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Conversion of Biovolume Measurements of Soil Organisms, Grown Under Various Moisture Tensions, to Biomass and Their Nutrient Content

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Direct microscopic measurements of biomass in soil require conversion factors for calculation of the mass of microorganisms from the measured volumes. These factors were determined for two bacteria, five fungi, and a yeast isolated from soil. Moisture stress conditions occurring in nature were simulated by growth in two media using shake cultures, on agar plates, and on membranes held at 34, 330, and 1,390 kPa of suction. The observed conversion factors, i.e., the ratio between dry weight and wet volume, generally increased with increasing moisture stress. The ratios for fungi ranged from 0.11 to 0.41 g/cm^3 with an average of 0.33 g/cm^3. Correction of earlier data assuming 80% water and a wet-weight specific gravity of 1.1 would require a conversion factor of 1.44. The dry-weight specific gravity of bacteria and yeasts ranged from 0.38 to 1.4 g/cm^3 with an average of 0.8 g/cm^3. These high values can only occur at 10% ash if no more than 50% of the cell is water, and a specific conversion factor to correct past data for bacterial biomass has not yet been suggested. The high conversion factors for bacteria and yeast could not be explained by shrinkage of cells due to heat fixing, but shrinkage during preparation could not be completely discounted. Moisture stress affected the C, N, and P content of the various organisms, with the ash contents increasing with increasing moisture stress. Although further work is necessary to obtain accurate conversion factors between biovolume and biomass, especially for bacteria, this study clearly indicates that existing data on the specific gravity and the water and nutrient content of microorganisms grown in shake cultures cannot be applied when quantifying the soil microbial biomass.

The biomass of microorganisms does not represent a major fraction of the organic and inorganic nutrient reservoirs of most ecosystems. Models of carbon (C), nitrogen (N), and phosphorus (P) transformations, however, have shown the biomass to be a major sink-source of nutrients controlling the dynamics of the system (10, 29, 33). Soil P redistribution is closely associated with the cycling of bacteria and cell debris (13). In P cycling, plants and microorganisms utilize pools of labile inorganic P often produced by microbial mineralization (10). Nitrogen cycling calculations show that the N contained in the soil biomass is often greater than the requirements for an annual crop (27), and mineralization-immobilization rates have a strong influence on nitrogen fertility (7). In the case of C, Jenkinson (16, 18) found that the biomass constituted only 2.5% of the total soil organic C, but 12% of the ^{14}C from decomposing labeled ryegrass tissue was found in the biomass after 1 year of incubation. Changes in the biomass had a large effect on C mineralization.

Plate counts are known to greatly underestimate the size of populations in natural systems (5, 32). Microscopic techniques for biovolume measurements have not been widely used because they are exceptionally time consuming (J. A. van Veen, Ph.D. thesis, Free Reformed University, Amsterdam). Alternate chemical techniques such as adenosine 5'-triphosphate measurements (2, 21), incubation, and CO_2 respirometry after $CHCl_3$ treatment (16, 17) or after substrate and antibiotic addition (1) have been recently suggested. These indirect techniques, however, require standardization and comparison with microscopic measurements.

Microscopic methods have recently been made easier by the availability of epifluorescence microscopes and a range of fluorescent dyes and immunofluorescent techniques. These allow for separation of organisms on a morphological and cell wall composition basis, but do not over-

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come all methodological problems inherent in microscopic measurements (6, 20). Interpretation of microscopic measurements requires a knowledge of the relationship between the biovolume observed under the microscope and the biomass and nutrient content of the microorganisms.

Conversion from biovolume to biomass usually utilizes arbitrary factors such as 80% (wt/wt) water and a specific gravity of 1.1. These values have been obtained from a range of earlier work relating media composition to the composition of a number of microbial types (11, 30, 31). They have not included studies of the effect of growth under different soil conditions such as varying moisture stresses.

To obtain data for conversion of biovolume measurements to biomass, we grew soil isolates (two bacteria, a yeast, and five filamentous fungi) under shake and plate conditions and on cellulose membranes where the microorganisms were subjected to moisture stresses normally encountered in the soil. Conversion of biovolume to biomass was obtained by weighing known volumes of microbial biomass, followed by nutrient analysis.

