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

# The fate of primed soil carbon between biomass immobilization and respiration is controlled by nutrient availability.

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**ABSTRACT**

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

## *1.* Introduction

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

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

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

In the literature, a positive PE is defined as an acceleration of the SOM ~~mineralization~~ turnover and not only SOM mineralization, including therefore incorporation of SOM-C into the microbial biomass as well as its release via respiration. But as it leads to an increase in the $CO_2$ flux to the atmosphere over a certain period, it is sometimes ~~regarded rightly or wrongly as a net~~ considered as increasing the loss of C from SOM [24,9]. However, PE, as a part of SOM turnover is just an element of the whole C budget. This means that if conditions favoring PE, also favor new carbon storage, the net effect on soil C is not necessarily negative. In addition, the PE is the result of the activity of heterotrophic microorganisms as for regular SOM decomposition. This means that the C assimilated during the PE is partly

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

The PE generated by "nutrient mining" may indeed lead to the remobilisation of a SOM pool with a long residence time, by the activity of K-strategists known to allocate more assimilated C to energy generation ($CO_2$ release) than to biomass synthesis [20,25,26,27]. Therefore, whether this PE drives the system towards a net loss of C from the soil depends on its feedback on the entry of fresh C into the soil. In contrast, the "stoichiometric PE" (1) targets a dynamic pool of SOM rather than a stable~~ilizing~~ one, (2) rather involves r-strategist populations known to preferentially allocate the assimilated C into their biomass: therefore this type of PE can be regarded as an acceleration of the transformation of plant OM into microbial OM and thus contributes to long term C sequestration in the soil [28,29,30]. In this case, during a stoichiometric PE event, more "primed" C would be integrated into microbial biomass than released as $CO_2$, resulting in a decrease in the specific respiration rate, also called metabolic quotient $qCO_2$ (i.e. Respired C/Biomass C ratio, [31]).

The aim of the present study was to evaluate the C allocation ~~balance~~ between $CO_2$ evolution and microbial biomass synthesis during a "stoichiometric" PE event. Therefore, the specific respiration rate was measured in a microcosm incubation experiment where soils were successively amended with $^{13}C$- and $^{12}C$-labelled complex OM. $^{13}C$-enriched wheat straw was added to a naturally nutrient-poor tropical soil, to create a dynamic labelled pool of SOM. After one week of incubation, non-labelled wheat straw was added to the soil with the aim of generating a stoichiometric PE on the $^{13}C$-enriched decomposing straw. Over a 13-day incubation period, $^{13}C$ and $^{12}C$ were monitored in released $CO_2$ and in microbial biomass

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

## 2. Materials and methods

### *2.1. Soil sample description*

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

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

### *2.2. Soil incubation conditions*

The experiment was designed to induce and observe a stoichiometric priming effect. For this purpose, two factors were crossed: (1) soil wheat amendment: no wheat, $^{13}C$ wheat residues (±S13) at day 0, $^{12}C$ wheat residues (±S12) at day 7 and (2) soil nutrient content (nutrient supply or not, ±N) leading to 8 modalities (Table 1, Fig S1), with 6 replicates per modality. Forty-eight 160-ml flasks were filled with 16 g of dry soil. To reactivate microbial communities, the soil water content was adjusted and maintained to 39.5% of water content using sterile deionized water, for a 14-day pre-incubation period. Every 3 days, flasks were opened to renew the gas phase using a large 50 mL syringe. After this pre-incubation period, the 24 +S13 flasks were amended with 4 mg of $^{13}C$ powdered wheat residue (wheat: leaves and stems, 97% $^{13}C$) per gram of dry weight soil. The $^{13}C$ wheat straw was grown in a continuous 99%$^{13}CO_2$ atmosphere as described in [34]. Then, 12 non-amended and 12 wheat-amended +N microcosms were enriched with nutrient solution at a rate of 0.125 mL (g incubated soil)$^{-1}$ (6.86 mg $NH_4NO_3$ $mL^{-1}$; 7.12 mg $NaH_2PO_4$ $mL^{-1}$; 3.06 mg Kcl $mL^{-1}$; 4.01 mg $MgSO_47H_2O$ $mL^{-1}$; 4.41 mg $CaCl_2$ $mL^{-1}$; 0.06 mg $O_2Si_xH_2O$ $mL^{-1}$) in order to bring 300 mg N, 200 mg P, 200 mg K, 200 mg S, 65 mg Mg, 150 mg Ca per kg of dry soil [32]. Soil water content of -N flasks was adjusted using sterile water (0.125 mL $g^{-1}$ incubated soil). The soil microcosms were incubated in the dark for 13 days at 28°C. After 7 days of incubation, 4 mg

