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## ***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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Received and published: 1 September 2012

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 eukaryotic and prokaryotic microorganisms. The phenomenon of cyanide-resistant respiration is

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Interactive Discussion

Discussion Paper



Interactive  
Comment

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  $\gamma$ -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.
- 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.
- 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 CO<sub>2</sub> emission detected in long term can indicate slow microbial re-activation after irradiation.
- 4) P8676 Lines 21-23 and P8677 Lines 16-18. The findings that “cumulated CO<sub>2</sub> emissions from the irradiated-soil represented 17 to 59 % of that measured in non-irradiated-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.
- 5) Additionally, it is not clear how sterile conditions were maintained during 350 days considering regular CO<sub>2</sub> measurements and necessity of soil aeration.
- 6) I find that Fig.3 is very good illustration that Exomet per se does not exist as glucose

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

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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Interactive comment on Biogeosciences Discuss., 9, 8663, 2012.

**BGD**

9, C3701–C3703, 2012

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