MATERIALS AND METHODS

Microorganisms utilized. Two bacteria (*Arthrobacter globiformis* and *Enterobacter aerogenes*) and one yeast (*Cryptococcus albidus*) were used. Fungi included *Trichoderma harzianum*, *Penicillium* sp., *Fusarium oxysporum*, and sterile dark and hyaline forms. The organisms had been isolated from a Chernozemic soil during studies involved with the International Biological Programme (22, 26).

Media. Bacteria were grown in nutrient broth or nutrient agar (Difco) and fungi on potato dextrose broth, potato dextrose agar (Difco), or a soil extract medium. Soil extract was made by autoclaving and extracting a fine sandy loam (Bradwell) with 1 liter of water per kg of soil. After extraction, 0.4 g of K_2HPO_4, 0.4 g of NH_4NO_3, and 1 g of glucose were added per liter of extract. The pH was adjusted to 7.4 for bacteria and 5.6 for fungi. Agar plates were prepared by adding 1.5% agar (Difco).

Growth conditions. Organisms were grown in shake culture, on agar plates, and at three moisture stresses at room temperature. To obtain the different moisture stresses, polyethylene glycol with a molecular weight of 20,000 (PEG 20,000; Fisher Scientific) was dissolved in the liquid medium at concentrations of 14 and 27 g per 100 g of solution. The microorganisms were grown on a cellulose membrane with a pore diameter that excluded material having molecular weight greater than 10,000 (Visking). This was placed on a petri dish filled with the polyethylene glycol-nutrient solution. Care was taken that the contact between the membrane and the solution was maximal. Bacteria were also grown at 34 kPa of tension by growing them on a 0.2-μm-pore-diameter polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.) held at the appropriate tension by a water column.

After 5 days of incubation, the microorganisms were harvested and washed twice with a 0.85% NaCl solution that had been filtered (0.2-μm pore size) and autoclaved. After washing and centrifuging, the organisms were taken up in 50 ml of the NaCl solution and used as stock solution. A 1:10 dilution of the stock solution was used for counting and weighing.

Osmotic potential. The osmotic potentials of the polyethylene glycol solutions as well as of the agar plates were determined with a thermocouple psychrometer. A concentration of 14 g of polyethylene glycol per 100 g of solution yielded an osmotic tension of 330 kPa (3.25 ± 0.1 atm); 27 g of polyethylene glycol per 100 g of solution resulted in a tension of 1,394 kPa (13.75 ± 0.25 atm). To measure the osmotic potential that microorganisms are subjected to during growth on an agar plate, the percentage loss of water from the agar versus the osmotic potential was determined during the incubation period.

Microscopic measurements. Numbers of bacteria and yeasts were determined using fluorescein isothiocyanate staining and fluorescence (3), counting 20 fields on each of four smears. They were also determined with the Levy bacterial chamber with a depth of 0.1 mm, counting 16 fields per sample. Volumes of 50 representatives of each organism type were determined on heat-fixed fluorescein isothiocyanate-stained slides and in wet preparations utilizing glyceraldehyde as a fixing agent. Fungi were observed using water-soluble aniline blue (BDH Chemicals, England) as the staining agent (28).

Microscopic determinations were carried out with a Carl Zeiss microscope equipped with epifluorescence. Bacterial counts and volume measurements were carried out at a magnification of 1,560, the yeast and fungi at a magnification of 625. Bacterial rods were assumed to be cylinders with spherical ends. After the length and the width were measured, the volume was calculated by adding the volumes of the two half-spheres with radium equal to 0.5× the width of the rod, plus length equal to the length of the rod minus 2× the radium of the half-spheres, i.e., the width of the rod.

Weights. The weights of the microorganisms were determined by filtering 10 ml of a 1:10 dilution through a 0.2-μm Nuclepore filter. The filters were prewashed in sterile, filtered (0.2 μm) water and dried to constant weight. The filters plus microorganisms were dried at 110°C to constant weight. Four replicates of the solution with microorganisms as well as the NaCl solution only were used.

Nutrient analysis. The C content of freeze-dried preparations was determined with a wet combustion procedure (24). The reaction mixture of $K_2Cr_2O_7$ and H_2SO_4 was previously mixed and cooled instead of added separately, and the heating time was extended to 45 min.

For N and P determination, freeze-dried microorganisms were digested in 1 ml of concentrated H_2SO_4 and 2 to 4 drops of H_2O_2 at 225°C for 1.5 h. The digest was transferred to 50-ml flasks and diluted to volume (34). Solution N and P were analyzed with a Technicon AutoAnalyzer II. The ash content of dried cultures was determined after heating at 550°C for 2 h.