of unlabelled ($^{12}C$) powdered wheat residue (leaves and stems) per gram of dry soil were added to the 24 +S12 flasks. At day 3, day 7 (before $^{12}C$-straw addition), day 10 and day 13, a 6 mL volume of gas phase was sampled in Exetainer evacuated tubes for total $CO_2$ and $^{13}CO_2$ measurements. After sampling, gas phases were renewed in different rooms, depending on their $^{13}C$ enrichment modality, to avoid $^{13}C$ contaminations of unlabeled modalities. At day 10 and day 13, 3 flasks per modality were harvested for microbial biomass analysis, in order to see whether the $^{12}C$-straw input of day 7 had modified the incorporation of $^{13}C$-straw C into the microbial biomass, 3 or 6 days after the new substrate supply.

### *2.3. $CO_2$ and $^{13}CO_2$ measurements*

The collected gas phases were analysed by IRMS "Isotope Ratio Mass Spectrometry" using a mass spectrometer (Euro-EA Vector Element Analyzer, France ; coupled with an IsoPrime Mass Spectrometer, Italy ) to quantify the total C and relative abundance of $^{13}C$ in the $CO_2$ samples. Total mineralized C per gram of soil (Total $CO_2$-C) between two sampling dates and its $^{13}C$-Wheat component (S13min $CO_2$-C) were calculated using the following equations:

**(1)**

$$\text{Total } CO_2\text{–C } (\mu g\ C\ g^{-1}\ \text{dry soil}) = \frac{\left(\left(\frac{\left(\left(\frac{Q}{Vp}\right) * Vm\right)}{T}\right) * D\right)}{M}$$

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

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

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

with Total CO2-C = total mineralized C (µg C $g^{-1}$ dry soil), A% = $^{13}C$ relative abundance of the amended flask (%), Ac = Atmospheric $^{13}C$, i.e. mean of $^{13}C$ abundance measured in 3 non-amended modalities (µg C $g^{-1}$ dry soil), 0.97 = $^{13}C$ enrichment of straw C.

$^{13}C$-Wheat induced PE was calculated as follows:

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

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

$^{12}C$-Wheat mineralization was calculated as follows:

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

Calculation details for the different variables are given in table 1. As S13PE and S12min are the results of subtracting fluxes measured from independent replicated flasks. Means and Standard errors were calculated on all the 9 possible combinations between the 3 replicate flasks per treatment. S12min $CO_2$-C actually includes $CO_2$-C resulting from a potential PE generated by the $^{12}C$ straw on SOM, which cannot be discriminated as both OM have a similar isotopic signature.

### *2.4. Microbial Biomass Carbon*

Microbial biomass Carbon (MBC) was assessed using the fumigation-extraction method [35] in three flasks per modality on day 10 and day 13. Shortly, 15g of each soil microcosm were separated into two equal fractions, one being subjected to Chloroform ($CH_3Cl$) fumigation. Total soluble carbon was then extracted from both fractions with 30 mL of potassium sulphate solution ($K_2SO_4$, 0.025M). Two mL of the obtained solution were concentrated by evaporation and resolubilization in 120µL of deionized water. Adequate volumes were finally sampled and dried for elementary analysis (EA Eurovector analyzer, Italy) coupled with isotopic mass spectrometry (Mass Isoprime, Elementar, France) [36]. MBC feeding on the different SOM pools were calculated as follows:

**(5)**

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

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

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

**(6.1)**

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

**(6.2)**

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

with SOM MBC corresponding to the Total MBC quantified in both control conditions (with or without nutrients but no wheat addition – Table 1). Like for $CO_2$, S12MBC might include MBC produced from SOM by the effect of $^{12}$C-straw inputs, as both MBC cannot be differentiated by their isotopic signature.

### *2.5. Specific respiration*

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

**(7)** $$qCO_2\ (\mu g\ C\ g^{-1}\ dry\ soil) = \frac{(Total\ CO_2{-}C\ )_t}{(Total\ MBC)_t}$$

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

### *2.6. Statistical analyses*

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

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

(i) Y ~ Time * Nutrient (Time) + (1|Nutrient)

(ii) Y ~ (Time + I(Time$^2$ )) * Nutrient + (Time + I (Time$^2$)) + (1|Nutrient)

(iii) Y ~ I(Time$^2$) * Nutrient + I (Time$^2$) + (1|Nutrient)

(iv) Y ~ I(Time$^2$) + Nutrient + I (Time$^2$) + (1|Nutrient)

with Y = mean of cumulative $CO_2$-C emitted during incubation (µg $CO_2$-C $g^{-1}$ dry soil), Time = fixed factor of time (days), and Nutrient = nutrient supply considered as random factor.

