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

3

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21

22 **Keywords**

23 13C; CNP; Microbial Stoichiometry; Priming; Soil respiration; Tropics

24 **Abstract**

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

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

49

50 1. Introduction

51 Soil nutrient availability and stoichiometry have strong influences on soil carbon (C) cycling
52 through their impact on the decomposition and formation of soil organic matter (Reed et al., 2011;
53 Cotrufo et al., 2013; Poeplau et al., 2016). Different elemental C, nitrogen (N), phosphorus (P)
54 stoichiometric ratios of plants (*ca.* C:N:P=3144:45:1; Cleveland and Liptzin (2007)), soil (*ca.*
55 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;
56 Cleveland and Liptzin (2007)) involved in molecular transformations during decomposition are assumed
57 to define the relationship between nutrients and C cycling (Sturner and Elser, 2002; Manzoni et al.,
58 2012; Sinsabaugh et al., 2013; Zechmeister-Boltenstern et al., 2015). The maintenance of fixed ratios of
59 elements in various organic substrates forms the basis of the Environmental Stoichiometry theory and
60 provides a mechanistic understanding of biogeochemical transformations (Sturner and Elser, 2002;
61 Spohn, 2016). Due to stoichiometric constraints, an increased availability of C and N in ecosystems due
62 to global change should subsequently lead to increased demands for other macro-nutrients, such as P
63 and potassium (K), thereby causing an imbalance between nutrient availability and nutrient demands in
64 natural ecosystems (Peñuelas et al., 2012). Ecosystem nutrient and C enrichment from global change
65 often corresponds with increased plant productivity and organic substrate inputs to the soil in the form
66 of litter (LeBauer and Treseder, 2008; Gill and Finzi, 2016). Therefore, disentangling the direct responses
67 of soil microbial activity to nutrient additions from the indirect responses via plant feedbacks *in situ*
68 is not straightforward. In order to better predict how nutrient enrichment affects soil C cycling, more
69 information is needed on the role of microbial C:N:P stoichiometric constraints to C cycling in direct
70 response to nutrient enrichments as compared to addition of plant inputs.

71 Unlike temperate and northern ecosystems that are mainly N limited, ecosystems in the tropics
72 are generally limited by low P availability due to the old age, strong weathering and high reactivity of Fe
73 and Al oxide rich soils (Walker and Syers, 1976; Vitousek and Farrington, 1997; Turner and Wright, 2014;
74 Grau et al., 2017). Relatively less is known about the role of K in C cycling, although evidence for possible
75 K limitation of tropical systems has also begun to emerge (Doetterl et al., 2015; Sardans and Peñuelas,
76 2015). Given the essential role of nutrients in microbial functioning, human-induced changes in the

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

81 Microbial physiology is critical to ecosystem C cycling because microbial biomass and residues
82 contribute significantly to the formation of persistent soil organic matter (SOM), while microbial
83 respiration leads to immediate loss of C from the soil (Cotrufo et al., 2013; Kallenbach et al., 2016).
84 Quantifying the partitioning of C from decomposing substrates into microbial biomass and respiration
85 allows us to mechanistically link microbial activities with soil CO₂ fluxes and C sequestration at the
86 ecosystem scale (Cotrufo et al., 2015; Soong et al., 2015; Campbell et al., 2016). Nitrogen additions tend
87 to increase microbial C use efficiency due to the C and N co-limitation of microbial growth (Sinsabaugh
88 et al., 2013). While N additions have been found to decrease microbial C respiration leading to an
89 increase in relative C retention in biomass (Spohn et al., 2016), P additions tend to stimulate respiration
90 activity relative to microbial biomass (Hartman and Richardson, 2013). Understanding the degree to
91 which microbial respiration and growth are coupled with microbial N and P constraints would help to
92 advance our understanding of how to integrate nutrients into models of C cycling (Reed et al., 2015).

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

105 In this study, we investigate how mineral nutrient additions themselves, or in combination with
106 organic matter inputs, affect the decomposition of soil organic matter and leaf litter, and the
107 partitioning of C into microbial biomass and CO₂ production. We also examine the microbial biomass C,
108 N and P responses to mineral nutrient additions alone or in combination with a labile source of litter C.
109 We hypothesized that microorganisms in this tropical forest mineral soil would respond most strongly to
110 the addition of P, but that the presence of labile litter would also enhance microbial activity and nutrient
111 uptake by providing a complex source of labile organic matter to the soil. This would help to explain the
112 direct impact of N, P and K availability on microbially mediated soil C dynamics *versus* indirect effects via
113 higher net primary productivity and organic matter inputs to the soil. To test these hypotheses, we
114 incubated soil from the lowland tropical Amazon rainforest of French Guiana amended with either a
115 labile, C-rich ¹³C labeled leaf litter (i.e., an organic substrate containing C and nutrients), additions of
116 mineral N, P and K, or their combinations. Over the course of a 30-day laboratory incubation we
117 measured microbial C cycling by quantifying the fate of decomposing SOM and litter C into CO₂ fluxes
118 and microbial biomass. We also measured the organic and mineral substrate addition effects on
119 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).