RESULTS

Comparison of the fluorescein isothiocyanate smears with phase-contrast observation of the undried organisms in a bacterial counting chamber indicated that unclumped organisms showed similar counts for bacteria and yeasts. Under certain cultural conditions, *E. aerogenes* and occasionally *Arthrobacter* formed clumps. Under these conditions, fluorescein isothiocyanate smear technique accounted for 80% of the number found utilizing the counting chamber (data not shown). However, this clumping, attributed to extracellular polymers such as polysaccharides, has not been found to be a major factor in soil smears after treatment with a Waring blender.

The greatest potential contributor to the error in biovolume measurements was considered to be the range in size of the organism. *A. globiformis* showed a fivefold range in size under most growth conditions, with no significant difference between heat-fixed and wet-fixed preparations (Table 1). The average volumes for the different treatments ranged from 0.43 to 0.87 μm^3; the average volume of the organisms growing on the more complex but lower nutrient content soil extract was two-thirds the volume of those grown in nutrient broth. No significant size differences were found for the effect of moisture stress.

Data for *E. aerogenes* and the yeast *C. albidus* did not show similar large size differences between different media. However, the range in size of individual organisms was again great (Table 2). These data confirm the observation of Jenkinson et al. (19), who indicated that an accurate volume measurement is a prerequisite for biomass determinations when conducting microscopic counts. The average volume of soil organisms in situ (2, 4, 12) is smaller than the average shown in Tables 1 and 2. The tabular data agree with the data of Luria (23). However, they fall within the lower ranges observed for most of the growth conditions.

TABLE 1. *Average and variation in volumes of A. globiformis measured on heat-fixed smears and in wet-fixed preparations after growth under different conditions*

Growth conditions[a]	Vol (μm^3)			
	Heat-fixed smears		Wet-fixed smears	
	Range	Avg vol	Range	Avg vol
Shake culture, NB	0.23–1.48	0.65	0.19–0.98	0.65
Shake culture, SE	0.19–0.98	0.51	0.23–0.98	0.52
3.25 atm, NB	0.32–2.1	0.80	0.32–2.1	0.85
3.25 atm, SE	0.10–1.1	0.42	0.10–1.5	0.50
13.75 atm, NB	0.34–2.1	0.83	0.32–2.1	0.87
13.75 atm, SE	0.23–1.5	0.69	0.23–1.5	0.69
Agar plate, NA	0.32–1.98	0.81	0.23–1.1	0.62
Agar plate, SEA	0.14–0.71	0.43	0.19–0.98	0.44

[a] NB, Nutrient broth; SE, soil extract; NA, nutrient agar; SEA, soil extract agar.

TABLE 2. *Average and variation in volumes of E. aerogenes and C. albidus grown under different conditions as measured on heat-fixed smears*

Growth conditions[a]	Vol (μm^3)			
	E. aerogenes		*C. albidus*	
	Range	Avg vol	Range	Avg vol
Shake culture, lab medium	0.2–1.5	0.70	13–106	47
Shake culture, SE	0.3–1.5	0.76	5–94	40
3.25 atm, lab medium	0.2–0.9	0.58	10–80	33
3.25 atm, SE	0.2–0.6	0.39	5–56	27
13.75 atm, lab medium	0.2–1.5	0.70	10–89	38
13.75 atm, SE	0.2–1.2	0.58	4–63	27
Agar plate, lab medium	0.2–1.2	0.46	8–55	27
Agar plate, SEA	0.2–1.0	0.46	7–49	19

[a] Lab medium is nutrient broth (or agar) for *E. aerogenes* and potato dextrose broth (or agar) for *C. albidus*. SE, Soil extract; SEA, soil extract agar.

Early work with pure cultures (30, 31) indicated that the specific gravity of bacteria averaged 1.1 with 80% water on a weight basis. At a specific gravity of water of 1, the volume of water in a cell of 1 μm^3 would be $(1.1 \times 0.80)/1 = 0.88$ μm^3. The dry matter weighing 0.22 μg would occupy 0.12 μm^3, and the specific gravity of the water-free cell contents would equal 1.83. In this paper, we have not attempted to measure the moisture content of microorganisms because we feel that this would be an impossible factor to consider under field conditions. The biovolume of a large number of organisms was determined by microscopic observation, and the biomass was determined by weight of a known number of washed cells. Division of the biomass by the biovolume results in the conversion factors shown in Tables 3 and 4. These values represent the dry weight of the microorganisms divided by the original volume, and therefore they are an expression of the specific gravity of the organisms on a moisture-free basis. The literature values of specific gravity (1.1 and 80% water, wt/wt) can be transformed to a similar conversion factor. Since 12% of the cell is material with a specific gravity of 1.83, the conversion factor for 80% water on a weight basis and a wet-weight specific gravity of 1.1 would be 0.22.

