Published 1996

12 Role of Microbial Biomass Carbon and Nitrogen in Soil Quality[1]

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Microbial biomass in soil is the living component of soil organic matter. Many models of organic matter formation include microbial biomass as a precursor to the more stable fractions of organic matter (Parton et al., 1987). Because as much as 95% of the total soil organic matter is nonliving and, therefore, relatively stable or resistant to change, decades may be required to observe a measurable change in soil organic matter. Microbial biomass has a turnover time of <1 yr (Paul, 1984) and therefore, responds rapidly to conditions that eventually alter soil organic matter levels. Thus, the size of the microbial biomass may indicate degradation or aggradation of soil organic matter (Powlson et al., 1987; Sparling, 1992).

As an active component of soil organic matter, soil microbial biomass is involved in nutrient transformations and storage. Nutrients released during turnover of the microbial biomass are often plant available. In native terrestrial ecosystems, where internal cycling of N predominates, microbial biomass is responsible for transforming organic N to plant-available forms. Agricultural systems that rely upon internal sources of N require microbial biomass and its activity to supply N to the crop. In fertilized systems, microbial biomass can be a significant source and sink of N. Carbon contained within the microbial biomass is

[1] This work was partially supported by NSF grant BSR-9011662 to Kansas State University. Contribution no. 95- 392-B from Kansas Agric. Exp. Stn., Manhattan, KS.

Methods for Assessing Soil Quality, SSSA Special Publication 49.

stored energy for microbial processes. Therefore, microbial biomass C may indicate potential microbial activity.

Because of its rapid turnover, microbial biomass is a sensitive indicator of changes in climate (Insam et al., 1989; Insam, 1990), tillage systems (Lynch & Panting, 1980; Carter, 1991), crop rotations (Anderson & Domsch, 1989; Campbell et al., 1991), and pollutant toxicity (Chander & Brookes, 1991a,b, 1993). Microbial biomass content is a function of other soil properties, including pH, texture, and soil water content. Microbial biomass varies with soil texture, probably because of the effect of texture on aggregate formation, thus protecting organic C (Schimel, 1986; Burke, 1989; Gregorich et al., 1991; Zagal, 1993). Microorganisms also play a major role in the formation and maintenance of soil aggregates and structure (Tisdall & Oades, 1982). Microbial biomass C and soil aggregate stability are strongly related (Ross et al., 1982; Haynes & Swift, 1990; Robertson et al., 1991). Because microbial biomass integrates soil physical and chemical properties and responds to anthropogenic activities, it may be considered a suitable biological indicator of soil quality.

The importance of microbial biomass to soil has generated interest from the research community resulting in the development of several analytical procedures. The most widely used procedure is some form of soil fumigation with chloroform. Fumigation results in lysis of the microbial cells. The traditional method involves incubation of fumigated soil for 10 d where the surviving microorganisms convert the organic C and N of lysed cells to CO_2 and to NH_4^+, respectively. This procedure is known as the fumigation-incubation (FI) technique (Jenkinson & Powlson, 1976; Voroney & Paul, 1984). A more recent alternative to the conventional FI procedure is known as the fumigation-extraction (FE) technique (Brookes et al., 1985; Vance et al., 1987a; Amato & Ladd, 1988; Sparling & West, 1988). In this method, the C and N released from cells by fumigation are extracted with a dilute salt solution (0.5 *M* K_2SO_4) and measured directly. Both methods are now widely used.

Direct counts of microorganisms also can be made by separating the cells from soil, staining with fluorescent dyes, and counting by microscopic observation (Schmidt & Paul, 1982). Microbial biomass C and N can then be estimated by converting cell counts and their estimated C, N, and water content to biomass C and N equivalents. These techniques require moderately priced equipment, skilled personnel, and are time-consuming.

Substrate-induced respiration (SIR) is an alternate technique that relies upon the response of the microbial population to the addition of a readily degraded substrate such as glucose (Anderson & Domsch, 1978; Beare et al., 1990). This method provides an indication of the activity of the microbial biomass and hence the size of the physiologically active biomass. Disadvantages of this technique include increased sensitivity to sample handling, the need to standardize soil water content and temperature, and the inability to measure microbial biomass N.

