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Field Interpretation of Microbial Biomass Activity Measurements

E. A. PAUL AND R. P. VORONEY

University of California, Berkeley, California 94720, and University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

Recent ecological research has produced a great deal of information concerning the pool sizes and nutrient dynamics of a variety of ecosystems (4, 9, 32). The basic processes, which are reasonably well understood, are identical in all ecosystems since they are controlled by the general laws of thermodynamics. The biological control mechanisms, however, require further definition: they have received extensive investigation from a genetic and cellular viewpoint (10, 26), but it has proved difficult to extrapolate in vitro information from the enzyme and organism level to that of the ecosystem. Further advances will depend on new information and concepts concerning the flux rates and controls of the intertwined processes in the transformation of elements such as C, N, S, and P.

Nutrient transformation kinetics and soil organic matter characteristics cannot be determined for each of the many soil-sediment-plant interactions in nature. Knowledge of the critical controls must be obtained so that extrapolation to the range of ecosystems in nature is feasible. The close relationship between C availability, soil biomass size and activity, and nutrient transformations means that further understanding or possible management of these transformations must involve a knowledge of biomass size and turnover.

INTERPRETATION OF BIOMASS AND ACTIVITY MEASUREMENTS

Estimates of the microbial biomass have usually involved treatment of the biomass as a single component, although it is known that a diversity of populations with different biochemical characteristics are present. Techniques have included microscopic measurements to determine values for total or active biovolumes (38, 40), which can be converted to biomass. The flush of decomposition during incubation after $CHCl_3$ fumigation (chloroform fumigation incubation method [CFIM]) also yields biomass when appropriate conversion factors are used (17, 28). Measurements of the biomass by analysis for specific constituents such as ATP, hexosamines, and nucleic acids (10, 18) has provided much useful information. Other techniques include measurement of the incorporation or respiration of radioactive and nonradioactive substrates (J. L. Smith, B. L. McNeal, and H. H. Cheng, submitted for publication).

The majority of biomass measurements have focused on C and N; however, S and P contents also have been measured (5, 14, 37). Microbial S and P measurements have the advantage in that the extraction can be carried out immediately after $CHCl_3$ treatment, eliminating the lengthy delay and the unknown mineralization-immobilization rates that arise during incubation. Extraction of C or N constituents immediately after $CHCl_3$ treatment has to date not proved successful. A relatively small percentage of the cell constituents has been found extractable. This fraction will at times correlate well with other biomass techniques, but tracers show it to represent a diversity of soil constituents.

METHODOLOGY

None of the present techniques for biomass measurements yields unequivocal results in the broad diversity of habitats that are encountered. The fact that measurements have at times been made without regard to the underlying assumptions in the methodology indicates the need for caution in use of the data derived from such estimates relative to nutrient cycling information. Simultaneous use of two measurement systems yielding complementary data should overcome a number of specific problems. Examples of the simultaneous use of two different techniques include the expression of colony-forming units on agar plates as a percentage of the biovolume measured microscopically to determine an activity coefficient. ATP is dependent on the activity of the organisms and on factors such as P concentration and biomass size. A comparison of ATP contents to biomass measured either microscopically or by the CFIM (34), or respiratory response technique (2), should also yield estimates of activity relative to total biomass.

Measurements of biomass C by the CFIM are limited by the question of what constitutes an appropriate control to calculate the actual amount of CO_2-C attributable to mineralization of the killed population. The microbial popula-

tion developing after $CHCl_3$ treatment is different from that in the soil before treatment (20, 39). The presence of readily available soil organic constituents and the speed with which these substrates are attacked affects the interpretation. Data showing the effect of four different methods of calculation involving the use of control during incubation are shown in Table 1. The biomass C values for which CO_2 evolved from controls was subtracted from the flush (A and B) are probably underestimates, and those calculated without the use of any control (C) can, under some conditions, be overestimates. The use of the 10- to 20-day data (D) from the fumigated treatment as the control resulted in much lower estimates of both biomass C and N. Column 2 indicates that a soil incubated with glucose plus NO_3^--N showed an increased biomass C ranging from 50 to 98% greater than with NO_3 alone, depending on the method of calculation.

