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1 ~~Whether Priming Effect generated by stoichiometric decomposition leads to~~
2 ~~an extra net C loss to the atmosphere or accelerates C incorporation into the~~
3 ~~microbial biomass depends on the nutrient status of soil.~~

4 **The fate of primed soil carbon between biomass**
5 **immobilization and respiration is controlled by nutrient availability.**
6

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12

13 **Key words:** Soil organic matter; Fresh organic matter; Priming effect; Microbial biomass;
14 Specific respiration
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23 **ABSTRACT**

24 A positive Priming Effect (PE) is defined as an acceleration of the decomposition of soil
25 organic matter (SOM) by a fresh organic matter (FOM) input. But in the literature many
26 studies present this phenomenon as an extra loss of carbon from SOM to the atmosphere.
27 SOM is actually a mixture of pools with different turnover rates, and microorganisms
28 generating PE are heterotrophic. Therefore, whether PE is in fact an extra loss of C depends
29 on the targeted SOM pool (high or low turnover) and the ratio between the incorporation of
30 the primed carbon into microbial biomass vs its mineralization. ~~A ^{13}C -wheat residue was~~
31 ~~added to a naturally~~ nutrient-poor tropical soil (enriched or not with a cocktail of nutrients);
32 was incubated and submitted to two successive wheat-straw inputs with differential ^{13}C
33 enrichment, in order to observe the PE of one straw on the other, and to measure the
34 specific respiration of the different carbon pools. ~~to create a recent labelled pool of SOM~~
35 ~~(supposed to have high turnover). After one week of incubation, non-labelled wheat straw~~
36 ~~was added to soil with the aim to generate a stoichiometric PE on the ^{13}C -enriched~~
37 ~~decomposing straw. Isotopic enrichment of respired CO_2 and microbial biomass were~~
38 ~~assessed to evaluate the specific respiration of the different carbon pools.~~ In our nutrient-
39 poor soil, nutrient enrichment intensified soil basal respiration while it lowered straw
40 mineralization. Our study showed that, fresh and primed C allocation between microbial
41 biomass and CO_2 were similar and depended on the nutrient status of the soil. We did not
42 observe any impact of freshly amended C on the fate of the previous amendment, but
43 conversely we observed an impact of the previous organic input on the decomposition rate
44 of the following one, as well as the allocation of the liberated C toward biomass rather than
45 CO_2 .

46

47 1. Introduction

48 Soil organic matter (SOM) is characterized by carbon (C) of diverse forms and ages and by
49 diverse nutrient concentrations [1,2,3]. The decomposition of SOM pools is mainly the result
50 of the activity of heterotrophic microorganisms. The first steps of the decomposition process
51 correspond to the depolymerization of macromolecules by microbial extracellular enzymes
52 and take place in the soil solution. Molecules smaller than 600 KDa can be assimilated by
53 microorganisms and then be allocated *via* the intracellular metabolism, between energy
54 generation (leading to C mineralization) and biomass synthesis (largely leading to C
55 stabilization [4]). ~~It has recently been put forward that~~ Long-term stabilized SOM mainly
56 corresponds to microbial-derived organic matter (OM) that is protected from enzymatic
57 activities by mineral associations [5]. In this way, microbial biomass synthesis contributes to
58 the long-term C stabilization in soils [6,7,8,9]. C stabilization in the soil is, therefore, linked to
59 organic matter mineralization [10,11].

60 The supply of fresh organic matter (FOM) to the soil may stimulates the microbial
61 ~~mineralization~~ decomposition of SOM by the so-called priming effect (PE) phenomenon [12].
62 Two different mechanisms, based on the interactions between FOM- and SOM-feeding
63 microbial populations, have been proposed in the literature for PE generation [13]. The first
64 one involves the increase in extracellular enzymes released by FOM decomposers, which
65 might accelerate the breakdown of SOM [14]. Because of enzyme specificity, this process
66 should target a SOM pool that shares similar structures with FOM, thus a pool of vegetal
67 signature, with a higher C:N:P than the microbial biomass [15]. This process has been called
68 “stoichiometric PE” because it follows similar nutrient demands of microorganisms to FOM
69 decomposition. In this case, nutrient input should increase the PE intensity, as it

70 simultaneously increases FOM ~~mineralization~~ decomposition. The second process
71 corresponds to the use of FOM catabolites as an energy source by SOM-feeding populations,
72 in order to decompose a nutrient-rich SOM pool ~~and recover nutrients~~ for nutrient demand
73 [16]. This process would more specifically target a stable ~~leilizing~~ pool of SOM [17], as long-
74 term C storage has a predominant microbial origin, and has been called “nutrient mining PE”
75 [20]. Conversely to the first one, the “nutrient mining PE” intensity decreases with nutrient
76 input. Both processes could hide a competition for nutrients between two microbial
77 functional guilds, the FOM-decomposers and the SOM-miners [13]. “Stoichiometric PE” is
78 ~~more important~~ predominant during the first steps of FOM decomposition, as FOM-
79 decomposers use the nutrients available in the soil solution, and is thereafter followed by
80 “nutrient mining” when the soil solution becomes nutrient depleted [15,21]. FOM-
81 decomposers involved in the “stoichiometric PE” have been qualified as r-strategists, as
82 opposed to K-strategists involved in the “nutrient mining PE” [21]. The balance between the
83 two processes is thought to be driven by the nutrient concentration of the soil solution [21],
84 labile C availability [18] and the richness of FOM in available nutrients [20,21,22, 23].

85 In the literature, a positive PE is defined as an acceleration of the SOM ~~mineralization~~
86 turnover and not only SOM mineralization, including therefore incorporation of SOM-C into
87 the microbial biomass as well as its release via respiration. But as it leads to an increase in
88 the CO₂ flux to the atmosphere over a certain period, it is sometimes ~~regarded rightly or~~
89 ~~wrongly as a net~~ considered as increasing the loss of C from SOM [24,9]. However, PE, as a
90 part of SOM turnover is just an element of the whole C budget. This means that if conditions
91 favoring PE, also favor new carbon storage, the net effect on soil C is not necessarily
92 negative. In addition, the PE is the result of the activity of heterotrophic microorganisms as
93 for regular SOM decomposition. This means that the C assimilated during the PE is partly

94 allocated to respiration and partly to incorporation into microbial biomass. Therefore, the PE
95 can in fact contribute to the gain of future new SOM when its allocation ratio is in favor of
96 biomass incorporation compared to the baseline of SOM decomposition, as microbial
97 biomass is the precursor of SOM via the death of microbes and the entombing effect [5].

98 The PE generated by “nutrient mining” may indeed lead to the remobilisation of a
99 SOM pool with a long residence time, by the activity of K-strategists known to allocate more
100 assimilated C to energy generation (CO₂ release) than to biomass synthesis [20,25,26,27].
101 Therefore, whether this PE drives the system towards a net loss of C from the soil depends
102 on its feedback on the entry of fresh C into the soil. In contrast, the “stoichiometric PE” (1)
103 targets a dynamic pool of SOM rather than a ~~stable~~ ~~ilizing~~ one, (2) rather involves r-strategist
104 populations known to preferentially allocate the assimilated C into their biomass: therefore
105 this type of PE can be regarded as an acceleration of the transformation of plant OM into
106 microbial OM and thus contributes to long term C sequestration in the soil [28,29,30]. In this
107 case, during a stoichiometric PE event, more “primed” C would be integrated into microbial
108 biomass than released as CO₂, resulting in a decrease in the specific respiration rate, also
109 called metabolic quotient qCO₂ (i.e. Respired C/Biomass C ratio, [31]).