122

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 ¹³C/¹²C 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).

143

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* ¹³C 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 CO₂ flux
158 measurements, for a total of 52 incubation units.

159 Mineral N was added at a rate of 367 g N/ kg soil and mineral P additions were added at a rate
160 of 195 g P/ kg soil. This is equivalent to approximately two times the annual natural N input from litter at
161 the site ($6.5 \text{ g N m}^{-2} \text{ y}^{-1}$), and fifty times the natural P input at the field site ($0.14 \text{ g P m}^{-2} \text{ y}^{-1}$), and is
162 equivalent to previous fertilization experiments at this site (Barantal et al., 2012; Fanin et al., 2014).
163 While these nutrient addition rates are somewhat greater than natural inputs they help to stimulate
164 existing mechanisms and therefore better identify them. Though this methodology may push the
165 microorganisms into a situation not faced in the field, this is a classical approach used to better
166 understand natural processes (Benton et al., 2007). Mineral nutrient treatments were added in 1 ml
167 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
168 the +NK treatment, 0.0214 g of KH_2PO_4 for the +PK treatment, 0.0117 g KCl for the +K treatment, and
169 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
170 the Control treatment. We used KH_2PO_4 as our P source because it is soluble, C-free and had no effect
171 on soil pH, therefore, we did not have a P-only nutrient addition. In an attempt to isolate the P-only
172 nutrient affects, and assuming additive responses of nutrient combinations, we added +K (as KCl) in an
173 equivalent amount as is contained in the KH_2PO_4 and KNO_3 additions in order to help differentiate the +K
174 from +PK effects. Thus, mineral K was added at a rate of 246 g K/ kg soil.

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

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

198

199 2.4 CO_2 fluxes

200 Soil CO₂ flux and C-isotopic composition were determined on days 1, 2, 3, 5, 7, 10, 15, 21 and 30
201 of the incubation. Each day, the incubation bottles were closed and both initial and final concentrations
202 of ¹²C- and ¹³C- CO₂ were measured using a Picarro G2131-i cavity ring-down spectrometer (Picarro inc.,
203 Santa Clara, CA, USA). The analyzer was calibrated for high enrichments and we utilized a specially
204 designed discrete sampling system (Dickinson et al., 2017). To measure CO₂ fluxes, the bottles were
205 closed for 24 hours for the first three time points, in order to capture the initial high CO₂ fluxes from the
206 litter, and for 2 hours for the rest of the time points. The CO₂ concentration was measured at the start
207 and end of each closure period and flux rate was calculated as the accumulation of CO₂ over the given
208 time period. After each measurement, we ventilated the bottles with CO₂-free air until the
209 concentration of CO₂ was close to ambient. Between measurements the bottles were loosely covered
210 with parafilm to prevent drying but allow for some gas exchange. All flux calculations were corrected for
211 the exact volume of each bottle minus the volume of the sample. The concentration of CO₂ never
212 exceeded 3% during any of the incubation periods. Flux rates were integrated between sampling dates
213 to estimate total cumulative CO₂ respiration. We used the initial soil and litter isotopic values as end
214 members in a two-end member isotope-mixing model to calculate the amount of soil-derived or litter-
215 derived C contributing to the measured CO₂ fluxes from the Soil & Litter units (see section 2.6 for
216 details).

217

218 *2.5 Microbial biomass and chemical analyses*

219 After 30 days we removed the samples from each incubation jar, weighed them, homogenized
220 them and subsampled them for chemical analysis. We sieved the soils to 2 mm and picked the remaining
221 litter pieces out of the soil before any soil analyses. One aliquot of soil was oven dried at 70 °C for 72
222 hours to measure gravimetric soil water content. The remaining soil was sub-sampled into 5 g aliquots
223 and either extracted with an 0.25 M HCl and 0.03 M NH₄F solution for available P (Bray and Kurtz, 1945),
224 or with 0.5 M K₂SO₄ for extractable C and N (Brookes et al., 1985), or fumigated with CHCl₃ for 72 hours
225 and similarly extracted for available P or extractable C and N. The HCl and NH₄F solution was chosen for
226 the P extraction because F⁻ promotes P desorption in these Al³⁺ rich soils, and promotes P desorption.
227 We filtered all extracts over pre-wetted Whatman #40 ash-less filters. We extracted the soils and began
228 fumigations on fresh soils within 48 hours after the last CO₂ flux measurement on day 30 of the
229 incubation. The difference between C, N and P contents of the fumigated and non-fumigated soils was
230 used as a measurement of microbial biomass C, N and P, respectively (modified from Brookes et al.
231 (1985) due to different solution for P extractions). This difference between fumigated and non-
232 fumigated samples was not corrected for extraction efficiencies, and thus should be considered as a
233 proxy for microbial biomass values in this soil.

