

Interactive comment on “An unknown respiration pathway substantially contributes to soil CO₂ emissions” by V. Maire et al.

V. Maire et al.

fontaine@clermont.inra.fr

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With these comments I would like to support the study of Maire et al., which traced the pathways of exo-enzymes in soil at the quantitative level. Activity of soluble and immobilized enzymes was distinguished and at least three enzymes pools were revealed according to their persistence in soil. These findings are very relevant and helpful for understanding and modeling the enzymes-mediated processes in soil. The MS of Maire et al. represents a brave attempt to disentangle the intriguing phenomenon of respiration sustainable to biocide treatments of soil. This phenomenon is similar to those known in soil microbiology as cyanide-resistant respiration which always occurs in soil even after application of combinations of strong inhibitors effective against eu- and-prokaryotic microorganisms. The phenomenon of cyanide-resistant respiration is

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explained by reliable protection of microbial cells within soil micro-aggregates and by extremely strong resistance of dormant microbial forms such as endospores which can survive under strong antibiotic treatments. That is why more or less appropriate sterilization of soil sample requires at least three autoclaving procedures with 3-5 days soil recovering under optimal moisture and temperature between autoclaving to provoke germination and activation of resistant microbial forms. From this point of view some uncertainties occur in the experimental design in study of Maire et al. 1) Compared with autoclaving the -irradiation at 45 kGy applied in the study of Maire et al. is relatively mild treatment as at least double irradiation treatment at 100 kGy is required for reliable soil sterilization.

Authors : We really appreciate your support and your recognition of efforts done to identify this unknown metabolism.

The dose of 45 kGy is all except a moderate treatment. For comparison, a dose of 25 kGy is used in pharmacology and to sterilize medical equipment in order to assure sterility at 100%. Concerning living soils, there are many works showing that γ -irradiation at 20 kGy eliminates all cultivable bacteria, actinomycetes and fungi (e.g. McNamara et al., 2003). We have more than doubled this dose for this experiment. None known microorganisms can support such dose. Moreover, soils were conditioned in small quantity (5, 10 or 15 g) favoring a deep penetration of gamma ray. We have tested several methods of sterilization (see Appendix) and none of them is as efficient as irradiation.

2) The statement that Exomet persist in the long-term (>100 days) without microbial production of new enzymes (P8673 Lines 12-13) is not convincing as spores germination of slow growing oligotrophic microorganisms can occur after weeks and months of soil incubation and such slow growth can contribute to the respiration detected in study of Maire et al.

Authors: Efficiency of γ -irradiation to kill soil organisms and maintenance of micro-

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cosm sterility were analyzed in detail during our experiments. This analysis was based on combination of complementary microbiological and molecular methods (TSA-FISH, transmission electron microscopy, microbial biomass measurement, culture on different media), simulation of contamination and determination of method sensitivity. All methods converge towards the same conclusion: absence of any microorganisms. We invite all interested people to consult our appendix where all these investigations are detailed. Oligotrophs are characterized by slow growth, low population density and low rates of metabolism. Thus, even there are some rascal microorganisms hidden by minerals they cannot contribute to observed respiration fluxes in irradiated soils (This question has been addressed, see Appendix).

3) P8677 Lines 11-12. The statement that “soil stabilization of respiratory enzymes released by ancient generations of microbial populations” is also not convincing as CO₂ emission detected in long term can indicate slow microbial re-activation after irradiation.

Authors: this issue of sterility has already been discussed.

4) P8676 Lines 21-23 and P8677 Lines 16-18. The findings that “cumulated CO₂ emissions from the irradiated-soil represented 17 to 59 % of that measured in nonirradiated-soil” and that “50% of Exomet were resistant to chloroform” – correspond very well to the values of conversion factor for fumigation-extraction method suggesting again incomplete soil sterilization.

Authors: this parallel is very interesting, but there is no doubt on our soil sterilization. However, have ever you thought how the fumigation can induce a rapid flush of respiration though it strongly reduces microbial biomass thereby the actors of soil respiration. I think that the EXOMET largely contribute to this fumigation-induced soil respiration.

5) Additionally, it is not clear how sterile conditions were maintained during 350 days considering regular CO₂ measurements and necessity of soil aeration.

Authors: All manipulations of microcosms were conducted under sterilized conditions

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(manipulation under sterile hood). Ventilation of incubated microcosms was made with filtered (0.22 μ m) air. Our local Institute has a long experience of working in sterile conditions since many researches have been done with axenic animals.

6) I find that Fig.3 is very good illustration that Exomet per se does not exist as glucose addition (S+G) did not cause respiratory increase as compared with irradiated soil (S). If pool of oxidative enzymes exists in soil these enzymes would decompose added glucose.

Authors: Soil enzymes in sterilized soils do not respond to glucose supply simply because there are not limited by substrate C. Indeed, irradiation released large amount of soluble C from the killed microbial biomass (See appendix).

This was confirmed by enzymes addition with yeast extract (S+G+YE) which strongly increased respiration.

Authors: We do not see the cause-effect link. The sole difference between S+G and S+G+YE is the supply of yeast extract containing respiratory enzymes. The key result here is that enzymes without cellular organization can carry out a complete oxidative metabolism.

Despite these concerns I find the study of Maire et al. on extracellular metabolism very progressive, bringing fresh ideas in the science and motivating research community to further progress. I do believe that publication of this study will induce further development of soil science as well as very fruitful discussion.

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