Net mineralization of N in a fumigated soil is usually much higher than in an unfumigated soil. The problem of whether to subtract a value representing CO_2 evolved from an unfumigated sample is not as serious in measuring biomass N as in measuring biomass C. However, the C/N ratio of the microbial population is variable; this can alter the rates of net N mineralization. R. P. Voroney and E. A. Paul (Soil Biol. Biochem., in press) overcame this problem by correcting the k_N value used to calculate biomass on the basis of the ratio of CO_2-C to NH_4^+-N produced in the CFIM. This has been found to range from a low of 1.3:1 in composted wheat straw (42) to >300:1 in a forest floor litter. The data for biomass N, when corrected for mineralization, gave much more consistent values than the data for biomass C (Table 1). The linear equation utilized for the calculation in Table 1 has more recently been found to be curvilinear. This results in higher k_N values for microorganisms with higher N contents (R. P. Voroney, Ph.D. thesis, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1983). The curvilinear relationship has been found to give more meaningful estimates of the C/N ratios of biomass under both field and laboratory conditions.

MYCORRHIZAL FUNGI

The roles of the ecto- and endomycorrhizal relationships are still largely unknown factors in relating microbial biomass and activity under field conditions. Work with legumes which constitute a tripartite symbiotic system of plants, bacteria, and fungi showed that although mycorrhizal biomass and nodule biomass contributed only 2 to 6% of the total plant weight, the respiration by the symbionts accounted for 12 to 20% of the C photosynthesized (19). Similar data are available for ectomycorrhizal pine (Table 2). Short roots which include the ectomycorrhizal fungi contributed <0.2% of the total plant weight of *Pinus taeda* at both 6 and 10 months. However, the presence of mycorrhizal fungi increased beneath-ground respiration two- to threefold (C. P. P. Reid, F. A. Kidd, and S. A. Ekwebelam, Plant Soil, in press). Depending on the cultural conditions, plant age, etc., mycorrhizal fungi can act either as symbionts or as parasites. In the above examples, the presence of the mycorrhizal fungi increased net photosynthesis on a unit weight basis, indicating that plants could compensate for the needs of the microbial partners.

Mycorrhizal fungi contributed ≈1% to total ecosystem biomass in both 23- and 180-year-old

TABLE 1. Comparison of estimates of the soil microbial biomass C and N obtained by four different methods of calculation (Voroney and Paul, in press)

Method of calculation	Biomass C and N (μg g^{-1} of soil)			
	NO_3^- amended soil		Glucose plus NO_3^- amended soil	
	C[a]	N[b]	C[a]	N[b]
(A) $(C_{F(0-10)} - C_{UF(0-10)})/k_C$	483		715	
(B) $(C_{F(0-10)} - C_{UF(10-20)})/k_C$	513		829	
(C) $C_{F(0-10)}/k_C$	600		1,193	
(D) $(C_{F(0-10)} - C_{F(10-20)})/k_C$	396		734	
(A) $(N_{F(0-10)} - N_{UF(0-10)})/k_N$		132		179
(B) $(N_{F(0-10)} - N_{UF(10-20)})/k_N$		134		163
(C) $N_{F(0-10)}/k_N$		139		208
(D) $(N_{F(0-10)} - N_{F(10-20)})/k_N$		79		29

[a] k_C (22°C) = 0.41.
[b] k_N (22°C) = 0.39 − 0.014 (C_F/N_F)
= 0.3 NO_3^- amended soils
= 0.24 glucose + NO_3^-.

TABLE 2. Net photosynthesis and carbon distribution in *Pinus taeda* (adapted from Reid et al., in press)[a]

Determination	Seedling age			
	6 months		10 months	
	NM	M	NM	M
Weight (g)	1.3	2.0	2.5	4.8
Net photosynthesis (mg of CO_2-C g^{-1} h^{-1})	0.65	1.25	0.68	1.55
^{14}C distribution (%)				
Needles and stems	67	30	67	55
Shoot respiration	5	6	6	7
Woody roots	20	34	14	14
Short roots	0.17	0.2	0.2	0.12
Beneath ground respiration	8	30	12	24

[a] NM, Non-mycorrhizal; M, mycorrhizal.

fir (*Abies amabilis*) (45) in western Washington. They were said to account for ≈15% of the annual net primary production in these stands. Sclerotia accounted for the largest proportion of the total mycorrhizal fungal production. These, together with sporocarps and mycorrhizal sheaths, represented a larger portion of the N, P, and K cycling than that returned annually through the litter fall.