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

240

241 2.6 Data analysis

242 For statistical analysis of CO₂ fluxes, we used a repeated measures mixed effects model with
243 mineral nutrient addition, litter addition and their interaction as fixed factors and individual sample as a
244 random factor in order to account for the repeated sampling in the analysis of CO₂ fluxes. For the
245 cumulative CO₂ fluxes used in the biomass-to-respiration ratio and priming calculations, and for all
246 microbial biomass data, we used a generalized linear model with mineral nutrient addition, litter
247 addition, and their interaction as fixed effects. We then analyzed the mineral nutrient effect in Soil Only
248 or Soil & Litter treatments separately using a Tukey *post hoc* analysis to make pairwise comparisons
249 between mineral nutrient addition effects within the mineral only and organic plus mineral nutrient
250 treatments. Normality and homogeneity of variance was checked for each analysis and an log
251 transformation was used when necessary to fit the assumptions of the parametric models. Statistically
252 significant differences were defined by p-values <0.05. All statistics were done using the nlme (Pinheiro
253 et al., 2017) and multcomp (Hothorn et al., 2008) packages in RStudio version 0.99.892.

254 In the Soil & Litter treatments, both litter and SOM decomposition contributed to microbial
255 biomass and CO₂ fluxes. Therefore, we used the isotopic mixing model to quantify the contribution of
256 litter-derived C and soil-derived C to CO₂ flux and microbial biomass.

257 (1)
$$f_A = \frac{atom\% \ ^{13}C_x - atom\% \ ^{13}C_B}{atom\% \ ^{13}C_A - atom\% \ ^{13}C_B}$$

258 Where f_A is the proportion of soil-derived C (A), and $atom\% \ ^{13}C_A$ is the percent ¹³C of starting soil, $atom\%$
259 $^{13}C_B$ is the percent ¹³C of the added residue (B), and $atom\% \ ^{13}C_x$ is the percent ¹³C measured from the
260 “Soil & Litter” sample. The f_A -value was then multiplied by the total CO₂ flux, or total microbial biomass,
261 in order to calculate the amount of soil-derived C in the CO₂ flux or microbial biomass C. The amount of
262 litter-derived C was calculated as the total CO₂ or microbial biomass pool minus the soil-derived
263 contribution.

264 The microbial biomass-to-respiration ratio was used as an indication of C partitioning during
265 decomposition. This ratio tells us proportionally how much C from litter or SOM decomposition is
266 retained in the microbial biomass at the end of the incubation, versus how much is lost as CO₂ flux to the
267 atmosphere. It was calculated separately for litter- and soil-derived C, based on isotopic partitioning
268 using equation 1. Therefore, the amount of litter C in microbial biomass was the final amount of litter-
269 derived C in the microbial biomass after 30-days, while the amount of soil-derived C in microbial
270 biomass at the end of the incubation was calculated as the difference between the final amount of soil-
271 derived C in microbial biomass minus the mean microbial biomass in the pre-incubated, starting soil.
272 This measure of microbial biomass gives us a net microbial biomass C production value, including both
273 growth and turnover, over the 30-day incubation. CO₂ fluxes were integrated between sampling points
274 to calculate the total litter- or soil-derived CO₂ respiration over the 30-day incubation. We used a 0.45
275 correction factor to account for the efficiency of the extraction for the microbial biomass C assimilation
276 value in the biomass-to-respiration ratio (Jenkinson and Powlson, 1976). Although this correction factor
277 is not specific to this soil, it is likely that the chloroform fumigation extraction method does not quantify
278 all microbial biomass C so a 0.45 correction factor helps to put our microbial biomass C measures on a
279 more comparable scale with respiration C values in the biomass-to-respiration ratio.

280 The effect of the litter addition and nutrient treatments on priming of SOM was calculated as
281 the difference between the cumulative CO₂ flux of soil-derived cumulative CO₂ fluxes per g of soil from
282 the Soil & Litter treatment and the Soil Only treatment.

283

284 3. Results

285 3.1 SOM respiration as affected by mineral and organic nutrient additions

286 The litter addition increased the overall measured CO₂ flux by an order of magnitude, while
287 nutrient addition and the interaction between nutrient addition and litter addition did not have a
288 significant effect on CO₂ fluxes in the Soil & Litter treatment (Figure 1b, Table 2). Mineral nutrient
289 additions significantly affected the total soil CO₂ flux only in the Soil Only treatment in the absence of
290 organic substrate amendment (Table 2). In the Soil Only treatments, the +NPK mineral nutrient addition
291 had significantly greater soil CO₂ fluxes than the Control, +K, +N and +NK treatments ($p < 0.05$; Figure 1a).
292 When we partitioned the total CO₂ flux from the Soil & Litter treatment into soil-derived and litter-
293 derived CO₂ flux, there was no significant effect of mineral nutrient additions on either soil-derived or
294 litter-derived CO₂ fluxes. Litter-derived C constituted approximately 90% of the total CO₂ flux in the Soil
295 & Litter treatments. This disproportionately large contribution of litter *versus* soil to the CO₂ flux
296 resulted in somewhat large uncertainty in the soil-derived CO₂ fluxes in the Soil & Litter treatment, as
297 seen in the large error bars in Figure 1.