110 The aim of the present study was to evaluate the C allocation ~~balance~~ between CO₂
111 evolution and microbial biomass synthesis during a “stoichiometric” PE event. Therefore, the
112 specific respiration rate was measured in a microcosm incubation experiment where soils
113 were successively amended with ¹³C- and ¹²C-labelled complex OM. ¹³C-enriched wheat
114 straw was added to a naturally nutrient-poor tropical soil, to create a dynamic labelled pool
115 of SOM. After one week of incubation, non-labelled wheat straw was added to the soil with
116 the aim of generating a stoichiometric PE on the ¹³C-enriched decomposing straw. Over a 13-
117 day incubation period, ¹³C and ¹²C were monitored in released CO₂ and in microbial biomass

118 in order to estimate the specific respiration rate related to each organic pool. As the
119 stoichiometric PE is usually positively correlated to soil nutrient concentration, incubations
120 were performed with or without soil enrichment with a cocktail of nutrients (N, P, K, S, Mg
121 and microelements) in order to verify the nature of the PE generating process. We
122 hypothesized that: (1) nutrients would foster the decomposition of SOM and ^{13}C and ^{12}C -
123 labelled wheat straw independently by favorizing r-strategist populations and therefore by
124 decreasing their specific respiration; (2) ^{13}C -labelled straw would first “stoichiometrically”
125 prime the decomposition of SOM at day 7, and ^{12}C -labelled-straw (added at day 7) would
126 “stoichiometrically” prime the decomposition of ^{13}C -labelled straw at day 13, with both PE
127 being enhanced in nutrient-rich conditions ; and (3) the primed OM pools (first SOM , then
128 ^{13}C -straw) would show similar or even lower specific respiration rates than those of non-
129 primed SOM and ^{13}C straw respectively. **If confirmed this last hypothesis would prove that**
130 **PE generated by stoichiometric decomposition is only an acceleration of SOM decomposition**
131 **mineralization with no balance switch to respiration and therefore no extra loss of C**
132 **compared to basal soil respiration.**

133

134 **2. Materials and methods**

135 *2.1. Soil sample description*

136 The soil sample (0-10 cm) was collected in Autumn 2018 next to the city of Imerintsiatosika,
137 20 km west of Antananarivo (-18.978671, 47.327678) from a natural savannah. The soil is a
138 Ferralsol (according to the FAO nomenclature), with a silty clay texture (Silt 57.7%, clay
139 28.9%), with a C content of 29 g kg⁻¹ of soil, a C:N of 10 and a naturally low available
140 phosphorus content (0.47 mg.Kg⁻¹ soil). Its full physical and chemical characteristics are
141 described in Raminoarison et al. [32]. Two kilograms of soil were air-dried, sieved (2mm),

142 and coarse plant residues were manually removed. Soil was pre-wetted and dried at 65°C for
143 48h to eliminate microfauna as recommended by Franco et al. [33]. Microfauna was
144 eliminated in order to reduce the turnover of the microbial biomass and improve the
145 accuracy of the specific respiration ratio (Biomass/respiration). The soil sample was air-dried
146 again.

147

148 2.2. Soil incubation conditions

149 The experiment was designed to induce and observe a stoichiometric priming effect. For this
150 purpose, two factors were crossed: (1) soil wheat amendment: no wheat, ¹³C wheat residues
151 (\pm S13) at day 0, ¹²C wheat residues (\pm S12) at day 7 and (2) soil nutrient content (nutrient
152 supply or not, \pm N) leading to 8 modalities (Table 1, Fig S1), with 6 replicates per modality.
153 Forty-eight 160-ml flasks were filled with 16 g of dry soil. To reactivate microbial
154 communities, the soil water content was adjusted and maintained to 39.5% of water content
155 using sterile deionized water, for a 14-day pre-incubation period. Every 3 days, flasks were
156 opened to renew the gas phase using a large 50 mL syringe. After this pre-incubation period,
157 the 24 +S13 flasks were amended with 4 mg of ¹³C powdered wheat residue (wheat: leaves
158 and stems, 97% ¹³C) per gram of dry weight soil. The ¹³C wheat straw was grown in a
159 continuous 99%¹³CO₂ atmosphere as described in [34]. Then, 12 non-amended and 12
160 wheat-amended +N microcosms were enriched with nutrient solution at a rate of 0.125 mL
161 (g incubated soil)⁻¹ (6.86 mg NH₄NO₃ mL⁻¹; 7.12 mg NaH₂PO₄ mL⁻¹; 3.06 mg KCl mL⁻¹; 4.01
162 mg MgSO₄7H₂O mL⁻¹; 4.41 mg CaCl₂ mL⁻¹; 0.06 mg O₂Si_xH₂O mL⁻¹) in order to bring 300 mg N,
163 200 mg P, 200 mg K, 200 mg S, 65 mg Mg, 150 mg Ca per kg of dry soil [32]. Soil water
164 content of -N flasks was adjusted using sterile water (0.125 mL g⁻¹ incubated soil). The soil
165 microcosms were incubated in the dark for 13 days at 28°C. After 7 days of incubation, 4 mg

166 of unlabelled (¹²C) powdered wheat residue (leaves and stems) per gram of dry soil were
 167 added to the 24 +S12 flasks. At day 3, day 7 (before ¹²C-straw addition), day 10 and day 13, a
 168 6 mL volume of gas phase was sampled in Exetainer evacuated tubes for total CO₂ and ¹³CO₂
 169 measurements. After sampling, gas phases were renewed in different rooms, depending on
 170 their ¹³C enrichment modality, to avoid ¹³C contaminations of unlabeled modalities. At day
 171 10 and day 13, 3 flasks per modality were harvested for microbial biomass analysis, **in order**
 172 **to see whether the ¹²C-straw input of day 7 had modified the incorporation of ¹³C-straw C**
 173 **into the microbial biomass, 3 or 6 days after the new substrate supply.**

174

175 2.3. CO₂ and ¹³CO₂ measurements

176 The collected gas phases were analysed by IRMS "Isotope Ratio Mass Spectrometry" using a
 177 mass spectrometer (Euro-EA Vector Element Analyzer, France ; coupled with an IsoPrime
 178 Mass Spectrometer, Italy) to quantify the total C and relative abundance of ¹³C in the CO₂
 179 samples. Total mineralized C per gram of soil (Total CO₂-C) between two sampling dates and
 180 its ¹³C-Wheat component (S13min CO₂-C) were calculated using the following equations:

181

182

183

184

185 (1)

$$\text{Total CO}_2\text{-C } (\mu\text{g C g}^{-1} \text{ dry soil}) = \frac{\left(\left(\left(\frac{Q}{V_p} \right) * V_m \right) \right)}{T} * D}{M}$$

186

187 with Q = C measured by IRMS (μg), V_p = sampled volume (6ml), V_m = gas phase
 188 volume (120 ml), T = period of gaz accumulation (min) variable between flasks, M =

189 soil dry mass (16 g), D = standardized period for all flasks between sampling dates
190 (min). Means and standard errors were calculated on 3 replicate flasks.

191

192 **(2)**
$$S13min\ CO_2-C\ (\mu g\ C\ g^{-1}\ dry\ soil) = \frac{(Total\ CO_2-C * A\%) - A_c}{0.97}$$

193

194 with Total CO₂-C = total mineralized C (μg C g⁻¹ dry soil), A% = ¹³C relative abundance
195 of the amended flask (%), A_c = Atmospheric ¹³C, i.e. mean of ¹³C abundance
196 measured in 3 non-amended modalities (μg C g⁻¹ dry soil), 0.97 = ¹³C enrichment of
197 straw C.