We recommend the fumigation techniques because of their ease of use, existence of a large database, lower equipment costs, and the ability to measure mineralizable C and N from the unfumigated soil samples. The FI technique permits use of existing resources in many laboratories and also gives an estimate of

C and N mineralization as discussed by Parkin et al. (1996, this publication). Laboratories with resources and/or soil conditions that limit the use of FI (low pH, too low or high soil water content) should consider the FE technique. The reader should be aware of the advantages and disadvantages of other existing techniques and development of new techniques to measure microbial biomass in soils. More detailed reviews of the various techniques for estimating microbial biomass are available in the literature (Jenkinson & Ladd, 1981; Jenkinson, 1988; Hulm et al., 1991; Horwath & Paul, 1994).

RECOMMENDED PROCEDURE

Equipment and Reagents: Fumigation Incubation–Extraction

Materials

ethanol-free chloroform

Note: All work must be done in an adequate fume hood because chloroform has carcinogenic and volatile properties. Commercially available ethanol-free chloroform preserved with heptachlor epoxide has obtained similar results to that of purified chloroform (Voroney et al., 1991).

vacuum pump
vacuum desiccator
125 mL Erlenmeyer flasks
canning jars (1 L capacity)
2 *M* KCl

Procedure

Soil Storage and Preparation

After sampling, the soil should be kept moist and cool. The soil can be stored overnight at 15°C or at 4°C for up to 10 d. Freezing and complete drying is not recommended because of their potential biocidal effects. The soil sample is normally sieved through a 2- to 6-mm sieve to improve sample uniformity. A 2-mm sieve is preferable due to conformity with the other soil quality analyses. Soil samples that are water-saturated should be partially dried before sieving.

Weigh out duplicate soil samples, usually 25 to 50 g, into 125 mL Erlenmeyer flasks. A third sample should be weighed for gravimetric soil moisture determination (105°C for 24 h). Soil water content can affect the determination of microbial biomass. Soil samples should be adjusted to optimum soil water content to maximize the response after $CHCl_3$ fumigation. Horwath and Paul (1994) recommend 55% of water-holding capacity (WHC). If water-holding capacity is known, then water is added to field-moist soil or removed from (by air-drying at 5 to 15°C) field-moist soil to attain 55% WHC. Alternatively, Doran recommends adjusting the soil water content to 55% water-filled pore space (WFPS). To do this, the volume of field-moist soil is measured and the soil is packed to a known

bulk density, usually 1.1 g cm^{-3}. The following equation can be used to calculate the water-filled pore space:

$$\text{Soil water-filled pore space} = \frac{((\%\ \text{soil water content}/100,\ \text{g g}^{-1}) \times \text{bulk density, g cm}^{-3}))}{(1 - (\text{soil bulk density, g cm}^{-3}/\text{particle density, g cm}^{-3}))}$$

If not directly measured, the density of mineral soil particles is commonly assumed to be 2.65 g cm^{-3}. Water then is added to adjust the WFPS to 55%.

Changes in the size of the microbial biomass may result from rewetting of dry soil, releasing organic C and N available for mineralization. To avoid this pulse of microbial activity, the samples should be pre-incubated for 7 to 10 d at 22 to 25°C after adjustment of soil water. Pre-incubation also may overcome storage effects.

Chloroform–Fumigation

After the pre-incubation period, one set of the duplicate soil samples is placed in a vacuum desiccator. The flasks need to be labeled with a pencil, because the chloroform will dissolve ink. The desiccator is lined with a moist paper towel to prevent drying of the sample during fumigation. A beaker containing 50 mL of ethanol-free chloroform and antibumping granules is placed in the desiccator. The desiccator is evacuated until the chloroform boils vigorously for approximately 30 s; air then is allowed to pass into the desiccator to distribute the chloroform into the soil. This process is repeated three times. After the fourth evacuation, the chloroform is allowed to boil for 2 min., and the desiccator valve is closed. The samples are fumigated for 18 to 24 h for the FI procedure and 24 to 48 h for the FE procedure. For most soils, 24 h is suitable; for FE however, 48 h of fumigation improves the "kill" in some soils and may be the method of choice where the optimum period of fumigation is not known. After the fumigation period, the paper towel and the chloroform are removed, and the desiccator is evacuated eight times for 3 min, letting air pass into the desiccator after each evacuation.