298 The combined litter addition and mineral +NK treatment led to a greater loss of soil C to CO₂ flux
299 ($t_{17} = 2.85$; $p = 0.0110$), and the soil C losses were significantly greater than in the correspondent Soil Only
300 +NK treatment (Figure 1a). This indicates priming of SOM decomposition by the combined litter and +NK
301 nutrient treatment interaction. The litter addition did not lead to a significant increase in soil-derived
302 CO₂ flux, or priming, in any of the other nutrient treatments (Figure 1a).

303

304 3.2 Microbial biomass carbon

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

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

319 and mineral nutrient addition in their effect on soil-derived microbial biomass C (Table 2; Figure 2 white
320 bars). Within the Soil & Litter treatment, mineral nutrient addition had a significant effect on soil-
321 derived microbial biomass (Table 2) with the +NK treatment having more soil-derived microbial biomass
322 C than the Control, +K, +N and +NPK treatments ($p < 0.05$). The +PK treatment also tended to have larger
323 soil-derived microbial biomass C on average, but it was not statistically significantly different from the
324 other treatments (Figure 2). The nutrient effect on litter-derived microbial biomass was the same, with
325 the +NK treatment having larger litter-derived microbial biomass than the Control, +K, +N and +NPK
326 treatments ($p < 0.05$) and the +PK treatment not being significantly different from any other treatment
327 (Figure 2). Soil and litter-derived C contributed equal amounts to the microbial biomass at the end of the
328 30-day incubation across all Nutrient treatments (Figure 2). This shows that after 30 days, litter derived
329 C made up approximately half of the microbial biomass C in the Soil & Litter treatment.

330

331 *3.3 Microbial carbon partitioning*

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

342

343 *3.4 Microbial biomass nitrogen and phosphorus*

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

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

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

364

365 **4. Discussion**

366 *4.1 Nutrient effects on soil organic matter decomposition*

367 Our results provide evidence for both organic matter and mineral nutrient effects on SOM
368 decomposition in this tropical soil. Microbial respiration and biomass responded to the addition of a
369 labile litter substrate, indicating a clear response to labile organic matter, which was mediated by
370 nutrient availability. Rapid decomposition of leaf litter under hot and humid conditions in tropical soils
371 can lead to a strong C limitation as seen in the low C content of this top-soil (Table 1). Moreover, it is
372 possible that the labile litter addition effect masked any potential nutrient addition effects in the Soil &
373 Litter treatment on SOM or litter decomposition. However, in the absence of the litter addition, the
374 addition of N, P and K in combination significantly increased the mineralization of soil C to CO₂ as
375 compared to the control. No other single or dual nutrient addition had any significant effect on soil
376 respiration, although the +PK treatment slightly increased CO₂ flux, indicating a tri-NPK limitation of
377 mineral soil respiration.

378 Although we did not have a P-only nutrient addition, we found evidence to support the
379 hypothesis that P stimulates soil CO₂ fluxes. In the Soil Only treatment, both nutrient treatments
380 containing P had the highest cumulative CO₂ production and were not significantly different from one
381 another. The +NPK treatment had the largest CO₂ flux, and was significantly greater than the Control,
382 +N, +K and +NK treatments. The +PK treatment had significantly greater CO₂ flux than the +K treatment.
383 If we assume additive effects of nutrients on microbial activity, we would then deduce that P is the
384 nutrient responsible for the increased CO₂ production in the +PK and +NPK nutrient treatments. Without
385 a true +P treatment, however, this remains an interpretation.

386 Soil microorganisms in tropical mineral soils may be both C and NPK co-limited. Previous studies
387 on the same tropical French Guianese soils have demonstrated the impact of N and P additions on litter
388 decomposition rates and microbial communities (Barantal et al., 2012; Fanin et al., 2014; Fanin et al.,
389 2016). However, our results clearly demonstrate the overwhelming importance of fresh litter inputs on
390 soil respiration and microbial biomass. Tropical forest leaf litter varies widely in composition
391 (Hattenschwiler et al., 2008). While the *A. gerardii* litter used in our study had a slightly greater P
392 content than most tropical forest leaf litter, we used it here to highlight the difference in mineral
393 nutrient effects *versus* organic matter and mineral nutrient effects on microbial activity in this mineral
394 tropical top soil. While Fanin et al. (2014) found that P fertilization alone affected microbial community
395 structure and cellulose paper decomposition, they also found strong synergistic effects of C and N
396 additions along with P fertilization. Similarly, in our tropical soil incubation the addition of labile organic
397 matter led to a large increase in microbial activity with no significant nutrient addition effects, while
398 nutrient additions alone did stimulate microbial activity.

