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Soil microbial biomass estimates using 2450 MHz microwave irradiation

CHARLES W. HENDRICKS and NANCI PASCOE
Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR 97333, USA and Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA

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Abstract

The effect of microwave irradiation on two soils of different carbon content was evaluated with the intent of investigating the potential of using this treatment to estimate of soil biomass. After irradiation for various time intervals, the soil was incubated at 25 ± 0.1°C for 10 days. The amount of CO_2-C produced with the two different soils remained constant, and little or no difference was observed for microwave treatment ranging from 1–20 min (4.2×10^4–8.5×10^5 J). When the CO_2-C results of this study were used to calculate biomass-C estimates, the Sierra and Garden soils were 29.5 ± 3.0 and 26.9 ± 3.9 mg C 100 g^{-1} dry soil, respectively. This compared with values of 35.8 ± 0.7 and 28.8 ± 1.2 mg C 100 g^{-1} dry soil obtained by chloroform fumigation. These results indicate that, for the soils tested, a sufficient number of microorganisms survived microwave irradiation to metabolize nutrients released from killed cells during the controlled heating process. This approach appears to provide biomass estimates comparable to chloroform procedure without the use of fumigants.

Introduction

The transformation of nutrients in soil is directly related to the size and activity of the soil microbial biomass (Jenkinson and Ladd, 1981; Paul and Voroney, 1984). The varied activities of these organisms and their importance to nutrient cycling, soil fertility, and general health of terrestrial ecosystems are well known (Brookes *et al.*, 1985; Erdman and Monson, 1986; Sparling *et al.*, 1986), and a variety of techniques are available to measure the numbers, composition, and activity of these organisms. These include: chloroform fumigation (Jenkinson and Powlson, 1976), substrate induced respiration (SIR) (Anderson and Domsch, 1978), direct microscopy (van Veen and Paul, 1979), and ATP determination (Nannipieri *et al.*, 1978). While these techniques provide biomass estimates in terms of the amount of carbon (biomass-C) contained in the organisms, procedures are available to measure biomass nitrogen, phosphorus, and sulfur (West *et al.*, 1986).

Of the various procedures to measure soil microbial biomass, chloroform fumigation is the more widely used and the results are accepted worldwide (Jenkinson and Ladd, 1981). Fundamental to the technique is the use of chloroform as the biocide and extreme care must be exercised in handling this toxic chemical. As a result, there is a continuing interest in investigating other ways of manipulating soil to provide estimates that are comparable to those obtained with chloroform.

Studies over the last several years have shown that microwave energy is an effective means of controlling microorganisms in soil (Ferriss, 1984). While its mode of action is thermal [Vela *et al.*, 1976], in many respects its selective and progressive destruction of soil microorganisms is similar to that seen with some chemical biocides (Ou *et al.*, 1985). Recent studies by Speir *et al.* [1986] have shown that the effects of low level microwave irradiation (approx. 5.4×10^4 J) on microorganisms in soil, including observed increases in extractable mineral N, were similar to that achieved by chloroform

fumigation. At intermediate irradiation times, the extractable N was closely related to a decrease in microbial biomass, indicating that the N was of microbial origin.

In this study, we have examined the effects of controlled and sustained microwave energy on soil with the intent of determining whether the resulting destruction of microorganisms could be used to estimate microbial biomass. The objectives of this research were to evaluate the ability of microbial populations native to soil to survive sustained microwave irradiation, to use this information to calculate a biomass estimate, and to compare these data with standard biomass procedures accepted in the literature.

Materials and methods

Soil

Two types of soil and 27 different samples were used in this study. One type was a Mollic Haploxeralf of the Argonaut series from an oak-grass savannah in the lower Sierra foothills of California. The other was a fine silty Argiaquic Xeric Agialbolls (Amity series) that is used in plant growth research at EPA's Environmental Research Laboratory in Corvallis, Oregon. Both sites typically receive 60 cm annual rainfall. Soil chemical analysis for the two soils, respectively, are as follows. Sierra: pH, 5.9; organic carbon, 2.6%; phosphate, 2.1 ug g^{-1}; and nitrogen, 0.22% (Hendricks *et al.*, 1987); Garden: pH, 5.9; organic carbon, 4.8%; phosphate, 7.8 ug g^{-1}; and nitrogen, 0.18% (U.S. EPA, 1983). The carbon/nitrogen ratio for the Sierra and Garden soils was 12 and 27, respectively.

