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Unusual isotopic composition of C-CO_2 from sterilized soil microcosms: a new way to separate intracellular from extracellular respiratory metabolisms.

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The mineralization of organic C requires two main steps. First, microorganisms secrete exoenzymes in soil in order to depolymerize plant and microbial cell walls and release soluble substrates for microbial assimilation. The second step of mineralization, during which C is released as CO_2, implies the absorption and utilization of solubilized substrates by microbial cells with the aim to produce energy (ATP). In cells, soluble substrates are carried out by a cascade of respiratory enzymes, along which protons and electrons are transferred from a substrate to oxygen. Given the complexity of this oxidative metabolism and the typical fragility of respiratory enzymes, it is traditionally considered that respiration (second step of C mineralization process) is strictly an intracellular metabolism process.

The recurrent observations of substantial CO_2 emissions in soil microcosms where microbial cells have been reduced to extremely low levels challenges this paradigm. In a recent study where some respiratory enzymes have shown to function in an extracellular context in soils, Maire et al. (2013) suggested that an extracellular oxidative metabolism (EXOMET) substantially contributes to CO_2 emission from soils. This idea is supported by the recent publication of Blankinship et al., 2014 who showed the presence of active enzymes involved in the Krebs cycle on soil particles.

Many controversies subsist in the scientific community due to the presence of non-proliferating but morphologically intact cells after irradiation that could substantially contribute to those soil CO_2 emissions. To test whether a purely extracellular oxidative metabolism contribute to soil CO_2 emissions, we combined high doses of gamma irradiations to different time of soil autoclaving. The presence of active and non-active cells in soil was checked by DNA and RNA extraction and by electronic microscopy.

None active cells (RNA-containing cells) were detectable after irradiation, but some morphological intact cells were observed by microscopy. These “ghost” cells were completely destroyed by the irradiation-autoclaving combination releasing large amount of soluble C. The soil respiration (O_2 consumption and CO_2 production) was reduced by irradiation and autoclaving but not stopped, suggesting the presence of an EXOMET. The delta 13C of CO_2 released in the irradiated-autoclaved soil was strongly depleted (-70‰ indicating that this extracellular metabolism induced a substantial isotopic fractionation. Our findings suggest that two main oxidative metabolisms co-occur in soils: cell respiration and EXOMET. The isotopic fractionation induced by the EXOMET open perspectives for its quantification in non-sterilized living soils.