399 Partitioning the decomposition of soil organic C to microbial biomass *versus* CO₂ flux is critical to
400 understanding soil C sequestration during decomposition of organic substrates. Respiration is the main
401 loss pathway of C from the soil, while microbial biomass contributes to the formation of persistent SOM
402 (Mambelli et al., 2011; Kallenbach et al., 2016). The microbial biomass-to-respiration ratio was
403 significantly lower with the +NPK addition due to both greater soil-derived CO₂ fluxes and smaller C
404 retention in microbial biomass. This shift in C partitioning, along with the low microbial biomass P
405 content in the Soil Only +NPK treatment, indicate increased turnover of microbial biomass. Previous soil
406 incubations have found P additions to increase microbial biomass specific respiration, or qCO₂ (Hartman
407 and Richardson, 2013), yet here we found that the tri-nutrient effect in the +NPK treatment decreased
408 the net biomass-to-respiration ratio, while the +PK and +NK treatment did not. This could be due to the
409 differential P demands by various microorganisms at different developmental stages or a shift in the
410 microbial community (Elser et al., 2003). Here, it appears that all three N, P and K nutrients were needed
411 for this outcome, pointing again toward the need for a greater understanding of the interactive effects
412 of nutrients on microbial functioning and carbon cycling to inform coupled C-nutrient cycling models
413 (Huang et al., 2018; Wang et al., In Review).

414 Along with litter addition, the +NK treatment primed SOM decomposition via a larger and more
415 active microbial community as seen in the larger microbial biomass and greater CO₂ flux as compared to
416 the Soil Only +NK treatment. This stimulation of SOM decomposition uniquely occurred in the +NK and
417 litter addition treatment indicating a C, N, K stimulation of SOM decomposition. It is not entirely clear
418 from our dataset why this treatment alone resulted in a positive SOM priming effect. One explanation
419 could be a shift to a larger and more active microbial community. Another explanation could be
420 enhanced SOM decomposition to acquire organic P when C, N and K were provided in excess.
421 Microorganisms can obtain organic P via enzymatic activity during SOM decomposition and inorganic P
422 through acidification and complexing agents. Therefore, the stimulation of SOM decomposition to
423 obtain limited P resources is only one potential P access pathway. Mining of SOM for P via enzymatic
424 activity could be more likely in P limited tropical soils than in more N limited temperate ecosystems
425 where P is more abundant (Craine et al., 2007; Dijkstra et al., 2013). In these highly weathered, Eastern
426 Amazonian soils it is likely that almost all of the soil P is divided between organic and occluded forms
427 (Walker and Syers, 1976). Thus, when provided with C-rich labile organic matter and +NK nutrients, the
428 enhanced decomposition of SOM measured here indicates a potential mining of SOM for organic P. If
429 ecosystem C, N and K enrichment is likely to stimulate plant primary productivity (Reich et al., 2006), our
430 results suggest that increased organic matter inputs to the soil in combination with N and K enrichment
431 could possibly cause enhanced decomposition of SOM in tropical soils due to priming.

432 The approximately ten-fold greater biomass-to-respiration ratio of soil-derived C compared to
433 litter C reflects the difference in microbial metabolism of organic matter of contrasting quality. Isotopic
434 partitioning of the CO₂ flux allowed us to see that there was no significant mineral nutrient effect on
435 litter-derived CO₂ fluxes or on microbial partitioning between biomass and respiration. While the +NK
436 treatment did lead to larger microbial biomass, slightly greater CO₂ fluxes from the same treatment did
437 not lead to significant change in C partitioning. The nutrient content of the decomposing litter itself,
438 which was being rapidly decomposed due to its high lability, may have masked any effects of the mineral
439 nutrient additions indicating a strong C limitation to microbial activity in this tropical soil. Due to the
440 high C-to-nutrient ratio of leaf litter compared to microbial biomass, much more C is lost as CO₂ rather
441 than retained in biomass during litter decomposition than during SOM decomposition, which is