Data for *C. albidus* grown in shake culture (Table 3) show the average specific gravity on a dry-weight basis to be 0.44. Growth under moisture stress resulted in a doubling of this value. Conversion factors for *T. harzianum* grown in

TABLE 3. *Conversion factors for determining biomass from biovolume for A. globiformis, E. aerogenes, C. albidus, and T. harzianum for different growth conditions*

Growth conditions[a]	Conversion factor (g/cm³)			
	A. globiformis	*E. aerogenes*	*C. albidus*	*T. harzianum*
Shake culture, lab medium	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.23 ± 0.03
Shake culture, SE	0.8 ± 0.2	0.7 ± 0.1	0.38 ± 0.04	0.11 ± 0.02
3.25 atm, lab medium	0.9 ± 0.3	0.9 ± 0.1	0.8 ± 0.2	0.30 ± 0.1
3.25 atm, SE	0.9 ± 0.3	0.6 ± 0.1	0.9 ± 0.2	0.04 ± 0.19
13.75 atm, lab medium	1.0 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	0.41 ± 0.1
13.75 atm, SE	1.3 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.30 ± 0.1
Agar plate, lab medium	1.2 ± 0.3	1.0 ± 0.2	1.4 ± 0.2	0.18 ± 0.01
Agar plate, SEA	1.4 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	0.21 ± 0.05

[a] Lab medium is nutrient broth (or agar) for *E. aerogenes* and potato dextrose broth (or agar) for *C. albidus*. SE, Soil extract; SEA, soil extract agar.

TABLE 4. *Conversion factors for four fungi grown under different conditions to calculate biomass from biovolume*

Fungi	Conversion factor (g/cm³) under growth conditions[a]:		
	Shake culture, PDB	Plate, PDA	34 kPa, PDB
Sterile hyaline	0.11 ± 0.04	ND	ND
Sterile dark	ND	0.3 ± 0.1	ND
Penicillium sp.	0.1	0.2	0.3
Fusarium oxysporum	0.2	0.2	0.4

[a] PDB, Potato dextrose broth; PDA, potato dextrose agar. ND, Not determined.

shake culture or on agar are low, ranging from 0.11 to 0.23 g/cm³. However, the average factor for this organism when subjected to osmotic tensions was 0.325 g/cm³. Growth of *Penicillium* and *Fusarium* isolates under a stress of 34 kPa (1/3 atm) yielded a factor of 0.35 g/cm³ (Table 5) with normal laboratory conditions, again resulting in lower densities.

The two bacterial isolates yielded very high conversion values even under nonstressed growth conditions (Table 3). The data in Table 3 also show a general increase in weight per unit volume for all the organisms as the growth conditions were altered from shake culture to growth under increasing moisture stresses. Although the laboratory media had altered the size of the individual organisms (Tables 1 and 2), there was no consistent effect on the weight per unit volume that could be attributed to substrate.

The contents of C, N, P, and ash in Table 5 are within the wide range of values found in the literature (9, 15, 23, 30, 36). However, most values for the P content of the bacteria and the yeasts and the C content of *A. globiformis* are somewhat below the average values reported, i.e., 2 to 6% P and 40 to 55% C of the dry weight.

The effect of moisture stress on the nutrient content of the various organisms seems to be fairly consistent for a particular strain with respect to the two growth media. They differ for different organisms, with the ash content generally increasing when the organisms were grown under higher moisture stress.

All organisms were grown on soil extract under the 3.25- and 13.75-atm moisture stress, and therefore nutrient content comparisons can be made for the different organisms. *A. globiformis* had an average N content of 7.3%, *E. aerogenes* 9.6%, *C. albidus* 2.0%, and the filamentous fungus, *T. harzianum*, contained 4.0% N on a dry-weight basis. The bacteria were grown on nutrient broth rich in organic N and on soil extract supplemented with inorganic N. The N content under these conditions could not be expected to represent the N content of organisms in soil under N-starved conditions, but could represent conditions where free mineral N is found. Similarly, microbial P values under soil conditions are very sensitive to levels of available P (B. Chauhan, J. Stewart, and E. Paul, Trans. 11th Cong. Soil Sci., 1:17, 1978).

