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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.

V. Maire et al.

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With this work S. Fontaine and his co-authors challenge the broadly accepted understanding that respiration is carried out by endo-enzymes inside cells, through a series of well thought and generally well executed laboratory experiments. The strength of this work is in its logic (and pretty comprehensive) structure. With a sequence of experiments, the authors test/demonstrate: 1) the existence of an extracellular oxidative respiration pathway, that they name Exomet; 2) the stabilizing role of the soil matrix on Exomet; 3) the relative contribution of Exomet to total soil respiration and 4) the resistance of Exomet to factors that affect cellular respiration (i.e., high temperature, autoclaving and chloroform fumigation). The validity of this work is based on the realized sterility of the soils and water samples. The authors made a significant effort

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Interactive Discussion

Discussion Paper

in proofing this true and convincing data on the sterility are also reported in the Supporting material. The ms is generally well written. The results from this work, given the significance (16-48%) of the measured exomet relative to total heterotrophic soil respiration for the analyzed soils, call for new research to text exomet in more soils – they tested 4 - but also to better understand generally the role of enzyme stabilization in soil processes. In my opinion, this is a very interesting and paradigm-shifting work that deserves publication. However, I do have a few comments/suggestions that will require some revision: 1) Authors should expand the discussion, in particular by providing some more in depth interpretation of the observed results. For example, the potential controls of Exomet are not at all discussed. The authors found that different enzymes had a different degree of stabilization (Why? What are the controls?), as well as that soils differing in land use and clay content showed different Exomet, with the lowest been found in the sandy soil under cropping management (again, why?). I understand that the data are limited and that there is a high risk for speculation, but I suggest that the authors point out a few treads that new research should follow.

Authors: we really appreciate that the referee recognizes the quality and the originality of work done. This work is the outcome of three years of intensive research involving five laboratories on a new and risky subject. It is important for us (and the research system) that this risk-taking is recognized and supported by the community. We completely agree that our discussion was too synthetic, but this paper historically had another format. We have taken time to reanalyse our results at light of referee advices. This permitted us to point out new findings about difference between soils and between enzymes and controls of EXOMET (soil pH, different types of soil particles cell mortality etc). This also permitted us to clarify what remains to be done and to give some priorities on some key processes. All these points have been taken into account in the new version of our manuscript, expanding our discussion section from 365 to 1207 words. We sincerely thank the referee for his contribution to this new discussion.

2) I do not follow the basic assumptions behind the experiment to quantify the relative

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contribution of soluble and soil-immobilized enzymes to total enzyme activity (P8670 section 2.3.3). The authors assume that the activity after 5 minutes it's only from soluble enzymes, why? I understand that it will take longer for the enzymes to stabilize, but that would just say that it is from the total enzymes still all in solution. After that time, the enzymes remaining in solution decline, while those which stabilize remain active.

Authors: We agree that the approach developed to separate activity of soluble and soil-immobilized enzymes was not sufficiently explained. Of course the duration of the incubation is not the only difference between the two methods designed to quantify activities of soluble and soil-immobilized enzymes. Activity of soluble enzyme (SolEnz) was quantified after their extraction from soil. For extraction, 80 mg soil samples were mixed with the 300 μl of the buffer solution containing substrates, co-factors and intermediate enzymes (See section 2.3b) and shacked during 5 mn. Samples were then centrifuged at 11,000 x g during 3min. The supernatant containing soluble enzymes, co-factors and substrates was transferred into a micro-plate where activity of soluble enzyme activity was measured during 3 min. The production rate of NADPH (for GHK and G6PI) and the consumption rate of NADH (for MDH) consecutive to the activity of soluble enzymes were quantified by spectrometry at 340 nm. For measurement of total enzyme activity (TotalEnz), the enzymatic reaction was made into the soil, that is, in presence of soluble and soil-immobilized enzymes. To this end, soil-enzyme mixture was incubated with substrates, cofactors (See section 2.3b) during 45 min. At different times between 5 and 45 min of incubation with substrates, independent samples were harvested and centrifuged at 11,000 x g during 3min. The NADPH concentration in the supernatant was determined by spectrophotometry at 340 nm. The production of NADPH (for GHK and G6PI) or the consumption of NADH (for MDH) during the 45 min incubation of soil with substrates corresponded to the activity of total enzymes. Finally, activity of soil-immobilized enzymes was estimated by difference (ImmEnz = TotalEnz - SoluEnz). This section has been completely rewritten in the new version of the manuscript (P9-10).

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My concern is further demonstrated by the fact that the initial activity of soluble enzymes is in fact higher than the total (Fig. 2) which obviously does not make sense.

Authors: in theory, yes the activity of soluble enzymes should not be higher than the total. However, the activity of soluble enzymes are increased when their extracted from the soil. This effect can easily be explained by the fact that soil is not an environment favourable for enzyme activities (substrates, co-factors may be absorbed to minerals and not available for enyzmes, their diffusion is constrained etc). This effect, which induces an overestimation of activity of soluble enzymes, does not question our main findings: only the activity of soil-immobilized enzymes is maintained in the long term (activity of soluble enzymes is close to zero after 15 days of incubation).

