http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (7): 629-639, 2003
ISSN 1028-8880
© 2003 Asian Network for Scientific Information

Microbial Biomass in Agricultural Soils – Determination, Synthesis, Dynamics and Role in Plant Nutrition

F. Azam, S. Farooq and A. Lodhi
Nuclear Institute for Agriculture and Biology, P.O. Box 128, Faisalabad, Pakistan

Abstract: Microbial biomass is a small but labile component of soil organic matter and plays an important role in cycling and other nutrient elements. Because of its importance in the functioning of different ecosystems, synthesis/dynamics of microbial biomass and its role in plant nutrition under different ecosystem conditions has assumed greater significance. In the same perspective, developing quick and convenient methods of determination have been of immense research interest for the least few decades. As a result significant improvements have thus far been made although no single method is devoid of snags. This paper presents I) a critical evaluation of methods to determine microbial biomass and ii) a review on the formation/dynamics of microbial biomass and its role in plant nutrition. Since carbon © and nitrogen (N) are the major elements, special attention has been given to these while discussing microbial biomass.

Key words: Carbon, methods, microbial biomass, nitrogen, plant nutrition

Introduction

Microbial biomass is a small but labile and living component of soil organic matter that is involved in most biogeochemical processes in terrestrial ecosystems (Paul and Voroney, 1980). It interacts with ecosystem productivity by regulating nutrient availability, determining soil C storage, and contributing to the atmospheric CO_2 from respiration. As such, microbial biomass is the main agent that controls the flow of C and cycling of nutrient elements in terrestrial ecosystems. The large size of the soil microbial biomass implicates it as a major nutrient sink during C immobilization (growth) and as a source during mineralization (decay). It consists of bacteria, fungi, actinomycetes, and protozoa etc. 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 biomass as the living part of soil organic matter excluding plant roots and soil animals larger than 5×10^3 um^3. The importance of microorganisms in ecosystem functioning has led to an increased interest in determination of soil microbial biomass. As a result, a wide variety of methods have been proposed and practiced over the last 3-4 decades. This paper presents I) a critical evaluation of methods of determining microbial biomass and ii) a review on the formation/dynamics of microbial biomass and its role in plant nutrition. Since carbon © and nitrogen (N) are the major elements, special attention has been given to these while discussing microbial biomass.

Methods of determining microbial biomass: The increased interest in the role of soil microorganisms in nutrient and energy flow relationships in natural as well as man-manipulated environments has emphasized the need for simple and objective methods for measuring the size of microbial populations in soils. Among the methods currently available are those involving I) direct counting, in which microorganisms are variously stained (Babiuk and Paul, 1970) and observed by different optical methods (Frankland, 1974), ii) soil extractions in which enzymes (Skujins, 1967), cell components peculiar to cell walls of certain groups of microorganisms (Miller and Casida, 1970), or components characteristic to living cells (Lee *et al.,* 1971) are extracted and quantitatively assayed, iii) disintegration of the soil followed by separation and collection of the microbial cells (Balkwill *et al.*, 1975), iv) measurement of respiratory activities of substrate-supplemented habitats and thus the relative proportions of actively metabolizing biomasses are used to (Anderson and Domsch, 1978), v) ultra-violet absorption (Turner *et al.*, 2001), vi) microwave radiation that leads to the release and determination of microbial C and N, vii) measuring heat output or microcalorimetry as an index of initial and final energy states of the system irrespective of the types of organisms or reaction pathway (Sparling, 1981), viii) ninhydrin reaction (Amato and Ladd, 1988), ix) chloroform fumigation incubation (CFIM) in which C bound in microbial biomass is released by microbial mineralization and thereby provides a means for calculating its biomass (Jenkinson and Powlson, 1976),

x) chloroform fumigation extraction (Brookes *et al*., 1985; Vance *et al*., 1987) in which C and N that becomes extractable following fumigation is measured, and finally xi) chloroform extraction in which soil samples are extracted with K_2SO_4 + $CHCl_3$ and the extractable N determined as a measure of biomass N (Azam *et al*., 1989a). Different methods available for quantitative determination of microbial biomass in soil and their advantages/disadvantages have been reviewed by Jenkinson and Ladd (1981) and Martens (1995). In this paper, a brief account of the commonly used and convenient methods is being provided.

Substrate-induced respiration method (SIRM): This method involves incubation of soil samples with an excess of easily oxidizable C source (25-4000 ug g^{-1} soil) and measuring the respiratory activity (CO_2 evolved) after one hr of incubation in all soils at 22 °C (Anderson and Domsch, 1978). A conversion factor of 1 ml CO_2 hr^{-1} to 40 mg biomass C was found to be realistic. Short period of incubation ensures the determination only of the prevalent and not the newly developed biomass. The authors claimed to obtain reliable estimates of biomass from soils of widely different origin e.g., from forests and agricultural sites. It was emphasized, however, that temperature of incubation plays a crucial role in final estimates and ay deviation from 22 °C will require fresh standardization of the method.

Substrate-induced respiration method is based on the premise that at increasing levels of easily degradable substrate like glucose and casamino acid the initial respiration rate of a soil increases in parallel to the biomass content. The fundamental assumption is that I) initial respiration rate in glucose-amended soil is the same for different sections of the microbial population and ii) biomass measurements by CFIM are correct. This method gives quick and direct information on the comparative estimates of biomass in different soils. However, quantitative measurement of the biomass is not possible. However, one of the advantages of SIRM is the apportioning of biomass into fungal and bacterial components by using inhibitors specific to either group; fungal and bacterial contributions to soil respiration being estimated at 22 and 78% (Anderson and Domsch, 1973). The method can be applied to hundreds of samples simultaneously within a day and is well suited to determine biomass C in acid forest soils as well as for soils rich in organic matter content. The disadvantages include I) an intricate respirometery system that requires continuous flushing of the incubation vessels for the determination of CO_2 at short intervals (e.g., an infrared gas analyzer) ii) the fact that the method does not give direct estimates of nutrients contained in the biomass, iii) indirect estimates of nutrients contained in biomass are generally erroneous, and iv) the need for determining the concentration of substrate to achieve maximum initial respiration response for each soil. In spite of such shortcomings, SIRM can conveniently be used for comparing microbial activity of soils especially for assessing the effect of soil additives. A semi- or fully automated respirometric system may, however, be required for the ease of determinations and data handling. This factor alone makes the method unsuited for most labs especially in developing countries.