Potato dextrose broth utilized for fungal studies is a medium more representative of conditions in many soil types than is the rich nutrient broth used for bacteria. Under these conditions, both the *C. albidus* and the *Trichoderma* showed lower but variable N and P levels (Table 5). The high C values for *C. albidus* have been attributed to lipids; observations showed that large portions of the cell did not fluoresce upon staining with fluorescein isothiocyanate.

DISCUSSION

Bacteria and yeast grown on agar plates showed a high weight-to-volume ratio. The possibility that this might be due to increased moisture stresses as the plates dried out was investigated. However, water loss from the agar, which was observed to be proportional to the thickness

TABLE 5. *C, N, P, and ash contents of microbes grown under different conditions*

Organism	Growth conditions[a]	Content (% dry wt)			
		C	N	P	Ash
A. globiformis	Shake culture, NB	34.8 ± 0.2	8.0 ± 0.3	1.93 ± 0.01	2.1
	3.25 atm, NB	37.1 ± 0.3	7.9 ± 0.2	1.53 ± 0.01	6.6
	13.75 atm, NB	35.6 ± 0.2	7.2 ± 0.2	1.98 ± 0.06	5.6
	Agar plate, NA	39 ± 2	8.9 ± 0.2	1.38 ± 0.05	ND[b]
	Shake culture, SE	38 ± 1	ND	ND	ND
	3.25 atm, SE	36 ± 2	8.1 ± 0.02	1.12 ± 0.01	ND
	13.75 atm, SE	38 ± 3	6.46 ± 0.07	1.86 ± 0.02	ND
E. aerogenes	Shake culture, NB	36.4 ± 0.3	11.1 ± 0.2	1.98 ± 0.01	7.5
	3.25 atm, NB	40.3 ± 0.6	11.1 ± 0.9	1.56 ± 0.04	8.9
	13.75 atm, NB	48 ± 2	9.1 ± 0.3	1.45 ± 0.07	26.0
	Agar plate, NA	41.6 ± 0.8	9.7	1.52	ND
	Shake culture, SE	40 ± 1	ND	ND	ND
	3.25 atm, SE	39 ± 2	10.4 ± 0.2	1.53 ± 0.02	8.7
	13.75 atm, SE	47.2 ± 0.5	9.1 ± 0.2	1.11 ± 0.03	ND
C. albidus	Shake culture, PDB	55 ± 2	2.0 ± 0.1	0.46 ± 0.04	2.4
	3.25 atm, PDB	60 ± 2	0.74 ± 0.03	0.091 ± 0.001	0.8
	13.75 atm, PDB	66 ± 1	0.48 ± 0.04	0.094 ± 0.005	1
	Agar plate, PDA	64 ± 4	1.55 ± 0.05	0.28 ± 0.02	13.4
	Shake culture, SE	44.0 ± 0.01	5.1 ± 0.3	1.21 ± 0.08	1.5
	3.25 atm, SE	55.0 ± 0.07	2.0 ± 0.1	0.34 ± 0.04	0
	13.75 atm, SE	63.7	ND	ND	ND
T. harzianum	Shake culture, PDB	43.9 ± 0.8	1.16 ± 0.02	0.15 ± 0.04	0.5
	3.25 atm, PDB	43.4 ± 0.1	2.76 ± 0.03	0.19 ± 0.01	0.9
	13.75 atm, PDB	37.3 ± 0.7	3.93 ± 0.1	0.38 ± 0.01	ND
	Agar plate, PDA	ND	ND	ND	ND
	Shake culture, SE	41 ± 1	ND	ND	ND
	3.25 atm, SE	38 ± 1	3.3 ± 0.2	0.229 ± 0.003	ND
	13.75 atm, SE	38 ± 2	4.7 ± 0.01	0.39 ± 0.07	ND

[a] NB, Nutrient broth; NA, nutrient agar; SE, soil extract; PDB, potato dextrose broth; PDA, potato dextrose agar.

[b] ND, Not determined.

of the agar layer, never exceeded 30% during the 5 days of incubation. This resulted in a moisture stress of less than 101 kPa (1 atm). A more appropriate explanation for the high conversion factors might be contamination caused by the harvesting procedure, which involved gentle scraping of the organisms from the agar plates.