198

199 ¹³C-Wheat induced PE was calculated as follows:

200 **(3)**
$$S13\ PE\ (\mu g\ C\ g^{-1}\ dry\ soil) = Total\ CO_2-C - SOM\ CO_2-C - S13min\ CO_2-C$$

201 with SOM CO₂-C = Total CO₂-C of the respective non-amended treatment (Table 1)

202 ¹²C-Wheat mineralization was calculated as follows:

203 **(4)**
$$S12min\ CO_2-C\ (\mu g\ C\ g^{-1}\ dry\ soil) = Total\ CO_2-C - SOM\ CO_2-C$$

204 Calculation details for the different variables are given in table 1. As S13PE and S12min are
205 the results of subtracting fluxes measured from independent replicated flasks. Means and
206 Standard errors were calculated on all the 9 possible combinations between the 3 replicate
207 flasks per treatment. *S12min CO₂-C actually includes CO₂-C resulting from a potential PE
208 generated by the ¹²C straw on SOM, which cannot be discriminated as both OM have a
209 similar isotopic signature.*

210

211 2.4. *Microbial Biomass Carbon*

212 Microbial biomass Carbon (MBC) was assessed using the fumigation-extraction method [35]
 213 in three flasks per modality on day 10 and day 13. Shortly, 15g of each soil microcosm were
 214 separated into two equal fractions, one being subjected to Chloroform (CH₃Cl) fumigation.
 215 Total soluble carbon was then extracted from both fractions with 30 mL of potassium
 216 sulphate solution (K₂SO₄, 0.025M). Two mL of the obtained solution were concentrated by
 217 evaporation and resolubilization in 120μL of deionized water. Adequate volumes were finally
 218 sampled and dried for elementary analysis (EA Eurovector analyzer, Italy) coupled with
 219 isotopic mass spectrometry (Mass Isoprime, Elementar, France) [36]. MBC feeding on the
 220 different SOM pools were calculated as follows:

221

$$222 \quad (5) \quad \text{Total MBC } (\mu\text{g C g}^{-1} \text{ dry soil}) = \frac{\left(\left(\frac{C_F - C_{NF}}{k_{EC}} \right) * d \right)}{m}$$

223

224 with C_F = C from fumigated solution (μg), C_{NF} = C from unfumigated solution (μg),
 225 k_{EC} = extraction efficiency coefficient (0.45; [34]), d= dilution factor (15), m = dry soil
 226 mass (7.5gr)

227

228 To calculate ¹³C-wheat derived MBC (S13 MBC), we considered that there is no isotopic
 229 fractionation during the incorporation of C into microbial biomass. Therefore, the same
 230 equation as (2) was applied to MBC. ¹²C-Wheat-derived MBC (S12 MBC) was calculated as
 231 follows, (depending on whether ¹³C-wheat was added (6.1) or not (6.2):

232

$$233 \quad (6.1) \quad \text{S12 MBC } (\mu\text{g C g}^{-1} \text{ dry soil}) = \text{Total MBC} - \text{SOM MBC} - \text{S13 MBC}$$

234

235

$$236 \quad (6.2) \quad \text{S12 MBC } (\mu\text{g C g}^{-1} \text{ dry soil}) = \text{Total MBC} - \text{SOM MBC}$$

237

238 with SOM MBC corresponding to the Total MBC quantified in both control conditions
239 (with or without nutrients but no wheat addition – Table 1). Like for CO₂, S12MBC
240 might include MBC produced from SOM by the effect of ¹²C-straw inputs, as both
241 MBC cannot be differentiated by their isotopic signature.

242

243 2.5. *Specific respiration*

244 The cumulative specific respiration was calculated as follows at each sampling date.

$$245 \quad (7) \quad q\text{CO}_2 \text{ (}\mu\text{g C g}^{-1} \text{ dry soil)} = \frac{(\text{Total CO}_2\text{-C})_t}{(\text{Total MBC})_t}$$

246

247 with t = corresponding to day 10 or day 13, and Total CO₂-C = corresponding to
248 cumulative mineralization between day 0 and t.

249

250 2.6. *Statistical analyses*

251 All statistical analyses were performed with R 3.5.2 [37,38]. The significance of the results
252 was accepted at the probability threshold $p < 0.05$.

253 A linear mixed effects model analysis was applied to the cumulative averages over time
254 (Y) (lmer, package lme4, [39]). Several models were tested, defining time as a fixed effect (a
255 factor common to microcosms) and nutrient effect as a random effect.

$$256 \quad (i) \quad Y \sim \text{Time} * \text{Nutrient} + (1 | \text{Nutrient})$$

$$257 \quad (ii) \quad Y \sim (\text{Time} + I(\text{Time}^2)) * \text{Nutrient} + (\text{Time} + I(\text{Time}^2)) + (1 | \text{Nutrient})$$

$$258 \quad (iii) \quad Y \sim I(\text{Time}^2) * \text{Nutrient} + I(\text{Time}^2) + (1 | \text{Nutrient})$$

$$259 \quad (iv) \quad Y \sim I(\text{Time}^2) + \text{Nutrient} + I(\text{Time}^2) + (1 | \text{Nutrient})$$

260 with Y = mean of cumulative $\text{CO}_2\text{-C}$ emitted during incubation ($\mu\text{g CO}_2\text{-C g}^{-1}$ dry soil),
261 Time = fixed factor of time (days), and Nutrient = nutrient supply considered as random
262 factor.

263 The choice of model was made parsimoniously using Akaike's information criterion (AIC) [40].

264 On the chosen model, a two-factor ANOVA was applied to measure the effect of time and
265 nutrient treatments.

266 Student's t-tests were performed to test the effect of nutrient and wheat addition for each
267 mineralization and at each date (t.test, package stats, [37]). The normality of the residues
268 was verified with the Lilliefors test (lillie.test, package nortest; [41]), and homogeneity with
269 the Fisher variance comparison test (var.test, package stats).

270 A polynomial regression mode, followed by an ANOVA, were performed to measure
271 the intensity of the relationship between the mineralization of ^{13}C wheat (S13 min) and the
272 resulting Priming Effect on SOM (S13 PE).

273 Moreover, to measure the effect of nutrient supply on mineralization of ^{13}C wheat
274 and mineralization of basal soil, the ratio of the first to the next was calculated.

275 A two-factor ANOVA, followed by a Tukey-HSD test, was applied to distinguish
276 significantly different means, according to time and modalities for microbial biomass (MBC)
277 and specific respiration ($q\text{CO}_2$).

278 The propagation of standard errors through calculations was carried out using an online
279 tool [42].

280

281 **3. Results**

282 **3.1. Mineralization of organic matter and Priming Effect**

283 The basal mineralization of soil organic matter (SOM min) remained constant until
284 the end of the incubation (p-value > 0.05) but was faster when nutrients were supplied (p-
285 value < 0.01) (Fig. 1A, Table S1). Conversely, ¹³C wheat residue mineralization was faster,
286 without nutrient supply (p-value < 0.01) (Fig. 1C). It was constant up to 10 days and
287 increased between day 10 and day 13 (p-value < 0.001). The increase was higher without
288 nutrient supply. The intensity of PE generated by ¹³C-wheat residues decreased from day 3
289 to day 7 and strongly accelerated (p-value < 0.05) from day 10 to day 13 (Fig. 1B), but was
290 not impacted by nutrient supply (Fig. 1B; p-value > 0.1).

291 The input of ¹²C wheat residues on day 7 induced an increase in the mineralization of
292 ¹²C-OM (¹²C straw itself and possible PE on SOM which cannot be verified, *as both organic*
293 *matters have a similar isotopic signature*) (Fig. 1A*D*). This input did not modify the
294 mineralization of ¹³C wheat residues (S13 min, p-value > 0.1, Fig 1C), but the presence of ¹³C
295 straw, previously added, increased the intensity of ¹²C wheat residue mineralization *and*
296 *possibly its own PE* (S12 min, p-value < 0.01) (Fig. 1D*E*).

297 3.2. Microbial Biomass Carbon (MBC)

298 After 10 days of incubation, and due to large standard errors, most treatments showed
299 similar MB¹²C (i.e. *SOM-derived MBC non-labelled microbial biomass corresponding to SOM*
300 *in -S12 flasks or SOM-derived MBC plus ¹²C wheat-derived MBC in + S12 flasks*) *in all*
301 *treatments to MB¹²C in the control microcosms* (p-value < 0.05), even when ¹²C-enriched
302 wheat straw was added 3 days before (Fig. 2A). Nutrients and/or ¹³C-wheat straw supply
303 tended to reduce SOM-derived MBC, though not significantly. Only the treatment with both
304 straw amendments but without nutrient supply (-N +S13 +S12) showed a significantly higher
305 MB¹²C. At the same time, ¹³C wheat-derived MBC (S13MBC) was not significantly affected
306 either by soil nutrient status or by subsequent fresh wheat addition. On day 13, MB¹²C was

307 significantly the same in all treatments (p-value > 0.05), except when nutrients and ¹³C
308 wheat straw were added together, in which case the biomass was slightly lower. S13MBC
309 was globally equal to, or lower than that registered on day 10.

