

## ***Interactive comment on “An unknown respiration pathway substantially contributes to soil CO<sub>2</sub> emissions” by V. Maire et al.***

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Maire et al present a provocative manuscript showing CO<sub>2</sub> efflux from sterile soils. They then go on to propose a mechanism by which stabilized extracellular enzymes, perhaps even in intact membranes, are able to perform respiration. Overall, I think this is an elegant study, with important implications. However, I think the writing could be improved. First, I think the authors should be clear in distinguishing observations from speculation. They present strong evidence for abiotic CO<sub>2</sub> flux, and even that extracellular enzymes are involved. However, the mechanism is indeed unknown, and may not even be respiratory. This should be reflected in the title- I would delete the work 'respiratory'.

Authors: we really appreciate that the referee recognizes the quality and the originality  
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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 agree that our result and discussion sections were not enough separated. We also largely enriched our discussions of results, expanding our discussion section from 365 to 1207 words (see below for details). Title : we agree that the term “respiration” was not entirely adapted because typically reserved for living organisms. We therefore propose the following title “An unknown oxidative metabolism substantially contributes to soil CO<sub>2</sub> emissions”.

Structurally, the introduction is too brief and doesn't really identify the problem that this study addresses- are there cases in which CO<sub>2</sub> flux is not explained by biological processes?

Authors: We completely agree that our introduction was too synthetic, but historically this paper had another format. The introduction of the manuscript has been rewritten and extended from 418 to 690 words. This new introduction includes some backgrounds on mineralization process and clearly separate role of endoenzymes and exoenzymes in C mineralization process.

One aspect where the paper could be improved is in discussing the implications of this proposed mechanism for ecosystem function. In particular, I would ask them to consider how respiration is modeled in ecosystem models, and whether this mechanism is implicit, and whether the models would need to be modified.

Authors: We agree that implications of this Exomet for ecosystem functioning deserved to be more discussed. 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). Concerning the issue of ecosystem functioning, we think that living respiration and Exomet should be considered separately when studying effects

of environmental factors on the C cycle because they do not obey to the same laws and respond differently to environmental factors. Soil microorganisms have tight physiological constraints comprising specific environmental conditions (temperature, moisture, absence of toxics) and needs in energy and nutrients. These constraints explain why soil CO<sub>2</sub> emissions are controlled by availability of fresh energy-rich C and nutrients to soil microorganisms (Fontaine et al., 2003; Blagodatskaya et al., 2007). In contrast, the Exomet-carrying enzymes have no physiological constraints and are highly resistant to toxics, high temperature and pressure due to their protection by soil particles (section section 3.4). This Exomet can explain why substantial part of soil CO<sub>2</sub> emissions can be maintained in soils exposed to factors lethal for most soil microorganisms (Chloroform, high temperature, pressure, and irradiation) (Peterson, 1962; Ramsay and Bawden, 1983; Lensi et al., 1991; Trevors, 1996; Kemmitt et al., 2008). Exomet can also contribute to the flush of CO<sub>2</sub> emission from soils submitted to freeze-thaw or wet-dry cycles since these treatments promote microbial death (Henry, 2007). All this information have been included in the new version of discussion that expended from 365 to 1207 words.

Concerning the issue of modelling, we think that the discovery of this metabolism is too fresh for including in the manuscript discussions on how this mechanism could be taken into account in models. We need to accumulate results on this metabolism in order to confirm its existence in different ecosystems and to quantify its importance. Nevertheless, we develop some aspects here. Most models of soil C dynamics describe soil respiration as a fraction of soil organic matter breakdown and assume first order decomposition kinetics of organic carbon ( $dC/dt = -KC$ ). Consequently they assume first order kinetics of soil respiration and EXOMET is implicitly comprised in the constant decay rate. Nevertheless this constant represents a complex mixture of C solubilisation rate, C respiration rate of living micro-organisms and Exomet that do not allow predicting the C cycle in a changing environment. Others models are micro-organisms centred and de facto do not represent Exomet mechanism.

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The authors state that this can be a substantial fraction of total respiration. The calculations for this finding were somewhat unclear to me.

Authors: sorry there were a typo in equations proposed to quantify the EXOMET. The second equation should be read as follows: . This correction should greatly facilitate your understanding of method of quantification.

Beyond that, I would question whether this statement is supported- we don't know how well this process would compete with intact microbes-

We agree that we don't know well Exomet would compete with intact microbes. However, we have paid attention to this point repeatedly in the new version of the manuscript. In the method section (P12) we discuss the fact that Exomet and living respiration are not likely to be in competition for organic substrates. Indeed, Exomet may have preferential access to organic substrate since Exomet-carrying enzymes are adsorbed on soil particles including organic matter. Moreover, most of the soil sites where Exomet can proceed are deprived of microorganisms. Indeed, enzymes responsible for Exomet may diffuse in most soil pores whereas living soil microorganisms due to their size occupy less than 0.5% of the soil pore space (Paul and Clark, 1989). In the new version of discussion, we encourage further experiments to explore the possible interactions between living respiration and EXOMET like competition for soluble substrate.

whether turnover time of these enzymes would increase with biotic activity, etc.

