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Microwave irradiation of soil for routine measurement of microbial biomass carbon

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Abstract Microwave irradiation was evaluated as a non-toxic alternate to chloroform fumigation for routine measurement of soil microbial biomass C. Microwave energy was applied to moist soil to disrupt microbial cells. The flush of C released was then measured after extraction or incubation. Microwave irradiation at 800 J g^{-1} soil was optimal because this level resulted in an almost instantaneous rise in soil temperature (≥ 80 °C), an abrupt reduction in microbial activity, maximal release of biomass C, and minimal solubilization of humic substances. Both incubation-CO_2 titration and extraction-colorimetry methods were used on separate 20-g subsamples to compare the labile C in the microwave-treated and untreated soil samples. The incubation-titration method was also used to measure C in chloroform-fumigated soil samples. Averaged across soils, the chloroform fumigation yielded 123.3 ± 5.1 mg CO_2-C kg^{-1}. Microwave irradiation yielded 93.6 ± 3.9 mg CO_2-C kg^{-1} soil determined by incubation and 52.4 ± 2.4 mg C kg^{-1} soil determined by extraction, accounting for 76% and 42% of the net flush of C measured by the chloroform fumigation. Microwave-stimulated net flushes of C were correlated closely ($r^2 = 0.974$ for incubation or 0.908 for extraction) with microbial biomass C measured by the chloroform fumigation. Little correlation was found with the total soil organic C ($r^2 = 0.241$ for incubation or for 0.166 extraction). Mean efficiency factors for incubation (K_{MI}) or extraction (K_{ME}) were used to calculate microbial biomass C from net flushes of C between microwaved and unmicrowaved soils. Values of K_{MI} and K_{ME} were not affected by soil pH, bulk density or clay contents. Extraction of microwaved soil by $0.5M$ K_2SO_4 proved to be a simple, fast, precise, reliable, and safe method to measure soil microbial biomass C.

Key words Microwave irradiation · Microbial biomass C · CO_2 evolution · K_2SO_4 extractable C · Chloroform-fumigation incubation

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Introduction

Soil microbial biomass C (C_{TMB}), although a small fraction (<5%) of total organic C (C_{org}), is a sensitive indicator of soil quality (Kennedy and Papendick 1995). Of the methods available, chloroform fumigation (CF) is the most commonly used method of estimating C_{TMB} (Vance et al. 1987). In the CF method, the fumigated soil is either extracted (CFE) or incubated (CFI) to measure the flush of C (net increase in C compared to unfumigated soil) under the assumption that non-biomass C is not affected by fumigation (Jenkinson and Powlson 1976; Vance et al. 1987). However, release of some non-biomass C compounds has been reported (Badalucco et al. 1992). The accuracy of biomass C estimation by the CFI method may be reduced in strongly acidic soils (Vance et al. 1987), in soils containing large amounts of freshly added organic substrates (Ocio and Brooks 1990), in waterlogged soils (Inubushi et al. 1991), and in O horizons (Scholee et al. 1992). The CFI method is time consuming, requires appropriate controls, and involves several more steps compared to the relatively simple CFE method (Vance et al. 1987). Although the CFE method is somewhat faster and has proved to be useful where the CFI technique does not work, both methods require extreme care in the handling of the biotoxic chloroform. As such, there is a need for alternate methods to measure C_{TMB} that are

quick, simple, safe and produce results comparable to those obtained with the CFI or CFE methods.

Microwave (MW) irradiation, a non-ionizing electromagnetic energy (EM) partially sterilizes soil, controlling pathogenic microbes and nematodes (Ferris 1984; Ou et al. 1985). In MW heating, absorbed energy causes rotation of dipole water molecules and conductive migration of dissolved ions in an EM field to generate heat almost instantly by molecular friction (Neas and Collins 1988). Although many reports indicate that microbial cells are killed by MW energy because of the rise in media temperature, dry or lyophilized organisms are not greatly affected even by extended exposures of MW irradiation (Vela and Wu 1979; Ou et al. 1985). Cell constituents other than water or polar/ionic molecules do not absorb sufficient MW energy to kill microbial cells (Stuchley and Stuchley 1980).

Microbial cells are 70–90% water by weight. The cytoplasm is composed of an aqueous solution, the cytosol, and a variety of insoluble particles suspended in a complex gel-like matrix (Lehninger et al. 1993). When exposed to MW energy, polar/ionic molecules of the cytosol oscillate rapidly and eventually volatilize due to the heat of friction. McClean et al. (1981) reported that this effect is greater on the moisture bound to crystalline biomolecule layers than on bulk free water. High temperature and vapor pressure affect the permeability and stability of the cell membrane and cause mechanical rupture of the cell. Disruption of microbial cells releases various intracellular C compounds, so extraction of MW-treated soil by a suitable extractant may release the intracellular C from lysed or dead microbial cells.

In the present study, the effect of short-term MW irradiation on the flush of soil C was examined with the objectives: (1) to determine whether changes in evolution of CO_2-C or extractable C resulting from microwaving of soil could serve as an alternative to the CFI method to measure C_{TMB}, and (2) to determine the lethal dose of MW energy to soil microbes and whether MW irradiation enhances the extractability of humic C in addition to C from the lysed microorganisms.

Materials and methods

Soils used

To test the proposed MW method for C_{TMB} estimation, 62 soils were collected from sites under forests, grass/pastures, and various agricultural management practices (Table 1). Total organic C ranged from 4.6 to 32.9 g kg^{-1}, pH ranged from 4.8 to 7.5, bulk density ranged from 1.07 to 1.48 g cm^{-3}, and clay content ranged from 100 to 350 g kg^{-1} soil. Fourteen cores (1.9 cm diameter) were randomly collected within a 5 m × 5 m quadrant at each site (or from each replicated experimental plot sampled) and composited. The cores were mixed in the field immediately after a site was sampled, and placed in sealed plastic bags. Soils were transported on ice in a dark cooler. The soil was sieved to pass a 2-mm mesh to remove stones, roots, and large organic residues. Field-moist homogenized soil samples were stored in polyethylene bags and kept under short-term refrigeration at 4 °C before analysis.