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

448

449 *4.2 Microbial biomass stoichiometry*

450 Across all our treatments, CN ratios of microbial biomass communities were constrained
451 between 3.5 and 8 while microbial CP ratios varied between 4 and 28 (Figure 4). This was in spite of the
452 fact that the mass of the N additions was nearly twice as large as the P additions (367 g N/ kg soil and of
453 195 g P/ kg soil). This demonstrates the much greater potential variability in microbial biomass P content
454 and CP ratios than N content CN ratios in this tropical soil. Although we cannot say whether this resulted
455 from a shift in the microbial community or a direct flexibility in cellular P content (Fanin et al., 2013;
456 Kaiser et al., 2014), we can conclude that the same community within a given treatment combination
457 often had a greater divergence in C:P than C:N ratios from the Control (Figure 4). While the addition of
458 NH_4NO_3 and KH_2PO_4 could have acidifying properties, the soil used here had a pH of 3.99 and was not
459 likely further acidified. The variability of microbial biomass P content relative to C and N may be a key
460 aspect in the complex role of microorganisms in soil C cycling, particularly in P limited tropical biomes.
461 The production of organic acids and phosphatase enzymes by bacteria, fungi and actinomycetes could
462 allow bacteria and fungi to immobilize P even in the absence of organic matter inputs (Jones and
463 Oburger, 2011). Tropical foliar P content is also responsive to P fertilization, while foliar N content is not
464 responsive to N fertilization (Elser et al., 2003; Mayor et al., 2014; Wang et al., 2017). The potential
465 implications of this are that both plant and microbial variability in cellular P content could be adaptive to
466 seasonal or temporal changes in P availability. However, the immobilization of available P by
467 microorganisms, particularly in the presence of available C, could also mean that fast growing
468 microorganisms could possibly compete with plants for available P particularly when litter inputs or root
469 exudation is high. The lack of a microbial P response in the Soil Only, +NPK treatment along with its
470 greater CO_2 flux and lower microbial biomass points toward high microbial turnover in this treatment
471 rather than P accumulation. This could account for the lack of a microbial biomass P response in this
472 treatment if high turnover inhibits the accumulation of microbial biomass P.

473 One possible mechanism for the difference in N and P limitation effects on SOM priming could
474 be the de-coupling of microbial P uptake from SOM decomposition and microbial growth (Dijkstra et al.,
475 2013). A large portion of P is occluded in minerals while most soil C and N is in organic matter (Gerard,
476 2016). Carbon and N are both required to build and maintain microbial cell walls and enzymes, which
477 both have rather constrained stoichiometry, explaining why microbial biomass C:N ratios show little
478 variation. Phosphorous, in contrast, controls the rate of metabolic processes but may be less intrinsically
479 linked to microbial biomass (Elser et al., 2003). Thus, while microbial respiration rates may be responsive
480 to P additions, microbial growth is more responsive to C and N additions (Hartman and Richardson,
481 2013). Moreover, the ability to store P in non-organic forms could allow microorganisms to thrive in
482 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 ¹³C 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 CO₂ 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 CO₂ flux was highest, which points to enhanced microbial biomass turnover. In contrast,
516 litter addition and +NK fertilization stimulated both biomass production and soil CO₂ 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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532