310 In the absence of nutrients, the presence of ¹³C wheat residues, added previously (T0),
311 increased the incorporation of C from ¹²C wheat in microbial biomass measured on day 10
312 (S12 MBC, p-value < 0.05) (Fig. 2B). Three days later, while S12 MBC increased in the non ¹³C
313 wheat enriched condition, it drastically decreased in the ¹³C wheat enriched modality (p-
314 value < 0.05). Negative values mean that even SOM-feeding microbial biomass decreased,
315 due to a non-identified mortality factor.

316 3.3. *Specific respiration (qCO₂)*

317 Specific respiration of microbes measured on day 10 are presented on figure 4. The qCO₂
318 was the lowest in the control condition (-N -S13 -S12, p-value < 0.05) while it increased in
319 conditions supplemented with nutrients or with ¹³C-wheat residue (Fig. 3A). The qCO₂
320 derived from ¹³C-wheat decomposition was higher in the modality where no nutrients were
321 added (p-value < 0.05). The presence of ¹³C-wheat residues lowered the qCO₂ derived from
322 ¹²C-wheat residue decomposition (p-value < 0.05) (Fig. 3B).

323

324 4. Discussion

325 4.1. *Priming Effect generation process*

326 The addition of ¹³C straw stimulated the mineralization of unmarked SOM-C following two
327 phases. A first peak **after** three days which, according to the literature, could correspond to
328 apparent PE (cf. review by Kuzyakov et al. [12]). This is a brief increase in respiration caused
329 by the renewal of biomass components **of the active part of** microbial communities that are
330 about to **break-down-feed on the labile part of** ¹³C wheat. The next peak, which appeared

331 after 7 days and intensified after 10 days, should partly correspond to the real PE, which is
332 considered as the stimulation of SOM mineralization. We cannot exclude that part of this PE
333 comes from the pool substitution of microbial species having longer turnover times. But as
334 (1) the cumulated PE over the 13 days period of incubation was equal to the C-MB of the
335 control condition, and (2) the microbial turnover time is usually about 30 days
336 [Blagodatskaya et al. 2011, 43], we can conclude that at least half of the measured PE came
337 from the SOM and not from the microbial biomass pool. Therefore, in the present study the
338 addition of ¹³C-wheat generated a PE on the SOM, but its intensity was not impacted by soil
339 nutrient status.

340 Stoichiometric PE is usually distinguished from PE generated by "nutrient mining" because of
341 its positive correlation to nutrient enrichment, as for the mineralization of the substrate
342 which induced the PE [18,40]. Mineralization of vegetal FOM is often limited by nutrients
343 [13,21]. Therefore, as stoichiometric PE is the consequence of the activity of enzymes
344 released against FOM, it should be limited by nutrients as well. However, in the present
345 study, nutrient enrichment increased basal soil respiration but decreased ¹³C wheat
346 mineralization (Fig. 1A and 1C), suggesting that in our microcosms microbial activity was
347 limited by nutrient availability and microorganisms decomposed ¹³C wheat both for its
348 nutrients and for its carbon. Fresh organic matter from wheat is usually considered as a
349 source of easily-available C (*i.e.* energy) for microorganisms, rather than as a source of N and
350 P [13]. Nevertheless, Nicolardot *et al.* [44] showed that younger wheat residues had a lower
351 C:N ratio than mature residues. In the present study, the wheat residues were 97% ¹³C
352 enriched and were therefore harvested at a young stage. Furthermore, the high level of ¹³C
353 enrichment could confer a lower decomposability to this organic matter, its C being less
354 available than that contained in a part of the SOM. Therefore, opportunistic populations

355 feeding on easily available substrates (not participating in the breakdown of FOM) could
356 have *commensally* fed on ^{13}C -straw metabolites when the soil solution was nutrient-
357 depleted, and on dissolved SOM when the soil solution was nutrient-enriched. This
358 hypothesis could explain why the soil nutrient status did not change the intensity of PE as it
359 would not change the pool of enzymes released against FOM.

360

361 In short, in the present study, nutrient enrichment did not change the intensity of the PE
362 (Fig. 1B), but the mineralization of ^{13}C wheat residue *appeared to be less nutrient-limited*
363 *than that of the soil itself. ~~was not nutrient limited either.~~* Therefore, the present case does
364 *not correspond to processes generally described in the literature, where PE is generated by*
365 *complex but always nutrient-poor FOM. ~~the term “stoichiometric” does not properly qualify~~*
366 *the PE generated by the ^{13}C wheat straw on SOM after 7 days of incubations. ~~But We will still~~*
367 *continue to call it by this term to make reference to the short-term PE generated by r-*
368 *strategist enzymes as described in the literature [21].* Contrarily to what was expected, the
369 ^{12}C wheat straw addition did not generate any PE on the ^{13}C wheat-straw mineralization
370 during the time of the experiment (see discussion below).

371

372 4.2. *Organic Matter pool targeted by the Priming Effect*

373 ~~Our objective was to confirm that a stoichiometric PE, generated by the release of enzymes~~
374 ~~against new FOM, targeted a recent SOM pool with a nature closed to that of incoming FOM.~~
375 ~~Therefore, ^{12}C wheat residue was added at day 7, to see whether it could stimulate the~~
376 ~~mineralization of the ^{13}C from the wheat straw added at T0.~~ Our results showed that the
377 mineralization of ^{13}C wheat residue was not modified by the subsequent addition of
378 unmarked straw (Fig. 1C). Therefore, enzymes released against ^{12}C residues appeared not to

379 affect the mineralization of ^{13}C straw. The first possible explanation is that FOM-directed
380 enzymes do not target a recent SOM pool, unlike already shown in the literature [21]. The
381 second explanation is a possible artefact linked to the high level of ^{13}C enrichment (97%) of
382 the wheat straw used in the present case. This level was chosen to ensure the detection of
383 all the C flows derived from this pool during the rest of the experiment. Therefore, while
384 enzymes directed against ^{13}C -wheat straw appeared to generate a PE on some pre-existing
385 SOM (see section 4. 1), enzymes directed against ^{12}C straw may not be suitable for such an
386 isotope-enriched substrate.

387 At the opposite, the decomposition of ^{13}C wheat residues had a positive effect on the
388 mineralization of ^{12}C straw (and its possible own PE on SOM) at day 10 in the absence of
389 nutrient addition (Fig. 1DE). These results suggest that the supply of ^{13}C wheat residues,
390 seven days earlier, could have increased the soil enzyme pool and/or brought some
391 nutrients which induced a kind of “priming effect” on the future mineralization of ^{12}C straw.
392 Razanamalala et al [45,46] suggested that a frequent supply of fresh organic matter would
393 maintain the communities of decomposers responsible for the stoichiometric PE. This
394 mechanism would avoid the PE by nutrient mining, which targets an older OM, and
395 therefore destocks a more stabilized carbon. Our study shows that a frequent supply of fresh
396 OM could also maintain a higher level of mineralization of each new arriving FOM, including
397 its own short-term PE on SOM that we could not evaluate here. In order to evaluate the ^{12}C -
398 straw derived PE, we should have used a third carbon label like ^{14}C . But the PE generated by
399 the ^{13}C straw hardly reached 12% of the ^{13}C straw mineralization. This means that the C
400 included in the flux we called ^{12}C -straw mineralization and which actually resulted from ^{12}C -
401 straw induced PE on SOM should correspond to a minor part compared to that originating
402 from the straw itself.

403

404 4.3. Carbon ~~balance~~ allocation between mineralization and immobilization.

405 On day 10, the specific respiration of non-amended microcosms was higher when
406 nutrients were not limiting (Fig. 3A), due to both a higher CO₂ evolution and a lower
407 microbial biomass (Fig. 1 and Fig. 2A). Malagasy soils are naturally poor in nutrients and
408 labile carbon. Nutrient enrichment, without labile C input, may have selected communities
409 with a k-strategy, able to decompose recalcitrant SOM to balance their C:N ratio [21,47] and
410 having thus a higher catabolic activity that limits their growth [48].