The other set of duplicate samples serves as the control or unfumigated sample. In the FI procedure, the samples are incubated under similar conditions as the fumigated samples except they are not evacuated or treated with $CHCl_3$. In the FE procedure, the controls samples are extracted immediately as described later.

Incubation Procedure

The fumigated and unfumigated samples are placed into airtight containers, a few milliliters of water are added to the bottom of the jar to prevent desiccation, and the containers are incubated for 10 d in the dark at 22°C (room temperature). Canning jars (1 L) make excellent containers for this purpose. A vial containing 1 mL of 2 *M* NaOH is placed in the jar to trap respired CO_2. To account for CO_2

in air, blanks are CO_2 traps in jars without soil maintained during the incubation period. After 10 d, $BaCl_2$ is added at an equivalent amount of the initial NaOH concentration (1 mL of 2 *M* $BaCl_2$). The excess NaOH is titrated with 0.1 *M* HCl to pH 7 or to a phenolphthalein endpoint. The volume of acid required to neutralize a blank minus that required to neutralize the sample is used to calculate the amount of CO_2 respired. One milliliter of 2 *M* NaOH will trap 12 mg CO_2–C. For soils with high microbial biomass, such as grassland soils or soils amended with organic materials, greater volumes of NaOH may be required (2 to 5 mL). The accumulated CO_2 in the headspace also can be measured by gas chromatography or with an infrared gas analyzer, if available. To determine the amount of microbial biomass N, the soil is extracted with 2 *M* KCl at a solution to soil ratio of 4:1. The solution is shaken for 30 min and filtered with acid-washed Whatman no. 42 filter paper. The filtrate then is analyzed for NH_4^+ and NO_3^-.

Extraction Procedure

To each of the fumigated and nonfumigated sample flasks, add 100 mL of 0.5 *M* K_2SO_4, stopper and shake for 30 min. After shaking, the solutions are filtered through preleached or acid-washed Whatman no. 42 filter paper. The filtrates are then analyzed for their concentrations of total C and N by wet chemical procedures. Organic C in the filtrates is measured by a dichromate digestion procedure (Jenkinson & Powlson, 1976; Vance et al., 1987a). Briefly, 8 mL of the filtrate is boiled gently under reflux (30 min) with a dichromate oxidizing reagent (2 mL 66.7 m*M* $K_2Cr_2O_7$ + 70 mg HgO + 15 mL [2 parts H_2SO_4 (98%) + 1 part H_3PO_4 (88%)]), allowed to cool and diluted with 20 to 25 mL of H_2O. The excess dichromate is determined by back-titration with ferrous ammonium sulphate (33.3 m*M*) in 0.4 *M* H_2SO_4, using 25 m*M* 1,10-phenanthroline-ferrous sulphate-complex solution as an indicator. The organic C concentration is calculated assuming that 1 mL 66.7 m*M* $K_2Cr_2O_7$ is equivalent to 1200 µg C. To avoid the cost of dichromate disposal, soluble C also may be analyzed on a commercial soluble C analyzer.

Total N in the filtrate can be determined by the method of Cabrera and Beare (1993). Briefly, 5 mL of the filtrate is combined with 5 mL of a persulfate oxidizing reagent (50 g low-N $K_2S_2O_8$ + 30g H_3BO_4 + 100 mL of 3.75 *M* NaOH made up to 1 L in H_2O) in a 15 mL pyrex glass tube sealed tightly with a teflon-lined screw cap. The tubes are weighed, autoclaved for 30 min at 120°C, allowed to cool to room temperature and reweighed. Any loss in weight (i.e., water) is used to correct the measured N concentrations. The digested samples are then analyzed for their NO_3^-–N concentrations using standard colorimetric procedures (Keeney & Nelson, 1982).