The choice of model was made parsimoniously using Akaike's information criterion (AIC) [40]. On the chosen model, a two-factor ANOVA was applied to measure the effect of time and nutrient treatments.

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

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

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

A two-factor ANOVA, followed by a Tukey-HSD test, was applied to distinguish significantly different means, according to time and modalities for microbial biomass (MBC) and specific respiration ($qCO_2$).

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

## *3.* Results

### *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, $^{13}C$ 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 $^{13}C$-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 $^{12}C$ wheat residues on day 7 induced an increase in the mineralization of
292 $^{12}C$-OM ($^{12}C$ 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 $^{13}C$ wheat residues (S13 min, p-value > 0.1, Fig 1C), but the presence of $^{13}C$
295 straw, previously added, increased the intensity of $^{12}C$ 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 $MB^{12}C$ (i.e. SOM-derived MBC ~~non-labelled microbial biomass corresponding to SOM~~
300 in -S12 flasks or SOM-derived MBC plus $^{12}C$ wheat-derived MBC in + S12 flasks) in all
301 treatments ~~to $MB^{12}C$ in the control microcosms~~ (p-value < 0.05), even when $^{12}C$-~~enriched~~
302 wheat straw was added 3 days before (Fig. 2A). Nutrients and/or $^{13}C$-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 $MB^{12}C$. At the same time, $^{13}C$ wheat-derived MBC (S13MBC) was not significantly affected
306 either by soil nutrient status or by subsequent fresh wheat addition. On day 13, $MB^{12}C$ was

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

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

### *3.3. Specific respiration ($qCO_2$)*

Specific respiration of microbes measured on day 10 are presented on figure 4. The $qCO_2$ was the lowest in the control condition (-N -S13 -S12, p-value < 0.05) while it increased in conditions supplemented with nutrients or with $^{13}C$-wheat residue (Fig. 3A). The $qCO_2$ derived from $^{13}C$-wheat decomposition was higher in the modality where no nutrients were added (p-value < 0.05). The presence of $^{13}C$-wheat residues lowered the qCO2 derived from $^{12}C$-wheat residue decomposition (p-value < 0.05) (Fig. 3B).

## ***4.*** **Discussion**

### *4.1. Priming Effect generation process*

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

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

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

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

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

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

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

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

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

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

On day 10, the specific respiration of non-amended microcosms was higher when nutrients were not limiting (Fig. 3A), due to both a higher $CO_2$ evolution and a lower microbial biomass (Fig. 1 and Fig. 2A). Malagasy soils are naturally poor in nutrients and labile carbon. Nutrient enrichment, without labile C input, may have selected communities with a k-strategy, able to decompose recalcitrant SOM to balance their C:N ratio [21,47] and having thus a higher catabolic activity that limits their growth [48].

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

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

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

## *5.* Conclusion

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

**Acknowledgment**

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604

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607
608 Figure legends
609
610 Figure.1: Cumulative average curves of mineralized carbon as a function of incubation time
611 (µg CO2-C $g^{-1}$ dry soil) for (A) Basal Soil Organic Matter mineralization, (B) Priming Effect on
612 SOM generated by the addition of $^{13}C$ enriched wheat straw, (C) mineralization of $^{13}C$
613 enriched wheat straw, (D) Total $CO_2$ released and (E) deduced mineralization of $^{12}C$ wheat
614 straw. Modality Codes corresponding to each curve are indicated on the graphs with solid
615 lines and broken lines representing nutrient enriched and non-enriched conditions,
616 respectively. The error bars represent the standard errors of the cumulative averages.

617
618 Figure 2: Microbial biomass Carbon MBC (µg C $g^{-1}$ dry soil) with standard error bars derived
619 from (A) SOM, $^{13}C$-enriched wheat straw and SOM + $^{12}C$ wheat straw, measured after 10
620 days and 13 days of incubation according to the different treatments, and from (B) $^{12}C$ wheat
621 straw at 10 and 13 days. Letters indicate the significance of the differences (ANOVA), Latin-
622 letters correspond to $^{12}C$ enriched-MB data (SOM or SOM+$^{12}C$-straw) and Greek-letters to
623 $^{13}C$ enriched-MB data.