Also, if the soluble enzyme activity is not quantified at subsequent times, where does the dynamic shown in the figure comes from? The authors should reconsider their interpretation of this experiment or, if I missed something – which I think I did -, do a better job at clarifying the assumptions and procedure used.

Authors: I hope it is clear now.

3) The level of CO2 reached in the microcosms is very high for lab incubations, often C3756 exceeding 10% (Fig 3). This may have inhibited CO2 diffusion (and possibly production) from soils to the atmosphere in the microcosms where CO2 was accumulated in the headspace (where it was trapped in soda it should have not affected CO2 efflux). In fact the authors observed in one case that the O2 had limited respiration. This inhibition would, if happened, actually represented an underestimation of Exomet. However, the authors need to discuss this potential problem in their analyses.

Authors:The concentration of CO2 in incubated flasks has always been maintained at a level < 3% but at two dates during the yeast extract incubation experiment. For this experiment, such a respiratory activity was unexpected and we left the CO2 concentrations reaching very high levels. Finally, this error was not one since it permitted us to observe the shift from partial fermentative metabolism to pure oxidative metabolism

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when the concentration of O2 rose again. The estimation of EXOMET in the four soils was based on a specific incubation where the concentration of CO2 was always maintained below 3%. Therefore, estimation of EXOMET was not affected by a problem of O2 diffusion. We have specified in the new version of the manuscript that the concentration of CO2 in atmosphere of flask was maintained below 3% (P14) but in the yeast extract incubation experiment.

Specific comments: P8666 L2: Delete soil names and change in “The five soils presented textures ranging from .” Authors: done P8666 L4: add “(Table 1)” after crops. Authors: done P8666 L4: Replace here and throughout the text “the soil of” with “the soil from” Authors: done P8667 L12: Add reference for Biuret method Authors: done P8667 L13: U MDH – this is for specialists – clarify Authors: done P8667 L29: The experimental units are defined “microcosms”, but what are they: jars (as for one of the following exp)?, vials? How big? Air tight? Dark, clear? The authors need to provide a clear description of the physical structure of the microcosms. Authors: done P8667 L29: add the sentence: “, following the methods described in section 2.6.” after incubation Authors: done P8668 L13: Why for this experiment incubation was at 20C, while all others were carried out at 30C? Authors: Because this experiment has been conducted before we launch this project on EXOMET. It’s even this result that has triggered our reflexion on this subject. P8668 L22: Add the sentence “repeating the above experiment but only . . .” between “content” and “using” Authors: done P8670 L24: Add reference for the calculation of half life. Authors: done P8671 L18: Correct “RI=” with “Ri=” in the second equation. Authors: Apologies! This error has been corrected. P8671 L21: Indeed it is simple algebra, but for clarity the authors could add after Rx “Thus, k can be obtained from . . ..”. Authors: Sorry we do not understand your advice. Could you check and reformulate your sentence please? P8673 L15: add the sentence: “, following the methods described in section 2.6.” after measured Authors: done. P8674 L2: Was titration done manually or automatically, please specify, and in the latter case add the model of the instrument used for titration. Authors: CO2 emissions were quantified by gas chromatography. Trapped CO2 was used to mea-

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sure delta 13C. P8674 L3: To my knowledge CO2 in air is measured by either IRGA or by Gas chromatography (with an appropriate detector). What do the authors mean by “Gas spectrometry”? Please clarify the method and provide the model for the specific instrument used. Authors: excuse for this typo. P8674 L21: Why native stabilized enzyme do not benefit from glucose additions? Are they not C-limited? This result have several potential implication for soil C cycling in the natural environmental and the authors should do a better job at highlighting this result and discuss it. Authors: The irradiation has released large amount of C substrate from the killed microbial biomass (See supplementary info 1 and 4). Thus, enzymes in irradiated soils are completely flooded by C substrates and do not respond to supplemental input of C. Consequently, this effect does not deserve to more interpreted. P8675 L21: Correct “incbation” with “incubation” Authors: done P8675 L24: Add “sterile” to read “from sterile control soil..” Authors: done P8676 L14: See general comment (1) above. This is a large variation – the authors should discuss it and speculate on some possible explanations. Authors: it’s right, this result deserved better attention. Thank you for your contribution. P8676 L23: Provide actual CO2 values besides percentages. Authors: we disagree with this proposition. This form permits to simplify presentation of respiration results that would be unreadable amounts were done for each treatment (4 soils x 2 treatments: irradiation versus no irradiation). P8677 L2: Again, see general comment (1) above. The authors should discuss the variability observed between soils and speculate on some possible explanations. A more general discussion may also fit well at P8678 L5, before “Finally”. Authors: done Table 1: Order the soils by a criterion (e.g. alphabetic, land use, clay content), any would do as long as there is one. Soro is not spelt correctly. It is a Danish name and the correct spelling is “Sorø”, please correct. Delete “(-)” after pH. The SI unit for CEC, is centimol which is written “cmol+” and not “Cmol”, please correct. Authors: done Fig.3 To my knowledge if CO2 is given as a concentration, % or ppm are used as units. Thus I suggest deleting “atm” from the y-axis labels. Authors: done

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