411 The addition of ¹³C wheat residues in the absence of nutrient supply also led to a higher
412 specific respiration of ¹²C-SOM (Fig. 3A), resulting from a higher respiration and a lower
413 biomass. However, when ¹³C-wheat straw and nutrients were added together, SOM specific
414 respiration was lower. ~~The specific respiration of ¹³C-straw followed similar trends.~~ This
415 suggests that ~~under nutrient limitation, decomposition of ¹³C-straw was ensured by slow~~
416 ~~growth rate—organisms K-strategist—following a K-strategy, in the same way as the PE~~
417 ~~generated on SOM. The PE generated by K-strategists under nutrient limitation has been~~
418 ~~defined as a “nutrient mining” PE [21], because K-strategists were supposed to use the~~
419 ~~energy of FOM to mine SOM for nutrients. But in the present context, it seems that SOM~~
420 ~~mineralization is rather more nutrient limited than the amended ¹³C-wheat straw. Therefore,~~
421 ~~we propose that PE generated by K-strategist enzymes directed against ¹³C-wheat helped to~~
422 ~~decompose the SOM, as described with r-strategists in the “stoichiometric” process can be~~
423 ~~ensured following r or K-strategy depending on the nutrient status of the soil and that of the~~
424 ~~FOM. But more attention should be given in future studies to PE generated by recalcitrant~~
425 ~~but nutrient rich FOM.—to the effect produced on SOM mineralization of inputs of~~
426 ~~recalcitrant but nutrient rich organic matter.~~

427 In these poor soils (suffering from strong colimitations), inputs of either C or nutrients
428 induced K-strategies to mine the SOM to complete their needs. But when C and nutrients are
429 brought together, the system might ~~switch to populations with favor~~ higher growth rates
430 and lower specific respiration, because they are more competitive when substrate C:N is
431 lower [25]. The specific respiration of ¹³C-straw respected this hypothesis (Fig. 3B). Therefore,
432 in these soils the ~~stoichiometric~~ short-term PE appeared to follow the same rules as FOM
433 mineralization, generated following r- or K-strategies depending on the nutrient status. And
434 its contribution to ~~the balance~~ the allocation between C fixation in the microbial biomass vs
435 C respired to the atmosphere is also similar to the FOM mineralization itself ~~strongly~~
436 ~~depended on soil nutrient status~~.

437 As previously stated, we observed that ¹²C-wheat straw mineralization was higher when
438 soil was previously amended with ¹³C-straw (Fig. 1DE), but the ¹²C microbial biomass thought
439 to mainly feed on this FOM was also enhanced on day 10 (Fig. 2B). The resulting specific
440 respiration was lower than in absence of ¹³C-straw amendment, showing that these
441 microorganisms favored the immobilization of C from unlabeled straw into their biomass (Fig.
442 3B). This was observed in the condition without nutrient supply (i.e. no biomass, and thus no
443 qCO₂, could be measured on the condition combining ¹³C-wheat, ¹²C-wheat and nutrient
444 supply), and thus in which the ¹³C-wheat straw addition selected for K-strategies~~sts~~.
445 Therefore, a first organic carbon input, even unaccompanied by nutrients, appeared
446 sufficient to enhance the soil conditions ~~via the action of K-strategists~~ and allow a better use
447 efficiency of C arriving in a following input ~~via the action of r-strategists~~.

448

449 5. Conclusion

450 First, our study showed that, in our Malagasy ferralsol, C mineralization through the short-
451 term PE probably generated by stoichiometric decomposition ~~the so-called “stoichiometric~~
452 ~~decomposition”~~ of fresh wheat-straw residue followed the same allocation balance as the
453 fresh carbon itself. When the soil was nutrient depleted, fresh or SOM C seemed to be
454 mostly respired, whereas in the presence of an adequate nutrient supply, a larger fraction
455 was integrated into microbial biomass. We further propose that under nutrient limitation,
456 the fresh carbon ~~inputs are decomposed by K-strategists known to allocate~~ rather used to
457 ~~generate more energy~~ to nutrient acquisition ~~than to create new biomass used to growth.~~
458 Such ~~K-strategist~~ activity may improve soil nutrient conditions, allowing the carbon arriving
459 ~~with the~~ following organic input to be ~~used by r-strategists and its C to be~~ preferentially
460 allocated to microbial biomass (Fig 4). Nutrient and enzyme monitoring and microbial
461 genetic composition analysis would help to verify our postulation. Anyhow, our results
462 showed that repeated C inputs have strong consequences on the dynamics and the fate of
463 the different pools of C in the soil, and merit to be deeply investigated in the future.

464

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470

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Figure legends

Figure.1: Cumulative average curves of mineralized carbon as a function of incubation time ($\mu\text{g CO}_2\text{-C g}^{-1}$ dry soil) for (A) Basal Soil Organic Matter mineralization, (B) Priming Effect on SOM generated by the addition of ^{13}C enriched wheat straw, (C) mineralization of ^{13}C enriched wheat straw, (D) Total CO_2 released and (E) deduced mineralization of ^{12}C wheat straw. Modality Codes corresponding to each curve are indicated on the graphs with solid lines and broken lines representing nutrient enriched and non-enriched conditions, respectively. The error bars represent the standard errors of the cumulative averages.

Figure 2: Microbial biomass Carbon MBC ($\mu\text{g C g}^{-1}$ dry soil) with standard error bars derived from (A) SOM, ^{13}C -enriched wheat straw and SOM + ^{12}C wheat straw, measured after 10 days and 13 days of incubation according to the different treatments, and from (B) ^{12}C wheat straw at 10 and 13 days. Letters indicate the significance of the differences (ANOVA), Latin-letters correspond to ^{12}C enriched-MB data (SOM or SOM+ ^{12}C -straw) and Greek-letters to ^{13}C enriched-MB data.

Figure 3: Mean specific respiration ($q\text{CO}_2$) \pm SE of microbial populations feeding on (A) SOM- or ^{13}C -enriched wheat straw and (B) ^{12}C -enriched wheat-straw, at 10 days of incubation and depending on modalities. Latin-letters correspond to ^{12}C -enriched OM data (A: SOM and B: ^{12}C -wheat straw) and Greek-letters to ^{13}C -enriched OM data to indicate the significance of the differences (ANOVA).

Figure 4: Schematic representation of the mechanism of priming effect generation by stoichiometric decomposition and its control by the soil nutrient status.