624 Figure 3: Mean specific respiration ($qCO_2$) ± SE of microbial populations feeding on (A) SOM-
625 or $^{13}C$-enriched wheat straw and (B) $^{12}C$-enriched wheat-straw, at 10 days of incubation and
626 depending on modalities. Latin-letters correspond to $^{12}C$-enriched OM data (A: SOM and B:
627 $^{12}C$-wheat straw) and Greek-letters to $^{13}C$-enriched OM data to indicate the significance of
628 the differences (ANOVA).

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

631

Figure.1

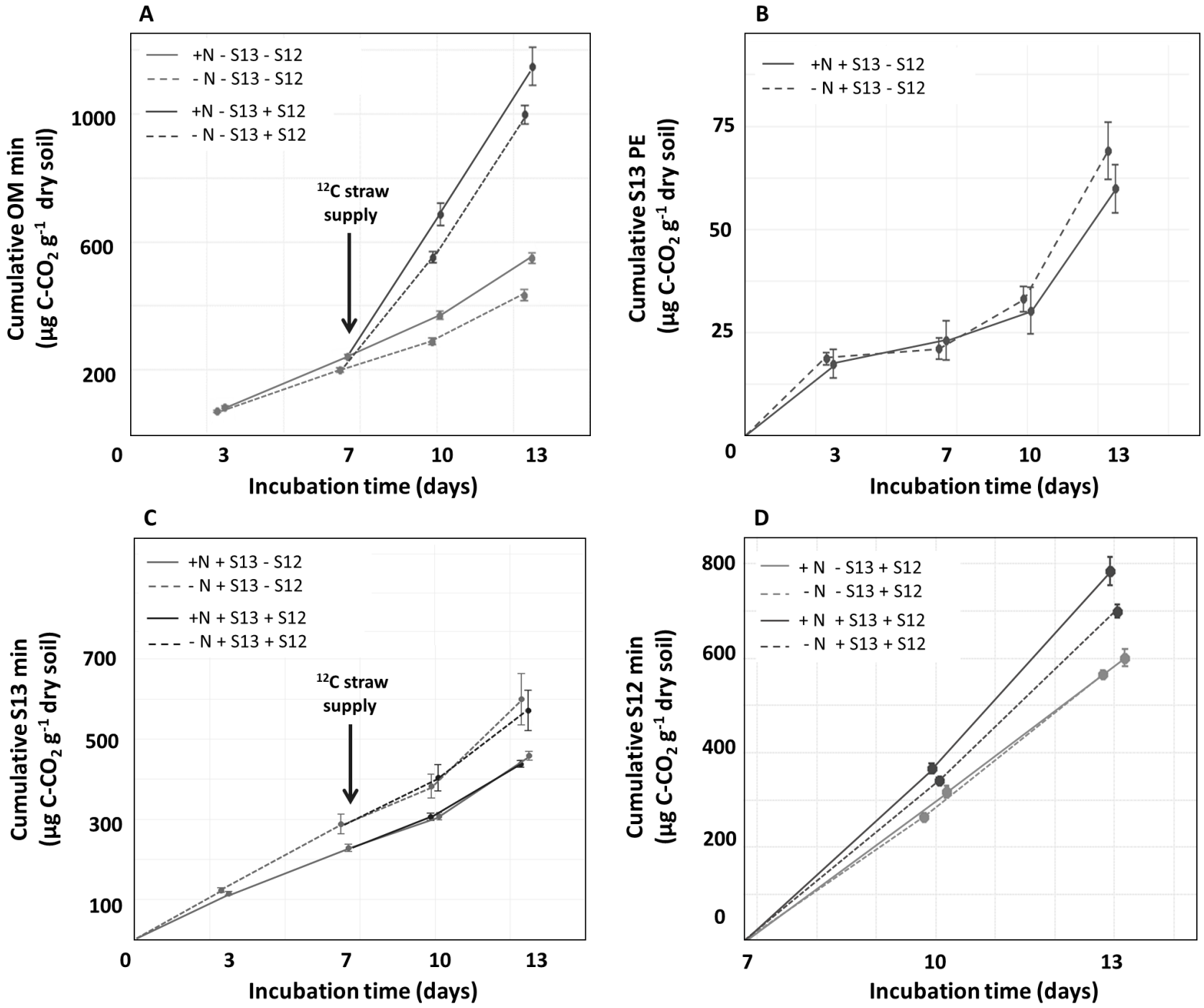

A
+N - S13 - S12
- N - S13 - S12
+N - S13 + S12
- N - S13 + S12
12C straw supply
Cumulative OM min (µg C-CO2 g-1 dry soil)
Incubation time (days)
B
+N + S13 - S12
- N + S13 - S12
Cumulative S13 PE (µg C-CO2 g-1 dry soil)
Incubation time (days)
C
+N + S13 - S12
- N + S13 - S12
+N + S13 + S12
- N + S13 + S12
12C straw supply
Cumulative S13 min (µg C-CO2 g-1 dry soil)
Incubation time (days)
D
+ N - S13 + S12
- N - S13 + S12
+ N + S13 + S12
- N + S13 + S12
Cumulative S12 min (µg C-CO2 g-1 dry soil)
Incubation time (days)

Figure.1: Cumulative averages curves ± standard error, of mineralized carbon during incubation for each organic matter pool and according to the different modalities (µg C-CO2 cumulative g-1 dry soil ± 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 ± prior presence of $^{13}C$ straw.