Chloroform fumigation-incubation method: The method involves $CHCl_3$ fumigation of moist soil samples for 0-20 days at 25°C and determining CO_2-C evolved, O_2 consumed or N mineralized in fumigated versus unfumigated soil (Jenkinson and Powlson, 1976b). Briefly, the soil samples are placed in a desiccator that contains acid washed $CHCl_3$ (ethanol free) and the desiccator evacuated to an extent that vigorous bumping of $CHCl_3$ is achieved. After 24 hrs under vacuum in dark, $CHCl_3$ is removed by repeated evacuations and the soil samples incubated for 0-10 or 10-20 days without or with the addition of *ca* 1% unfumigated moist soil to revive microbial activity. The incubation is carried out in a way to trap CO_2 evolved from the soil. A similar set of unfumigated samples is also incubated. The difference of CO_2-C evolved, O_2 consumed, or NH_4-N accumulated in fumigated and unfumigated samples is used to calculate biomass C and biomass N by using the expression: $B = F/K$, where B is the biomass, F is the flush or difference in fumigated and unfumigated soil samples in terms of CO_2-C evolved or N mineralized, and K is the proportion of biomass C or N mineralized (*KC* and *KN*, respectively).

This has been the most widely used method and hence subject to immense scrutiny as well. It is based on the observations that moist soil samples subjected to wetting-drying, freezing-thawing, physical disruption, irradiation or fumigation show a flush of CO_2 evolution and N mineralization upon incubation under laboratory conditions (Jenkinson, 1966; Powlson and Jenkinson, 1976; Marumoto *et al*., 1977). Such an effect is reportedly substantial following fumigation of soil with chemicals to control soil-borne plant pathogens (Jenkinson and Powlson, 1976a,b). When a soil is exposed to a volatile fumigant, the fumigant removed, and the soil incubated, respiration rate of the fumigated soil is greater than that of the unfumigated control. This effect has been attributed to I) exposure of otherwise inaccessible organic matter to microbial attack (Rovira and Graecan, 1957), ii) release of cellular components (Marumoto *et al*., 1977), and iii)

partial or complete sterilization followed by mineralization of dead microbial cells by surviving or newly inoculated microorganisms (Jenkinson, 1966). Thus over a short period, fumigated soil consumes more O_2 and evolves more CO_2 than untreated soil. According to Jenkinson (1966) the flush of activity following fumigation is mainly due to the decomposition of dead microorganisms and is related to the size of soil biomass.

One of the limitations of the CFIM may be the qualitative change in the microbial population following fumigation (Martin, 1963). Shift in species composition is also exhibited through a change in C/N ratio of the biomass; narrowing of C/N ratio means more bacterial biomass and vice versa (Grace *et al*., 1993). The major assumption in CFIM that mineralization of non-biomass material is unaffected by fumigation has generally been found invalid especially for soils having a higher basal respiration. Shields ***et al.*** (1974) suggested that fumigation (with chloroform e.g.) not only killed the microorganisms but altered certain extracellular microbial metabolites rendering them susceptible to decomposition. Using ^{15}N methodology, Azam ***et al.*** (1989b) observed significant increase in the extractability of non-biomass N (unlabelled N) following fumigation suggesting that CFIM may give over-estimates of biomass.

The fraction of biomass C and N mineralized following fumigation/incubation i.e., *KC* and *KN* is the single most important in biomass determinations by CFIM. However, it varies widely depending upon the incubation conditions and the type of soil. To begin with, Jenkinson (1966) proposed a *KC* factor of 0.3 that was later modified to 0.5 (Jenkinson, 1976). Adams and Laughlin (1981) reported value of 0.55 based on several organisms, while a weighted average of 0.41 was reported by Anderson and Domsch (1978). Like *KC*, *KN* is also reported to vary over a wide range depending upon the type of microorganisms studied. Generally, 24-59% of the bacterial N may be mineralized under aerobic conditions yielding *KC* factor of 0.24-0.59, while for fungal biomass, net N mineralization is not observed and hence the contribution to flush of mineral N may be negligible or even negative (Jenkinson, 1976; Adams and Laughlin, 1981; Marumoto, 1984; Voroney and Paul, 1984). Under anaerobic conditions, Voroney and Paul (1984) determined *KN* factor of 0.2-0.3 depending on the C/N ratio of decomposing biomass. Inubushi and Watanabe (1987) found this factor to be 0.43 and attributed this to higher C/N ratio of the decomposing biomass in anaerobic than aerobic soil understandably because of the preponderance of bacterial biomass in the former and fungal biomass in the later. Grace *et al.* (1993) used *KC* and *KN* factors of 0.21 and 0.22, respectively, while Voroney and Paul (1984) used *KN* of 0.3.

In most of the studies, *KC* or *KN* factor has been determined by subjecting the laboratory-raised microbial material to decomposition/mineralization and hence may not represent the native microflora of a particular test soil. Voroney and Paul (1984) were the first to determine *KC* or *KN* of biomass developed in soil ***in situ*** by incubating soil samples with ^{14}C-labelled glucose and ^{15}N-labelled KNO_3. They assumed (and rightly so) that at the time of subjecting the treated soils to $CHCl_3$ fumigation, the entire ^{14}C and ^{15}N in soil was in microbial cells and associated metabolites. The authors determined a *KC* and *KN* of 0.41 and 0.42, respectively. Azam ***et al.*** (1988) used NH_4-N rather than NO_3-N for ***in situ*** determination of *KN* in soil containing variable amounts of biomass. The values of *KN* thus determined varied from 0.19 to 0.42 and increased with the amount of glucose and N applied.

In spite of the limitations discussed above, CFIM has been the most widely employed method of determining biomass in samples originating from diverse ecological conditions. It has prompted extensive research on microbial biomass vis-à-vis its role in ecosystem functioning. In addition, the underlying principles helped develop new and less time-consuming methods of biomass determination that are described below.

Chloroform fumigation-extraction method (CFEM): Some of the shortcomings of CFIM for the determination of biomass N, particularly those arising from changes that occur during incubation as a result of immobilization-remineralization turnover, were apparently overcome by the method of Brookes *et al*. (1985). This method involves direct extraction of $CHCl_3$-fumigated as practiced in CFIM. Total N and NH_4-N extracted after $CHCl_3$ fumigation is closely related with mineral N flush obtained with CFIM, while a *KN* factor of 0.54 can be used for calculating biomass N. Cheng and Virginia (1993) suggested that CFEM works better for biomass N than biomass C. Recently, Bailey ***et al.*** (2002) compared CFEM with extractable phospholipids fatty acids and found a good correlation between the two.