Soil samples from both locations were collected from a depth of 0–4 cm. The soils were moistened and incubated for 5 days at room temperature (25–27°C), and then screened through a 2-mm sieve. Aliquots of the prepared soil were stored at 5 ± 2°C until used.

Microwave treatment

Samples (300 g) of prepared soil were placed into glass microwave dishes and introduced into a model MW8055XP, 700 watt microwave oven (Whirlpool Corporation, Benton Harbor, MI 94022)* equipped with a mechanical turntable. Samples were irradiated at full power for periods up to 20 min. To minimize heat buildup within the soil for those samples irradiated for 5–20 min, the soil was irradiated for 2.5 min, stirred, and then cooled for 1 min. Using this 2.5 min 'burst' procedure, the measured temperature of the soil never rose above 87°C. Total calculated energy (Joules = Watts × seconds) exposure was estimated as 0, 4.2×10^4, 2.1×10^5, 4.2×10^5, 6.3×10^5, 8.5×10^5 J at 0, 1, 5, 10, 15, and 20 min, respectively.

Soil incubation experiments

To measure the respiration of developing microbial cultures, large Mason jar lids were modified to accept a small rubber septum so that the gas phase could be periodically sampled for CO_2 with a gas-tight syringe. Gas samples were withdrawn through the septum at specific intervals and manually injected into a gas chromatograph (Hewlett-Packard, Model 5840A). This system utilized a thermoconductivity detector and two Poropak Q columns. CO_2 standards of known concentration and purity (1.00%; 99.99% – Union Carbide Corp., Linde Div.) were used in the initial calibration of the system, and control standards were run after every fifth sample.

Samples of each soil (15 g) were irradiated in the microwave oven for 1–20 min and placed into 960 ml Mason jars. These soil cultures were moistened to 55% water holding capacity (WHC), capped, and incubated at 25 ± 1°C for periods up to 20 days. The cultures, including untreated control samples, were run in triplicate.

Biomass determination

Three standard biomass estimation techniques were used to compare data derived from microwave irradiation: the direct cell counting techniques (van Veen and Paul, 1979), chloroform

* Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

fumigation (Jenkinson and Poulson, 1976), and the substrate induced respiration method (SIR) (Anderson and Domsch, 1978).

Samples of the prepared soil were used for both direct cell count and respiratory response methods. Bacterial numbers in the samples were estimated by the fluoroscein isothiocyanate (FITC) staining technique and used in biomass-C calculations [van Veen and Paul, 1979]. Fungi were observed by staining with trypan blue (Kormanik and McGraw, 1982) and converted to biomass-C by calculating the biovolume (Ingham and Klein, 1982). This procedure assumes a dry weight specific gravity of 0.33 g dry weight cm^{-3} and a carbon content of 47 percent. Bacterial numbers and fungal hyphal lengths were measured using an eyepiece grid in the microscope, and 20 fields were counted from each of two smears prepared from duplicate soil samples.

Biomass estimates using the SIR method were conducted in 62 ml vaccine bottles using 10 g (dry weight) of soil. Then, β-D-glucose (500 ug g^{-1} soil) was added to each of three bottles. The cultures were incubated at 22 $\pm$ 1°C for 3 h, and the head space CO_2 was sampled hourly and measured by GC analysis.

Chloroform fumigation biomass estimates were run in triplicate using 15 g (dry weight) of dry soil from each site. After removing the chloroform vapors by venting the sample in a chemical fume hood, the samples were brought to 55% WHC and sealed in Mason jars fitted with a rubber septum. The soil cultures were incubated at 25 $\pm$ 1°C for 10 days and then measured for CO_2 content.

A protocol similar to that used for chloroform fumigation was used in describing biomass estimates by treating the soil with microwave irradiation. Soil samples were irradiated for 1–20 min as described above and 15 g (dry weight) aliquots (in triplicate) were placed in Mason jars. After 10 days incubation at 25 $\pm$ 1°C, the cultures were sampled for CO_2 production by GC analysis.