Microbial biomass C measurement by chloroform fumigation incubation

The C_{TMB} was measured by the CFI method (Jenkinson and Powlson 1976). Microbial biomass C measured as CO_2-C was calculated as follows:

$$C_{TMB}\ (mg/kg) = (CO_2\text{-}C_{fum} - CO_2\text{-}C_{unfum})/K_C = F_C/K_C, \quad (1)$$

where F_C is the "flush" of CO_2, i.e., evolution of CO_2 in fumigated soil minus the evolution of CO_2 in unfumigated soil, and K_C is the fraction (0.45) of the C_{TMB} mineralized as CO_2 for 10 day incubations at 25 °C as determined by Jenkinson and Powlson (1976).

Microwave irradiation of soil for microbial biomass C measurement

A 650-W household-type microwave oven was used, with energy at high power supplied by a magnetron operating at 2450 MHz in the continuous mode. The energy output into the MW oven cavity was determined by measuring the rise in temperature of 1000 ml distilled water (initial temperature 22 °C) in a 2-l Pyrex glass beaker placed at the center of the cavity and heated continuously at full power for 2 min (Neas and Collins 1988):

$$P = C_p K \Delta T m/t \quad (2)$$

where P is the apparent power absorbed by the water sample (J s^{-1}); C_p is the heat capacity of water (J ml^{-1} $°K^{-1}$); K is a factor (4.184) to convert thermal chemical cal ml^{-1} $°K^{-1}$ to watts (J s^{-1}); ΔT (°C) is the difference between final temperature and initial temperature of water; m is the mass of the water (g); and t is the duration (s) of MW energy application. Using this equation, the MW oven output was calculated as 640-W (J s^{-1}).

To determine an optimum MW energy level, experiments were conducted to relate MW energy application to temperature rise, loss of moisture, changes in metabolic activities, and flushes of C from soils. About 10 g oven-dried equivalent (ODE) of field-moist soil was taken in a 50-ml glass beaker and adjusted to 80% water-filled porosity (WFP) by adding distilled deionized water as needed. Three soils were used: a sandy loam, a silty loam, and a clay loam. The beakers were covered with small glass petri dishes and MW energy was applied at 0, 200, 400, 800, and 1600 J g^{-1} ODE soil. These levels were achieved by 0, 30, 60, 120, and 240 s of MW energy application. A mechanical turntable was used to ensure an even absorption of MW energy by the soil samples. To minimize heat pockets within the moist soil, those samples irradiated at 800–1600 J g^{-1} received a series of 400 J g^{-1} bursts with stirring in between for uniform mixing. Using this 400 J g^{-1} "burst procedure," soil temperature never rose above 88 °C. Soil temperature was measured with a thermistor probe inserted into the soil mass after each MW burst. The soils were allowed to cool down and the moisture content before and after MW treatment was determined to calculate moisture loss. Control samples remained at room temperature and were not exposed to MW irradiation. Microbial activity, extractable C, and optical density of MW or field-moist soil extracts were measured to determine the effects of MW irradiation on soil biological and chemical properties.

To determine C_{TMB}, soil samples were adjusted to 80% WFP and microwaved for a total energy exposure of 800 J g^{-1} ODE soil. The flushes of C were then measured by incubation or direct extraction of post-microwave soil. Our decision to use 800 J g^{-1} ODE soil was based on the results of the initial experiment as presented in this paper.

Microwaved soil incubation

About 20 g ODE of MW soil was placed in 50-ml glass beakers and water was added to adjust the soil moisture to 60% WFP. The procedures for incubation and measurement of flush of CO_2-

Table 1 Soil characteristics, microbial biomass and flushes of carbon (*CT* Conventional tillage, *OR* organic rotation, *NT* untilled, *RiT* ridge tillage, *MP* moldbold plow, *CD* chisel disk, C_{org} total C, *LIP-A* low input animal based rotation, *LIP-CG* low input cash crop based rotation, *RT* reduced tillage, *Conv* conventional herbicide and fertilizer use, *MLC* mature low compost, *MHC* mature high compost, *IHC* immature high compost, C_{TMB} microbial biomass C, C_{EXTMW} net flush of extractable C from 0.5*M* K_2SO_4 extraction of microwaved soil, K_{MI} fraction of the microbial biomass C mineralized as CO_2 over 10 days incubation of microwaved soil at 25 °C, K_{ME} fraction of the microbial biomass C by 0.5*M* K_2SO_4 extraction of microwaved soil, CO_2-C_{MW} net flush of CO_2 from microwaved soil incubation, *b* bulk density

