Microbial Activity in Organic Soils as Affected by Soil Depth and Crop†

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The microbial activity of Pahokee muck, a lithic medisaprist, and the effect of various environmental factors, such as position in the profile and type of plant cover, were examined. Catabolic activity for [7-¹⁴C]salicylic acid, [1,4-¹⁴C]succinate, and [1,2-¹⁴C]acetate remained reasonably constant in surface (0 to 10 cm) soil samples from a fallow (bare) field from late in the wet season (May to September) through January. Late in January, the microbial activity toward all three compounds decreased approximately 50%. The microbial activity of the soil decreased with increasing depth of soil. Salicylate catabolism was the most sensitive to increasing moisture deep in the soil profile. At the end of the wet season, a 90% decrease in activity between the surface and the 60- to 70-cm depth occurred. Catabolism of acetate and succinate decreased approximately 75% in the same samples. Little effect of crop was observed. Variation in the microbial activity, as measured by the catabolism of labeled acetate, salicylate, or succinate, was not significant between a sugarcane (*Saccharum officinarum* L.) field and a fallow field. The activity with acetate was insignificantly different in a St. Augustine grass [*Stenotaphrum secundatum* (Walt) Kuntz] field, whereas the catabolism of the remaining substrates was elevated in the grass field. These results indicate that the total carbon evolved from the different levels of the soil profile by the microbial community oxidizing the soil organic matter decreased as the depth of the soil column increased. However, correction of the amount of carbon yielded at each level for the bulk density of that level reveals that the microbial contribution to the soil subsidence is approximately equivalent throughout the soil profile above the water table.

The Everglades agricultural area consists of approximately 300,000 hectares of organic soils (Histosols), of which approximately 200,000 hectares are currently under cultivation (2). The soils were formed primarily from the remains of decayed sawgrass and related marsh plants which accumulated under the swampy conditions that prevailed over the last 5,000 years (10). This accumulation of organic matter as peat was reversed when the soils were drained. At that point the previously anaerobic conditions became aerobic. This stimulated the microbial oxidation of the peat, thereby resulting in the conversion of the peat to muck. Currently, instead of accumulating organic matter, as was the case before drainage, the soil is being lost at a rate of about 3 cm of surface elevation per year. This loss of surface elevation is termed subsidence.

Because of the value of this type of soil in crop production and recreation, both to Florida and

to other regions where such soil deposits exist, preventing or decreasing the subsidence is of utmost importance. This is particularly important in Florida in that the rich, productive muck soils are underlain by an impervious limestone layer (10). Several physical factors, such as compaction, erosion, and burning, can contribute to this loss of soil, but the primary cause of the subsidence of the soils of Florida, as they currently exist, is biological. Microbiological oxidation of the soil organic matter accounts for 58 to 73% of the observed subsidence rate (13). The only known effective means of reducing subsidence is through the prudent manipulation of the water table. The subsidence rate is linearly proportional to the depth of soil (from the surface) to the water table (8, 10). For example, by using the formula of Stephens (10), which was derived from data collected by measuring the subsidence rate of Everglades Histosols with water tables ranging from 45 to 76 cm, an increase in the depth of the water table from 45 to 60 cm results in an increase in the subsidence rate of 1 cm/year. Presumably the mechanism of protection

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of the soil organic matter by water table manipulation is the conversion of that portion of the soil column below the water table to the anaerobic conditions that prevailed before drainage.

Since microbial oxidation is the major cause of subsidence, the linear effect implies that the microbial contribution to subsidence is reasonably constant with depth of soil. This is a viable hypothesis since Waksman and Purvis (14) demonstrated high numbers of aerobic bacteria in drained Everglades Histosols collected from depths as great as 360 cm. Oxidation of the soil organic matter at such depths would be in direct opposition to the situation in mineral soils, where the majority of the microbial activity is confined to the top few centimeters of the soil profile (1, 9). Since the Histosols of South Florida contain approximately 82% organic matter, organic carbon would not be considered to be a limitation to microbial growth deep in the soil profile. Thus, oxygen can be postulated to be the greatest limitation to the microbial growth below the surface of organic soils.

The observation of the effect of water table on subsidence, the need for the development of alternative means of controlling soil subsidence, and the need to better understand those factors controlling the rate of oxidation of soil organic matter, both in mineral and organic soils, prompted this study. The objective of this work was to determine the microbial oxidation rate of the soil organic matter *in situ* and to observe the various environmental factors, such as soil depth and crop, which affect this process.

MATERIALS AND METHODS

The soil used in this study was Pahokee muck (a lithic medisaprist) collected at Belle Glade, Fla. To determine the variation of the microbial activities throughout the soil profile, soil samples were collected at various depths from a 5-hectare fallow (bare) field. Surface samples (0 to 10 cm) were composite samples of the field. Because of compaction of the soil and interference by the water table, it was not possible to collect representative samples at different depths in the field with a core sampler. Accordingly, soils deep in the profile were sampled by digging a trench (2 by 2 m) in the fallow field to the indicated depth. Composite samples were collected at each depth. Surface soils were used to determine the effect of cropping on the microbial activity. Composite soil samples were collected from the fallow field, an adjacent field of St. Augustine grass [*Stenotaphrum secundatum* (Walt) Kuntz], and a nearby sugarcane (*Saccharum officinarum* L.) field. All samples were placed in sterile plastic bags and immediately transported to the laboratory, where they were diluted for microbial analysis. Generally, the samples were diluted immediately upon return to the laboratory, but when storage was necessary, the soils were stored at 4°C. No sample was stored longer than 5 days. To allow for less variation

in the samples, those soils used to study the catabolism of radioactive substrates were sieved through a 2-mm (surface soils) or 6-mm (subsurface samples) grid before analysis. The larger sieve was needed for the subsurface samples because of the greater moisture content of these soils.