Calculations

Microbial biomass C (mg C kg^{-1}) is calculated from the equation:

$$\text{Microbial biomass C} = (F_C - UF_C)/K_C$$

where, F_C = CO_2 released from the fumigated sample (mg CO_2 – C kg^{-1}) in 10 d

UF_C = CO_2 released from the unfumigated sample (mg CO_2 – C kg^{-1}) in 10 d

K_C = fraction of biomass C mineralized to CO_2 or extracted

For FI procedure, $K_c = 0.41$

For FE procedure, $K_c = 0.35$

According to the approach by Shen et al., (1984) microbial biomass N (mg N kg^{-1}) is calculated from the equation:

$$\text{Microbial N} = (F_N - UF_N)/K_N$$

where, F_N = flush of inorganic N ($NH_4^+ + NO_3^-$) released by fumigation (mg N kg^{-1})

UF_N = inorganic N released (mg N kg^{-1})

$K_N = 0.68$

To compare different ecosystems or agricultural management systems, microbial biomass should be expressed on a volumetric basis for a given depth of soil. This is done by:

Microbial biomass (kg C or N ha^{-1} $depth^{-1}$) = mg biomass C or

N kg^{-1}soil × [bulk density (g cm^{-3})] × [sampling depth (cm)] × (0.10)

Cautions

One assumption of the fumigation–incubation technique is that the surviving microbial population will use the organic C released from the killed cells within a 10-d period. Therefore, an active microbial population must be present to use this C. Soil water content is a critical factor. The soil water effect can be avoided by preincubation of the soil after adjusting the water content to 55% of water-filled pore space. Acid soils or subsoils also are known for limited microbial response after fumigation (Vance et al., 1987b). In this situation, an alternative method needs to be considered. Reinoculation of fumigated soil before incubation can be accomplished by adding 0.2 g of unfumigated soil to 50 g of fumigated soil (Vance et al., 1987b; Horwath & Paul, 1994). The fumigation–extraction procedure also has been successfully used in these situations. Any persistent organic toxin or heavy metal also may inhibit the microbial response after fumigation, thus causing an underestimation of microbial biomass.

INTERPRETATION OF RESULTS

Microbial biomass is very dynamic in soil and responds to weather, crop input, and season (McGill et al., 1986; Bristow & Jarvis, 1991; Garcia & Rice, 1994). This variation is accentuated in cropping systems that include tillage, fertilizer applications, and irrigation (McGill et al., 1986). As much as a 40% change in the values can be realized in native ecosystems (Garcia & Rice, 1994) and in

agricultural systems (Buchanan & King, 1992; Van-Gestel et al., 1992). Therefore, the microbial biomass value is subject to the time of sampling and the antecedent soil water and temperature conditions, as well as plant dynamics. Single-point in time samples should be taken before tillage, nutrient addition, and planting or postharvest to account for seasonal changes in microbial biomass. Depending upon objectives, sampling should generally be conducted in early spring or late fall in temperate regions. In native or perennial systems, sampling should be done before initiation of plant growth. To determine if a soil is aggrading, degrading, or at equilibrium, it is important to establish a baseline or reference condition. Due to its dynamic nature, the amount of microbial biomass at any one time cannot indicate whether soil organic matter; (i.e., soil quality) is increasing, decreasing, or at equilibrium. Monitoring microbial biomass with time can provide information on the changes in amount and nutrient content of the microbial biomass. Relating microbial biomass to other soil parameters has been successful in assessing changes in soil quality as discussed in the next section. Variation in microbial biomass also can be attributed to variations in soil type or landscape position. Variations in biomass C as large as 35% can be expected at the field scale (Cambardella et al., 1994). Soil sampling from homogenous soil types and the use of composite samples can reduce variation considerably and make the desired comparisons more clear.

