Published in final edited form as:

Soil Biol Biochem. 2018 October ; 125: 286–289. doi:10.1016/j.soilbio.2018.07.023.

Cellular and non-cellular mineralization of organic carbon in soils with contrasted physicochemical properties

Kéralval Benoit^{1,2,*}, Fontaine Sébastien², Lallement Audrey², Revaillet Sandrine², Billard Hermine¹, Alvarez Gaël^{2,3}, Maestre Fernando⁴, Amblard Christian¹, and Lehours Anne-Catherine¹

¹Université Clermont Auvergne, CNRS, Laboratoire Microorganismes : Génome et Environnement, F-63000 Clermont-Ferrand, France

²INRA, UR874 (Unité de Recherche sur l'Ecosystème Prairial), 5 Chemin de Beaulieu, 63039 Clermont-Ferrand, France

³Clermont Université, VetAgro Sup, BP 10448, F-6300 Clermont-Ferrand, France

⁴Departamento de Biología y Geología, Física y Química Inorgánica, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Calle Tulipán Sin Número, 28933 Móstoles, Spain

Abstract

It has been recently demonstrated that soil organic carbon (SOC) mineralization is supported by intracellular respiration of heterotrophic microorganisms and by non-cellular oxidative processes. However, little is known about the prevalence and drivers of non-cellular SOC mineralization among soils. In this study, untreated and gamma-irradiated soils sampled along a latitudinal gradient and exhibiting contrasted physicochemical properties were incubated in order to quantify potential non-cellular SOC mineralization and to identify its sensibility to soil properties. In sterilized and unsterilized soils, CO₂ emission mirrored O₂ consumption signifying the presence of several coupled redox reactions transferring electrons from organic C to intermediate acceptors and to O₂. This supports the idea that non-cellular mineralization results from extracellular oxidative metabolisms catalyzed by soil enzymes and/or abiotic catalysts. Our findings also show that non-cellular SOC mineralization is ubiquitous and contributes to 24 % of soil respiration on average. Cellular and non-cellular SOC mineralization are positively linked but the contribution of non-cellular processes to soil CO₂ emissions increases with dissolved organic carbon concentration.

Keywords

Mineralization processes; EXOMET; Heterotrophic respiration; dissolved organic carbon

*to whom correspondence should be addressed, Benoit KERAVAL, Address : LMGE, UMR CNRS 6023, Université Blaise Pascal, 1 impasse Amélie Murat, CS 60026, 63178 Aubière Cedex, France, Phone : +33 (0)4 73 40 74 60. benoit.keraval@gmail.com.

Conflict of Interest

The authors declare no conflict of interest.

Representing one of the largest terrestrial source of CO₂ flux, soil organic carbon (SOC) mineralization significantly impacts atmospheric CO₂ concentration and climate (Lal & Kimble, 2000; Paterson & Sim, 2013). SOC mineralization is traditionally viewed as a two-step process. First, microorganisms secrete extracellular enzymes to convert insoluble SOC polymers into compounds assimilable by microbial cells. In the second step, during which carbon (C) is released as CO₂, assimilated compounds are carried out by an oxidative metabolism including many coupled reactions, enzymes and cofactors as well as required redox and pH conditions. Given this complexity, it is taught that respiration (second step of the C mineralization process) is strictly an intracellular process. However, recurrent observations of persistent substantial CO₂ emissions in soil microcosms where sterilization treatments reduced microbial activities to undetectable levels challenged this paradigm (e.g., Peterson, 1962; Lensi et al, 1991; Maire et al, 2013; Blankinship et al, 2014; Kéroual et al, 2016). Different non-cellular processes (Fig. S1) have been proposed as potential contributors to these CO₂ emissions such as (i) the partial degradation of aromatic compounds induced by reactive oxygenated species and/or metals (Fe³⁺, Mn⁴⁺) (Majcher et al, 2000; Wang et al, 2017), (ii) the extracellular decarboxylation of the metabolites of the Krebs cycle supported by some decarboxylases released during cell lysis and retaining their activities in soil (Maire et al, 2013; Blankinship et al, 2014) and (iii) the complete mineralization of organic compounds supported by an extracellular oxidative metabolism (EXOMET) (Maire et al, 2013; Kéroual et al, 2016). EXOMET differs from the two other processes in its complexity. Whereas (i) and (ii) involve single catalytic reactions, EXOMET implies numerous coupled redox reactions capable of complete conversion of organic C into CO₂ with an electron transfer to O₂.

