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Biogeosciences Discussions

Interactive comment on "An unknown respiration pathway substantially contributes to soil CO_2 emissions" *by* V. Maire et al.

Anonymous Referee #1

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The ms consists on the results of 3 studies evaluating importance of exoenzymes to CO2 release from soil. The individual studies are probably well done, despite they are based on numerous unproven assumptions. Also the enzymes were carefully chosen for the experiments.

My main problem is that the study pretends to show new pathways of organic C decomposition in soil and new processes contributing to soil CO2 emissions. To my knowledge, it is well known that exoenzymes contribute to the decomposition, hydrolysis, and oxidation or organic substances outside of microbial cells. This is not new and should be not tried to sell as a new pathway of C cycle. However, I appreciate the authors that they devoted to this problem, because despite the general knowledge, there are only very few studies focused on the separation / evaluation of processes

occurring outside and inside soil microorganisms.

Therefore, it is much better to show to which portion this (well known) CO2 source contributes to the CO2 fluxes from various soils and how this contribution depends on the soil properties (if this is the case). For this evaluation of the contribution however, the underlying assumptions for the methods/experiments should be proven and clearly presented. Furthermore, the main part of the Results is written too concise. It is hard to evaluate the details.

The ms should be completely rewritten, including enlargement of the Results section, separate Discussion based on the results obtained, and should not overestimate the things that are already known.

General comments Using of terms: there are some terms that the authors use incorrectly. Such terms as 'metabolism', 'respiration' . . . are the terms for solely intracellular processes. It is not correct to expand these terms on extracellular processes, even they are biochemically/enzymatically driven. E.g. 'respiration' is not equal to 'CO2 production'.

The Introduction is very weak: it contains just $\sim$ 20 lines, and consists mainly on general words. There is no information, WHY and WHICH enzyme groups (and not only hydrolytic enzymes) are released extracellulary.

The Title should reflect the study. It should be clear from the Title that contribution of extracellular enzymes to CO2 production in soils were evaluated.

Section 2.3.3: the approach of separate evaluation of the activity of soluble and soil-immobilized enzymes in not clear. For soluble enzymes the soil was incubated 5 min and centrifuged thereafter. For the immobilized enzymes the soil was incubated between 5 and 45 min and centrifuged thereafter. Is the duration of the incubation the only difference? If yes, this is surely not enough to separate the activity of immobilized enzymes. This part needs extended explanation with all details and assumptions to

evaluate the plausibility. This is very important as a part of the main hypothesis should be proven by the results of this approach.

Acceptance of CO2 release from g-irradiated soil as Exomet is very questionable (8671/ 1. . .). G-irradiation surely kills the microorganisms. This leads to the outflow of endoenzymes into the soil. These artificially released enzymes surely may contribute to oxidation of organics and consequently CO2 release. However, may be important part of these enzymes were not present in the soil before g-irradiion? (Sorry, I just have seen that this issue is already mentioned by the authors in the next lines).

One topic regarding the enzyme stability is completely disregarded. As enzymes are easily available proteins, after their release into the soil, the most part of them will be decomposed and utilized as substrates by living microorganisms. So, in many cases when non sterilized soil was used, as well as under real soil conditions, the released enzymes will be not stabilized, but decomposed by other enzymes and microorganisms. This surely has effects on their activity and contribution to CO2.

The ms has nearly no Discussion. This is especially poor as it is submitted for the Journal 'Biogeosciences Discussions'.