EFFICIENCY OF MICROBIAL PRODUCTION

The extent to which microorganisms convert substrate to microbial biomass is an important ecological determinant in that this controls the relative rates of N, S, and P mineralization and immobilization. Laboratory estimates of microbial growth efficiency of 60% on substrates such as glucose (31) have been confirmed for glucose in soils under both N-sufficient and N-limiting conditions (13). The water-insoluble particulate fraction of seaweed has been found to be converted to bacterial C at an overall efficiency of 43% (35). We have measured similar efficiency values for a general soil population growing on mature wheat straw. In longer incubations in which both microbial biomass and other organic microbial products were formed, a yield of 0.15 for microbial biomass and a conversion efficiency of 0.37 for conversion of ^{14}C to microbial products indicated an overall conversion through microbial biomass as high as 50% (8).

MICROBIAL ACTIVITY

The term "activity" includes the many processes carried out by microbial enzymes. When interpreted in conjunction with biomass measurements, it often refers to net nutrient mineralization and immobilization rates. Smith et al. (submitted for publication) point out that the mineralization of elements such as C and N has kinetics which depend on the concentration of substrate and require microbial biomass as the reacting agent. This can best be described by a second-order equation. Traditionally, microbial biomass has been considered to be constant and at a maximum, thus reducing the equation to pseudo-first order. Use of first-order equations has proved possible in long-term models in which the microbial population could develop early enough in the incubation so that overall biomass sizes did not limit the final rates (44).

Studies to understand the role of microbial biomass other than as a source-sink for nutrients must recognize the different growth rates and activity coefficients of various segments of the population. These segments range from the zymogenous and autochthonous defined by Winogradsky many years ago to more recent segments characterized on an activity basis. J. L. Smith (personal communication) has recognized three population states: (i) the active biomass capable of growth and all metabolic functions, (ii) sustainable populations which are nongrowing but can dissimilate glucose and resume growth under favorable conditions, and (iii) dormant spores or other long-term resting populations. Estimates of the active population usually range from 10 to 40% of the total identifiable biomass (30, 32).

The recognition of large sustainable and dormant populations with maintenance energy and starvation characteristics vastly different from those usually measured in chemostats is a necessary prerequisite to an understanding of the functioning of microorganisms in nature. Some measurements of the adenylate energy charge (6) indicate that, even though little of the total biomass in soil can be in an active growth stage at any given time, ATP accounted for 77% of the total adenine nucleotides in a fresh soil. Other measurements have indicated a low adenylate energy charge, and more information is required concerning the activity state of in situ organisms.

In nature, the state of dormancy and the necessity of a long exposure to sufficient nutrient levels before response are major survival criteria (25). Physiological studies on the starvation-survival of a marine pseudomonas showed a miniaturization of cells during starvation (1). Extrapolation of this observation to soil could help explain the preponderance of very small bacterial cells in soil. Further understanding of microbial growth and production will depend on a better delineation of the microbial responses to what appears to be the general C-limiting condition in most terrestrial habitats.

POTENTIAL FOR USE OF BIOMASS ESTIMATES IN MANAGEMENT DECISIONS

Information on the size of the soil biomass has been used for determining: (i) the degradation, stabilization, and incorporation into biomass of plant C and N (21, 43); (ii) the effects of freezing and thawing (39); (iii) the effect of tillage (7, 12); (iv) the role of soil sampling, mixing, and grinding (23, 33); (v) the effect of climatic variations (36, 41); (vi) biomass in forest floors (11, 27); and (vii) the effect of faunal feeding (3, 46).

There is potential for improved management of soil and fertilizer nutrients through a better understanding and possible manipulation of biomass (E. A. Paul, Plant Soil, in press). Jenkinson and Ladd (16) published a model (Table 3) of C turnover, using data from (i) the carbon dating of the resistant soil organic matter, (ii) plant C inputs to a soil in equilibrium with its environment and management practices, e.g., the Rothamsted continuous wheat plots, and (iii) microbial biomass and production estimates based on substrate degradation rates measured with tracers. In their first-order model, microbial N was taken as one-sixth of the microbial C value of 570 kg ha^{-1}; it was calculated that the population of microorganisms had a turnover time of 2.5 years.

The chernozemic soil in Saskatoon under a wheat-fallow crop rotation had high reserves of organic C and N at C inputs somewhat similar to those at Rothamsted. The microbial N determined by using CFIM and a k_N depending on CO_2-C/NH_4^+-N produced during incubation represented 360 kg of N h^{-1} (a measured C/N ratio of 4.4). The turnover time of 6.8 years calculated on the basis of Jenkinson and Ladd's model shows the potential for stabilization of a largely inactive population in soils with high organic matter levels.