Irrespective of the mechanism, nothing is known about the prevalence and drivers of non-cellular SOC mineralization ($R_{\text{NON-CELLULAR}}$) in soils with contrasting physicochemical properties. The objectives of this study were to (i) generalize non-cellular SOC mineralization to many soils, (ii) quantify $R_{\text{NON-CELLULAR}}$ and its contribution to total SOC mineralization and (iii) identify soil properties that most influence $R_{\text{NON-CELLULAR}}$.

Twenty two soils (Table S1) with various physicochemical properties (Table 1, Fig. S2, Table S2) were collected in permanent grasslands along a latitudinal gradient from Europe to Southern Africa. Untreated and sterilized soils (*gamma*-irradiations) were incubated to measure potential $R_{\text{NON-CELLULAR}}$ and total SOC mineralization (R_{SOIL}), respectively (see Supplementary information). Soils were incubated at 20°C and at a water potential of -100 kPa. We used γ -irradiations at 45 kGy to sterilize soil samples because previous investigations combining various molecular, microscopic and biochemical techniques showed that such procedure reduce microbial biomass to undetectable levels, prevent any microbial re-colonization (MacNamara et al, 2003; Berns et al, 2008, Maire et al, 2013) and limit the impact of sterilization treatment on the physicochemical soil properties compared to the use of fumigants or thermal sterilization treatments (MacNamara et al, 2003). All manipulations were done under sterile conditions and sterility of soils was checked, at the end of incubation, using culture approach and propidium iodide staining coupled to flow cytometry (see Supplementary information).

We recorded O_2 consumption and CO_2 production over a 16-day incubation period in γ -irradiated and untreated soil microcosms (Figs 1a and 1b, Table S3). As none viable cells were detected in irradiated soil microcosms (Supplementary information, Table S4), we assumed that γ -irradiations allowed quantifying potential $R_{NON-CELLULAR}$ in sterilized soil microcosms. We acknowledge a possible contribution of carbonates to CO_2 emissions in alkaline soils (Serrano-Ortiz et al, 2010). However, like Maire et al (2013), we observed that the consumption of O_2 mirrored the production of CO_2 (Fig. S3) with no significant difference ($p < 0.05$) in the average value of the respiratory coefficient (CO_2/O_2) between R_{SOIL} and $R_{NON-CELLULAR}$ (1.2 and 1.05, respectively). Accordingly, we used O_2 consumption in untreated and γ -irradiated soils as a proxy of R_{SOIL} and $R_{NON-CELLULAR}$, respectively and we estimated the contribution of the cellular respiration ($R_{CELLULAR}$) to SOC mineralization.

In the wide range of soil types investigated, the contribution of $R_{NON-CELLULAR}$ to R_{SOIL} ranged from 13 to 50 %, with an average of 24 % (Fig. 1c). The close positive correlation between $R_{CELLULAR}$ and $R_{NON-CELLULAR}$ (Fig. 2a) could result from (i) the stimulation of $R_{NON-CELLULAR}$ by cell activities such as the secretion of extracellular enzymes solubilizing SOC releasing substrates for $R_{CELLULAR}$ and $R_{NON-CELLULAR}$, (ii) the liberation of intracellular enzymes and substrates after microbial cell lysis responsible for decarboxylation and/or performing EXOMET (Maire et al, 2013, Blankinship et al, 2014, Kéralval et al, 2016) and/or (iii) the dependency of $R_{CELLULAR}$ and $R_{NON-CELLULAR}$ on the same rate-limiting factors (*e.g.*, amount and assimilability of C, Figs. 2b and 2c). Pearson correlations partly accredited this last hypothesis as these two respiration pathways globally responded to the same environmental factors (Table S6). SOC, especially, was identified as a soil variable exhibiting a highly significant correlation with $R_{CELLULAR}$ and $R_{NON-CELLULAR}$ (Fig. 2b, 2c). However, $R_{CELLULAR}$ and $R_{NON-CELLULAR}$ exhibited a different level of sensibility to dissolved organic carbon (DOC) content (Fig. 2b, 2c), with $R_{NON-CELLULAR}$ having a higher sensitivity to DOC content than $R_{CELLULAR}$. Consistently, the contribution of $R_{NON-CELLULAR}$ to soil respiration (R_{SOIL}) increased with DOC content (Fig. 2d). These findings suggest that these two processes use, at least partly, different sources of carbon. Soil microorganisms would use the large stock of insoluble SOC for their respiration by using extracellular enzymes present on their wall or released in the vicinity of the cell (Burns et al, 2013). In contrast, $R_{NON-CELLULAR}$ would depend on soluble substrates that must diffuse between catalysts that are likely dispersed in soil pores; hence this respiration pathway may be limited by diffusive process and the concentration of substrates.