Figure.1

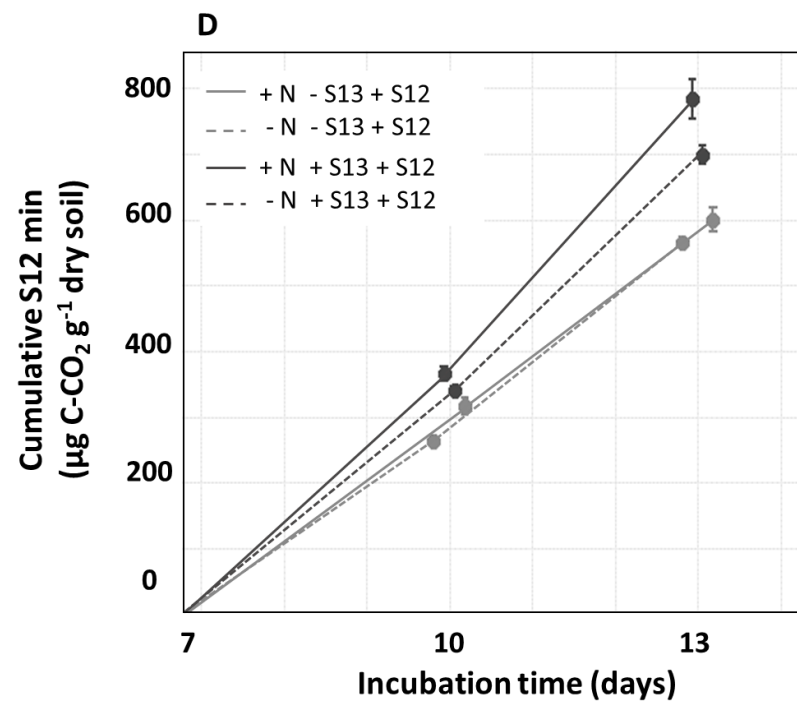
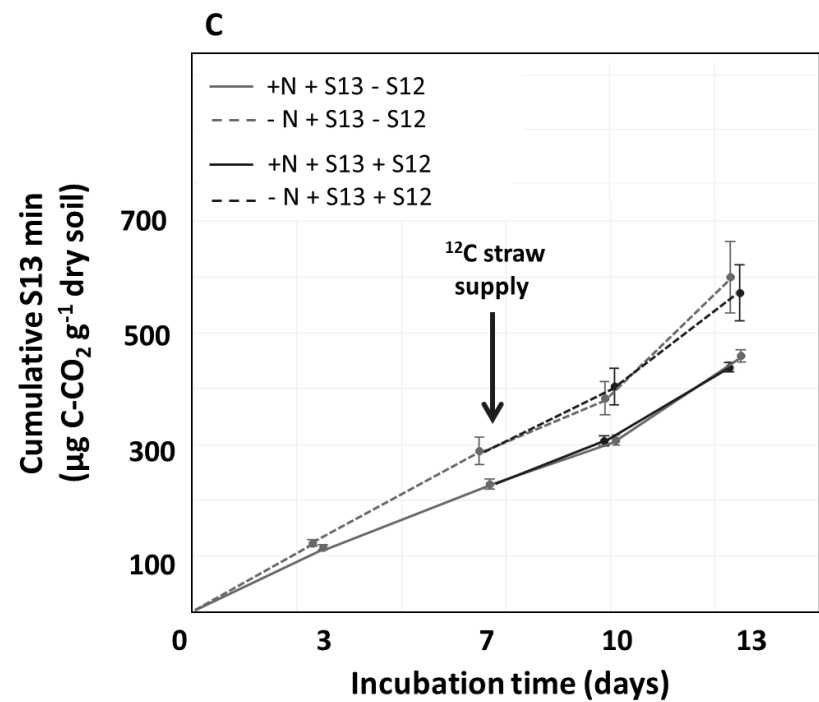
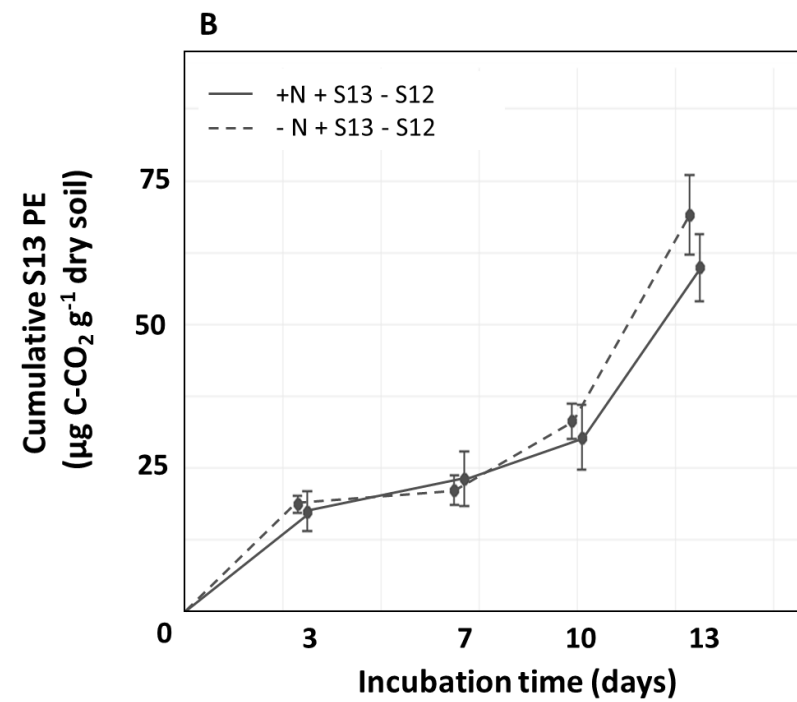
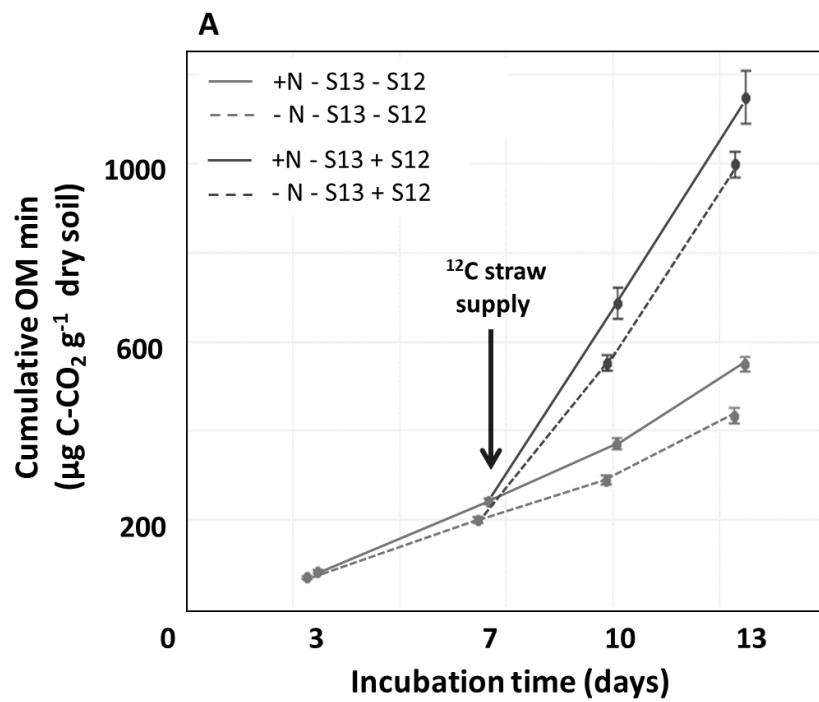


Figure.1: Cumulative averages curves \pm standard error, of mineralized carbon during incubation for each organic matter pool and according to the different modalities ($\mu\text{g C-CO}_2$ cumulative g^{-1} dry soil \pm SE). A: Total CO_2 released under control conditions, with or without ^{12}C wheat straw added at day-7. B : Priming Effect on SOM generated by the addition of ^{13}C wheat straw. C: Mineralization of ^{13}C straw, with or without ^{12}C wheat straw at day-7. D: Mineralization of ^{12}C wheat straw \pm prior presence of ^{13}C straw.

