments within the Soil Only treatment, lower case letters indicate significant differences between nutrient treatments within the Soil & Litter treatment and * indicates statistically significant differences between the Soil Only and Soil & Litter treatments within a nutrient treatment (i.e. positive priming due to the litter addition).

554

555 Figure 2



556

557 Figure 2. Soil- and Litter-derived microbial biomass carbon at the end of the 30-day incubation. Error
558 bars are standard errors for the average of four replicates of each mineral-nutrient treatment.

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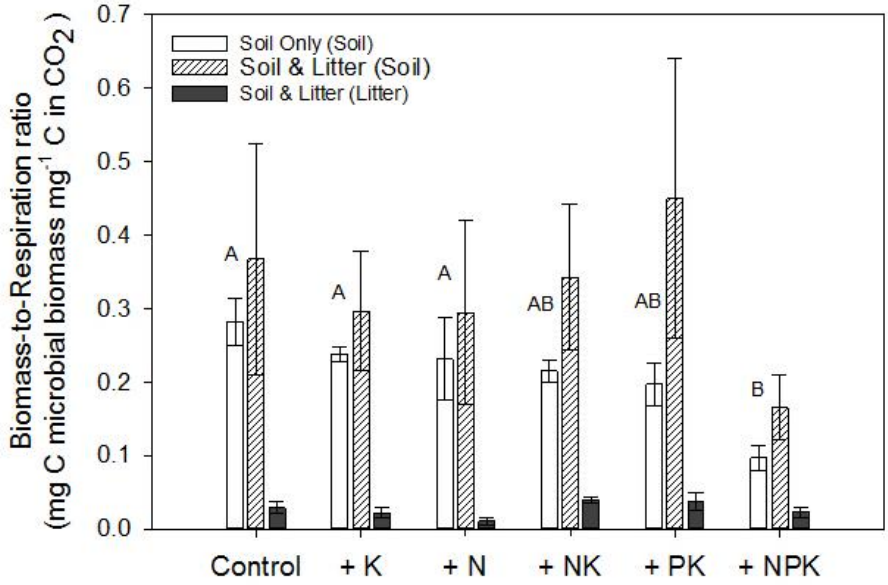
563 Figure 3



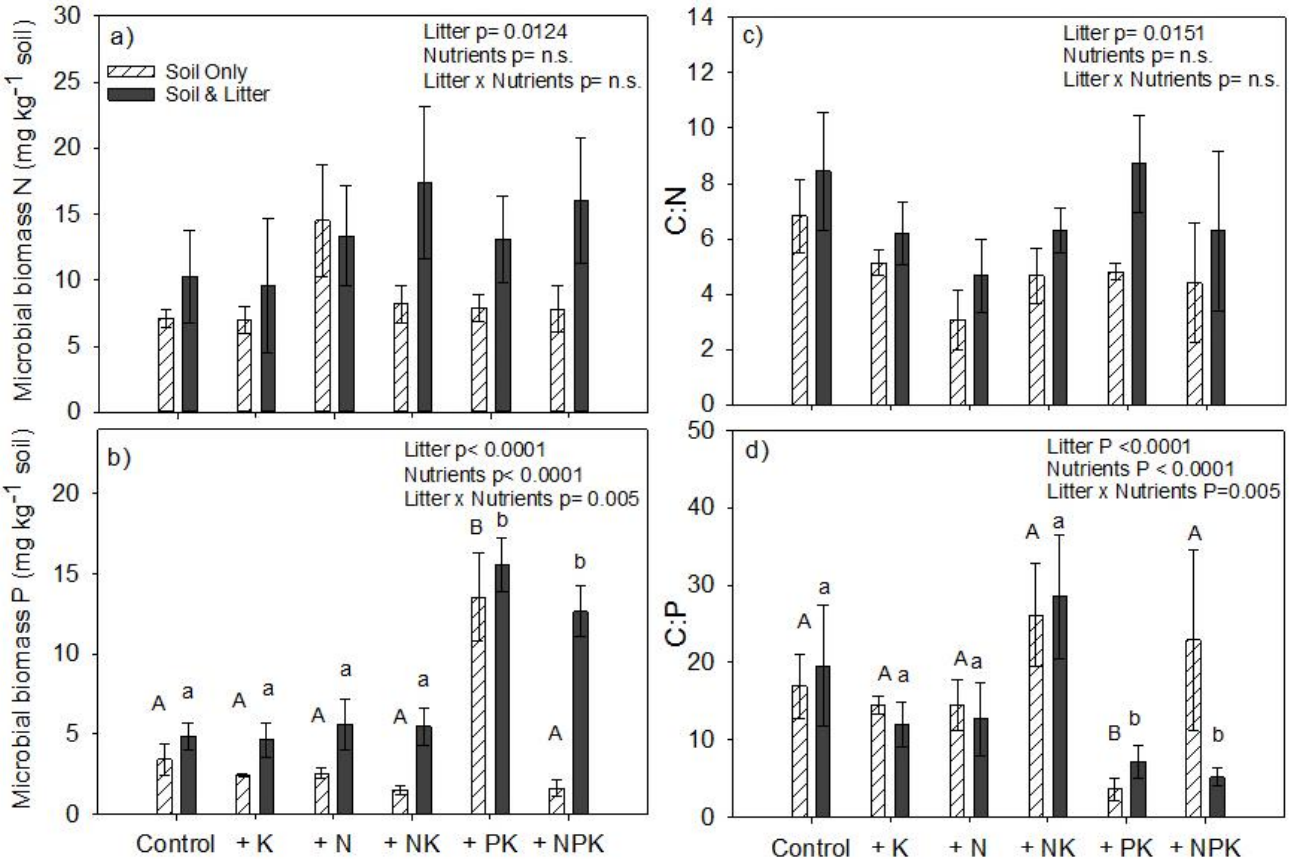
564

565 Figure 3. The Biomass-to-respiration ratio for soil-derived carbon in the Soil Only and Soil & Litter
566 treatments, and litter-derived carbon in the Soil & Litter treatments for the various nutrient additions.
567 Capital letters indicate significant differences between Nutrient treatments in the Soil Only treatment.
568 There were no significant differences between Nutrient treatments in the Soil & Litter treatment. Bars
569 are mean values of the mineral-nutrient treatments and error bars are standard errors (n=4).

570

571

572 Figure 4



573
574 Figure 4. a) Microbial biomass N, b) Microbial biomass P, c) Microbial biomass C:N, and d) Microbial
575 biomass C:P ratios at the end of the 30-day incubation. Different uppercase letters indicate statistical
576 significance between mineral nutrient additions within the Soil Only treatment, while lowercase letters
577 indicate differences within the Soil & Litter treatment ($p<0.05$). Bars are mean values and error bars are
578 standard errors (n=4).
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