The steady production of CO_2 over 16 days combined to a consumption of O_2 (Table S3) strongly suggests the presence of several coupled redox reactions transferring electrons from organic C to O_2 . This observation supports the idea that non-cellular mineralization mainly results from an EXOMET catalyzed by soil enzymes and/or abiotic catalysts. However, other processes such as single decarboxylation (*e.g.* by decarboxylases, reactive oxygenated specie and/or metals) may contribute to $R_{NON-CELLULAR}$ in sterilized soil. We therefore encourage further investigations using metabolomic approaches to identify potential metabolic intermediates and the chemical reactions in play.

Globally, our results show that $R_{\text{NON-CELLULAR}}$ is ubiquitous substantially contributing to CO_2 emissions ($24 \pm 3\%$) in many soils with contrasting physicochemical properties and can be estimated by measuring R_{SOIL} and DOC contents. Our estimation of $R_{\text{NON-CELLULAR}}$ contribution to R_{SOIL} (13–50 %, Fig. 1c) is in a similar range of that determined by Maire et al (2013) through a modeling approach. However, we acknowledge that methods used here and in Maire et al. (incubation of sterilized soils) can lead to an overestimation of $R_{\text{NON-CELLULAR}}$ because sterilization (i) increases DOC content (McNamara et al, 2003; Kéroual et al, 2016) and (ii) removes the possible competition for DOC between R_{CELLULAR} and $R_{\text{NONCELLULAR}}$. It is therefore crucial to develop a method of quantification without these biases. The recent discovery that $R_{\text{NON-CELLULAR}}$ has a specific ^{13}C isotope signature opens avenues toward a quantification based on isotopes without the use of sterilization (Kéroual et al, 2016).

The greater sensitivity of $R_{\text{NON-CELLULAR}}$ to DOC content compared to R_{CELLULAR} suggests that its contribution to soil CO_2 emissions may be larger in DOC-rich soils (Fig. 2d) such as permafrost, soils submitted to drying/rewetting cycles and rhizosphere soils (Nguyen, 2003; Frey & Smith, 2005). Soil water availability might be another important factor affecting this contribution due to its control on DOC diffusion and distribution in the different soil spaces (R_{CELLULAR} and $R_{\text{NONCELLULAR}}$ may occupy different soil pore size, see Maire et al, 2013). Further studies are required to directly study the effect of DOC content and soil water availability on the balance between R_{CELLULAR} and $R_{\text{NONCELLULAR}}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the project ‘*Adaptation and responses of organisms and carbon metabolism to climate change*’ of the program CPER (French Ministry of Research, CNRS, INRA, Région Auvergne, FEDER) and by the project EXCEED of the program PEPS (CNRS). Benoit Kéroual was supported by a PhD fellowship from the Région Auvergne and the FEDER. Maestre T Fernando acknowledges support from the European Research Council (ERC Grant Agreements 242658 [BIOCOM] and 647038 [BIODESERT]). We thank Victoria Ochoa for conducting laboratory analyses on our soil samples; Julien Pottier for his involvement in the statistical processing of our data; Andrew Thomas and Andrew Dougill for providing the soils from Botswana; and Aurélie and Anaïs Kéroual for providing the soils from Brittany.

References

- Blankinship JC, Becerra CA, Schaeffer SM, Schimel JP. Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. *Soil Biology and Biochemistry*. 2014; 71:68–75.
- Berns AE, Philipp H, Narres HD, Burauel P, Vereecken H, Tappe W. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *European Journal of Soil Science*. 2008; 59:540–550.
- Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology and Biochemistry*. 2013; 58:216–234.
- Frey KE, Smith LC. Amplified carbon release from vast West Siberian peatlands by 2100. *Geophysical Research Letters*. 2005; 32