This method has also been used for the determination of biomass C using K_2SO_4 or water as extractant. Jenkinson (1966) reported $CHCl_3$ fumigation led to an increase in the amount of C extractable with 0.5 M K_2SO_4. A close correlation between microbial biomass in soil and the extra C extractable due to fumigation was confirmed in subsequent studies of Vance ***et al.*** (1987) who proposed the relationship: biomass C = K_{EC} x E_C where K_{EC} is the extractability factor for C and E_C is organic C extracted from fumigated soil. They used a *KC* factor of 0.38. Nunan ***et al.*** (2000) determined biomass indirectly from the difference of organic C and organic N in K_2SO_4 extracts of

fumigated and unfumigated soil samples (used CFEM). Biomass C was calculated as 2.64 x E_C (extractable C in fumigated minus unfumigated soil) and N as 2.22 x E_N. Gregorich *et al.* (2000) found K_2SO_4 and water to be equally efficient in extracting biomass C. The problem could be the artifacts resulting from incomplete removal of $CHCl_3$ after fumigating the soil samples and thus leading to overestimates of biomass C. However, the method can conveniently be used in studies aimed at determining incorporation of ^{14}C from diverse plant residues into the microbial biomass and its subsequent dynamics. In such studies, ^{14}C-labelled plant material produced under controlled conditions is incorporated into the soil and biomass ^{14}C determined at desired time intervals (Smith *et al.*, 1995; Malik and Azam, 1980).

Like CFIM, CFEM is beset with the problem of increased susceptibility of non-biomass materials to extraction following fumigation leading to inflated values of nutrients contained in the microbial biomass. Studies by Azam *et al.* (1989b, referred to above) clearly demonstrated the release of non-biomass entities due to fumigation. In addition, the data obtained does not necessarily indicate the exact proportion of biomass N that is recovered by fumigation-extraction because the biomass content of the soils used in the study of Brookes *et al.* (1985) was not *kn*own. In addition, chloroform fumigation may work best for soils at lower moisture content in which case $CHCl_3$ is distributed fairly uniformly in the soil sample. In soil samples at higher moisture (e.g., flooded rice soils) biomass may not be exposed uniformly to the fumigant leading to underestimates. In spite of these short comings, CFEM followed CFIM closely as a method of choice for determining biomass and prompted further improvements as discussed below.

Chloroform extraction method (CEM): As a logical extension to CFEM, Azam *et al.* (1989a) suggested a chloroform extraction method for aerobically incubated soils on the pretext that if $CHCl_3$ vapours can extract microbial contents and render them degradable, $CHCl_3$ may be more efficient in doing so. They incubated a silt loam soil from Pakistan with increasing amounts of ^{15}N-labelled ammonium sulphate and glucose (C/N ratio of the additives being 30) in order to generate different levels of microbial biomass in soil *in situ*. At a stage when all of the applied ^{15}N was in organic forms (microbial biomass and products), the soil samples were analyzed for biomass N by CFEM. In comparison, soil samples were extracted for 1 hr with K_2SO_4 containing different amounts of $CHCl_3$. Biomass N determined with CFEM ranged between 14.6 and 30.6, while that extracted with 20% $CHCl_3$ was 21-65%. The difference in the extractability of biomass N as well as extractability ratios revealed that CEM is more selective than the CFEM. In addition, CEM is simpler and rapid as compared to CFEM while retaining the advantages of the later. Azam *et al.* (1989c) used CEM for the determination of biomass N in 18 agricultural soils from Punjab, Pakistan. The values obtained with CEM were significantly correlated ($r = 0.89$; $P < 0.01$) with those by CFEM. The biomass N determined by CEM accounted for 2.8 to 13.8% of the total soil N; an almost similar variation (2.6 to 14.8%) was observed for CFEM. Biomass N estimated by CFIM under anaerobic conditions accounted for 6.1 to 13.6% of the soil N and showed no significant correlation with values obtained by CFEM and CEM. With CEM it was possible to specifically differentiate between microbial and non-microbial origin of the extracted N. The results clearly demonstrated the extractability of unlabelled N that increased with the amount of C added to the soil. Hence, overestimates of biomass are obtained especially in the presence of increasing levels of an easily oxidizable C source. In spite of this deficiency, however, methods involving $CHCl_3$ in one or the other way still remain the most popular and widely used methods of determining microbial biomass.

Inubushi *et al.* (1991) introduced a modification of the CEM with specific objective of determining biomass in flooded (anaerobic) rice soils. A rigorous shaking of anaerobic soil samples with 10 μl $CHCl_3$ g^{-1} soil was proposed to facilitate uniform exposure of soil to the fumigant. This treatment was superimposed with 24 h fumigation in a desiccator as proposed in CFIM and the samples extracted with 0.5 M K_2SO_4 for the determination of extractable C and N for subsequent calculation of biomass values as suggested in CFIM and CFEM. According to Inubushi *et al.* (1991), the method is useful for determining microbial biomass in flooded or anaerobic soils. The method was further simplified by Gaunt *et al.* (1993) for use with waterlogged soils. One-step fumigation with $CHCl_3$ liquid in closed bottles was found as effective as fumigation in a desiccator under vacuum. Further improvements in the method for the sake of convenience and rapidity were proposed by Witt *et al.* (2000) for use with flooded rice soils and termed CFAP (chloroform-fumigation extraction at atmospheric pressure). In this method, the soil samples (35 g) are fumigated (so it is termed) by adding 2 ml of $CHCl_3$. and agitating for 24 h in the dark at 25°C followed by removal of the fumigant through evaporate in the fume hood at 25°C and extraction with 140 ml of 0.5 M K_2SO_4 and determination of C and N in the extract. Unfumigated samples were extracted straight away with 140 ml of 0.5 M K_2SO_4 Biomass C and N was calculated as the difference in extractable fractions between fumigated and unfumigated soil. Based on a

series of laboratory experiments, Witt *et al.* (2000) found CFAP to be a reliable alternative to the CFEM for measuring biomass C and N; the two methods showed highly significant correlation.