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

535

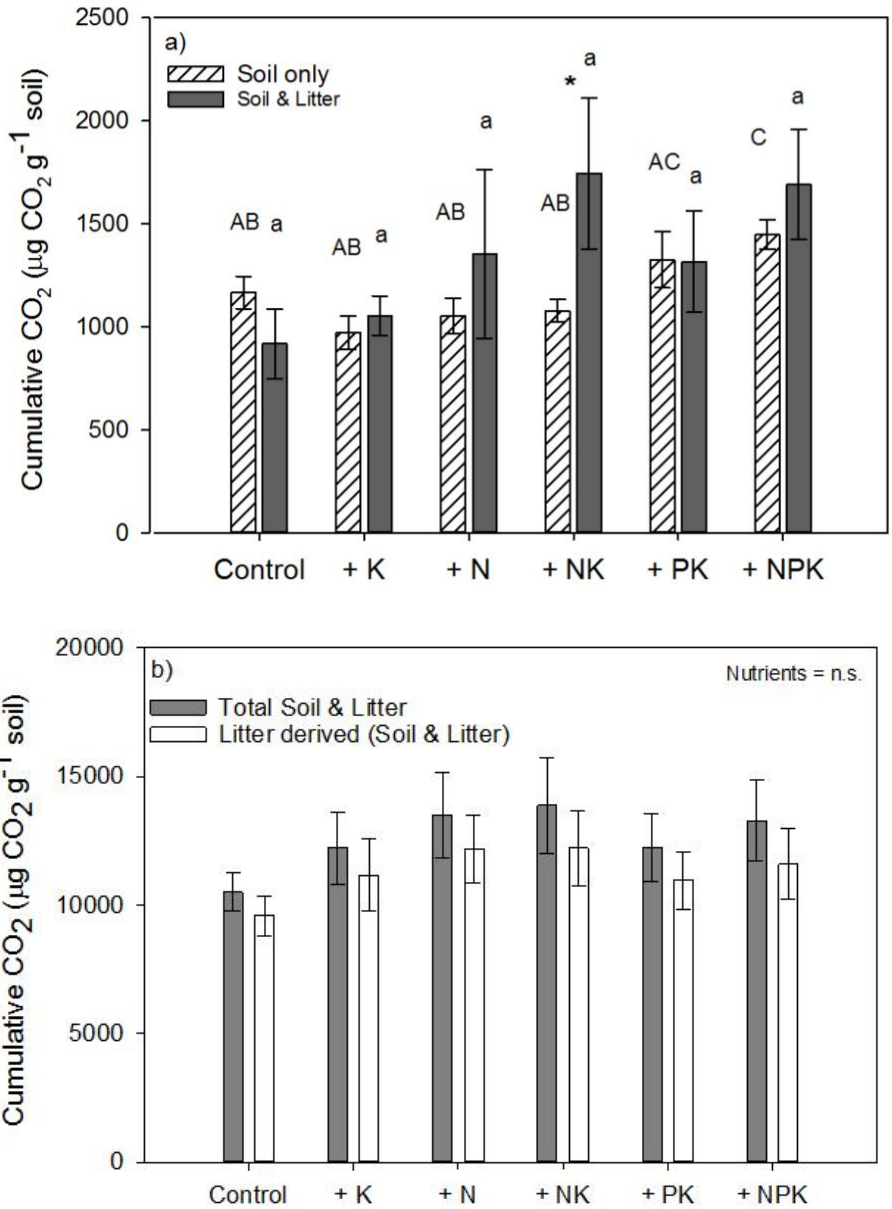
536

537 Table 2. Statistical summary of litter and nutrient addition treatment effects on CO₂ flux and microbial
 538 biomass carbon, nitrogen and phosphorus content. Statistically significant treatment effects with p-
 539 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

540

541 Figure 1



542

543

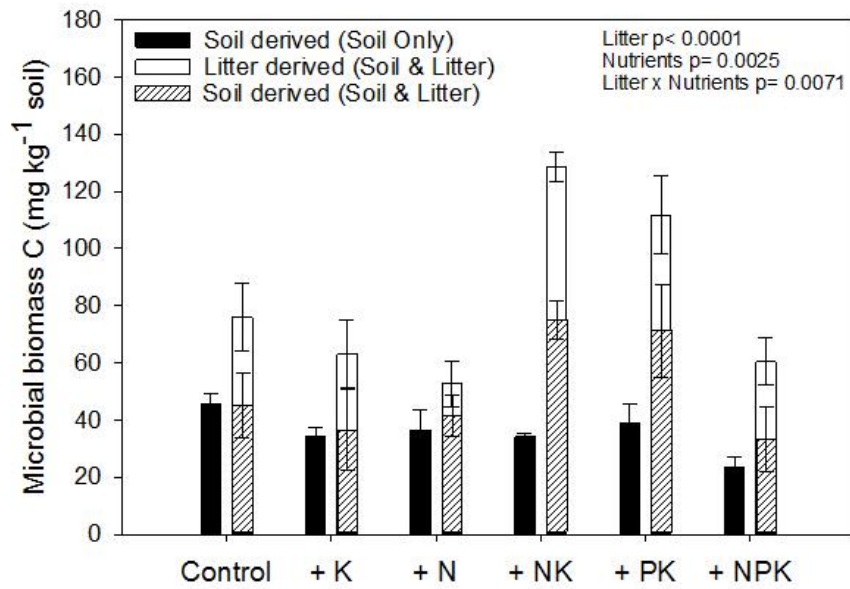
544 Figure 1. a) Cumulative soil derived CO₂ over 30 days in the Soil Only (striped bars) and Soil & Litter (grey
 545 bars) treatments, and b) Cumulative CO₂ from the soil and litter in the Soil & Litter treatments (grey
 546 bars) and litter derived only CO₂ (white bars) over 30 days. Bars are means of four replicates with
 547 standard error bars. Capital letters indicate significant differences between nutrient treatments within
 548 the Soil Only treatment, lower case letters indicate significant differences between nutrient treatments
 549 within the Soil & Litter treatment and * indicates statistically significant differences between the Soil
 550 Only and Soil & Litter treatments within a nutrient treatment (i.e. positive priming due to the litter
 551 addition).

552

553

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.