- Kéroual B, Lehours AC, Colombet J, Amblard C, Alvarez G, Fontaine S. Soil carbon dioxide emissions controlled by an extracellular oxidative metabolism identifiable by its isotope signature. *Biogeosciences*. 2016; 13:6353–6362.
- Kuzyakov Y. Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry*. 2006; 38:425–448.
- Lal, RJ, Kimble, JM. Pedogenic carbonates and the global carbon cycle. *Global Change and Pedogenic Carbonate*. Lal, R, Kimble, JM, Eswaran, H, Stewart, BA, editors. CRC Press; Boca Raton, FL: 2000. 1–14.
- Lensi R, Lescure C, Steinberg C, Savoie J-M, Faurie G. Dynamics of residual enzyme activities, denitrification potential, and physico-chemical properties in a γ -sterilized soil. *Soil Biology and Biochemistry*. 1991; 23:367–373.
- Maire V, Alvarez G, Colombet J, Comby A, Despinasse R, Dubreucq E, Joly M, Lehours A-C, Perrier V, Shahzad T, Fontaine S. An unknown oxidative metabolism substantially contributes to soil CO₂ emissions. *Biogeosciences*. 2013; 10:1155–1167.
- McNamara NP, Black HJJ, Beresford NA, Parekh NR. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. *Applied Soil Ecology*. 2003; 24:117–132.
- Majcher EH, Chorover J, Bollag J-M, Huang PM. Evolution of CO₂ during birnessite-induced oxidation of 14 C-labeled catechol. *Soil Science Society of America Journal*. 2000; 64:157–163.
- Nguyen C. Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie*. 2003; 23:375–396.
- Paterson E, Sim A. Soil-specific response functions of organic matter mineralization to the availability of labile carbon. *Global Change Biology*. 2013; 19:1562–1571. [PubMed: 23505211]
- Peterson GH. Respiration of soil sterilized by ionizing radiations. *Soil Science*. 1962; 94:71–74.
- Serrano-Ortiz P, Roland M, Sanchez-Moral S, Janssens IA, Domingo F, Goddérís Y, Kowalski AS. Hidden, abiotic CO₂ flows and gaseous reservoirs in the terrestrial carbon cycle: Review and perspectives. *Agricultural and Forest Meteorology*. 2010; 150:321–329. DOI: 10.1016/j.agrformet.2010.01.002
- Wang B, Lerdau M, He Y. Widespread production of nonmicrobial greenhouse gases in soils. *Global Change Biology*. 2017; doi: 10.1111/gcb.13753

Figure 1.

(a) O₂ consumption and (b) CO₂ production by γ -irradiated and untreated soil microcosms. Twenty two soils sampled along a latitudinal gradient were incubated (From S1 to B6 in x axis, see Table S1 for correspondence). (c) Contribution (in %) of $R_{\text{NONCELLULAR}}$ and R_{CELLULAR} to R_{SOIL} see SI). ns indicates that measured fluxes were not significantly different from 0 ($p < .05$).

Figure 2.

Linear regressions between (a) cellular (R_{CELLULAR}) and non-cellular ($R_{\text{NON-CELLULAR}}$) soil organic carbon (SOC) mineralization, (b) $R_{\text{NON-CELLULAR}}$, SOC and dissolved organic carbon (DOC) contents, (c) R_{CELLULAR} , SOC and DOC contents, (d) contribution (in %) of $R_{\text{NON-CELLULAR}}$ to R_{SOIL} and DOC. $R_{\text{NON-CELLULAR}}$ and R_{SOIL} were quantified using O_2 consumption in irradiated and non-irradiated soils, respectively. R_{CELLULAR} was estimated by the difference between R_{SOIL} and $R_{\text{NON-CELLULAR}}$.

Table 1

Main physicochemical properties of the twenty two soils sampled. (S1 to B6, see Table S1 for correspondence). OC: organic carbon, IC: inorganic carbon, DOC: dissolved organic carbon.

Soil references	OC	IC	DOC	PH	Sand	Clay	Silt
	mg.g ⁻¹	µg g ⁻¹			%		
B1	1.0	0.5	9.6	6.5	96.6	2.2	1.1
B2	1.4	0.9	13.8	6.2	95.6	3.3	1.1
B3	2.2	0.5	17.8	6.2	95.7	1.1	3.2
B4	2.1	0.7	11.3	6.2	96.6	0.0	3.4
B5	2.9	0.0	13.0	6.3	96.7	1.1	2.2
B6	1.6	0.7	9.7	6.0	92.3	2.2	5.5
S1	31.7	52.4	58.7	8.1	59.2	6.0	34.8
S2	20.4	79.2	62.1	8.2	44.4	6.2	49.4
S3	31.1	64.4	81.3	8.0	49.0	7.2	43.8
S4	43.2	67.8	63.4	7.7	48.9	6.2	44.9
S5	31.8	76.8	68.9	8.0	54.4	6.8	38.8
S6	20.3	83.1	49.4	8.1	49.4	3.9	46.8
S7	25.9	49.5	54.4	7.6	52.4	4.1	43.5
S8	17.8	94.7	48.1	8.1	49.8	9.2	41.0
S9	44.9	25.5	115.3	8.0	60.4	2.2	37.4
S10	28.3	66.5	63.8	8.1	59.4	5.8	34.8
S11	29.6	27.4	114.7	7.6	74.3	6.6	19.1
F1	21.9	0.0	84.3	6.4	70.0	6.8	23.3
F2	15.2	6.7	23.4	8.1	42.2	10.0	47.8
F3	21.5	0.6	26.9	6.0	32.2	3.3	64.4
F4	17.4	6.2	21.2	7.6	42.2	16.7	41.1
F5	34.8	0.0	41.9	6.0	55.6	13.3	31.1