The following formula was used in calculating biomass C for both the chloroform fumigated soil and the soil irradiated with microwaves (Paul and Voroney, 1984).

$$\text{Microbial Biomass-C (mg C 100 g}^{-1}\text{ dry soil)} = \frac{[CO_2\text{-C (Treated)}] - [CO_2\text{-C (Untreated)}]}{\text{Fraction of biomass C mineralized to } CO_2 \text{ in 10 days)}}$$

$$\text{or } B = \frac{T}{k_c}$$

The fraction of biomass-C mineralized to CO_2 (k_c) was taken to be 0.5 (Jenkinson, 1976).

Soil chemistry

To determine the effects of the biomass estimating techniques on the soils used in this study, samples from both sites were treated as follows: chloroform vapors, 24 h; microwave irradiation, 1–20 min (in 2.5 min bursts); and, for reference, the autoclave (20 min at 121°C). Ten g aliquots of the treated and untreated soils were extracted for N with 20 ml 0.5 M K_2SO_4 (Brookes *et al.*, 1985), for P with 20 ml of 0.5 *M* $NaHCO_3$ [Olsen and Dean, 1965], and for soluble organic C with distilled water. The samples were clarified by filtration through Whatman No. 40 paper and analyzed for ammonia and nitrate/nitrite, organic carbon, and phosphorus by autoanalyzer techniques (U.S. EPA, 1983).

Results

The use of microwave irradiation with an oven designed for home use is a versatile tool for manipulating the microbiology of soil. We found that glass cookware designed for the microwave oven could be used to process the soil used in this study and was superior to the plasticware we used initially. 'Hot spots' were noticeable during some of the early microwave runs which resulted in uneven heating of the soil. However, when the dish of soil was set on a wooden block on a plastic mechanical turntable within the oven, 'hot spots' were not observed. This technique coupled with limiting the irradiation period to 2.5 min resulted in minimal heat buildup by keeping the temperature of the soil between 83–87°C. No moisture loss was noted after the first min of microwave treatment.

Figures 1 and 2 show the accumulated respiratory CO_2-C over a 20-day period for Sierra and Garden soil, respectively. These data were collected from a single experimental run, but are typical of other data we collected during this study. The control cultures (untreated) of both soils produced

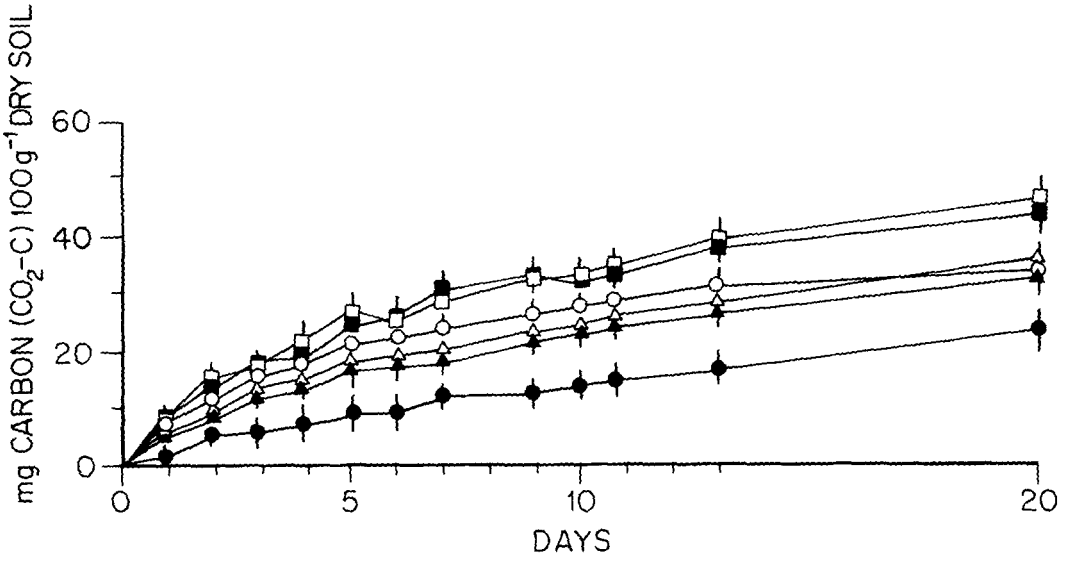

Fig. 1. Carbon dioxide (CO_2-C) produced in Sierra soil as a result of microwave irradiation for various lengths of time. The CO_2 flush is expressed as the amount of carbon produced by 100 g of dry soil, and the data points are a mean of three replicates ± SE. Symbols: ●, control (no microwave irradiation); △, 1 min; ▲, 5 min; ○, 10 min; □, 15 min; ■, 20 min.

similar amounts of CO_2 by the end of the 20th day; but the maximum growth rate occurred at day 2. While both treated soils produced more CO_2 than the untreated controls, some differences were noted. Maximum CO_2 production with Sierra soil (Fig. 1) occurred with 15 min microwave treatment, and was consistent through the course of the experiment. For the Garden soil (Fig. 2), maximum CO_2 was produced in cultures treated for 10 minutes.