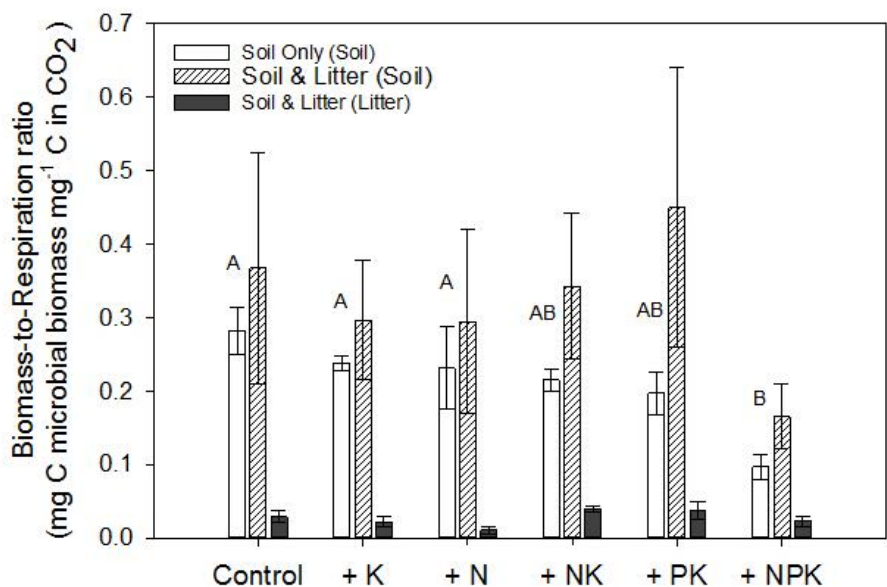
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563 Figure 3

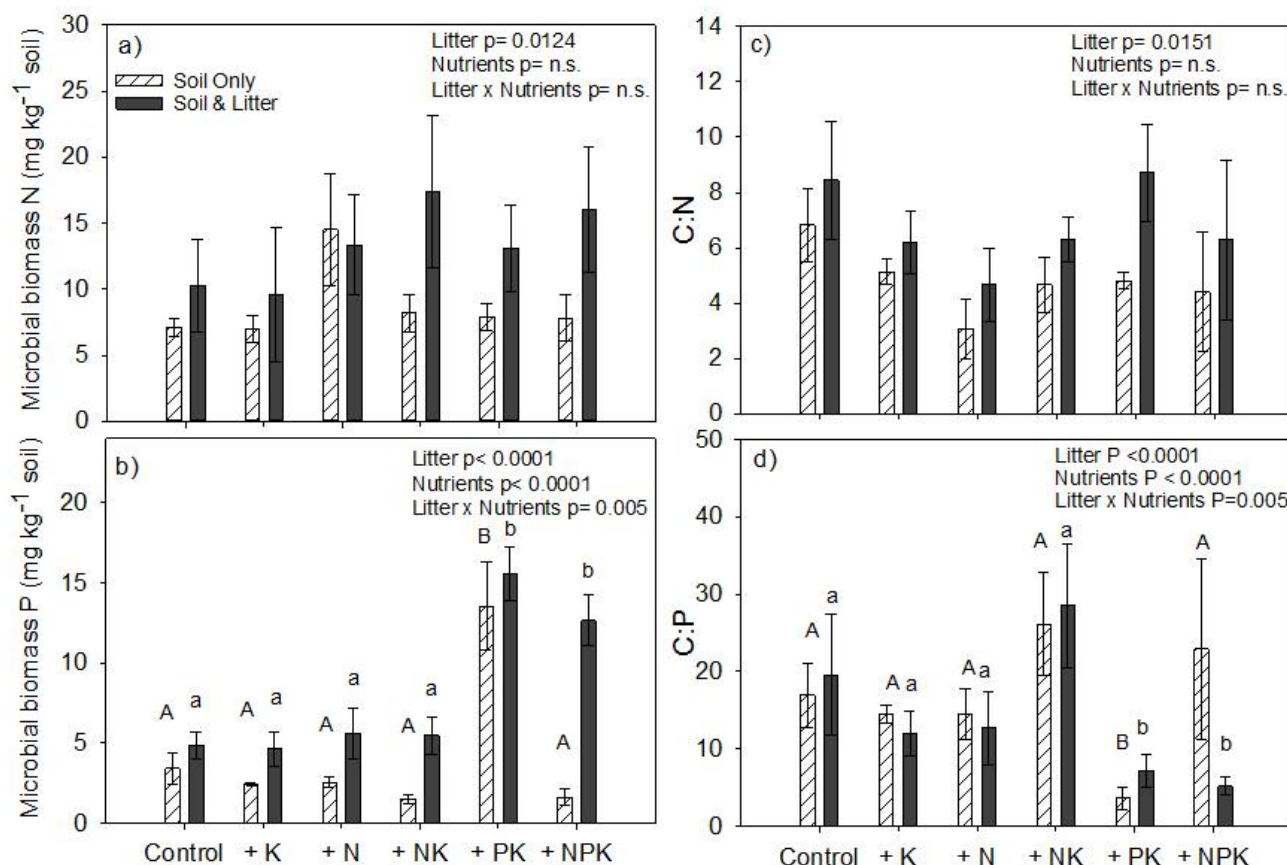


564
565 Figure 3. The Biomass-to-respiration ratio for soil-derived carbon in the Soil Only and Soil & Litter
566 treatments, and litter-derived carbon in the Soil & Litter treatments for the various nutrient additions.
567 Capital letters indicate significant differences between Nutrient treatments 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).

579
 580
 581

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