The error bars represent the standard error of the cumulative average

Figure.2

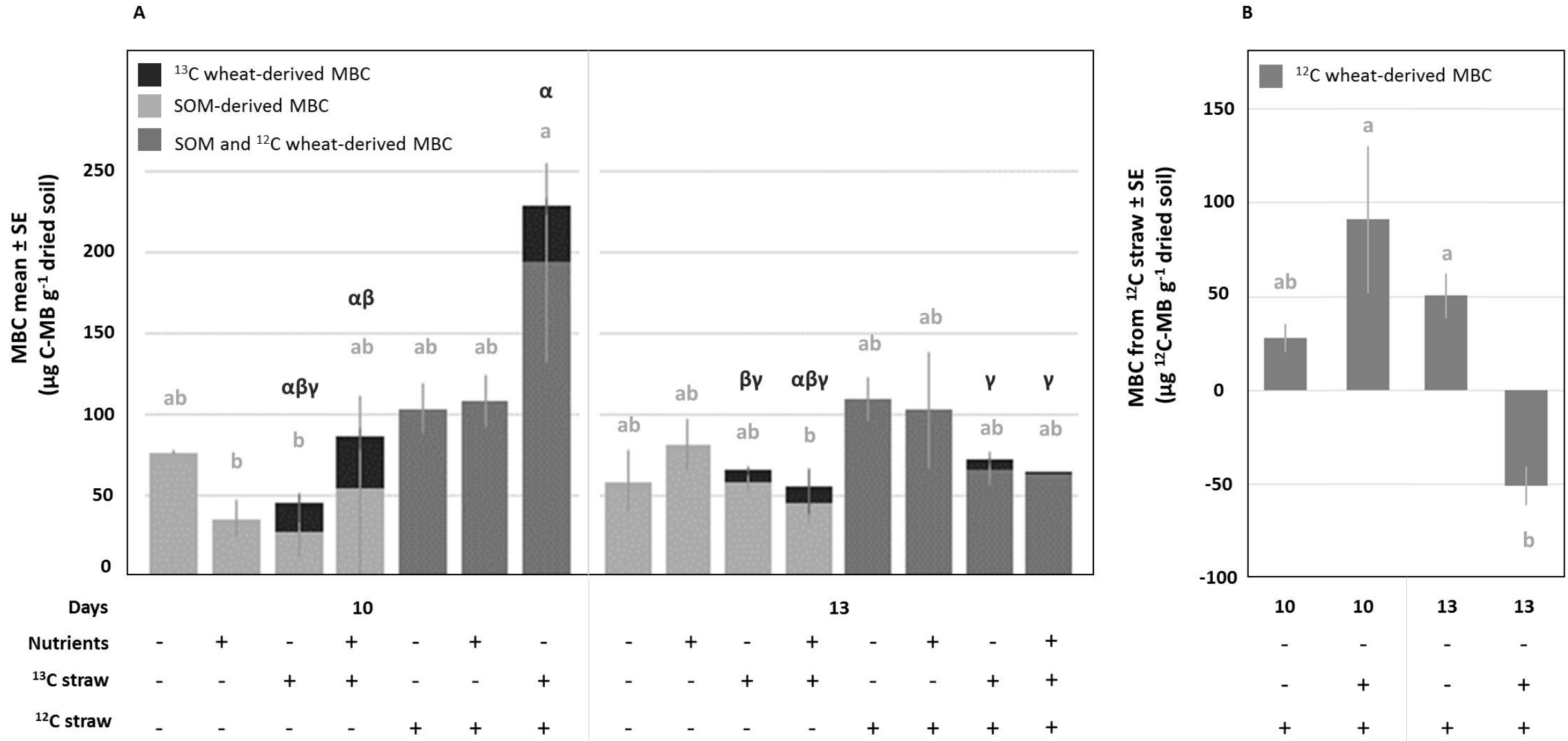
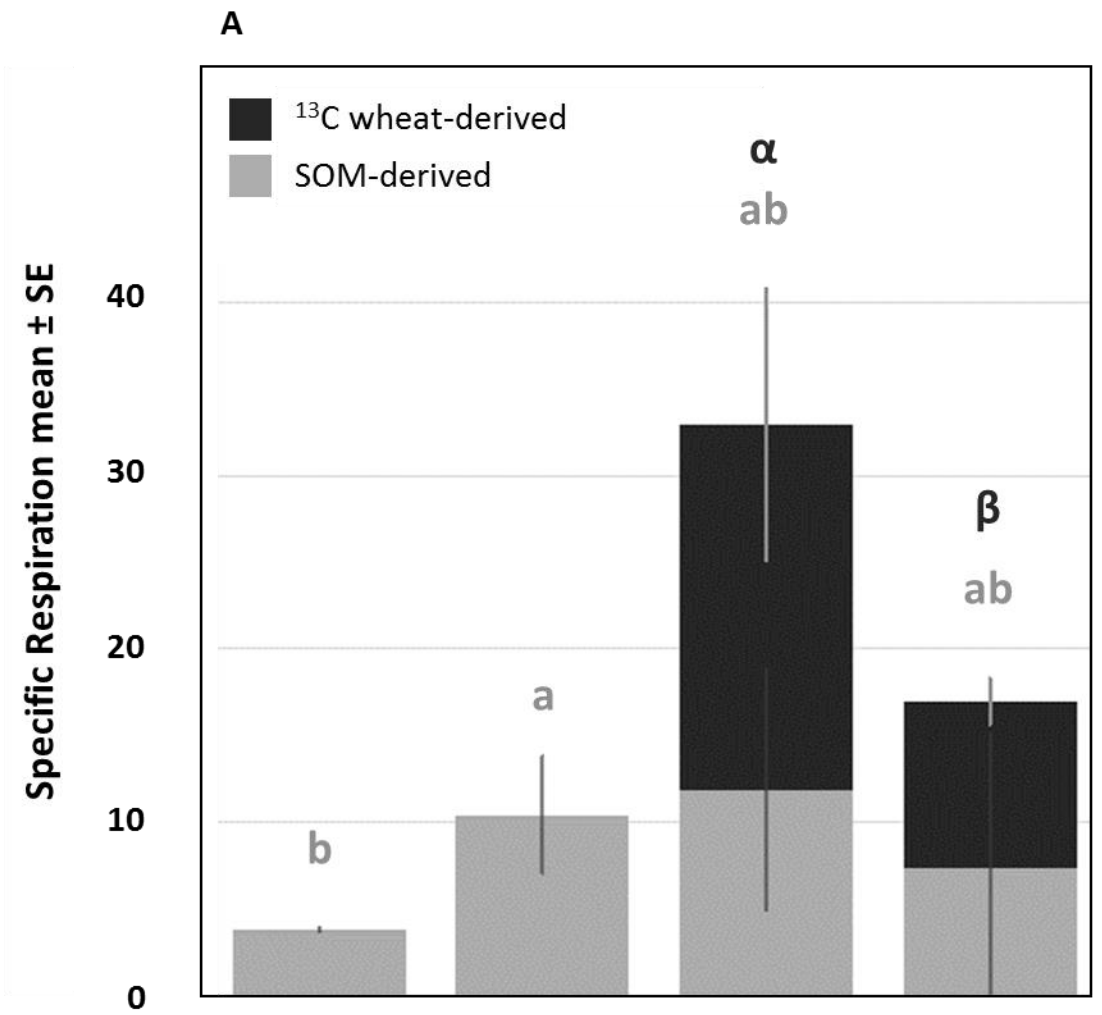


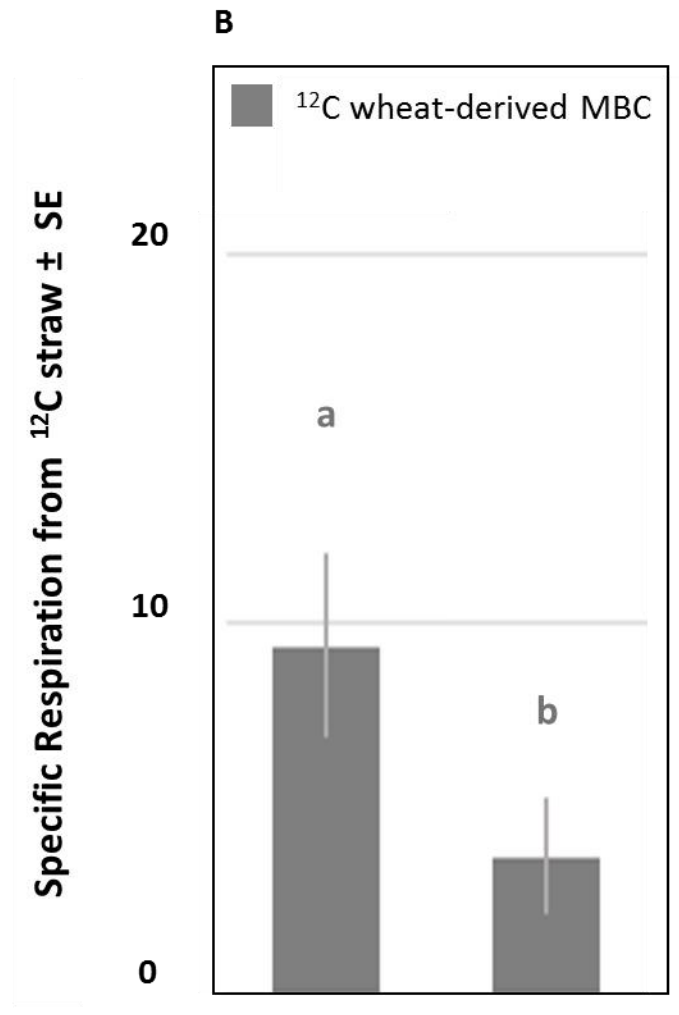
Figure 2 : Carbon microbial biomass ($\mu\text{g C-MB g}^{-1}$ dry soil \pm SE)