Figure 3 shows the mean respiratory CO_2-C produced on 10 days for three experimental runs. CO_2-C from chloroform fumigation is shown for comparison. These data demonstrate that microwave treatment is comparable to chloroform

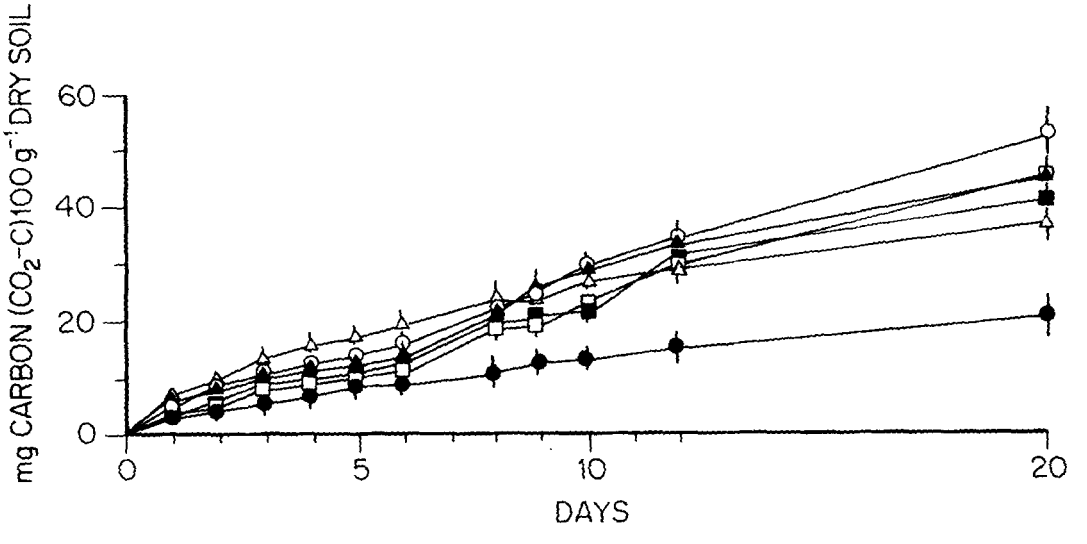

Fig. 2. Carbon dioxide (CO_2-C) produced in Garden soil as a result of microwave irradiation for various lengths of time. The CO_2 flush is expressed as the amount of carbon produced by 100 g of dry soil, and the data points are expressed as the mean of three replicates ± SE. Symbols: ●, control (no microwave irradiation); △, 1 min; ▲, 5 min; ○, 10 min; □, 15 min; ■, 20 min.