The error bars represent the standard error of the cumulative average

**Figure.2**

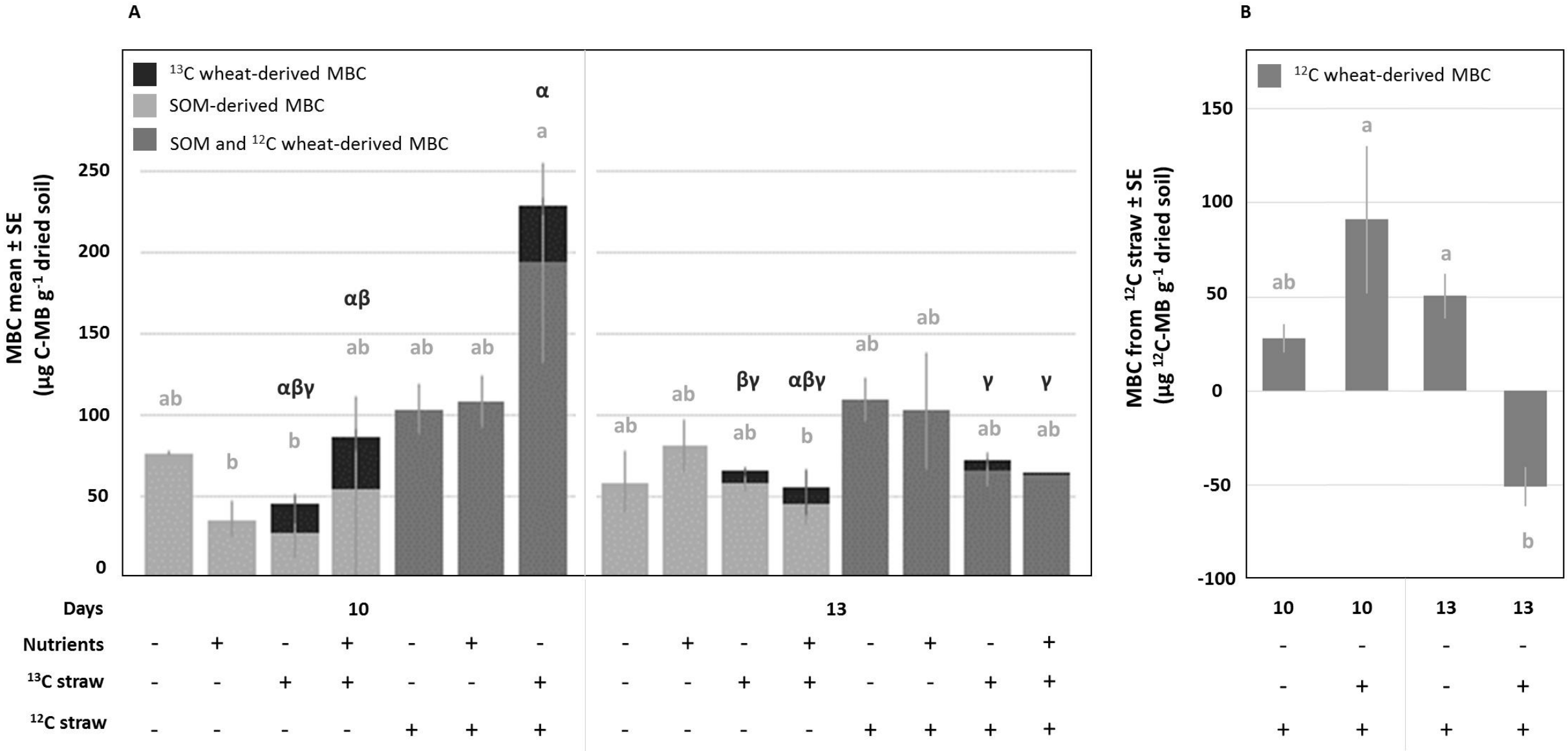
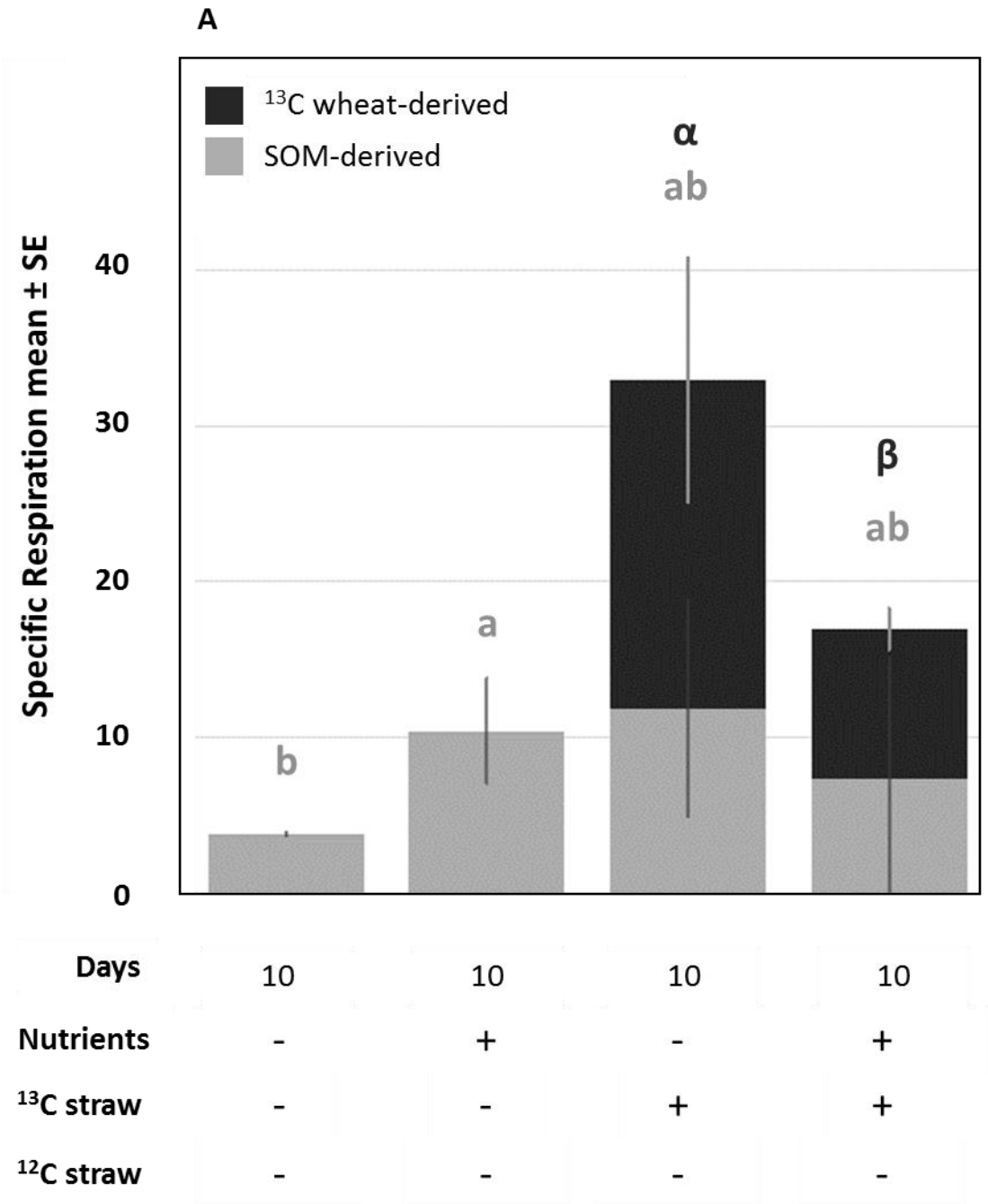

A
B
$^{13}$C wheat-derived MBC
SOM-derived MBC
SOM and $^{12}$C wheat-derived MBC
$^{12}$C wheat-derived MBC
MBC mean ± SE (µg C-MB g$^{-1}$ dried soil)
MBC from $^{12}$C straw ± SE (µg $^{12}$C-MB g$^{-1}$ dried soil)
250
200
150
100
50
0
-50
-100
α
αβ
αβγ
βγ
γ
a
ab
b
Days
10
13
Nutrients
$^{13}$C straw
$^{12}$C straw