Site, tillage and crops	pH_{H_2O} 1:2.5	C_{org} g kg^{-1}	Clay g kg^{-1}	b g cm^{-1}	C_{TMB}	CO_2-C_{MW} mg kg^{-1}	C_{EXTMW} mg kg^{-1}	K_{MI} CO_2C_{MW}/C_{TMB}	K_{ME} C_{EXTMW}/C_{TMB}
Maryland Coastal Plain soils, USA									
1-NT-Wheat/soy/corn	6.3	8.9	100	1.34	300.3	97.9	51.4	0.33	0.17
2-RT-Wheat/soy/corn	6.2	8.8	102	1.35	224.6	74.7	34.8	0.33	0.15
3-OR-Clover-soy/corn	6.5	8.3	103	1.24	280.5	95.3	47.5	0.34	0.17
4-CT-Continuous corn	6.3	7.4	101	1.38	152.1	54.3	29.4	0.36	0.19
5-NT-Continuous tall fescue	6.1	13.0	100	1.15	409.7	138.9	75.6	0.34	0.18
6-CT-Tomato	5.3	10.1	165	1.31	86.6	37.3	20.6	0.43	0.24
7-CT-Corn	5.6	10.0	160	1.34	52.7	23.7	13.9	0.45	0.26
8-CT-Beans	5.2	10.3	170	1.32	119.1	49.0	29.5	0.41	0.25
9-CT-Soybean/wheat	5.5	9.5	177	1.35	48.7	22.9	17.7	0.47	0.36
10-NT-Continuous tall fescue	5.9	17.8	131	1.18	198.3	83.8	52.9	0.42	0.27
11-NT-Continuous tall fescue	5.6	9.2	107	1.23	145.1	46.3	34.0	0.32	0.23
12-NT-Soybean/wheat	6.2	8.9	105	1.34	65.0	27.9	18.2	0.43	0.28
13-NT-Corn/corn	5.3	8.6	160	1.38	148.7	44.6	31.9	0.30	0.22
14-CT-Corn/soybean	5.4	7.1	156	1.32	108.5	30.6	23.0	0.28	0.21
15-RT-Corn/soybean/corn	5.8	6.3	163	1.30	145.9	46.2	35.7	0.32	0.24
16-NT-Continuous tall fescue	5.6	5.9	250	1.48	125.4	37.8	30.0	0.30	0.24
17-NT-Continuous tall fescue	5.0	17.1	180	1.26	500.6	161.9	80.6	0.32	0.16
18-NT-Continuous tall fescue	5.7	11.7	217	1.40	171.4	53.1	40.4	0.31	0.23
19-CT-Beans	5.1	7.9	271	1.27	102.1	34.7	25.5	0.34	0.25
Maryland Piedmont soils, USA									
20-NT-Soybean/corn	6.7	13.4	246	1.34	309.9	101.5	59.1	0.33	0.19
21-CT-Wheat/corn	6.4	17.6	230	1.27	195.9	69.0	43.2	0.35	0.22
22-CT-Alfalfa/wheat	6.3	14.0	251	1.29	427.9	157.2	101.9	0.37	0.24
23-CT-Wheat/alfalfa	7.2	15.71	239	1.26	428.9	146.0	92.3	0.34	0.22
24-NT-Orchard grass/clover	6.5	22.1	220	1.36	442.0	139.7	101.7	0.32	0.23
25-NT-Orchard grass/clover	6.9	32.9	140	1.07	830.3	284.9	180.2	0.34	0.22
26-NT-Low fertility (LF):corn	6.8	14.3	241	1.30	453.5	154.8	89.2	0.34	0.20
27-NT-LF and corn/vetch	7.0	12.8	238	1.21	310.7	102.2	55.2	0.33	0.18
28-NT-High fertility (HF):corn	6.7	12.6	235	1.28	466.9	155.0	88.8	0.33	0.19
29-NT-HF and corn/vetch	6.7	13.9	236	1.27	468.0	167.3	92.0	0.36	0.20
30-NT-Continuous tall fescue	7.0	16.8	251	1.46	507.1	176.4	102.5	0.35	0.20
31-CT-Continuous corn	6.2	16.3	255	1.23	269.3	96.3	55.5	0.36	0.20
32-NT-LF alfalfa	7.5	16.7	245	1.34	273.1	99.7	50.8	0.36	0.18
33-NT-HF alfalfa	7.2	18.2	251	1.33	349.2	126.6	74.0	0.36	0.21
Kutztown, Pa., USA									
34-CT-LIP-A:Soybean/wheat/corn	6.2	22.8	267	1.18	345.9	148.4	96.6	0.43	0.28
35-CT-LIP-CG:Wheat/corn/clover	6.0	23.6	269	1.26	206.6	98.0	69.9	0.47	0.34
36-CT-Conv-CG:Corn/soybean	5.9	24.2	272	1.27	220.4	110.8	68.9	0.50	0.31
37-MP-Conv:Corn/soybean	6.4	20.5	255	1.42	140.3	50.5	17.8	0.36	0.13
38-CD-Conv:Corn/soybean	6.2	20.4	259	1.33	172.4	71.0	33.9	0.41	0.19
39-RiT-Conv:Corn/soybean	6.2	20.0	260	1.36	211.2	75.7	39.4	0.36	0.18
40-NT-Conv:Corn/soybean	6.3	19.3	257	1.39	248.1	91.3	37.0	0.37	0.15
41-RiT-Corn/soybean/rye	6.2	22.5	256	1.38	249.9	90.6	38.6	0.36	0.15
42-NT-Corn/soybean/rye	6.2	24.2	259	1.29	303.9	115.1	55.2	0.38	0.18
43-CT-Control:Amaranth/oat	5.8	15.0	255	1.43	180.3	59.5	32.8	0.33	0.18
44-CT-MLC:Amaranth/oat	5.6	17.2	258	1.30	330.5	114.1	52.3	0.35	0.16
45-CT-MHC:Amaranth/oat	6.0	17.5	255	1.30	400.6	134.8	76.3	0.34	0.19
46-CT-IHC:Amaranth/oat/clover	5.9	19.0	257	1.30	380.1	130.1	71.6	0.34	0.19
47-CT-Fertilizer:Amaranth/oat	5.6	17.0	255	1.30	300.3	105.5	50.4	0.35	0.17