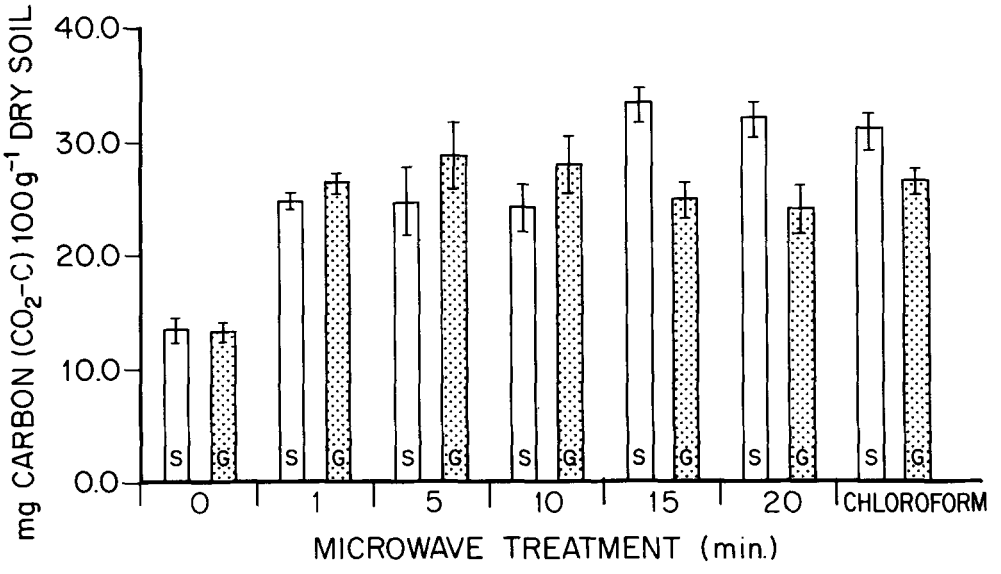

Fig. 3. Total amount of carbon dioxide produced in 10 days as a result of microwave irradiation of Sierra and Garden soil for various lengths of time. The CO_2 flush is expressed as the amount of carbon produced by 100 g of dry soil and the error *bars* indicate ± SE. Symbols: **S**, Sierra soil; **G**, Garden soil.

fumigation technique in the production of CO_2-C from these soils. The pattern of increased levels of CO_2-C production occurred at microwave treatment times similar to those observed in Figs. 1 and 2 when the experimental standard error for all observations is taken into account.

Biomass estimates using traditional techniques and the microwave irradiation data from this study are presented in Tables 1 and 2. The mean biomass estimates based on microwave treatment for the Sierra and Garden soils were 29.5 ± 3.0 and 26.9 ± 3.9 mg C 100 g^{-1} dry soil, respectively. This compared to 35.8 ± 0.7 and 28.8 ± 1.2 mg C 100 g^{-1} dry soil obtained by chloroform fumigation.

The nitrogen, phosphate, and organic carbon (OC) analyses were run to obtain insight into the effects of prolonged microwave treatment on the two soils. These results are shown in Table 3.

Table 1. Biomass estimates[a] using microwave irradiation on two different soils (Sierra and Garden)

Microwave treatment (min)	Soil	Mean mg CO_2 100 g^{-1} dry soil[b]	mg Biomass C 100 g^{-1} dry soil
1	Sierra	11.2 ± 0.6	22.5 ± 1.2
	Garden	13.4 ± 0.8	26.8 ± 1.6
5	Sierra	13.1 ± 1.8	26.1 ± 3.6
	Garden	15.4 ± 2.8	30.8 ± 5.6
10	Sierra	10.7 ± 2.2	21.4 ± 4.4
	Garden	15.8 ± 2.5	31.6 ± 5.0
15	Sierra	20.1 ± 2.5	40.1 ± 3.0
	Garden	11.8 ± 1.6	23.7 ± 3.2
20	Sierra	18.7 ± 1.5	37.4 ± 3.0
	Garden	10.9 ± 2.1	21.8 ± 4.2

[a] Represents the mean of 27 samples from each soil ± SE.
[b] Based on a 10-day incubation period.

Table 2. Comparison of various techniques to estimate biomass in two different soils

Soil	No. trials	Mean mg CO_2 100 g^{-1} dry soil[d]	mg Biomass C 100 g^{-1} dry soil
Glucose stimulation			
Sierra	18	NA[a]	64.0 ± 6.7[b]
Garden	18	NA	63.2 ± 12.2
Chloroform fumigation			
Sierra	6	17.9 ± 0.3	35.8 ± 0.7
Garden	6	14.4 ± 0.6	28.8 ± 1.2
Direct cell counts			
Sierra	2	NA	14.8 ± 3.1
Garden	2	NA	16.5 ± 3.9
Microwave[c]			
Sierra	27	14.8 ± 1.5	29.5 ± 3.0
Garden	27	13.5 ± 2.0	26.9 ± 3.9

[a] Not appropriate in method.
[b] Mean of analyses ± SE.
[c] Mean of analyses at all microwave treatment times.
[d] Based on a 10-day incubation period.