Figure 2 : Carbon microbial biomass (µg C-MB g-1 dry soil ± 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 ± 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 $^{12}C$-MB data and greek-letter for $^{13}C$-MB data

**Figure.3**


A
$^{13}$C wheat-derived
SOM-derived
Specific Respiration mean ± SE
0
10
20
30
40
α
ab
β
ab
a
b
Days
10
10
10
10
Nutrients
-
+
-
+
$^{13}$C straw
-
-
+
+
$^{12}$C straw
-
-
-
-

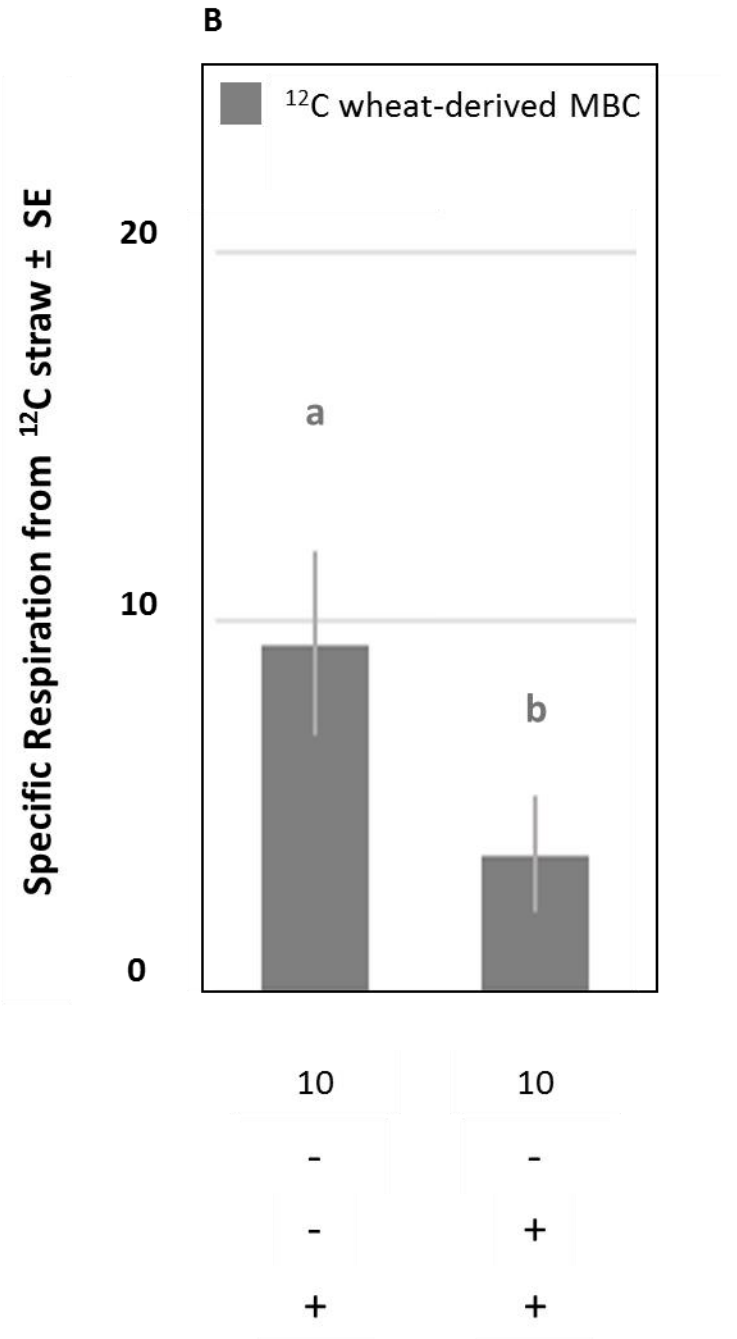

B
$^{12}$C wheat-derived MBC
Specific Respiration from $^{12}$C straw ± SE
0
10
20
a
b
10
10
-
-
-
+
+
+

Figure 3 : Specific respiration of $^{12}C$ and $^{13}C$ carbon ± 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 ± 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).