Table 1 Continued

Site, tillage and crops	pH_{H_2O} 1:2.5	C_{org} g kg^{-1}	Clay g kg^{-1}	b g cm^{-1}	C_{TMB}	CO_2-C_{MW} mg kg^{-1}	C_{EXTMW} mg kg^{-1}	K_{MI} CO_2C_{MW}/C_{TMB}	K_{ME} C_{EXTMW}/C_{TMB}
Bangladesh soils, Dhaka, Bangladesh									
48-NT-*Shorea robusta,* L.	4.9	9.7	150	1.24	271.0	87.1	43.2	0.32	0.16
49-NT-*Acacia auriculiformis,* L.	5.1	7.3	250	1.23	300.5	96.3	52.7	0.32	0.18
50-NT-*Acacia minjiri,* L.	4.8	18.2	251	1.13	375.2	120.0	60.9	0.32	0.16
51-NT-*Tektona grandis,* L.	5.0	3.9	350	1.40	120.0	25.4	18.6	0.21	0.15
52-CT-Sugarcane	5.2	7.2	200	1.41	322.6	99.3	58.2	0.31	0.18
53-CT-Jute/rice/mustard	6.0	7.5	210	1.35	194.4	57.2	30.8	0.29	0.16
54-NT-*Shorea robuste,* L.	4.9	7.0	210	1.20	247.9	75.5	39.7	0.31	0.16
55-CT-Rice/jute	4.8	21.0	301	1.24	173.0	46.4	26.4	0.26	0.15
56-CT-Cotton	5.3	14.8	290	1.41	180.0	54.7	30.7	0.30	0.17
57-NT-*Mullacana brucifera,* L.	5.7	19.5	290	1.38	419.4	131.9	103.6	0.31	0.25
58-CT-Rice/jute/maize	6.2	4.6	248	1.31	62.3	18.3	11.3	0.29	0.18
59-CT-Pasture (Napier grass)	5.6	11.4	260	1.19	412.8	142.1	74.6	0.34	0.18
60-NT-*Imperata cylindrica,* L.	5.5	13.8	251	1.17	375.4	124.5	73.8	0.33	0.20
61-CT-Rice/jute/vegetables	6.2	9.6	230	1.25	359.7	116.0	60.2	0.32	0.17
62-NT-*Mullacana brucifera,* L.	5.7	15.1	260	1.35	274.3	89.1	66.3	0.32	0.24

C were the same as for the CFI method. The C_{TMB} was calculated as:

$$C_{TMB} = CO_2\text{-}C_{MW}/K_{MI} \quad (3)$$

where CO_2-C_{MW} is the net flush of CO_2 (i.e., evolution of CO_2 from MW soil minus evolution of CO_2 from unmicrowaved soil, and K_{MI} is the fraction of the C_{TMB} mineralized as CO_2 over 10 day incubation of MW soil at 25 °C in the dark.

Microwaved soil extraction

Exactly 5 g ODE of MW and field-moist untreated soils were placed in 50-ml polycarbonate centrifuge tubes with 20 ml 0.5*M* K_2SO_4 (pH 7.0) and extracted by horizontal shaking at 250 rpm for 60 min. The soil suspension was centrifuged at 5000 rpm for 5 min, then filtered to obtain soil-free filtrate. A few drops of concentrated sulfuric acid (5 ml l^{-1}) were added to the filtrates in order to inhibit microbial decomposition of organic C. The acid treated filtered extracts were frozen until analyzed.

Organic C in these extracts was analyzed by the rapid oxidation spectrophotometric method of Heanes (1984). Sucrose C solutions were also digested and used to standardize the absorption readings. The C_{TMB} was calculated as:

$$C_{TMB} = C_{EXTMW}/K_{ME} \quad (4)$$

where C_{EXTMW} is the net flush of C from the difference between the extracted C in MW soil minus the extracted C in field-moist soil, and K_{ME} is the fraction of the C_{TMB} extracted by 0.5*M* K_2SO_4 as C_{EXTMW}.

Microbial activity in microwaved soil

To determine whether soil microbes were killed, microbial activity in the MW soil and non-microwaved field-moist soil was measured by arginine ammonification (Alef and Kleiner 1987) and dehydrogenase activity (Tabatabai 1994).

Optical density of soil extracts

To determine whether non-cellular humic C was solubilized by microwaving, the optical density of the 0.5*M* K_2SO_4 extracts from the MW soil was measured. To detect the presence of chromophore, i.e., yellowish-brown humic C compounds (Davis-Colley and Vant 1987), the optical density was measured at 410 nm by a spectrophotometer and compared to extracts from paired field-moist non-microwaved soils. Distilled deionized water was used as the blank.

Chemical and physical soil properties

Soil pH was determined in a 1:2.5 soil-water slurry using a combination glass electrode. Total organic C content was determined on finely ground air-dried soil samples using a LECO CHN-600 analyzer (LECO Corp., St. Joseph, Mich.). The gravimetric water content was measured by drying the soil with MW energy applied at 2000 J g^{-1} soil until a constant weight was obtained. The WFP was calculated as: $WFP = (\theta_g\ _b)/(1 - {}_b/{}_p)$, where θ_g is gravimetric water content, $_b$ is soil bulk density (g cm^{-3}), and $_p$ is soil particle density (assumed 2.65 g cm^{-3}). The particle size analysis was done by the pipette method (Day 1965).

Statistical analysis

Bi-variate linear regression was used to relate the flushes of CO_2-C_{MW} or C_{EXTMW} with C_{TMB} measured by the CFI method. Since neither C_{TMB} (x) and flushes (y) of CO_2-C_{MW} or C_{EXTMW} were fixed, reduced major axis bi-variate regression equations (Fluery 1991) were used to calculate model loss parameters and improve the fit of the regression line to the measures of C_{TMB} and the flushes of C from MW soil incubation or extraction.

Results and discussion

Effect of microwave irradiation on temperature rise, moisture loss, microbial activity, and extractable C in soil

The temperature in sandy loam, silt loam, and clay loam soils rapidly rose to approximately 82 °C when MW energy applied was ≥400 J g^{-1} soil (Fig. 1). When MW irradiation applied was increased to 800 J g^{-1}, more than 75% of the total soil moisture was lost. A further increase in MW energy did not increase the temperature but almost completely dried the soils. A rapid reduction in microbial activity occurred with MW energy applied at 400 J g^{-1} soil. Neither arginine ammonification nor dehydrogenase activity was detectable when MW energy applied was ≥800 J g^{-1} soil (Fig. 2, 3).