TABLE 3. C and N turnover in Saskatchewan wheat-fallow, Rothamsted continuous wheat, and Brazilian sugarcane soil-plant systems

Determination	Saskatoon	Rothamested	Brazil
Soil weight (t ha^{-1})	2,700	2,200	2,400
Organic C (t ha^{-1})	65	26	26
C inputs (t ha^{-1} yr^{-1})	1.6	1.2	13
Turnover of soil C (yr)	40	22	2.0
Microbial C (kg ha^{-1})	1,600	570	460
Microbial N (kg ha^{-1})	360	95	84
Microbial turnover time (yr)	6.8	2.5	0.24
N flux through microbial biomass (kg ha^{-1} yr^{-1})	53	34	350
Crop removal of N (kg ha^{-1} yr^{-1})	40	24	220

The spodosolic sugarcane soil of northeast Brazil (M. Lima, personal communication) had C and N contents similar to those of the continuous wheat plots in Rothamsted with a lower biomass C and N. The high input of C (13 t ha^{-1} $year^{-1}$) resulted in a calculated turnover time of 0.24 years. This was 28 times faster than that found in the chernozemic soil and demonstrates the situation where the biomass acts more as a catalyst and a short-term reservoir than as a major source-sink for nutrients. Although biomass N represented only one-third of the N removed by the crop, the estimated N flux through this biomass was 1.5 times that removed by the crop.

The biomass is large enough, under temperate conditions, to act as a significant temporary storage of nutrients (24). The net N mineralized during a 12-week period was found to be derived almost equally from the microbial biomass, a fraction consisting of microbial metabolites, and a stabilized N fraction with lower decomposition rates (29).

The general relationships between the size of the microbial biomass and N_Δ, the soil N mineralized during laboratory incubation, is shown in Table 4. One hundred soils from Saskatchewan ranging in organic N content from 0.13 to 0.32% produced an N_Δ of 87 to 157 $\mu g\ g^{-1}$; the biomass N in the same soils ranged from 53 to 102 $\mu g\ g^{-1}$. There was a close relationship between the N mineralized and the microbial biomass: $N_\Delta = 1.3$ microbial biomass N + 24 ($r^2 = 0.75$). It has also been shown that the ^{15}N atom % excess of the microbial N and of the mineralized N are identical during extended incubations (29), demonstrating that the biomass is closely related to the amount of net N mineralized and that the mineralizable N moves through the biomass on its way to NH_4^+.

The so-called priming action measured in tracer experiments with increased microbial activity was said to result from an increased attack on soil organic matter. Alternatively, priming can be explained by the internal turnover of the large microbial population. Tracer experiments often show an enhanced flux of nontracer (soil C and N) upon the addition of the tracer such as fertilizer N or substrate C. This phenomenon has been described as priming, as it was thought to result from the enhanced turnover of the native soil organic matter (15). The release of nonlabeled C and N from a large biomass as it cycles and incorporates the added tracer is a more logical explanation for this phenomenon.

Management techniques such as zero tillage (12), crop rotations, and intercropping (22) have potential for better utilization of nutrients and energy in crop production. These techniques also directly affect the size and turnover of the biomass. Further information is necessary to relate management to soil process controls. Productivity of forest sites has been related to N accumulation during anaerobic incubation (R. Powers, Agronomy Abstr. 1982, 270). We predict that mineralizable N under these conditions will be related to the size of biomass N and closely related fractions such as the microbial metabolites.

Differential management of agricultural residues has been shown to affect the microbial community and the associated microbial feeding faunal population (E. T. Elliott, K. Horton, J. C. Moore, D. C. Coleman, and C. V. Cole, Plant Soil, in press). This has the potential for significantly altering nutrient cycles. The possibility of increasing soil and fertilizer use efficiency through management of the biomass by cultivation, cropping practices, and possibly even faunal feeding definitely exists and should be further investigated (D. C. Coleman, R. E. Ingham, J. F. McClellan, and J. Trofymow, Proceedings of the Joint BMS/BES Meeting on Animal Microbial Interactions, in press).

TABLE 4. Relationship of microbial biomass and soil properties of 100 medium-textured soils[a]

Soil	Organic C (%)	Kjeldahl N (%)	N_Δ[b] ($\mu g\ g^{-1}$ of soil)	Microbial biomass[c] ($\mu g\ g^{-1}$ of soil)	
				C	N
Brown	1.32	0.13	87	660	53
Dark brown	1.80	0.18	117	750	74
Black	3.49	0.30	157	970	102
Dark grey luvisol	3.80	0.32	116	810	72

[a] Microbial biomass C ($\mu g\ g^{-1}$ of soil) = 0.017 organic C + 417 ($r^2 = 0.4$). Microbial biomass N ($\mu g\ g^{-1}$ of soil) = 0.029 soil N + 16 ($r^2 = 0.44$). N_Δ ($\mu g\ g^{-1}$ of soil) = 0.17 biomass C + 24 ($r^2 = 0.53$). N_Δ ($\mu g\ g^{-1}$ of soil) = 1.3 biomass N + 24 ($r^2 = 0.75$).
[b] Derived from 20-week incubation at 28°C.
[c] Measured by CFIM, 28°C; $k_C = 0.5$, $k_N = 0.4$.