Table 3. Nitrogen, phosphate, and organic carbon extracted from Sierra and Garden soils after treatment with microwave irradiation, the autoclave and chloroform vapors

Treatment time	Soil	
	Sierra	Garden
	$(NO_2$-$NO_3)$-N[a]	
Microwave (min)		
0	12.4 ± 0.6	100.0 ± 5.0
1	17.2 ± 0.9	104.0 ± 5.2
5	26.0 ± 1.3	106.0 ± 4.8
10	21.6 ± 1.1	114.0 ± 5.7
15	19.2 ± 1.0	115.0 ± 5.1
20	20.0 ± 1.0	116.0 ± 5.1
Autoclave (min)		
20	16.4 ± 0.8	120.0 ± 6.0
Chloroform (day)		
1	18.4 ± 0.9	112.0 ± 5.6
	NH_4-N	
Microwave (min)		
0	1.0 ± 0.1	1.0 ± 0.1
1	1.4 ± 0.1	1.2 ± 0.1
5	2.0 ± 0.1	2.8 ± 0.1
10	1.8 ± 0.1	1.6 ± 0.1
15	1.5 ± 0.1	2.0 ± 0.2
20	2.0 ± 0.1	5.2 ± 0.3
Autoclave (min)		
20	1.4 ± 0.1	1.5 ± 0.1
Chloroform (day)		
1	2.7 ± 0.1	1.6 ± 0.1
	Extractable-P	
Microwave (min)		
0	0.38 ± 0.02	3.4 ± 0.2
1	0.36 ± 0.03	4.2 ± 0.3
5	0.28 ± 0.02	4.8 ± 0.2
10	0.22 ± 0.02	4.2 ± 0.2
15	0.14 ± 0.03	4.2 ± 0.3
20	0.26 ± 0.02	3.8 ± 0.2
Autoclave (min)		
20	0.31 ± 0.02	6.1 ± 0.4
Chloroform (day)		
1	0.40 ± 0.04	5.8 ± 0.3
	Organic carbon	
Microwave (min)		
0	28 ± 2	48 ± 2
1	76 ± 4	108 ± 7
5	168 ± 10	108 ± 10
10	172 ± 14	238 ± 17
15	176 ± 14	250 ± 12
20	190 ± 15	310 ± 22
Autoclave (min)		
20	147 ± 9	296 ± 15
Chloroform (day)		
1	143 ± 7	163 ± 10

[a] Concentration of all constituents expressed in $\mu g\, g^{-1}$ dry soil.

Besides the obvious differences in nitrogen and phosphate levels of the two soils, microwave treatment had a significant effect on N and OC levels in the treated soils. Elevated levels of OC were observed, which increased 6–7 fold after 20 min. Corresponding increases in N, P, and OC were noted in samples of both soils that were subjected to the autoclave and chloroform vapors.

Discussion

Several methods exist to measure the size, activity, and composition of soil microbial biomass and to evaluate its importance to the cycling of nutrients and the fertility of soils (Jenkinson and Ladd, 1981; West *et al.*, 1986). Our current state of knowledge requires that these methods must be used with forethought since no one procedure appears to be adequate in all circumstances (Carter, 1986). Because biomass estimates are affected by a variety of factors, including the number and types of microorganisms present (Nannipieri *et al.*, 1978), incubation temperature (Anderson and Domsch, 1978), soil type and preparation (Sparling *et al.*, 1986), and storage of the soil samples (West *et al.*, 1986), it is remarkable that it is possible to collect comparative data. There are instances, however, where various biomass esti-

mating techniques yield results of doubtful value because of the nature of the soil sample, the habitat from which it came, or by a misunderstanding of what information a procedure can provide and its underlying assumptions (Paul and Voroney, 1984). In this study, 3 standard biomass estimating procedures were used to compare estimates derived from the use of microwave irradiation.

While none of the standard procedures used in this study gave unequivocal results, the estimate based on microwave irradiation closely approximated that based on chloroform fumigation. Some variation was observed with those obtained by the SIR or direct cell count techniques but these fluctuations were consistent with those reported by others (Nannipieri *et al.*, 1978; Sparling *et al.*, 1986).

It can be argued that the various biomass estimates obtained in this study for a given soil differ significantly if only the mean standard errors are considered. This is especially evident when Sierra soil data obtained by chloroform fumigation is compared with soil treated by microwave irradiation (Table 2). Likewise, SIR biomass estimates are 2–3 times higher than those derived by the other techniques for both soils. While sample to sample variation as noted by the standard error estimates is within acceptable ranges, we as soil scientists need to be concerned about our ability to precisely estimate microbial biomass. For the most part, comparative studies using more than 2 of the biomass techniques are rare, but there are a number of studies that speculate on the inherent variation in the fumigation technique (Voroney and Paul, 1984; Merck and Martin, 1987) or why fumigation data may differ from direct count estimates (van Veen and Paul, 1979; West *et al.*, 1986).