In comparison with extraction of field-moist soil, the MW irradiated soil consistently produced higher

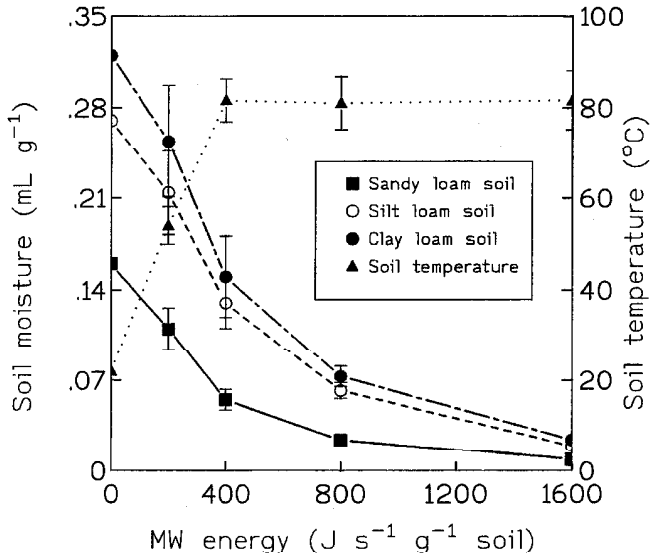

Fig. 1 Relationships among microwave energy (*MW*) application, temperature rise, and soil moisture loss

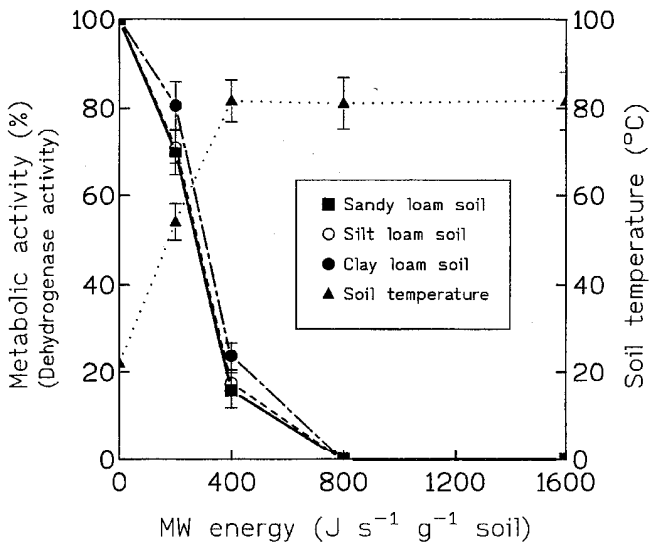

Fig. 3 Relationships among microwave energy (*MW*) application, temperature rise, and dehydrogenase activity (100% corresponds to 10.9, 11.9, and 11.2 mg triphenyl formazan kg^{-1} day^{-1} for sandy loam, silt loam, and clay loam soils, respectively)

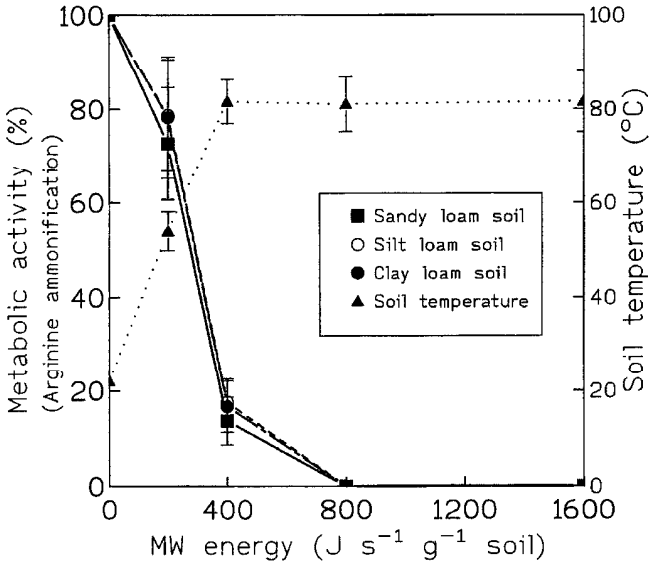

Fig. 2 Relationships among microwave energy (*MW*) application, temperature rise, and arginine ammonification (100% corresponds to 2.2, 2.9, 2.5 mg NH_4 kg^{-1} h^{-1} for sandy loam, silt loam, and clay loam soils, respectively)

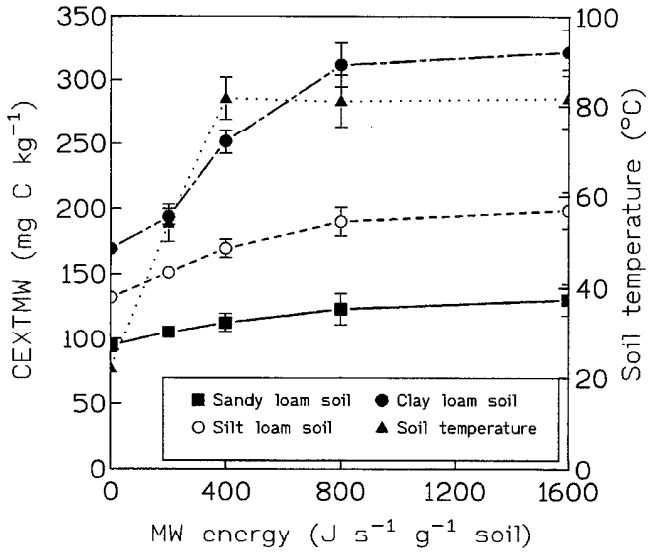

Fig. 4 Relationships among microwave energy (*MW*) application, temperature rise, and extractable C (C_{EXTMW}) in microwaved soil

amounts of extractable C (Fig. 4). Extractable C increased linearly with increasing MW energy up to 800 J g^{-1} soil, then leveled off at higher energy levels. Optical density was almost identical for extracts of both MW or field-moist non-microwaved soils (Fig. 5) except when MW energy was applied at more than 800 J g^{-1} soil. With 1600 J g^{-1} soil, the extract was light yellow.