The data for the fungi unequivocally show that shake culture results, where organisms are grown without moisture stress, cannot be applied to field conditions. The fungal data for the various stress conditions are also consistent enough to allow the calculation of an average specific gravity on a dry-weight basis. The range in fungal conversion factors, for organisms grown under conditions of 34 to 1,394 kPa of moisture stress, was 0.10 to 0.41 g/cm^3, with an average of 0.323. This is 44% higher than the literature values utilized in the calculations of Babiuk and Paul (3) and Clark and Paul (8). Correction of the earlier data would require a multiplication factor of 1.44 for most soil moisture conditions.

The high mass-to-volume ratios for the bacteria are difficult to explain. Jenkinson found a 20% drop in cell biovolume on heat fixing (19). We did not find any volume differences between wet- and dry-fixed organisms. Other aspects, such as shrinkage during preparation, could not be completely discounted and could account for the high conversion factors. However, shrinkage would not alter the calculation of soil biomass as long as the proportion of shrinkage was the same for the laboratory cultures and the organisms in the soil. The increase in the dry weight-to-volume ratio, i.e., the conversion factor, was related to increased ash content. In the case of *E. aerogenes* grown at 1,394 kPa, 25% of the total cell weight was ash. This could account for the high weight-to-volume ratio. The average conversion factor for the bacteria and yeasts is 0.8 g/cm^3. At 10% ash with a specific gravity of 2.65 and organic materials with a specific gravity of 1.55 (35), the void content that normally would be filled by water would be 51%. Orga-

nisms grown under moisture stress have not received much study; values such as this, although they appear very low, cannot be discounted.

The variation shown for these few bacteria and the yeast is not great and is independent of medium type, although affected by moisture stress. The average conversion factor of 0.8 g/cm^3 would raise the bacterial biomass values in previously reported studies from this laboratory by the factor of 0.8/0.22 = 3.63. The bacteria previously accounted for 20% of the soil biomass. The use of the conversion factors discussed in this paper would raise the bacterial-plus-fungal biomass estimates by a factor of 1.88, with bacteria accounting for 39% of the sum of bacterial plus fungal biomass.

Microscopic biomass measurements were earlier criticized as giving values that were far too high; however, the soil bacteria and fungi counted microscopically only accounted for approximately 50% of the biomass determined by other techniques such as chloroform or adenosine 5'-triphosphate (29). Jenkinson et al. have shown that filamentous fungi and bacteria account for approximately two-thirds of the total biovolume (19). We have also observed a large number of spherical forms ranging in size from 3 to 10 μm in diameter, but have not yet found adequate methods for staining and biovolume measurements. The inclusion of biovolume estimates for organisms other than bacteria and fungi and the use of biovolume-biomass conversion factors that take into account soil conditions would decrease the discrepancy between microscopic and chemical techniques.

The large number of replicates, the very accurate weighing, and the detailed microscopic measurements required in a study such as we report make it difficult for us to suggest that authors always measure biomass-to-biovolume ratios. However, this study has definitely indicated that shake culture data are not applicable to soil conditions. Conversion factors of 0.33, as found in this paper for fungi, are suggested.

It is difficult to accept the dry weight-to-biovolume ratio of 0.8 found for bacteria as reported earlier in this paper. Transformation of these data to a wet weight basis would result in a maximum possible water content of 51%. The specific gravity of the wet cell under these conditions would be 1.3 rather than the generally accepted value of 1.1 for laboratory-grown cultures. Faegri et al. (12), using $CsCl_2$ density gradients of organisms actually separated from soil, found a specific gravity of 1.3 for the soil bacteria. Our data therefore are in agreement with these findings.

Hansen et al. (14), in calculating the biomass of soil organisms, utilized a specific gravity of 1.3. They assumed a dry matter content of 17%, whereas Faegri et al. (12) assumed the dry matter to be 20%. According to our calculations, these water contents are not feasible; 80% (wt/wt) water of a 1-μm^3 organism weighing 1.3 μg would result in 1.04 μg of water. At normal temperatures the water would occupy a larger volume than the total cell of 1 μm^3. To confirm or reject the biomass-to-biovolume ratio of 0.5 to 0.8 on a dry-weight basis requires further work, and we look forward to other studies from our own and other laboratories.

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