Ninhydrin reaction method: In the CFIM, biomass is measured on the basis of CO_2-C evolved and NH_4-N accumulated in fumigated versus unfumigated soil samples. As a change in approach, Amato and Ladd (1988) proposed to use ninhydrin reactive C and N compounds released during fumigation-incubation as a measure of biomass. They specifically determined that fumigated soils retained protease but lost dehydrogenase activity as well as the ability to decompose glucose and immobilize NH_4-N during 10 days of incubation. They proposed to quantify ninhydrin reactive N compounds released in CFIM (10 days incubation at 25°C, extraction with 2N *K*Cl) and determine biomass N by using a multiplication factor of 21. Thus the method differs from original CFIM in that ninhydrin reactive C and N compounds rather than NH_4-N (or total mineral N) and CO_2 are taken into consideration while calculating biomass. Ocio and Brookes (1990) considered the method suitable for freshly amended soils (CFIM gives unreliable results for such soils) and found good correlation with CFEM and SIRM (next section). Sparling *et al.* (1994) concluded that the ninhydrin method can give a reliable estimate of biomass in organic materials as well as mineral soils. They determined a biomass C factor of 21 x NPC (ninhydrin positive carbon) and biomass N of 3.5 x NPC. They preferred to calculate biomass C by this method and biomass N from the soluble N flush (K_{EN} = 0.38). Van Gestel *et al.* (1993) determined biomass C indirectly by multiplying ninhydrin reactive extractable N of fumigated soils with 21 (Amato and Ladd, 1988); they used 2N *K*Cl for extraction.

As with original CFIM, the disadvantage of ninhydrin reaction method is the length of time (at least 10 days) required for obtaining biomass values, while it has the advantage of giving reliable results for freshly amended soils or soils rich in easily oxidizable C. However, the premise that fumigated soils retain protease activity but loose the ability to decompose organic matter and immobilize N would seem contradictory to the basis of CFIM that relies on microbial activities including C mineralization. These difficulties are fairly overcome if ninhydrin reactive compounds are measured using CFEM rather than CFIM. However, the values obtained are significantly lower compared to total extracted N (Inubushi *et al.*, 1991).

Microcalorimetry: Sparling (1981) proposed microcalorimetry as a method to assess microbial metabolism in soil on the pretext that the heat produced depends only on the initial and final energy states of the system and is independent of the types of organisms or reaction pathway. In addition, the total catabolic activity in the soil is closely related to the heat production, anabolic processes normally contribute a little to the heat. Sparling (1981) studied heat output from 12 soils and compared the results with CFIM and SIR, ATP, dehydrogenase and amylase, and basal respiration. Correlation of heat with some but not all methods was obtained. The rate of heat output from soil is closely related with the rate of respiration. However, microcalorimetric method has not achieved popularity to significant extent.

Microwave irradiation: Microwave irradiation is an effective biocidal treatment of soil which kills weeds, nematodes and microorganisms; the effect on microorganisms being probably entirely thermal (Vela and Wu, 1979), fungi being more susceptible (Wainwright *et al.,* 1980). Spier *et al.* (1986) were probably the first to use as microwave radiation as a soil treatment to measure microbial biomass, an approach akin to $CHCl_3$ fumigation. They found that both eukaryotes and prokaryotes are equally susceptible to killing effect of radiation. Generally 90 sec irradiation was enough to get an effect similar to that of $CHCl_3$ fumigation in terms of mineralization/extractability of C and N. In spite of its simplicity, this method has not found a wide spread acceptability.

Cellular contents: In theory, the soil biomass can be determined by measuring its own constituents. but there is some difficulty in the choice of constituent. Jenkinson and Ladd (1981) suggested that a suitable constituent should meet four conditions at the same time: I) it should be uniformly present at the same concentration in all biomass, ii) it should be present in the living cells alone and should not be adsorbed on soil organic matter/clay or if adsorbed should be readily degradable, iii) it should be extractable reproducibly and quantitatively, and iv) the method of its determination should be accurate and reproducible. No cell constituent fully meets these conditions. The constituents tried include, ATP, muramic acid, nucleic acid bases and N-acetylglucosamine (Jenkinson and Ladd, 1981). Of these constituents, ATP has been the most frequently used. It has been used as an indicator of microbial activity (Lee *et al.,* 1971) and as a means of calculating microbial biomass (Oades and Jenkinson, 1979; Jenkinson *et al.,* 1979). Jenkinson *et al.* (1979) and Sparling and Eiland (1983) found a close

relationship between ATP and biomass as measured by CFIM. Nannipieri *et al.* (1978) found soil P to be an important factor in determining ATP. Extractability and reliable determination have been the main limitations in using ATP as a measure of microbial biomass. However, Verstraete *et al.* (1983) further evaluated the procedure and found it 80% efficient for ATP added to the soil. They found the method rapid, specific for microbial biomass, and applicable to all soils. ATP is assayed from the amount of light emitted by the luciferin luciferase system. The procedure is extremely sensitive and 10-14 mol of ATP can be measured.

ATP contents vary widely according to the growth conditions of microorganisms. For example, under P deficiency (Nannipieri *et al.*, 1978), ATP content of biomass will be low, while it may rise in glucose-amended soils during and shortly after the period of maximum biological activity (Oades and Jenkinson, 1979). Values ranging 3 to 6 μmol g^{-1} dry biomass have been reported (Lee *et al.*, 1971, Oades and Jenkinson, 1979; Jenkinson *et al.,* 1979). According to Jenkinson and Ladd (1981), close agreement between the mean values for the ATP contents of prokaryotic and eukaryotic organisms grown in vitro and the measured values for the soil biomass strongly suggests that the concentration of ATP in the largely resting soil population is little different from that in actively growing organisms.

Concluding remarks: Each of the biomass methods mentioned has its limitations. The microscopic methods require skilled personnel and often present difficulties in distinguishing between living and dead cells. In addition a number of assumptions have to be made before the counts are converted into weights. The extraction methods often fail to fully extract the component of interest and the recovery often depends on the characteristics or type of extraction procedure used. Also, the quantity of particular cell component can vary considerably with growth conditions and within different members of the microbial community. The ratio of specific component to the actual biomass is therefore not necessarily constant. Jenkinson and Ladd (1981) suggested that more than one method may be used for biomass determination in acid soils or those undergoing rapid metabolic changes.