Cessation of microbial activity at 800 J g^{-1} soil may have resulted from the rise in soil temperature beyond 80 °C as well as from the rapid loss of moisture. As in the study by Ferris (1984), our data suggest that the biocidal effects are a function of the total amount of MW energy delivered and the presence of moisture, but not related to soil texture. This was expected because the range of specific heat capacities for soil mineral fractions is relatively narrow (Hillel 1980).

Microwave energy probably causes cell rupture as a high vapor pressure gradient in the core of the cell moves polar/ionic components of the cytoplasm outward to areas of lower vapor pressure, causing cell membranes to rupture and macromolecules to leak out

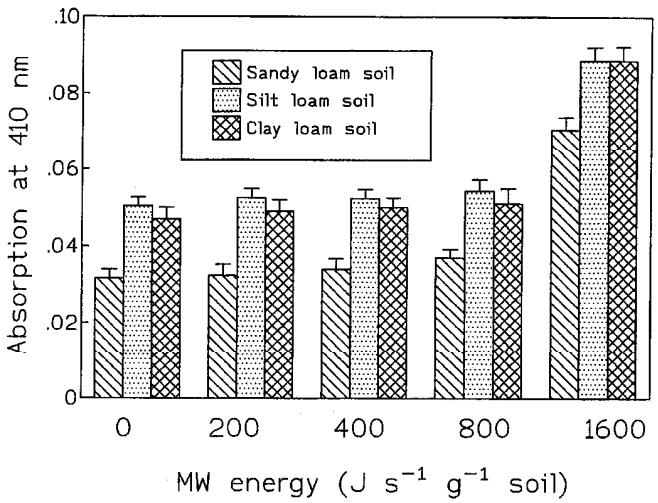

Fig. 5 Effect of microwave energy (*MW*) levels applied to soil on the optical density (410 nm) of potassium sulfate soil extracts

of the cytoplasm. Microwaves themselves also exert a mechanical effect (Neas and Collins 1988), which may stress or injure the microbial cells, and therefore, increase the extraction of intracellular C or lead to decomposition of the biomass C upon incubation. Such chemical constituents of living cells as proteins, enzymes, and nucleic acids are rapidly denatured at temperatures ranging between 50 °C and 90 °C (Lehninger et al. 1993), resulting in the rapid death of most microorganisms.

If the soil microbial biomass were not killed or the cell membranes of the organisms were not damaged, ruptured, or lysed during or following the MW irradiation periods, no C flush would have occurred (Speir et al. 1986), although the MW irradiation at 800 J g^{-1} soil was sufficient for complete reduction in metabolic activity. Although structurally complex non-biomass C may be stable in the short term at temperatures below 160 °C (Azuma et al. 1984; Erdman and Monson 1986), data in Fig. 5 suggest that microwaving at 1600 J g^{-1} disrupted non-biomass humic C at temperatures below 90 °C (Hendricks and Pascoe 1988; Puri and Barraclough 1993).

Despite the apparent cessation of microbial activity and increase in extractable C, MW irradiation at 800 J g^{-1} soil did not substantially breakdown non-biomass C, as indicated by the comparison of optical density of MW and non-MW soil extracts (Fig. 5). Therefore, we used MW irradiation at 800 J g^{-1} soil, in essence, to pasteurize the soil and measure C_{TMB}. Using the 400 J g^{-1} "burst" procedure, the soil temperature did not rise above 88 °C, even when a total of 1600 J g^{-1} MW energy was applied. Application of 800 J g^{-1} MW energy as a series of two "bursts" is used in our proposed method to minimize the effect of soil temperatures on the disruption of non-biomass C.

Calibration of flushes of C to estimate microbial biomass

Averaged across all soils, the mean flushes were 93.6 ± 3.9 mg CO_2-C kg^{-1} with MW soil incubation and 52.2 ± 2.4 mg extracted C kg^{-1} with MW soil extraction by 0.5*M* K_2SO_4, and 123.3 ± 5.1 mg CO_2-C kg^{-1} soil as measured by the CFI method (Table 1). Compared to the CFI method, the MW irradiated soil incubation produced a mean C flush of 76% as large and the extraction method produced a mean C flush of 42% as large.

As expected, the flushes of CO_2-C_{MW} and C_{EXTMW} from MW soil were closely related to C_{TMB} measured by the CFI method (Figs. 6, 7). The correlation between C_{TMB} and the CO_2-C_{MW} (r^2=0.974) was closer than the relationship between the C_{TMB} and the C_{EXTMW} (r^2=0.908). A close relationship (r^2=0.893) was also observed between the flush of CO_2-C_{MW} and that of C_{EXTMW} using MW soils (Fig. 8). The regression intercepts were not significantly ($P \leq 0.05$) different from zero. The close relationship between the methods suggests that the flushes of C upon incubation or extraction of MW soils were released from the same labile C pool of C_{TMB} that was mineralized as net flush of CO_2-C over 10 days incubation of chloroform fumigated soils. On the other hand, the flushes of C using MW soil did not closely relate to C_{org} (r^2=0.241 for CO_2-C_{MW} or 0.166 for C_{EXTMW}). This supports our conclusion that little non-biomass C is released during incubation or extraction of MW soil.