A : Means of quantities of ^{12}C and ^{13}C carbon in microbial biomass according to the different treatments at day-10 and day-13 of incubation. B : Amount of carbon from ^{12}C straw in microbial biomass \pm prior presence of ^{13}C straw. The error bars are the standard errors of mean, the grey ones for the ^{12}C carbon data and the black ones for the ^{13}C data. Letters indicate the significance of the differences (ANOVA), latin-letter correspond to $^{12}\text{C-MB}$ data and greek-letter for $^{13}\text{C-MB}$ data

Figure.3



Days	10	10	10	10
Nutrients	-	+	-	+
¹³ C straw	-	-	+	+
¹² C straw	-	-	-	-



Days	10	10
Nutrients	-	-
¹³ C straw	-	+
¹² C straw	+	+

Figure 3 : Specific respiration of ^{12}C and ^{13}C carbon \pm SE.

A : Specific respiration between the control modalities and addition of ^{13}C straw at 10. B : Amount of carbon from ^{12}C straw in Specific respiration \pm prior presence of ^{13}C straw.

The error bars is standard error of the ratios (black for ^{12}C carbon data and grey for ^{13}C carbon data). Latin-letters correspond to ^{12}C carbon data and greek-letters to ^{13}C carbon data to indicate the significance of the differences (ANOVA).

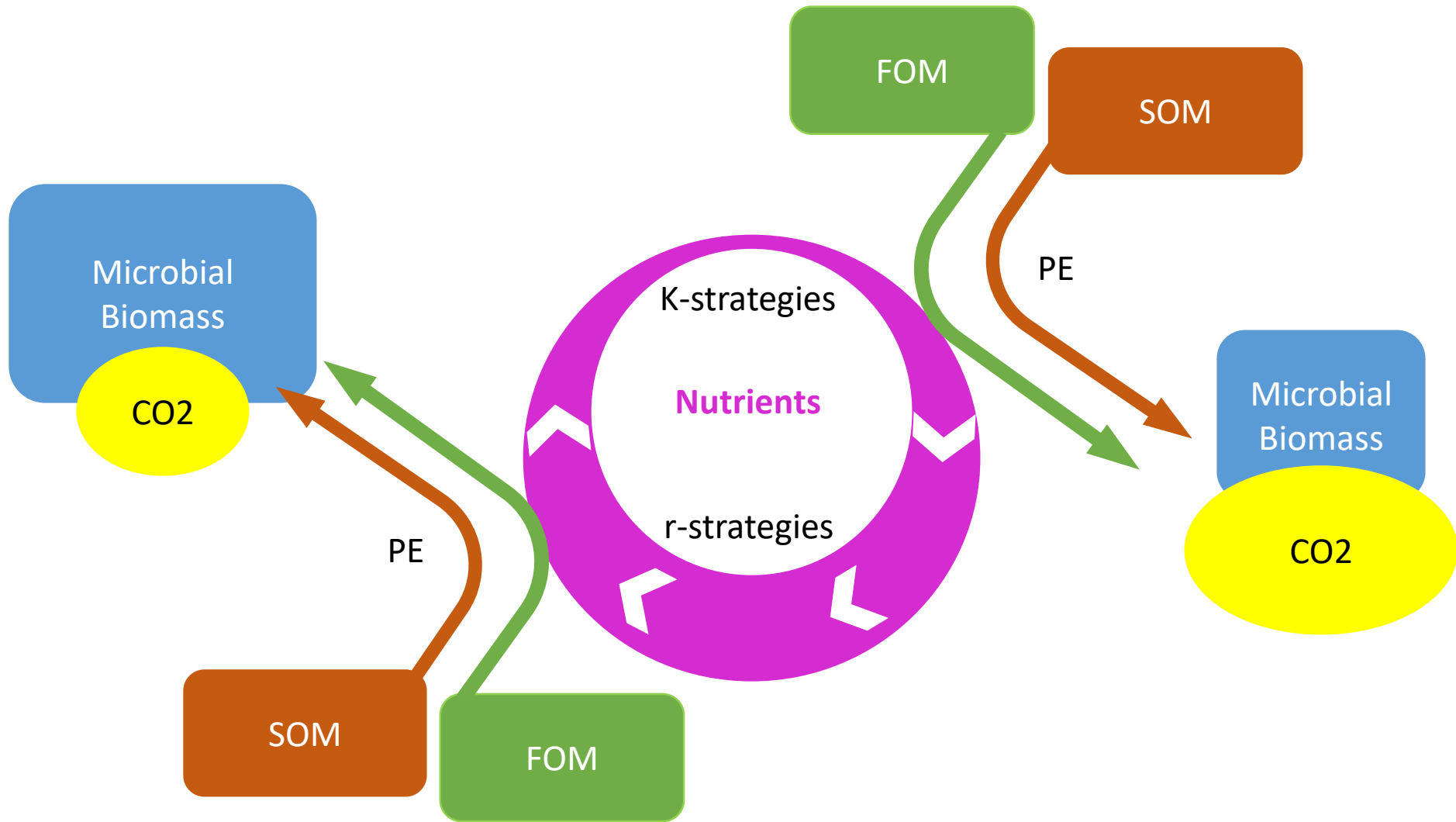


Figure 4 : Schematic representation of the mechanism of **short-term** priming effect generation by the FOM decomposition as controlled by the soil nutrient status.

Table 1: Modalities used for the different calculations (top left), measured and calculated C mineralization fluxes (top right) from the various OM pools and priming effect, and equations used for the calculations (bottom left and bottom right).

MODALITY CODE	TREATMENTS			C MINERALIZATION ACTIVITIES			
	Nutrient	¹³ C-Wheat straw	¹² C-Wheat straw	SOM min	S13min	S13 PE*	S12min*
	N	S13	S12				
1: [-N, -S13, -S12]	-	-	-	-N Tot CO ₂			
2: [+N, -S13, -S12]	+	-	-	+N Tot CO ₂			
3: [-N, +S13, -S12]	-	+	-		-N S13CO ₂	-N S13 PE	
4: [+N, +S13, -S12]	+	+	-		+ N S13CO ₂	+N S13 PE	
5: [-N, -S13, +S12]	-	-	+				-N S12 CO ₂
6: [+N, -S13, +S12]	+	-	+				+N S12 CO ₂
7: [-N, +S13, +S12]	-	+	+		-N S13 CO ₂		-N S12 CO ₂
8: [+N, +S13, +S12]	+	+	+		+ N S13 CO ₂		+N S12 CO ₂
*CALCULATION	S13 PE			S12 min			
3: [-N, +S13, -S12]	= Tot CO ₂ (3) - SOM min (1) - S13min (3)						
4: [+N, +S13, -S12]	= Tot CO ₂ (4) - SOM min (2) - S13min (4)						
5: [-N, -S13, +S12]				= Tot CO ₂ (5) - SOM min (1)			
6: [+N, -S13, +S12]				= Tot CO ₂ (6) - SOM min (2)			
7: [-N, +S13, +S12]				= Tot CO ₂ (7) - SOM min (1) - S13 min (7) - S13 PE (3)			
8: [+N, +S13, +S12]				= Tot CO ₂ (8) - SOM min (2) - S13 min (8) - S13 PE (4)			

Abbreviations: Tot CO₂: Total CO₂; SOM min: basal mineralization of soil organic matter; S13min: mineralization of the ¹³C labeled straw; S13 PE: Priming Effect induced by the ¹³C labeled straw on the soil organic matter; S12 min: mineralization of C thought to come from the non-labeled straw.