Synthesis and dynamics of microbial biomass from different substrates: Availability and use of isotopic techniques have greatly helped in studying the formation and breakdown of microbial biomass in soil. For example, it has become quite convenient to study the fate (including incorporation into the biomass) of^{4} C-labelled plant residues and components added to the soil. Similarly, microbial biomass can be isotopically labelled under laboratory conditions or in soil *in situ* to study its further transformations. Indirect evidence of the synthesis and breakdown of biomass can also be obtained by following immobilization-remineralization turnover of N, a process that is governed to a significant extent by the nature of carbonaceous materials available to soil microorganisms. With the background *kn*owledge of C/N ratio of the microbial biomass, the later can be quantified at any stage of the study from the amount of inorganic N transformed into organic forms (courtesy microorganisms). A major assumption while making such calculations will be that microbial immobilization is the main, if not the sole, process of consuming the applied N. The methods described above have been of great help to study the synthesis of microbial biomass and its subsequent fate in soil.

Generally 2-3% of the soil C is found in microbial biomass (Jenkinson and Ladd, 1981). However, values higher than this have often been reported (Azam *et al.*, 1989c). Relationship of other nutrient elements to C being *kn*own, quantitative information on other nutrients (e.g., N and P) contained in microbial biomass can easily be gathered as reported by Anderson and Domsch (1980). In most studies, not more than 60% of the substrate C is converted into biomass. For plant materials, the rate of synthesis of microbial biomass depends on the chemical constituents (Amato and Ladd, 1980). Substrates like glucose are rapidly metabolized within 1-2 days followed by a quick decline in the biomass which itself becomes a substrate for the succeeding microflora (Azam *et al.,* 1985; Haider and Azam, 1982). Stott *et al.* (1983) followed the fate in soil of ^{14}C-labelled lignins, polysaccharides and protein fractions for one year and determined 4.9-7.8 and 4.6-13.4% of the residual C from polysaccharides and proteins, respectively, in the biomass, while a clear decrease with time was evident. Nicolardot *et al.* (1994) studied carbon and nitrogen cycling through soil microbial biomass at various temperatures (4-28°C) for 140 days. Before incubation the soil was amended with $K^{15}NO_3$ and either ^{14}C-glucose or ^{14}C-holocellulose. More ^{14}C was found in biomass at lower temperature probably because of higher mineralization of biomass at elevated temperature. Some studies have indeed been reported on the synthesis *in situ* and breakdown of microbial biomass taking immobilization-remineralization of N as an index of biomass dynamics (Azam *et al.*, 1988, 1989a,b). In some other studies, the build-up and decrease of microbial biomass has been studied using ^{14}C and ^{15}N isotopes (Azam *et al.*, 1985; Haider and Azam, 1982; Ladd *et al.*, 1985; van Veen *et al.*, 1985). From these studies, it

appeared that the immobilization and release of nutrients e.g., N could serve as an indirect index of the persistence of the newly synthesized microbial biomass. In studies using ^{14}C-labelled lignins, polysaccharide and protein fractions 4.9-7.8% and 4.6-13.4% of the residual C from polysaccharides and proteins was found to be present in biomass after one year, a decrease with time was observed (Stott *et al.*, 1983).

While studying transformation of added C into biomass, attention needs to be given to the native organic C content of the soil. For example, Cerri and Jenkinson (1980) studied the transformation of ^{14}C-labelled plant residues into biomass and found less incorporation in acid than neutral soils. They attributed this difference to pH and clay but overlooked the significant effect of native organic matter that was 3 times higher in acid clay soil. These authors attributed difference in the transformation of added ^{14}C-labelled ryegrass to soil pH. However, a critical evaluation of their data will suggest that it was the amount of native soil C in the two soils that mattered. Acid soil had higher organic matter than neutral soil and thus less decomposition and incorporation of added material into microbial biomass. Net decomposition was similar in the two soils. There are indications that the CFIM cannot be applied to strongly acid soils with pH 4.5 and less (Jenkinson and Ladd, 1981). A strongly acid soil used by Powlson and Jenkinson (1976) did not give a flush and yet contained appreciable amount of biomass, while a similar soil used by Cerri and Jenkinson (1980) gave a flush.

Residence time of microbial biomass in soil may vary from days to years depending upon the quantity and quality of the carbonaceous substrate available and the soil conditions (Jenkinson and Ladd, 1981). Biomass developed at the expense of easily oxidizable C like glucose and sucrose being more labile compared to that synthesized from more complex materials (Azam *et al.*, 1986,1988; Stott *et al.*, 1983). Decomposition of microbial biomass and cells will mainly depend on the community structure that changes with the chemical composition of the substrate. For example, biomass of hyaline fungi developed at the expense of easily oxidizable C compounds will decompose rapidly as compared to melanoid mycelium. Indeed, the pure culture studies served as the basis for the development of CFIM of determining microbial biomass (Jenkinson, 1976; Jenkinson and Powlson, 1976b), the proportion of biomass C mineralized (*K* value) being an important part. As such, the earlier discussion related to *K* factor would suffice to understand at least the rate of decomposition of microbial material under different conditions. However, it varies from 0.21 to 0.55 depending upon the incubation conditions and the type of soil (Jenkinson, 1966, 1976; Adams and Laughlin, 1981; Anderson and Domsch, 1978; Grace *et al.*, 1993). As mentioned earlier, in most of the studies, *KC* factor has been determined by subjecting the laboratory-raised microbial material to decomposition/mineralization and hence may not represent the native microflora of a particular test soil. Voroney and Paul (1984) were the first to determine *KC* in soil *in situ* by incubating soil samples with ^{14}C-labelled glucose and determined a factor of 0.41.

Marumoto *et al.* (1982) studied the decomposition and mineralization of ^{14}C- and ^{15}N-labelled dried biomass from several bacterial and fungal species. After 10 days, up to 43% of the labelled C was released as CO_2 and up to 50 % after 28 days; up to 33% of microbial N was mineralized. In a ^{14}C-labelled soil, the amount of labelled ^{14}C mineralized during incubation after fumigation was greater than could be accounted for by the decrease in soil biomass (Shields *et al.*, 1974), suggesting that $CHCl_3$ fumigation caused the release of some non-biomass C.

The persistence of microbial biomass depends, like any other organic matter entity, on the phyico-chemical characteristics of the soil and management practices. For example, soil porosity also affects the decomposition of organic matter and the build-up of microbial biomass due to spatial distribution of substrate and microbes (Killham *et al.*, 1993). Ploughing and other soil operations involving physical disruption of soil are usually considered to accelerate the decomposition of soil organic matter including microbial biomass. Cultivation may cause a rapid increase in the size of microbial population that is quickly reduced as the sources of available C decrease and the soil dries out. Likewise, an increase in mineral N content following soil disturbance is due to the mineralization of biomass (Powlson, 1980; Sorenson, 1983). Cultivation also promotes the decomposition of recently synthesized microbial metabolites and enhances substrate depletion (Grace *et al.*, 1993).