Although there was a significant relationship between flushes of CO_2-C_{MW} or C_{EXTMW} and C_{TMB}, an

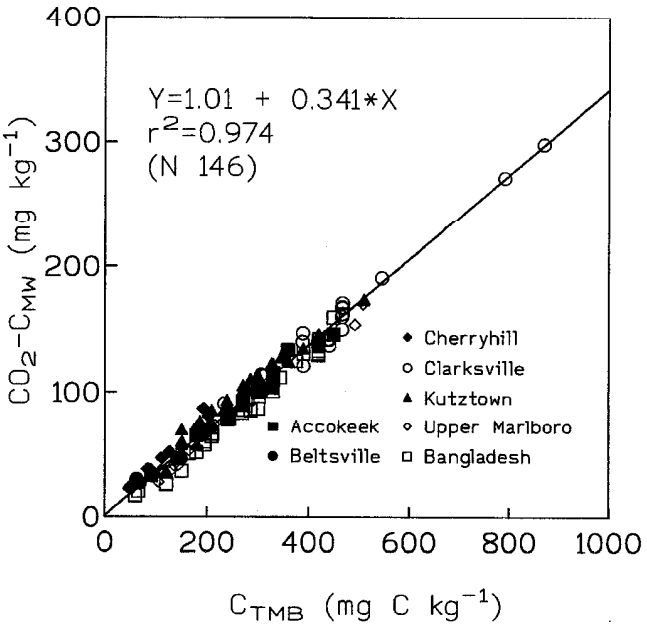

Fig. 6 Relationship between microbial biomass (C_{TMB}) measured by chloroform fumigation incubation and net flush of CO_2 (CO_2-C_{MW}) from microwaved soil incubation

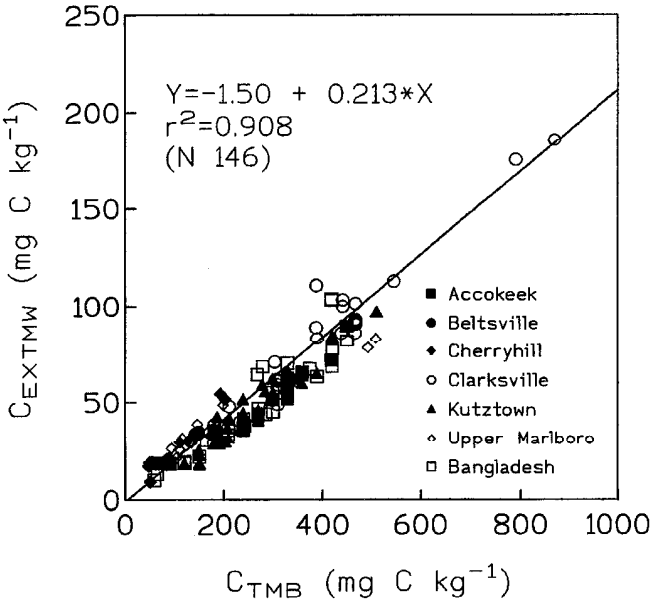

Fig. 7 Relationship between microbial biomass (C_{TMB}) measured by chloroform fumigation incubation and net flush of extractable C (C_{EXTMW}) from microwaved soil extraction

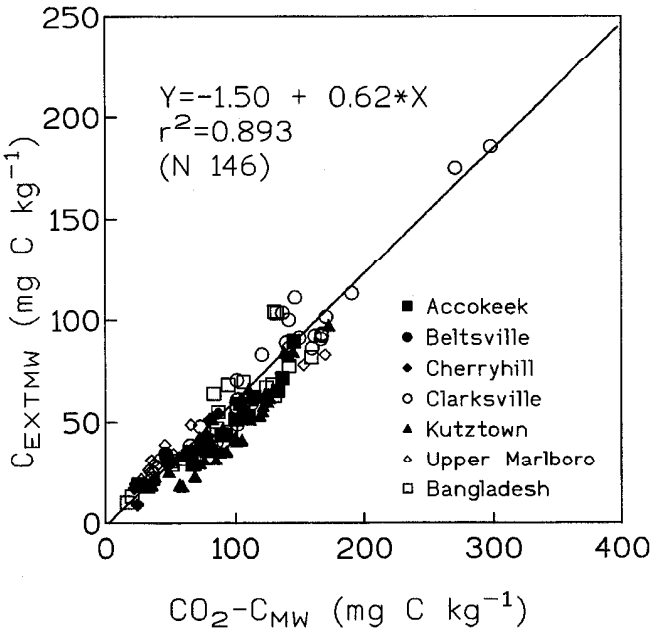

Fig. 8 Relationship between net flushes of C from incubation (CO_2-C_{MW}) and direct extraction (C_{EXTMW}) of microwaved soil

appropriate conversion factor is needed to convert the flushes of C into C_{TMB} as determined by CFI. The conversion factor thus corrects the fractional release or incomplete extraction of biomass C from MW soil. Based on reduced major axis bi-variate linear regression equations, mean incubation (K_{MI}) or extraction (K_{ME}) efficiency factors were calculated to estimate C_{TMB} from net flushes of C in MW soils as follows:

$$C_{TMB} = \text{flush of } CO_2\text{-}C_{MW}/0.341 \pm 0.004$$
$$C_{TMB} = \text{flush of } C_{EXTMW}/0.213 \pm 0.004 \quad (5)$$

Using the calculated K_{MI} or K_{ME} values, our results suggest that an average of 34.1% of the biomass C was measured by MW incubation and 21.3% by MW extraction, compared to 45% of the biomass C measured by the CFI method of Jenkinson and Powlson (1976).

