A high CO_2 pressure-based method for soil microbial cells disruption

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Soil microbial biomass (SMB), a small but highly dynamic living organic matter pool, plays a pivotal role in nutrient cycling and thus its size and activity are major determinants for soil fertility and quality. Indeed, SMB performs more than 90% of the key functions in the degradation and recirculation of organic matter and nutrients[1]. Moreover, due to its nature, SMB quickly responds to biotic and abiotic factors, thus becoming a sensitive indicator of most disturbances and changes occurring in the soil ecosystem[2]. For all these reasons, the study and characterization of soil microbial biomass (SMB) is essential for the assessment of soil quality. The two methods still widely used for determining microbial biomass are the chloroform-fumigation incubation (FI) and chloroform-fumigation extraction (FE) methods[3][4]. Both methods are based on the ability of chloroform ($CHCl_3$) in lysing soil microbial cells, so that nutrients held by them can be then determined by different techniques. The use of $CHCl_3$, however, rises several critical issues. Not to be forgotten, it is an alkyl halide toxic to humans and the environment[5]. Furthermore, $CHCl_3$ has been shown not to be very efficient in lysing the microbial cells. Badalucco et al. [6][7] demonstrated that amounts of phenol-reactive C and anthrone-reactive C represented higher proportions of total extractable C after $CHCl_3$ fumigation than before, thus arguing that some non-biomass sugars were solubilized during the 24 h fumigation. Later, Badalucco et al.[8], showed that the efficiency of $CHCl_3$ in lysing microbial cells appeared to be inversely related to the stability of soil aggregates, due to its lower diffusion in more clayey soils. Also, Toyota et al.[9] by using a sandy loam soil showed that approximately 10% of bacterial colony forming units survived a 5-day $CHCl_3$ fumigation. This percentage could have been much higher when fumigating a clayey soils. Finally, Alessi et al.[10] demonstrated that significant concentrations of $CHCl_3$ were adsorbed, and thus retained, by the clay fraction of soils. Therefore, the major problem in the indirect assessment of the microbial biomass inhabiting soil still persists and consists in that there is not yet a highly efficient, and possibly environmentally safe, disruption technique of microbial cells for subsequent extraction and quantification of intracellular matter.

Thus, the purpose of this study was to set up a new method, possibly more reliable and environmentally safe compared to the $CHCl_3$ based one, for lysing soil microbial cells. The method proposed here is based on a remarkable pressurization of the soil with gaseous CO_2, through the use of a steel reactor, followed by rapid depressurization via the gas release. Hereafter, we call this approach CO2HP (CO_2 – High Pressure). With increasing pressurization, it is expected that the microbial cells are gradually penetrated and filled with the gas. After being saturated by the gas, the applied pressure is suddenly released so that the absorbed gas will rapidly expand inside the cells; therefore, the cells are mechanically ruptured like a popped balloon. This technique, which was first reported by Fraser in 1951[11], has been implemented

by several workers, applying it on pure microbial cultures as a novel sterilization/inactivation method for heat-sensitive materials like foods[12][13] but never in soil. One agricultural and one forest soil were used to set up the CO2HP method. The ability of the new method in lysing the soil microbial cells was assessed by determining, in soils pressurized or fumigated by chloroform, either the CO_2-C released during 10-d incubation at controlled conditions or the KCl extractable C.

In order to evaluate the most efficient pressure and time of pressurization, soils were pressurized with CO_2 at pressures ranging from 400 to 800 psi and from 2 to 32 hours as duration. Results indicate that the new CO2HP method is more efficient than $CHCl_3$ in lysing soil microbial cells. The most efficient combination was found to be 600 psi for CO_2 pressurization and at 32 hours as duration.

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