RELEVANCE TO OTHER SOIL ATTRIBUTES

Expressing the size of the microbial biomass in relation to other parameters, such as total soil organic C and N, mineralizable C and N or respiration, may provide a measure of soil organic matter dynamics and thus soil quality. Soil microbial biomass comprises 1 to 4% of the total organic C (Anderson & Domsch, 1989; Sparling, 1992) and 2 to 6% of the total organic N (Jenkinson, 1988) in soil. The proportion of organic C as microbial biomass varies with climate, such that within a climatic region, this ratio will have an equilibrium value (Anderson & Domsch, 1989; Insam et al., 1989; Insam, 1990). Sparling (1992) suggested that trends in soil organic matter quality are more apparent with this ratio. Deviations from this ratio indicate long-term degradation or aggradation of soil organic matter (Anderson & Domsch, 1989). As with C, similar ratios of microbial biomass N to organic N can be calculated to alleviate the problems associated with the temporal dynamics of the microbial biomass. Beside differences in climatic regions, vegetation, soil mineralogy, and texture also can influence the ratio microbial biomass to organic C and N. Therefore, interpretation of the ratio should be defined within similar soil types, climatic regions, and cropping systems (Gregorich et al., 1994).

The ratio of microbial C to mineralizable C and microbial biomass N to mineralizable N also may be a useful index for soil quality. Mineralizable C and N may be easily obtained by including a second unfumigated control as described in the microbial biomass procedure except incubated for 20 d (Parkin et al., 1996, this publication). Mineralizable N also can be obtained by the procedures outlined in Drinkwater et al. (1996, this publication). These ratios have been used to

estimate the quality of the soil organic matter (Bonde et al., 1988; Rice & Garcia, 1994; Rice et al., 1994).

The rate of respiration (i.e., CO_2 production) from soil can be related to microbial biomass as an indicator of microbial activity. Under stress conditions, many soil microorganisms become dormant; the respiratory ratio (CO_2 production per unit of microbial biomass C, q CO_2) is indicative of general microbial activity (Anderson & Domsch, 1990). The q CO_2 can be calculated easily from the control (unfumigated soil) during the FI procedure or from CO_2 evolution (basal respiration) rate as discussed by Parkin et al. (1996, this publication). The value of q CO_2 is illustrated in the work by Dinwoodie and Juma (1988). They found that one soil had low microbial biomass but a high proportion of microbial C to soil organic C. The respiratory ratio, q CO_2, also was high, possibly indicating that more C was being lost and that greater care was needed to maintain soil organic matter levels.

Microbial biomass also is related to N mineralization (Doran, 1987; Myrold, 1987; Smith & Paul, 1990); however, this relationship is not as strong as the relationship between microbial biomass and soil organic matter. The relationship between microbial biomass and N mineralization can be influenced by N fertilization history and the contribution of nonliving microbial biomass to N mineralization.

APPLICATION

The following examples demonstrate the utility of microbial biomass measurements in soil quality assessment.

Rotations

In semiarid areas, continuous cropping reduces the decline in soil organic matter and microbial biomass compared with a wheat (*Triticum aestivum* L.)–fallow rotation (Campbell et al., 1991; Collins et al., 1992). Campbell et al. (1991) reported that the microbial biomass N was more useful in predicting a change in soil quality than microbial biomass C. In contrast, Jordan and Kremer (1994) reported microbial biomass C to be a better indicator of soil quality. This conflict demonstrates the need for both measures and careful interpretation with other measures for accurate assessment of soil quality. Comparison of corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] cropping systems grown continuously and in rotation with soybean [*Glycine max* (L.) Merr.] generally shows an increase in microbial biomass in the rotation (Table 12–1). However one soil, a sandy loam, showed a significant decrease in microbial biomass with rotation. This negative effect may have been due to less residue produced with the soybean crop on a lower organic matter soil.

Tillage

Reduction in tillage intensity will conserve plant residues and may eventually increase soil organic matter. Microbial biomass has been used as an early

Table 12–1. Effect of crop rotation on microbial biomass C and its relation to organic C.