CONCLUSIONS

Reasonable techniques for measurement of biomass size and activity now exist. Refinements of these techniques in the next few years will improve ease and accuracy of measurement. These measurements, when combined with tracer techniques and mathematical modeling, will help answer a number of questions concerning soil processes of importance to both agricultural soil management and the understanding of ecosystem functioning.

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Microbial Biomass and Activity Measurements in Soil: Ecological Significance

P. NANNIPIERI

Instituto Chimica Terreno, Consiglio Nazionale delle Ricerche, 56100 Pisa, Italy

Modern society's increasing demands on scientists for help in making decisions regarding land use and resource management have accentuated the importance of ecological knowledge. The promotion of ecological research has had a great importance to the development of microbial ecology. Microbiologists were forced out into the field, where they were faced with problems new to them, problems for which existing techniques were inadequate or nonexistent. The interest of the soil microbiologist was, therefore, shifted from the identification of isolates and the estimation of populations to the study of the role of microorganisms in soil processes.

Such increased interest has produced, in the past 20 years, numerous empirical observations on biomass and biological activity in various soil types and ecosystems, with respect to amendments, cultivation practices, and responses to environmental and climatic factors. The observations, however, have often produced conflicting and confusing results, not only because the methodologies employed were questionable but also because the motivation of the research and the meaning of the measured criteria have not been properly defined.

DETERMINATION OF BIOMASS

Microbial biomass has recently been defined as the living part of the soil organic matter with the exclusion of plant roots and soil animals larger than 5×10^3 μm^3 (16).

The need to characterize the mineral cycling and the energy flow in the ecosystem has led to the consideration of the microbial biomass in soil as an undifferentiated whole (16), without any consideration of the enormous diversity of microbes with varying abilities to survive in extreme environmental conditions. The approach of considering microbial biomass as an undifferentiated whole has been made possible by the availability of the fumigation technique which allowed the accurate estimation of the relative pool of nutrients (16). When a soil is fumigated with $CHCl_3$, the fumigant is removed, and the soil is inoculated with an untreated sample and incubated, the respiration rate of the fumigated soil becomes, after some hours, much greater than that of the unfumigated soil (17). Fumigation usually causes an immediate increase in the extractable inorganic P, sulfur, and NH_4^+ content of soil (6, 17, 32). Additional NH_4^+ is released when the soil is subsequently incubated. The "flush of decomposition," which is defined as the amount of CO_2 evolved (or N mineralized) by a fumigated soil in a given incubation time less the CO_2 evolved (or N mineralized) by the same amount of unfumigated sample in the same time, has been ascribed to the decomposition of killed organisms by the inoculated population. The size of the CO_2 or NH_4^+ flush can be related, by a simple expression, to the size of biomass C or N, respectively (16). It is also possible to determine the biomass P (6) and S (32) by use of the fumigation technique; however, the method has mainly been used to investigate the behavior of the microbial C and N in soil.

Experiments with labeled plant material added to soil have demonstrated the role of microbial biomass and activity in carbon cycling (27, 28). Decomposition rates corrected for microbial utilization of the substrate at reasonably high efficiencies (35 to 60%) have shown much faster transformation rates than are usually described in the literature (28).

Climatic conditions have been found to influence not only decomposition rates of labeled plant material added to soil but also the turnover and doubling time of the soil microbial populations (16). However, these determinations were carried out long after the addition of the fresh material and therefore did not take into consideration the active population present shortly after the addition of the carbonaceous material. In addition, the approach of considering the soil population as a homogeneous simple compartment model does not take into consideration the variety of turnover times that occur in the soil biomass. Methods for distinguishing various sections of the population, such as that devised by Anderson and Domsch (2) or direct counts based on selective staining procedures, can only give an estimate of bacterial and fungal biomass, without any indications of their nutrient content. The measurement of microbial biomass with methods based on specific biomass constituents, such as muramic acid, hexosamines, nucleic acid, etc., have provided, with the exception of the ATP technique, erroneous estimates (16).