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MINI REVIEW

Is the microwave irradiation a suitable method for measuring soil microbial biomass?

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Abstract Soil microbial biomass (SMB), the living part of soil organic matter, is used to quantify the total biomass of microorganisms present in the soil. The importance of studies about SMB has emphasized the need to identify methods which can measure the size of SMB. Among the methods currently available, chloroform-fumigation extraction and incubation are the most commonly used for estimation of SMB. However, several studies have proposed the microwave (MW) irradiation as a quick, simple and safe alternate method. There are different opinions about suitability of this method for measuring SMB. There is a question to do "Is the microwave irradiation a suitable method for measuring soil microbial biomass?" Most of the published papers comparing MW and chloroform-fumigation showed strong relationship between both methods. Therefore, we consider MW a suitable method for measuring SMB; however, it is necessary to calibrate the MW methods in different soils with a range of properties, such as clay content, to find an appropriate conversion factor in order to generate correct values for SMB with MW method.

Keywords Soil organic matter · Chloroform-fumigation · Soil microorganisms

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1 Introduction

Soil microbial biomass (SMB) is used to quantify the total biomass of microorganisms present in a soil (Brookes 2001). This living component of soil acts as an important ecological indicator and is responsible for the decomposition and mineralization of plant and animal residues in the soil (Marinari et al. 2006). Additionally, because it is living, the microbial biomass responds much more quickly to changes in soil conditions than does total soil organic matter (Brookes et al. 2008).

It consists of bacteria, fungi and actinomycetes (Darbar and Lazkian 2007). However, fungi and bacteria are the dominant organisms both with regards to biomass and metabolic activities (Anderson and Domsch 1973). Jenkinson and Ladd (1981) have defined soil microbial biomass as the living part of soil organic matter excluding plant roots and soil animals larger than 5×10^3 μm.

The importance of studies about SMB related to soil pollution, soil management and nutrients cycling in natural as well as manipulated environment has emphasized the need for simple and objectives methods for measuring the size of SMB. Among the methods currently available, chloroform-fumigation (CF) is the most commonly used for estimation of SMB. In the CF method, the fumigated soil is either extracted (Vance et al. 1987) or incubated (Jenkinson and Powlson 1976) to measure SMB. Fundamental to the technique is the use of chloroform as the biocide

and extreme care must be exercised in handling this toxic chemical (Hendricks and Pascoe 1988). Therefore, several studies have proposed the microwave (MW) irradiation as a quick, simple and safe alternate method to measure SMB (Wainwright et al. 1980; Ferris 1984; Speir et al. 1986; Hendricks and Pascoe 1988; Monz et al. 1991; Puri and Barraclough 1993; Islam and Weil 1998; Ferreira et al. 1999; Ruzek et al. 2009).

There are different opinions about suitability of this method for measuring SMB. For example, we submitted a manuscript for publication in European Journal of Soil Biology and the response of reviewer was "MW irradiation for killing the soil organisms (instead of chloroform) is not an appropriate one". However, we published another paper in the same journal using MW irradiation for killing microorganisms (Araújo et al. 2008). Additionally, others papers (Table 1) were published using MW irradiation (Ferris 1984; Speir et al. 1986; Hendricks and Pascoe 1988; Monz et al. 1991; Puri and Barraclough 1993; Ferreira et al. 1999; Wang et al. 2001; Liebig et al. 2002, 2004; Cox et al. 2004; Frank et al. 2006; Darbar and Lazkian 2007; Wakelin et al. 2008; Adeli et al. 2009; Gomoryova et al. 2009; Ruzek et al. 2009; Nunes et al. 2010; Matias et al. 2009; Leite et al. 2010; Souza et al. 2010).

We consider, there is a question to answer "Is the microwave irradiation a suitable method for measuring soil microbial biomass?" It is important because many researchers use regularly this method for measuring SMB and they need to publish their studies. With this point of view, we summarized the reports about MW method in order to understand its suitability for SMB estimation.