Results in Table 2 show that C_{TMB}, CO_2-C_{MW} and C_{EXTMW} were positively correlated with soil pH, but negatively correlated with bulk density, as might be expected. However, there were no significant correlations between values of K_{MI} or K_{ME} and soil pH, bulk density or clay content. Furthermore, we compared soils with pH>6 or clay content >25% to those with pH<6 or clay content <25% and found no difference in the mean values of K_{MI} or K_{ME} by *t*-test (data not shown). We therefore, conclude that the values of K_{MI} or K_{ME} are independent of soil type, and that the proposed MW method worked equally well on all soils tested despite variations in soil properties.

Conclusions

Because of its rapid biocidal characteristics and convenience of operation, the MW irradiation has potential as a routine alternative to conventional CF methods for the estimation of C_{TMB}. Measurement of C_{TMB} by the proposed MW soil incubation or extraction methods was in good agreement with the CFI method. Although the MW-incubation method gave a slightly closer correlation with the C_{TMB}, the method using extraction of MW soil by 0.5*M* K_2SO_4 was far more rapid and therefore more suitable for routine use in soil testing laboratories. Compared to the CFI method, the MW soil extraction was a faster, less laborious, and simpler method to estimate C_{TMB} without using a biotoxic chemical.

Table 2 Correlation between microbial biomass C measurements and selected soil properties (K_{MI} fraction of the microbial biomass C mineralized as CO_2 in 10 days incubation of microwaved (*MW*) soil at 25°C, C_{TMB} microbial biomass C measured by $CHCl_3$ fumigation incubation, CO_2-C_{MW} net flush of CO_2 from MW soil incubation, K_{ME} fraction of the microbial biomass C from 0.5*M* K_2SO_4 extraction of MW soil, C_{EXTMW} net flush of extractable C from 0.5*M* K_2SO_4 extraction of MW soil

Microwaved soil incubation or extraction coefficient	Soil pH	Bulk density (*r*)	Clay content
K_{MI}	0.20 ns	−0.11 ns	−0.15 ns
K_{ME}	0.01 ns	−0.07 ns	−0.12 ns
C_{TMB}	0.39 **	−0.41***	0.10 ns
CO_2C_{MW}	0.44 ***	−0.44 ***	0.10 ns
C_{EXTMW}	0.42 ***	−0.42 ***	0.11 ns

Proposed MW soil extraction method for the routine measurement of microbial biomass carbon

Reagents

Concentrated sulfuric acid (concentrated H_2SO_4): 18*M*.

Potassium dichromate ($K_2Cr_2O_7$): 0.17*M*: Dissolve 49.06 g $K_2Cr_2O_7$ in a mixture of 400 ml distilled water and 100 ml concentrated H_2SO_4. Dilute to 1000 ml mark with distilled water. Sucrose stock solution ($C_{12}H_{22}O_{11}$): Dissolve 0.981 g sucrose in 100 ml distilled deionized water. Pipette 0, 0.25, 0.50, 1.0, 2.0, 5.0, and 10.0 ml of this stock solution and dilute to 100 ml to prepare 0, 10, 20, 40, 80, 200, and 400 mg C l^{-1} solutions.

Microwave irradiation of soil

Place 10 g ODE of field-moist soil in 50-ml centrifuge tubes and adjust to approximately 80% WFP. Cover each tube with a cap that has a pin hole to allow gas to escape. Place the tube in a rack on a turntable to rotate for uniform absorption of MW energy by soil. Expose the samples to MW energy at 400 J g^{-1} ODE soil. After initial microwaving, stir the soil samples by tapping and rotating. Microwave for an additional 400 J g^{-1} ODE soil. Cool the soil. Prepare an identical set of soil samples in centrifuge tubes, but do not expose to MW. To all tubes add 25 ml of 0.5*M* K_2SO_4, then seal with hole-free caps and shake horizontally at 250 rpm for 60 minutes. Centrifuge the soil suspension at 5000 rpm for 5 minutes and filter the solution to obtain a soil-free extract.

Determination of extracted C (modified from Islam and Weil 1998)

Pipette 5.0 ml filtered extract in a 50-ml Erlenmeyer flask with 1 ml of 0.17*M* $K_2Cr_2O_7$ and 5 ml concentrated H_2SO_4. Place a short-stem 25-mm glass funnel in the mouth of each flask to aid in refluxing the dichromate-acid mixture. Microwave at 500 J ml^{-1} digestion mixture. Acid fumes, though minimal, should be vented from MW oven. After MW digestion, cool the flasks and adjust the volume to 30 ml with distilled deionized water. Repeat the above digestion and dilution procedures for sucrose C standards and a reagent blank solution. Measure the absorption at 590 nm for C standards and sample extracts using a spectrophotometer. Calculate the C_{TMB} as follows:

$$C_{TMB} = C_{EXTMW}/K_{ME}. \quad (6)$$

The C_{EXTMW} is the net flush of C (the difference between the extracted C in MW soil minus the extracted C in field-moist unmicrowaved soil), and K_{ME} is 0.213, the fraction of the C_{TMB} extracted by 0.5*M* K_2SO_4 as C_{EXTMW}.

Adjustment of microwave oven

In the proposed method, MW irradiation is used for pasteurizing soil and digesting the extract. The time settings and MW oven power have to be changed when loading the oven cavity with higher or lower amounts of soil or volumes of digesting mixture, or using a higher or lower powered MW oven. We used twelve 10-g samples in a 650-W MW oven. Microwave time (s) required for a total exposure of 800 J g^{-1} soil (400 J g^{-1} at each time) or 500 J ml^{-1} digestion mixture, can be calculated as follows:

$$\text{Time (s)} = (r*w)/(P) \quad (7)$$

where r is the MW energy to be applied (J g^{-1} ODE soil or J ml^{-1} digestion mixture), w is the total amount of soil (g) or volume of mixture (ml), and P is the power output of the MW oven (W or J s^{-1}).

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