Increased respiration or mineralization of N has been observed after compression and shearing, grinding, ultrasonic treatment, and chemical treatments like fumigation etc. (Jenkinson, 1976). Grinding more than doubled the respiration rate in two soils (Powlson, 1980); the increase being smaller in previously fumigated soil suggesting that the flush of decomposition is caused in part from killed organisms and in part from enhanced decomposition of non-biomass organic matter. Increased in mineralization following grinding is attributed to an enhanced exposure of substrates (Rovira and Greacen, 1957). The non-biomass material released by grinding contains a large proportion of polysaccharides, which are thought to stabilize soil aggregates and have a high C/N

ratio (Powlson, 1980).

Soil drying causes a rapid decrease in biomass, while drying and rewetting promote the turnover of C derived from added plant material mainly as a result of enhanced turnover of microbial products (Van Gestel *et al*., 1993). Short-term increase in the C and N mineralization in soil following physical treatments is believed to have a biological origin. This may primarily be due to a physical effect of desiccation upon soil organic matter getting exposed to microbial action because of aggregate fragmentation or increased porosity. Marumoto *et al.* (1977) demonstrated that cell cytoplasmic compounds become easily mineralizable when active organisms are dried at low temperatures, whereas the cell walls are more resistant to degradation. Factors other than soil characteristics that may determine decreases in biomass by drying are the inherent properties of soil microbial populations. Soil microorganisms are not homogeneous as they differ in age, type, physiological, and metabolic state. Sorenson (1983) studied the influence of stress treatments on the dynamics of microbial biomass and humus in soils with variable clay content. He used ^{14}C-labelled glucose, cellulose, hemicellulose, maize straw and barley straw. After 2 years 10% of the ^{14}C was in biomass determined by CFIM. Heating, grinding, drying etc. enhanced the mineralization of C and biomass.

Van Veen *et al.* (1985) observed decreases in biomass C and flushes of mineralization after desiccation and remoistening of soils. They found clays to exert a positive effect on the formation and persistence of biomass. Ladd *et al.* (1985) also suggested that heavier soils (clayey) retained higher biomass. This would suggest that factors other than clays may be involved. Sorenson (1983) studied the influence of stress treatments on the dynamics of microbial biomass and humus in soils with variable clay content. He used ^{14}C-labelled glucose, cellulose, hemicellulose, maize straw and barley straw. After 2 years 10% of the ^{14}C was in biomass determined by CFIM. Heating, grinding, drying etc. enhanced the mineralization of C and biomass.

Microbial biomass and plant nutrition: Soil microorganisms play a major role not only in the decomposition of organic matter but also serve as a sink for plant nutrients in soil that are made available slowly during the cropping period. Despite the small size, biomass makes a contribution to plant nutrition far greater than could be anticipated because of its labile nature. Portions of microbial biomass are always killed by the changing environmental conditions as discussed in the previous section. Since cells are readily mineralized by the surviving microflora (Jenkinson, 1976; Anderson and Domsch, 1978), it has been suggested that they contribute substantially to the pool of mobile plant nutrients in soil (Anderson and Domsch 1980). Studies have indicated that N from microbial cell walls and cell contents makes an essential contribution to the readily mineralizable organic N in soil (Marumoto *et al*., 1977).

Anderson and Domsch (1980) indirectly estimated the amount of nutrient elements in the microbial biomass of 26 soils. The average amount of N, P, K and Ca stored in the vegetative cells of microflora of arable soils (upper 12.5 cm) was estimated to be about 108, 83, 70 and 11 kg ha^{-1}, respectively. Jenkinson and Ladd (1981) determined biomass N and biomass P to be 95 and 11 kg ha^{-1} with a flux of 38 and 5 kg ha^{-1} yr^{-1}, respectively. They found a close relationship between biomass N and plant uptake of N; a similar relationship was found for P taking C/P ratio as 50. Azam *et al.* (1989c) found biomass N to be 64-186 kg ha^{-1} and it showed a significant correlation with N uptake by wheat plants. Witt *et al.* (2000) demonstrated a clear indication that biomass underwent a transition from sink to source of plant nutrients, flooded rice being the indicator crop.

Except for the studies just referred there are hardly any estimates of a direct impact of microbial biomass on plant nutrition. Nevertheless, sufficient indirect evidence is available to suggest a significant role of microbial nutrients in plant nutrition. For example, biomass C represents 2-3% of the total soil C that is equivalent to 44-66 tons ha^{-1}. A nitrogen equivalent of this amount will be 440-660 kg ha^{-1}. In view of the labile nature of microbial biomass, especially of the newly synthesized fraction, a reasonable proportion of the crop N may actually come from the biomass.

Microbial biomass is a small but labile component of soil organic matter and makes a significant contribution to the plant available nutrients. The most important aspect of microbial biomass is its dynamic nature that facilitates recycling of nutrients in the soil-plant system. Being the living component of soil matrix, microbial biomass may serve as a good index of biological status of a soil. In view of the importance of microbial biomass in organic matter dynamics and nutrient cycling, a variety of methods have been developed and used over the past 3 decades. The methods differ in reproducibility and convenience, but quite a few of them show reasonably comparable results. Of the available methods, those using $CHCl_3$ have been most popular and used extensively. Several modifications/ refinements have been made in the original CFIM and it appears that direct incorporation of $CHCl_3$ in the extractant may ultimately be the method of choice for extracting nutrients from the biomass and their subsequent quantification by appropriate methods.

References

Adams, T. and R.J. Laughlin, 1981. The effects of agronomy on carbon and nitrogen contained n the soil biomass. J. Agric. Sci. Cambridge, 97: 319-327.

Amato, M. and J.N. Ladd, 1980. Formation and distribution of isotope labeled biomass during decomposition of ^{14}C and ^{15}N-labelled plant material. Soil Biol. Biochem., 12: 405-411.

Amato, M. and J.N. Ladd, 1988. Assay for microbial biomass based on ninhydrin reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem., 20: 107-114.

Anderson, J.P.E. and K.H. Domsch, 1973. Quantification of bacterial and fungal contributions to soil respiration. Arch. Mikrobiol., 93: 113-127.

Anderson, J.P.E. and K.H. Domsch, 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem., 10: 215-221.