2 Microwave irradiation method

MW irradiation act as high frequency electric fields and will heat any material containing mobile electric charges, such as polar molecules in a solvent or conducting ions in a solid (Hoz et al. 2005). Polar solvents are heated as their component molecules are forced to rotate with the field and lose energy in collisions. Once that microbial cells are 70–90% of water and the cytoplasm is composed of an aqueous solution (Lehninger et al. 1993), these cells may be exposed to microwave energy and the polar/ionic molecules of the cytosol would oscillate rapidly and eventually would volatilize due of heat friction. Thus, high temperature and vapor pressure affect the permeability and stability of the cell membrane and cause mechanical rupture of the cell (Islam and Weil 1998).

MW is an effective biocidal treatment of soil which kills weeds, nematodes and microorganisms; the effect on microorganisms being probably entirely thermal, i.e., energy transfer from microwaves to the specimen material with a consequent rise in temperature (Vela et al. 1976). It was shown that bacteria, actinomycetes, and fungi became more susceptible to microwaves when irradiated in moist soil and, also, that the degree of susceptibility varied as a function of the physiological condition of the irradiated cells (Vela et al. 1976).

Table 1 Range of soil microbial biomass measured by MW method (SMB_{MW}, mg kg^{-1} soil) under different managements in tropical and temperate soils

Management (region)	Soil texture	SMB_{MW}	References
Organic farming (Brazil)	Clayey soil	80–200	Leite et al. (2010)
No tillage/Forest (Brazil)	Clayey soil	70–420	Matias et al. (2009)
Crop-livestock system (Brazil)	Clayey soil	280–570	Souza et al. (2010)
Crop rotation (USA)	Silt clay loam soil	75–127	Liebig et al. (2002)
Grassland/Wheat fallow (USA)	Loamy soil	300–600	Frank et al. (2009)
Conventional tillage/Grassland (Czech Republic)	Loamy soil	113–329	Ruzek et al. (2009)
Conventional tillage (Brazil)	Clayey soil	270–750	Nunes et al. (2010)
Compost amendment (Australia)	Clayey soil	190–550	Cox et al. (2004)
Grassland (Slovakia)	Loamy soil	361–1,270	Gomoryova et al. (2009)
Native forest (Iran)	Loamy soil	400–1,100	Darbar and Lazkian (2007)

3 Comparisons between SMB measured by CF and MW methods

The comparisons between CF and MW as methods for measuring SMB began about 25 years ago. Ferris (1984) probably was the first to use MW irradiation as a soil treatment to measure SMB, an approach akin to CF. The author reported that MW irradiation provides an effective means of killing soil microorganisms. Two years later, Speir et al. (1986) reported that the biocidal effect of MW radiation could be similar to that found by CF. They observed that both eukaryotes and prokaryotes are equally susceptible to killing effect of MW irradiation. Generally 90 s irradiation was enough to get an effect similar to that of CF method in terms of extractability of C and N.

Afterwards, Hendricks and Pascoe (1988) evaluated MW treatments ranging 1–20 min and concluded that MW method appears to provide SMB estimates comparable to CF method without the use of fumigants. They reported that there were not defined how suitable time to process soil samples without promote of disruption of non-biomass C. Variations in the time of MW radiation may contribute to different results. Low time generate insufficient energy to kill microorganisms. While, high time liberate sufficient energy to breakdown humified C.

In this way, Monz et al. (1991), compared MW and CF followed by direct extraction to estimate SMB. A 2 min irradiation time was used as the optimal treatment. Compared to the CF, MW irradiation resulted in 70.6 and 52.2% less extractable SMB C in unamended and amended soil, respectively. They concluded that MW is not as effective as CF for SMB estimates in the soils examined. However, Ferreira et al. (1999) compared MW irradiation and CF methods in Brazilian soils; they observed high correlation between MW and CF methods. The authors suggested that MW irradiation using 2 min may be used in the place of CF method. However, the above studies used few soils, with a limited range of soil properties, and therefore, may not applicable to a wider range of soils.

