Can the presence of Biochar negatively affect the ability of chloroform to lyse soil microbial cells?

S. Paliaga[1], L. Badalucco[1] D. Said-Pullicino[2] & V.A. Laudicina[1*]

[1]Department of agricultural, food and forest sciences, University of Palermo, Palermo, Italy
[2]Department of agricultural, forest and food sciences, University of Turin, Torino, Italy
**E-mail: vitoarmando.laudicina@unipa.it* (corresponding author)

Biochar is the solid product of the thermochemical decomposition of biomass at moderate temperatures (350–700 °C)[1] under oxygen-limiting conditions. It is nowadays utilized in various applications, for example, in the synthesis of new materials for environmental remediation, catalysis, animal feeds, adsorbent for odours, etc.[2]. In recent decades, interest has grown in the application of biochar as a soil amendment due to its beneficial effects on soil fertility and crop productivity. Biochar amendment is known to alter soil porosity, improve soil structure, increase soil surface area[3], cation exchange capacity, soil organic carbon content and soil microbial biomass[1].

The latter variable is one of the most widely adopted biological indicator for the evaluation of soil fertility status. In fact, the microbial component is the engine that governs energy transfers and nutrient transformations in the soil, thus playing a key role in its fertility. The most widely used methods for determining soil microbial biomass are the chloroform-incubation (FI) and chloroform-extraction (FE) methods[4][5], both relying on the ability of chloroform ($CHCl_3$) fumigation to lyse soil microbial cells and release their contents. Over the years, several critical issues related to the use of $CHCl_3$ have risen due to its toxicity to humans and the environment, as well as due to its not fully proved ability to lyse soil microbial cells. Toyota et al.[6] showed that approximately 10% of bacterial colony forming units in a sandy loam soil survived a 5-day $CHCl_3$ fumigation. This percentage was much higher when fumigating a clayey soils. Alessi et al.[7] demonstrated that significant concentrations of $CHCl_3$ were adsorbed, and thus retained by the clay fraction of soils thus negatively affecting the extractability of microbial-derived constituents. Such a controversial ability of $CHCl_3$ to lyse microbial cells may be even more critical when applied to soils amended with biochar. Indeed, biochar, due to its porous structure and high specific surface area can adsorb several volatile organic compounds, including $CHCl_3$[8]. Therefore, the aim of this study was to assess the ability of $CHCl_3$ to lyse microbial cells in soils amended with two different biochars (EG) and (NB). Treatments were: soil without biochar (control), soil amended with 16 g of EG or NB biochar per kg of air dry soil (corresponding to 20 t ha^{-1}) and soil amended with double amount of EG or NB biochar (corresponding to 40 t ha^{-1}). The ability of the $CHCl_3$ to lyse soil microbial cells in soils with or without biochar was assessed by quantifying either the amount of CO_2-C released during incubation or the extractable C and N in fumigated soils, and comparing with the corresponding amount of C obtained from soil pressurized with CO_2 (CO2HP). The latter is a new method, under evaluation, that causes lysis of soil microbial cells by high CO_2 pressurization and

subsequent rapid decompression. Since the CO2HP method is based on a physical approach, it should not be influenced by the presence of biochar in the soil samples being analyzed.
Results showed that the amount of CO_2-C emitted during the incubation of pressurized soils amended with biochar is higher than that of the same soils but fumigated, thus suggesting higher cell lysis efficiency of the CO2HP method than the $CHCl_3$ in soil amended with biochar. Moreover, extractable C and N results suggested that the ability of $CHCl_3$ depends on the type and concentration of biochar used.
$CHCl_3$ could be partly adsorbed and thus retained in the soil after fumigation and risks overestimating the C of the microbial biomass or does not allow for complete lysis of soil microbial cells.

Bibliography

[1] Brassard, P., Godbout, S., Lévesque, V., Palacios, J. H., Raghavan, V., Ahmed, A., Houge R., Jeanne T. & Verma, M., 2019. *Char and Carbon Materials Derived from Biomass*,109-146. Elsevier.

[2] Conte, P., Bertani, R., Sgarbossa, P., Bambina, P., Schmidt, H.P., Raga, R., Lo Papa, G., Chillura Martino, D.F. & Lo Meo, P., 2021. *Agronomy*, 11(4), 615.

[3] Hardie, M., Clothier, B., Bound, S., Oliver, G., & Close, D., 2014. *Plant and Soil*, 376(1), 347-361.

[4] Jenkinson, D. S., Powlson, D. S., 1976. *Soil Biology and Biochemistry*, 8, 209- 2013

[5] Vance, E. D., Brookes, P. C., Jenkinson, D. S., 1987. *Soil Biology and Biochemistry*, 19, 703-707

[6] Toyota, K., Ritz, K., Young, I.M., 1996. *Soil Biology and Biochemistry* 28, 1545-1547.

[7] Alessi, D.S., Walsh, D.M., Fein, J.B., 2011. *Chemical Geology*, 280 (1-2), 58-64

[8] Kumar, A., Singh, E., Khapre, A., Bordoloi, N., & Kumar, S., 2020. Sorption of volatile organic compounds on non-activated biochar. *Bioresource Technology*, 297, 122469.