Anderson, J.P.E and K.H. Domsch, 1980. Quantities of plant nutrients in the microbial biomass of selected soils. Soil Sci., 130: 211-216.

Azam, F., K. Haider and K.A. Malik, 1985. Transformation of ^{14}C labeled plant residues and components in soil in relation to immobilization and remineralization of ^{15}N fertilizer. Plant Soil, 86: 15-25.

Azam, F, K.A. Malik and F. Hussain, 1986. Microbial biomass and mineralization-immobilization of nitrogen in some agricultural soils. Biol. Fertil. Soils, 2: 157-163.

Azam, F., R.L. Mulvaney and F.J. Stevenson, 1989a. Synthesis of ^{15}N-labelled microbial biomass in soil in situ and extraction of biomass N. Biol. Fertil. Soils, 7: 180-185.

Azam, F., F.J. Stevenson and R.L. Mulvaney, 1988. Determination of *in situ KN* by chloroform fumigation-incubation method and mineralization of biomass N under anaerobic conditions. Plant Soil, 111: 87-93.

Azam, F., F.J. Stevenson and R.L. Mulvaney, 1989b. Chemical extraction of newly immobilized ^{15}N and native soil N as influenced by substrate addition rate and soil treatments. Soil Biol. Biochem., 21: 715-722.

Azam, F., M. Yousaf, F. Hussain and K.A. Malik, 1989c. Determination of biomass in some agricultural soils of Punjab, Pakistan. Plant Soil, 113: 223-228.

Babiuk, L.A. and E.A. Paul, 1970. The use of fluorescein isothiocyanate in the determination of bacterial biomass of grassland soil. Can. J. Microbiol., 16: 57-62.

Bailey, V.L., A.D. Peacock, J.L. Smith and H. Bolton Jr., 2002. Relationship between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipids fatty acid analysis. Soil Biol. Biochem., 34: 1385-1389.

Balkwill, D.L., D.P. Labeda and L.E. Casida Jr., 1975. Simplified procedures for releasing and concentrating microorganisms from soil for transmission electron microscopy viewing as thin-sectioned and frozen-etched preparations. Can. J. Microbiol., 221: 1040-1045.

Brookes, P.C., A. Landman, G. Pruden and D.S. Jenkinson, 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem., 17: 837-842.

Cerri, C.C. and D.S. Jenkinson, 1980. Formation of microbial biomass during the decomposition of ^{14}C-labelled ryegrass in soil. J. Soil Sci., 32: 619-626.

Cheng, W. and R.A. Virginia, 1993. Measurement of microbial biomass in arctic Tundra soils using fumigation extraction and substrate-induced respiration procedures. Soil Biol. Biochem., 25: 135-141.

Frankland, J.C., 1974. Importance of phase-contrast microscopy for estimation of total fungal biomass by the agar-film technique. Soil Biol. Biochem., 6: 409-410.

Gaunt, J.L., H.U. Neue, K.G. Gassman, D.C. Olk, J.R.M. Arah, C. Witt, J.C.G. Ottow and I.F. Grant, 1993. Microbial biomass and organic matter turnover in wetland rice soils. Biol. Fertil. Soils, 19: 333-342.

Grace, P.R., I.C. MacRae and J.K. Myers, 1993. Temporal changes in microbial biomass and N mineralization under simulated field cultivation. Soil Biol. Biochem., 25: 1745-1753.

Gregorich, E.G., B.C. Liang, C.F. Drury, A.F. Mackenzie and W.B. McGill, 2000. Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils. Soil Biol. Biochem., 32: 581-687.

Haider, K. and F. Azam, 1982. Turnover of ^{14}C-labelled plant components and ^{15}N-ammonium in soil. Z. Pflanzenernaehr. Bodenk., 146: 151-159.

Inubushi, K. and I. Watanabe, 1987. Microbial biomass nitrogen in anaerobic soil as affected by N-immobilization and N_2-fixation. Soil Sci. Plant Nutr., 33: 213-224.

Inubushi, K., D.S. Jenkinson and P.C. Brookes, 1991. Soil microbial biomass C, N and ninhydrin-N in aerobic and anaerobic soils measured by the fumigation-extraction method. Soil Biol. Biochem., 23: 737-741.

Jenkinson, D.S., 1966. Studies on the decomposition of plant material in soil II- partial sterilization of soil and and the soil biomass. J. Soil Sci., 17: 280-302.

Jenkinson, D.S., 1976. The effects of biocidal treatments on metabolism in soil. IV. The decomposition of fumigated organisms in soil. Soil Biol. Biochem., 8: 203-208.

Jenkinson D.S. and J.N. Ladd, 1981. Microbial biomass in soil: measurement and turnover. In: Soil Biochemistry (Paul, E.A. and J.N. Ladd, eds), Dekker, New York., pp: 415-471.

Jenkinson, D.S. and D.S. Powlson, 1976a. The effects of biocidal treatments on metabolism in soil . I. Fumigation with chloroform. Soil Biol. Biochem., 8: 167-177.

Jenkinson, D.S. and D.S. Powlson, 1976b. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem., 8: 209-213.

Jenkinson, D.S., S.A. Davidson and D.S. Powlson, 1979. Adenosine triphosphate and microbial biomass in soil. Soil Biol. Biochem., 11: 521-527.

Jenkinson, D.S., D.S. Powlson and R.W. Wedderburn, 1976. The effects of biocidal treatments on metabolism in soil. V. The relationship between soil biovolume, measured by optical microscopy, and the flush of decomposition caused by fumigation. Soil Biol. Biochem., 8: 189-202.

Killham, K., M. Amato and J.N. Ladd, 1993. Effect of substrate location in soil and soil pore-water regime on carbon turnover. Soil Biol. Biochem., 25: 57-62.

Ladd, J.N., M. Amato and J.M. Oades, 1985. Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope-labelled legume material and soil organic matter, decomposing under field conditions. Aust. J. Soil Sci., 23: 603-611.

Lee, C.C., R.F. Harris, J.D.H. Williams, D.E. Armstrong and J.K. Syers, 1971. Adenosine triphosphate in lake sediments. I. Determination. Soil Sci. Soc. Am. Proc., 35: 82-86.

Malik, K.A. and F. Azam, 1980. Effect of salinity on ^{14}C-labelled microbial biomass and its contribution to soil organic matter. Pak. J. Bot., 12: 117-127

Martens, R., 1995. Current methods for measuring microbial biomass C in soil: potentials and limitations. Biol. Fertil. Soils, 19: 87-99

Martin, J.P., 1963. Influence of pesticide residues on soil microbiological and chemical properties. Residue Rev., 4: 96-129.