Islam and Weil (1998) used MW irradiation method for SMB estimation in 62 soils collected from forests, grass/pastures and agricultural managements. In these soils, clay content and pH values ranged from 100 to 350 g kg^{-1} soil and 4.8 to 7.5, respectively. The authors applied 0, 200, 400, 800 and 1,600 J g^{-1} oven-dried equivalent soil that they were achieved by 0, 30, 60, 120 and 240 s of MW energy, respectively. The results showed that it is necessary 800 J g^{-1} soil to pasteurize the soil and measure SMB. The authors compared their results with CF method proposed by Jenkinson and Powlson (1976) and SMB by incubation from MW irradiated soil were closely related to SMB measured by CF incubation method. However, a conversion factor of 0.213 to convert the flushes of C into SMB was proposed by Islam and Weil (1998).

Wang et al. (2003) criticized MW method proposed by Islam and Weil (1998). The strong criticism focused on clay content (<50%) and pH values (<7.5) from soils evaluated by those authors. Wang et al. (2003) reported that it is very important to test the MW method using soils representing a wide range of properties. Previously, Wang et al. (2001) found high correlation between SMB estimated by MW incubation and extraction. However, there were not correlations with those method with substrate-induced respiration, CF extraction and CF incubations methods. These authors used 32 soils with different soil properties, to provide a wide range in pH, texture and total organic C. They observed values several times higher in MW than that estimated by others methods and concluded that the MW methods could not selectively and unequivocally quantify SMB. The main argument is Islam and Weil (1998) did not use clayey soils. According to Zagal (1989), clay content of soil has influences on SMB content estimated by MW irradiation and CF methods.

Afterwards, Weil and Islam (2003) used an argument based on the study of Ferris (1984) who suggested that the biocidal effect is a function of the total amount of MW energy delivered and the presence of moisture, but not related to soil texture. They reported that an adjustment to 80% water-filled pore space (WFPS) is required for MW method. Thus, the failure of Wang et al. (2001) was add water equivalent to 50% of sample dry weight, resulting in many of samples being irradiated while at >100% of WFPS.

Recently, Ruzek et al. (2009) evaluated MW and CF methods to estimate SMB of Cambisols, Luvisols and Stagnosols from Czech Republic. MW values varied from 113.8 to 329.4 mg C kg^{-1} soil and CF extraction values from 112.9 to 522.2 mg C kg^{-1} soil. A significant correlation was found between MW and CF methods. The authors concluded that MW method for SMB may substitute CF method,

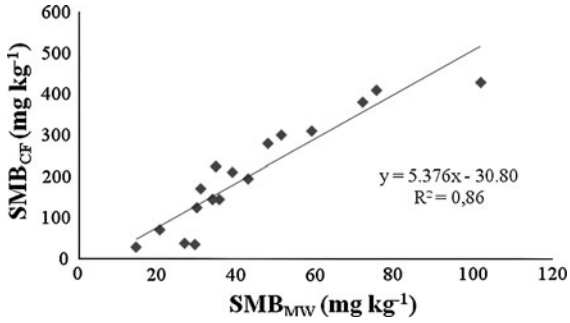

Fig. 1 Relationship between soil microbial biomass (*SMB*) measured by chloroform fumigation (*CF*) and microwave irradiation (*MW*) in tropical and temperate soils. * Data selected from Hendricks and Pascoe (1988), Monz et al. (1991), Islam and Weil (1998), Ferreira et al. (1999), Andrea and Hollweg (2004), Ruzek et al. (2009)

especially to exclude the use of chloroform. However, they used soils with clay <30% and pH < 7.0 similar with soils used by Islam and Weil (1998).

Papers comparing SMB measured by chloroform fumigation (CF) and microwave irradiation (MW) were already published and they showed a strong relationship between both methods (Fig. 1). However, we observed that the values found using MW is usually 30–40% lower than in CF method. Comparing methods for estimation of SMB, Andrea and Hollveg (2004) evaluated MW and CF and compared different conversion factor used in these methods. They observed that the values of SMB obtained by MW method using conversion factor proposed by Vance et al. (1987) were different those obtained by CF method, showing the necessity that to use a different conversion factor. Therefore, the conversion factor proposed by Islam and Weil (1998) it seems to be the most appropriate to convert C into SMB by MW method.

4 Conclusion

We consider MW a suitable method for measuring SMB. Most of the published papers comparing MW and CF showed strong relationship between both methods. However, it is necessary to calibrate the MW methods in different soils with a range of properties, such as clay content, to find an appropriate conversion factor in order to generate correct values for SMB with MW method.

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