It is right that our results show the stabilisation rate of G6PI was lower in non-irradiated soil than in irradiated soil (Tables 2 and 3). However, our method quantifies the EXOMET induced by enzymes that are already stabilized on soil minerals. This pool of enzymes has been built when soil was not irradiated, that is, in real conditions.

Further, although this process is abiotic, it would only be decoupled from biology for short periods of time- it relies on living microbes to produce the respiratory enzymes,

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and to produce enzymes to depolymerize organic matter. What are the implications for system energy dynamics, DOC production, etc for a molecule respired through Exomet vs microbes? Can this mechanism explain other observations that have been difficult to explain- perhaps respiration following freeze-thaw or wet-dry?

Authors: As explained above, the issue of EXOMET consequence on soil and ecosystem functioning is now discussed in the new "Discussions and perspective" section.

It would be helpful to offer some comments at the end on what the remaining and follow up questions are regarding Exomet.

Authors: We have dedicated a complete paragraph on this issue in the new version of the manuscript. We paste this text here for your information. Further experiments are necessary to better understand the molecular mechanisms at play and predict the Exomet across soils. Processes leading to microbial death and release of endoenzymes in soils (virus infection, predation, cell death due to stress like drought) must be identified. In marine and freshwater ecosystems, viral infection of microbial cells may be an important way by which endoenzymes are released in the environment since between 10 and 40% of bacterial cells is lysed by viruses (see the review Weinbauer, 2004; Colombet et al., 2006). To our knowledge, such quantification does not exist for soil ecosystems. The endozymes stabilized by soil particles (humus, minerals) could be identified by soil proteomics (Ogunseitan, O.A., 2006) whereas the chain of biochemical reactions involved in Exomet could be precisely characterized by metabolomics (Baudoin et al., 2001). Further experiments should also explore the possible interactions between living respiration and Exomet like competition for soluble substrate use (See section 2.4.a). Nevertheless, the Exomet is likely to be common in many soil types as consistent results were found in the five contrasted studied soils. Moreover, the reconstitution of intracellular metabolism outside the cell could occur in other environments (sediments, water) and may concern other metabolisms (methanisation, denitrification) since it only requires the presence of dead cells releasing endoenzymes. We therefore encourage research in other environments to quantify the role of intracellular

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metabolisms reconstituted outside the cell on global C cycle.

I would replace the final sentence of the current conclusion-which is pretty wild speculation, and illicit a laugh from this reviewer since these enzymes were produced by living microbes to begin with.

Authors: All theories developed to explain the origin of life have their part of speculations. When Miller and Urey demonstrated in 1959 that it is possible to produce a wide variety of organic molecule in conditions thought to be similar to those of the early earth, they open some doors to understand how life could have appeared. Nevertheless, the chemical structure of their amino acids etc is ridiculously simple compared to that of living organisms and I am sure that skeptics of this period underlined it. In this article, we try to modestly contribute to the issue of self-organization of life building block leading to first ancestral metabolism or life form. Previous studies have shown the role of soil minerals in the concentration and polymerization of amino-acids and sugars leading to the formation of a wide variety of molecules (proteins, polysaccharides) during the prebiotic period (Bernal, 1949; Miller and Urey, 1959). However, these models do not explain how you pass from macro-molecules to metabolism. Our results indubitably provide a part of response to this question with one example: the oxidative metabolism. Indeed, we show that complex biochemical reactions underpinning bioenergetics of life (respiration) can occur spontaneously in soil without compartmented living structure. Of course, all relevant organic molecules (comprising proteins with a catalytic function) must be present in soil, but this is the speculation part of our theory. Finally, we do not say glycolysis and the Krebs cycle have appeared in soil before the presence of the first cells. We know that these metabolisms have been optimized with evolution. However, on the basis of our observations, we say that the first ancestral oxidative metabolism may have occurred in soil before the presence of the first cells. This paragraph has been slightly modified in order to clarify our modest contribution to this big question for humanity.

Is this novel? You bet. Sure, others including Kemmit have suggested that respiration

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can be decoupled from microbial biomass or activity, but this takes it to a new mechanistic level. My own lab has observed respiration from yeast extract, but we never followed up on this observation.

Authors: Thank you for this information that will help us to convince referee 1.

This is a nice series of experiments carefully testing each mechanism. I think a paper like this is prone to criticism- there is always something that wasn't measured, always an alternative explanation for results- easy fodder for a skeptic. For example, the enzymes could be directly detected with proteomics, the enzymatic products could be measured.

Authors: This example has been included in our paragraph dedicated to perspectives.

But, the authors are careful in their wording to present this as a proposed mechanism that can be further tested. From a biochemical perspective, it is hard to imagine how this mechanism could persist for any length of time. Yet, I found the observations convincing. Just because it is hard to believe given our current paradigm does not mean it is wrong. I encourage the advancement of science by publishing this paper. Time will tell if this was a naïve idea with a simple explanation, or a breakthrough.

Authors: I am happy to see it is still possible to publish new ideas in scientific journals. Thank you for your openness and your contribution in popularising our work.

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