In the case of the SIR procedure, there has been work to suggest that respired CO_2 should be continuously removed from alkaline soil cultures to minimize loss of the gas (Martens, 1987). However, even when the respired CO_2 was estimated using a continuous flushing aeration train, error beyond the coefficient of variation was apparent, although the estimates more closely agreed with fumigation data. These observations do much to explain how variations between procedures is possible, but also point to the need of further, more definitive research.

Although there has been considerable controversy about some unknown but lethal microwave component (Chipley, 1980), there is now general agreement that heat is the mechanism of destruction for microorganisms (Vela *et al.*, 1976; Ou *et al.*, 1985). The heat produced can reach temperatures of 200°C and higher at elevated pressures in microwave chambers designed specifically for chemical hydrolysis (Kingston and Jassie, 1986), but the home oven is not capable of sustained operation under those conditions. The 2.5-min 'burst' procedure was used in this study to minimize the effect of soil temperatures that could be disruptive to the soil. Enough organisms survived microwave irradiation for up to 20 min to metabolize cellular constituents of those organisms that were killed and lysed. A variety of soil bacteria, actinomycetes, and fungi are capable of surviving 90°C for up to 30 minutes (Bollen, 1969), but Vela *et al.* (1976) have suggested that a significant portion of the viable population can grow from the 'heat shock' activation of bacterial and fungal spores in soil after microwave irradiation.

Obviously, biomass procedures that inadvertently include in the measurement part of the substrate or some component of the soil organic matter are prone to error and should not be used on a routine basis. For example, this is especially evident with added fresh substrate, but recent studies by Merckx and Martin (1987) have shown that plant roots in soils often degraded at faster rates in nonfumigated controls than in fumigated soil. The effects of microwave irradiation on soil organic material are not clearly defined, but there is information to suggest that the more complex chemical structures may be stable at temperatures below 160°C. Microwave heating at temperatures above 160°C have resulted in enhanced enzymatic susceptibility of lignocellulose wastes, but no substantial change in the crystallinity of cellulose was detected at temperatures below 230°C (Azuma, 1984). While starch polymers are susceptible to moderate levels of microwave energy (0.18 mA for 16, 18, and 20 minutes) (Khan *et al.*, 1979) and hemicellulose and lignin underwent acid-catalyzed

autohydrolysis (Azuma, 1984), agricultural lignocellulosic wastes that were irradiated at 700 watts for up to 20 minutes were not altered sufficiently to demonstrate increased biodegradation in rumen fluid (Erdman and Monson, 1986). The degree to which microwave irradiation may have disrupted the soil organic matter in this study is unknown, but the lack of substantial increases of C and N after irradiation suggest minimal disruption occurred (Speir *et al.*, 1986).

A variety of tillage practices (Sparling *et al.*, 1986; West *et al.*, 1986) and soil sterilants (Skipper and Westerman, 1973; Brookes *et al.*, 1986) are also known to increase the levels of extractable N, P, and C from soils with subsequent increases in biomass upon incubation. These observations are important not only in providing information on the effects of soil treatment, but also in understanding some of the physiological controls on the rate of nutrient cycling and biomass development in soils. Soil perturbations *in situ* and in the laboratory do result in biomass estimate inconsistencies, and these and other soil variables are probably compensated for within the k_c factor in the equation for calculating biomass from CO_2-C flush data (Voroney and Paul, 1984). In this study, we observed increasing levels of inorganic N and soluble organic carbon (OC) with irradiation time. Levels of P under the conditions tested did not increase. With the exception of OC levels following longer microwave exposure times, these data are in general agreement with the reports cited above and with our comparative data using autoclave and chloroform treatment. The increased levels of OC at the longer microwave periods (10–20 min) suggest some disruption of the soil organic fraction even though the soil treatment temperature was kept at 87°C and below. Therefore, irradiation times of less than 5 min are advisable.

The development of a microbial biomass procedure that would reliably estimate biomass with the precision of the chloroform fumigation technique would be of particular advantage in the soil microbiology laboratory. The microwave procedure is uncomplicated, and the only additional equipment needed is a microwave oven if the laboratory is already performing analyses with chloroform. Also, use of the microwave technique allows the laboratory to eliminate the use of chloroform chemical in the work environment. This consideration alone should encourage further evaluation of this procedure.

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