Specific remarks 8664/ 5 and 8665/ 10 this is very uncertain question: The microorganisms usually do not die. They can be grazed by soil animals (e.g. amoebae), but in this situation the most part of the microorganisms (including enzymes) will be digested. If the environmental conditions change or the substrate is exhausted, the microorganisms convert to the dormant state and build cysts or spores. So, one of the assumption of the study, that exoenzymes are released into the soil just 'by case' by dying probably is not very consistent. 8664/ 17 this is generally not correct: it is well known that exoenzymes strongly contribute to the decomposition of organics, and this is the main pathway of polymer decomposition. 8664/ 19 this is the chemical / thermal decomposition. In soils however, the glucose will be take up in microbial cells and will be decomposed by enzymes. 8664/ 25 the respiration is surely intracellular pro-

cess. However, there are various pathways of C oxidation and consequently of CO2 production independently on the intracellular oxidation. Extracellular decomposition of polymers is one of them. 8665/ 1 strong reduction does not mean that the respiration will be completely stopped. 8665/ 5 this is not really correct: besides hydrolytic enzymes also oxidising enzymes (oxidases, peroxidases) are present in soils extracellularly and these enzymes contribute to CO2 production. 8665/ 7 again: minimization does not mean absence 8665/ 26 because the soil from individual samplings were bulked together, all further analyses are expressed with analytical error/variation, and not the real variation in the field. 8667/ 13 what is UMDH? 8667/ 21-23 the amount of glucose added to the soil is very high! 8667/ 28 how CO2, O2 and 13CO2 were measured? Continuously, trapping, sampling at some periods? 8670/ 21 This equation consists on 6 (!) parameters. Using 6 parameters it is easy to fit any data points. Additionally, the parameters of such equation are interdependent. So, the comparison of these parameters is hardly possible unless half of the parameters will be estimated by independent approach. 8671/ 18 This model equation is not correct. It should be Rni = ... (not Rl)! Please check and correct! 8671/ 21 This is also big question. It is not really clear, does g-irradiation affect the exoenzymes or not? 8672/ 7 The exoenzymes may diffuse, but this process is really of very low importance, as they will be bound on clay minerals or SOM very fast. 8673/ 1 why it was necessary to sample the microcosms to estimate CO2 emission? The CO2 can be measured by GC or by trapping without microcosms' sampling. 8675/ 1 these high numbers are mainly the result of very high glucose amount added to the soil.

Fig 2 The approach is not clear for separation of the activity of total and immobilized enzymes. If I understood correctly, the activity of immobilized enzymes was calculated by difference. Consequently, all experimental errors are included in the activity of immobilized enzymes. Fig 2 An approximation of 8 points by equation with 6 parameters is incorrect (and the parameters are highly interdependent). For each parameter at least 2 points should be available.

8675/ 9-20 this paragraph is poor speculation. If this process sequence occurs within the cells, the individual steps are finely adjusted to each other spatially and timely. Outside of the cells it is hardly to believe that such or even comparable correspondence is possible in chaotic distribution of organelles after the cell died (if they died). 8676/ 6 I am not sure that this is the half life of the fast pool. In my view it is the half life of enzymes denaturation after there are released outside of the cells. 8676/ 14 The Fig shows only the decrease of the enzyme activity. It does not show the immobilization of enzymes. 8676/ 23 was the sterility proven at the end of incubation? 8677/ 10 was the sterility proven at the end of incubation? 8677/ 17 $CHCl_3$ does not completely kill microorganisms in soil. A part of microorganisms remains alive (e.g. Kemmitt et al. 2008) 8678/ 15 Again, the microorganisms actually not die from alone. Therefore, this delay is not really clear compared to what. 8678/ 22 The origin of life in soils, means in initial soils at the coasts and tidal locations, is generally accepted. However, the conclusion presented here (8678/ 28) is not correct, as the study was focused on the enzymes released by microorganisms (consequently microorganisms / life originated earlier than the enzymes released) and not on the origin of enzymes without microorganisms. May be it sounds as nice conclusion for the paper, but the study and the results are not connected with it. Table 2 is superficial, as the parameters of the non-linear regressions are highly interdependent, and it is not correct to calculate 6 parameters if only 8 experimental points are present. Figs 2,3,4 It is not clear what the error bars present. In any case, they show the variability of analytics, not of the real biological variability of the parameters, as the soil samples were mixed.

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