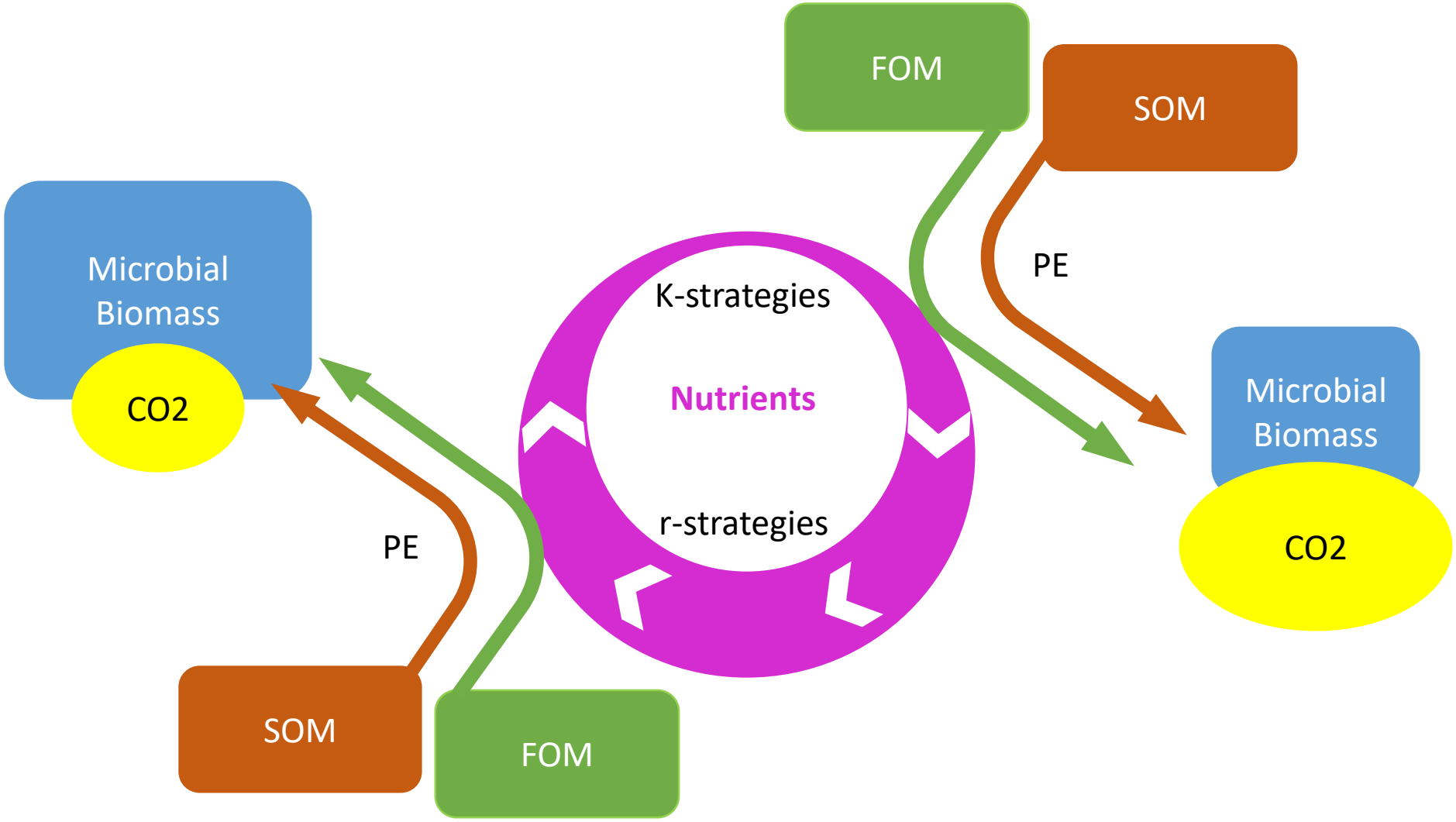

FOM
SOM
PE
Microbial Biomass
CO2
K-strategies
Nutrients
r-strategies
Microbial Biomass
CO2
PE
SOM
FOM

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).

| | TREATMENTS | | | C MINERALIZATION ACTIVITIES | | | |
|---|---|---|---|---|---|---|---|
| **MODALITY CODE** | 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 S13$CO_2$ | -N S13 PE | |
| 4: [+N, +S13, -S12] | + | + | - | | + N S13$CO_2$ | +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 $CO_2$: Total $CO_2$; SOM min: basal mineralization of soil organic matter; S13min: mineralization of the $^{13}$C labeled straw; S13 PE: Priming Effect induced by the $^{13}$C labeled straw on the soil organic matter; S12 min: mineralization of C thought to come from the non-labeled straw.