Previous crop	Soil texture	Microbial biomass rotation		%	Organic C rotation		%
		Monoculture	Soybeans	Difference†	Monoculture	Soybean	Difference
		mg C kg^{-1}			g C kg^{-1}		
Sorghum‡	SiCL	600	650	+ 8.3	14.8		0
Corn§	SiCL`	108	128	+18.5	16.7	15.6	6.6
Corn§	SL	115	105	-8.7	8.7	7.7	11.5

† Data from Roder et al. (1988).
‡ Data from Omay et al. (1992).
§ Percentage of change relative to continuous cropping of corn or sorghum.

indicator of the increase in soil organic matter. A decrease in tillage intensity results in a greater proportion of microbial biomass to organic C (Lynch & Panting, 1980; Carter, 1991; Table 12–2). This may indicate that organic matter is increasing (Carter, 1991), although soil organic C may not be significantly different between tillage systems. Angers et al. (1993) measured 1.2% organic C as microbial biomass in a plowed treatment compared with 3.5 to 5.1% in a minimum-tillage treatment after 11 yr.

Toxins

Microbial biomass has been used to indicate heavy metal toxicity from sludge applications (Chander & Brookes, 1991a, b, 1993; Table 12–3). High levels of Cu or Zn alone (1.4 times the permitted limits) decreased microbial biomass C by 12%, but Zn and Cu combined decreased microbial biomass C by 29 to 53%, thereby suggesting an interactive effect. The percentage of organic C as microbial biomass decreased by more than 50% in the heavy metal-contaminated soils compared with the controls.

Ecosystems

Microbial biomass is related to ecosystem types. Values for microbial biomass C range from 20 kg ha^{-1} in desert grasslands to 1340 kg ha^{-1} in tallgrass

Table 12–2. Effect of tillage on microbial biomass and soil organic C.

Crop	Tillage†	Microbial biomass C	Soil organic C	Microbial C / Soil organic C
		mg kg^{-1}	g kg^{-1}	%
Corn‡	P	120	19.6	0.61
	NT	237	21.5	1.10
Wheat/Barley‡	P	150	22.3	0.67
	NT	299	25.5	1.18
Wheat§	P	760	44	1.73
	NT	940	48	1.96

† P = plowed, NT = no-tillage.
‡ Carter, 1991.
§ Lynch and Panting, 1980.

Table 12–3. Effect of heavy metals on microbial and total organic C (adapted from Chander & Brooks, 1993).

Heavy metal	Application rates	Microbial biomass C	Organic C	Microbial C / Soil organic C
	kg ha^{-1}	mg C kg^{-1}	g kg^{-1}	%
Control	0	169	10.4	1.6
Zn	3000	108	15.5	0.7
Cu	3000	82	19.9	0.4
Ni	200	182	12.2	0.1
Zn/Cu	2300/1600	79	18.8	0.4
Zn/Ni	600/100	181	12.2	1.5

prairie (Gallardo & Schlesinger, 1992; Garcia & Rice, 1994; Zak et al., 1994). Forest ecosystems had intermediate values. Microbial biomass C also has been shown to be related positively to aboveground net primary productivity (Myrold et al., 1989; Zak et al., 1994). In the study by Zak et al. (1994), texture did not influence microbial C, but other studies within the shortgrass prairie region have reported a textural effect (Schimel, 1986; Burke, 1989).

CONCLUSION

Soil is a vital natural resource and maintaining its quality is essential to the survival of any society. Soil organic matter is a key indication of soil quality because it impacts other soil properties; however, organic matter levels in soil change slowly, on the order of decades. Microbial biomass is the dynamic, living component of soil organic matter. Therefore, microbial biomass may be an early indicator of the direction of change in soil organic matter levels. Unfortunately, since microbial biomass levels are affected by climatic variables, soil type, and season, direct interpretation of the values need to be done carefully. The ratio of C and N in microbial biomass relative to organic C and N may be a more useful parameter for assessing soil quality. The recommended procedure for measuring microbial biomass C and N uses the chloroform fumigation technique followed by incubation or direct extraction. The incubation technique has the advantage in that mineralizable C and N also can be measured simultaneously, which is another useful indicator of soil organic matter and its quality. In summary, microbial biomass and its relation to other soil properties is a useful measurement for assessing soil quality.

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