Marumoto, T., 1984. Transformation of C and N from microbial biomass in paddy soil. Plant Soil, 76: 165-173.

Marumoto, T., J.P.E. Anderson and K.H. Domsch, 1982. Decomposition of ^{14}C- and ^{15}N-labelled microbial cells in soil. Soil Biol. Biochem., 14: 461-467.

Marumoto, T., H. Kai, T. Yoshida and T. Harada, 1977. Chemical fractions of organic nitrogen in acid hydrolysates given from microbial cells and their cell wall substances and characterization of decomposable soil organic matter due to drying. Soil Sci. Plant Nutr., 23: 125-134.

Miller, W.N. and L.E. Casida Jr., 1970. Evidence for muramic acid in soil. Can. J. Microbiol., 16: 299-304.

Nannipieri, P., R.L. Johnson and E.A. Paul, 1978. Criteria for measurement of microbial growth and activity in soil. Soil Biol. Biochem., 10: 223-229.

Nicolardot, B., G. Fauvet and D. Cheneby, 1994. Carbon and nitrogen cycling through soil biomass at various temperatures. Soil Biol. Biochem., 26: 253-261.

Nunan, N., M.A. Morgan, J. Scott and M. Herlihy, 2000. Temporal changes in nitrogen mineralization, microbial biomass, respiration and protease activity in a clay loam soil under ambient temperature. Proc. Royal Irish Acad., 100: 107-114.

Oades, J.M. and D.S. Jenkinson, 1979. Adenosine triphosphate content of the soil microbial biomass. Soil Biol. Biochem., 11: 201-204.

Ocio, J.A. and P.C. Brookes, 1990. An evaluation of methods for measuring the microbial biomass in soils following recent additions of wheat straw, and the characterization of the biomass that develops. Soil Biol. Biochem., 22: 685-694.

Paul, E.A. and R.P. Voroney, 1980. Nutrient and energy flows through soil microbial biomass. In: Contemporary Microbial Ecology (Ellwood, D.C., J.N. Hedger, M.J. Latham and J.M. Lynch, eds), Academic Press, New York, pp: 215-237.

Powlson, D.S., 1980, The effects of grinding on microbial biomass and non-microbial organic matter in soil. J. Soil Sci., 31: 77-85.

Powlson, D.S. and D.S. Jenkinson, 1976. The effects of biocidal treatments on metabolism in soil. II. Gamma irradiation, autoclaving, air-drying and fumigation. Soil Biol. Biochem., 8: 179-188.

Rovira, A.D. and E.L. Greacen, 1957. The effect of aggregate disruption on the activity of microorganisms in soil. Aus. J. Agric. Res., 8: 659-673.

Shields, J.A., E.A. Paul and W.E. Lowe, 1974. Factors influencing the stability of labelled microbial materials in soils. Soil Biol. Biochem., 6: 31-37.

Skujins, J.J., 1967. Enzymes in soil. In: Soil Biochemistry (McLaren, A.D. and G.H. Peterson, eds), vol 1, Marcel Dekker, New York, pp: 371-414.

Smith, J.L., J.J. Halvorson and H. Bolton Jr., 1995. Determination and use of a corrected control factor in the chloroform fumigation method of estimating soil microbial biomass. Biol. Fertil. Soils, 19: 287-291.

Sorenson, L.H., 1983. Size and persistence of microbial biomass formed during the humification of glucose, hemicellulose, cellulose and straw in soils containing different amounts of clay. Plant Soil, 75: 121-130.

Sparling, G.P., 1981. Microcalorimetry andother methods to assess biomass and activity in soil. Soil Biol. Biochem., 13: 93-98.

Sparling, G.P. and Eiland, F., 1983. A comparison of methods for measuring ATP and microbial biomass in soils. Soil Biol. Biochem., 15: 227-229.

Sparling, G.P., S.A. Brandenburg and C. Zhu, 1994. Microbial C and N in revegetated whatbelt soils in Western Australia: Estimation in soil, humus and leaf litter using the ninhydrin method. Soil Biol. Biochem., 26: 1179-1184.

Spier, T.W., J.C. Cowling, G.P. Sparling, A.W. West and D.M. Corderoy, 1986. Effects of microwave radiation on the microbial biomass, phosphatease activity and levels of extractable N and P in a low fertility soil under pasture. Soil Biol. Biochem., 18: 377-382.

Stott, D., G. Kassim, W.M. Jarrell, J.P. Martin and K. Haider, 1983. Stabilization and incorporation into biomass of specific plant carbons during biodegradation in soil. Plant Soil, 70: 15-26.

Turner, B.L., A.W. Bristow and P.M. Haygarth, 2001. Rapid estimation of microbial biomass in grassland soils by ultra-violet absorbance. Soil Biol. Biochem., 33: 913-919.

Van Grestel, M., R. Mercxk and K. Vlassak, 1993. Soil drying and rewetting and the turnover of ^{14}C-labelled plant residues: first order decay rates of biomass and non-biomass ^{14}C. Soil Biol. Biochem., 25: 125-134.

Van Veen, J.A., J.N. Ladd and M. Amato, 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)glucose] and [^{15}NH$_4$)$_2$SO$_4$] under different moisture regimes. Soil Biol. Biochem., 17: 747-756.

Vance, E.D., P.C. Brookes and D.S. Jenkinson, 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem., 17: 703-707.

Vela, G.R. and J.F. Wu, 1979. Mechanism of lethal action of 2450-MHz radiation on microorganisms. Appl. Environ. Microbiol., 37: 550553.

Verstraete, W, H. Van de Werf, F. Kucnerowicz, M. Ialiwi, L.M.J. Verstraeten and K. Vlassak, 1983. Specific measurement of soil microbial ATP. Soil Biol. Biochem., 15: 391-396.

Voroney, R.P. and E.A. Paul, 1984. Determination of K_C and K_N in situ for calibration of the chloroform fumigation-incubation method. Soil Biol. Biochem., 16: 9-14.

Wainwright, M., K. Killham and M.F. Diprose, 1980. Effects of 2450-MHz radiation on nitrification, respiration and S-oxidation in soil. Soil Biol. Biochem., 12: 489-491.

Witt, C., J.L. Gaunt, C.C. Galicia, J.C.G. Ottow and H.U. Neue, 2000. A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. Biol. Fertil. Soils, 30: 510-519.