Moisture content was determined on each sample by drying the soil at 110°C to a constant weight. All moisture values are reported as a percentage of the wet weight of the soil.

Samples of sterile soil were prepared by dispensing 10 g of soil into a test tube (25 by 200 mm). The tubes were capped with aluminum foil and steam sterilized for 1 h on three alternate days.

Dilutions of the soil for microbiological analysis were prepared in 0.1% (wt/vol) sodium chloride. Total heterotrophic bacteria were assayed by plating appropriate dilutions on soil extract agar (12) by the spread plate method. The fungal population was determined with rose bengal streptomycin agar (6). The proportion of the microbial population capable of catabolizing aromatic compounds was determined by the catechol-soil extract agar method of Sundman (11). The number of organisms capable of cleaving the aromatic ring was then calculated by multiplying the population density by the proportion of the population possessing the trait. All assays were performed in triplicate.

Microbial activity in the various freshly collected soil samples was estimated by amending the soils with high-specific-activity ^{14}C -labeled substrate and examining the rate of $^{14}\text{CO}_2$ evolution during the first 60 to 120 min of incubation. To accomplish this, soil was sieved as indicated above and dispensed into test tubes (25 by 200 mm; 10 g/test tube). The soil was amended with 0.5 μCi of the substrate in 0.5 ml water. This was sufficient water to allow mixing of the substrate with the soil but not enough to greatly change the moisture status of the soil sample. CO_2 -free air was passed over the sample, and the $^{14}\text{CO}_2$ was collected in 0.5 M NaOH. Radioactivity was measured in Aquasol (New England Nuclear Corp., Boston, Mass.) with a liquid scintillation counter. The substrates were [7- ^{14}C]salicylic acid (60 mCi/mmol), [1,2- ^{14}C]sodium acetate (54 mCi/mmol), and [1,4- ^{14}C]succinic acid (25 mCi/mmol). All isotopes were supplied by New England Nuclear Corp.). Incubations were performed at room temperature (24 to 26°C) in triplicate. Rates of $^{14}\text{CO}_2$ evolution are expressed as a percentage of the ^{14}C added at zero time.

RESULTS

To relate the microbial activities to oxidation of the native soil organic matter, initial studies involved determination of the microbial activity of the fallow field soil. The catabolic rate (microbial activity) for the oxidation of salicylate, succinate, and acetate for the surface (0 to 10 cm) samples for this field are presented in Fig. 1. Data were collected from the end of the wet season (May through September) to the end of the dry season to provide an indication of the variability of the microbial activity. Salicylic acid was used as an indicator of aromatic ring

FIG. 1. Microbial activity in surface (0 to 10 cm) soil collected from a fallow field between August 1977 and May 1978. % $^{14}\text{C}_1$, Percentage of ^{14}C added at zero time.

catabolism, whereas succinate and acetate provided information on the rate of general intermediary metabolism. Little variation occurred in the rate of catabolism of these substrates from August through early January. Late in January a decrease of approximately 50% in all three activities was observed. This significant decrease in the rates was maintained throughout the remainder of the dry season.

That this activity resulted from microbial catabolism of the substrates and not spontaneous decomposition of the high-specific-activity substrates or chemical decomposition in the soil was demonstrated by the addition of the substrates to sterile soil or water. No detectable $^{14}\text{CO}_2$ was evolved from the sterile soil or water.

To study the variation of the microbial activity throughout the soil column, samples were collected at various depths of soil, as described above, at two times when the microbial activity was maximum in the surface samples and once when the activity of the surface samples had decreased. Salicylate catabolism was the most sensitive to the increased soil depth (Fig. 2). For this substrate, the greatest effect of depth was observed with the October sample. At that time a 90% decrease between the surface and the 60- to 70-cm level was noted. As the dry season progressed, this differential decreased. The variation in the catabolism of acetate and succinate was similar, but the effect of soil depth was less severe. For these substrates in October, the decrease in activity was approximately 25%.

By measuring the number of aromatic catabolizing bacteria in the various soil samples, it was possible to compare the potential for microbial catabolism of this substrate with the actual rate noted with the radioactive substrates. In the January sample, 8.5×10^5 , 5.4×10^5 , and 2.8×10^4 bacteria with the capability of cleaving aromatic rings were measured per cm^3 of soil in the 0- to 10-, 30- to 40-, and 60- to 70-cm samples, respectively. This corresponds to catabolic rates of 0.123, 0.100, and 0.049% of added ^{14}C per cm^3 of soil evolved as CO_2 with the 0- to 10-, 30- to 40-, and 60- to 70-cm samples, respectively. Since the rate of decrease in the number of bacteria between the surface and the 60- to 70-cm level was so much greater than the rate noted with radioisotopes, the implication is that many of the microbes detected in the surface samples were inactive.

This conclusion is supported by the comparison of the rates of $^{14}\text{CO}_2$ evolution per 1,000 bacteria (Fig. 3). For this study the total bacterial population was estimated by using soil extract agar. Comparable counts were obtained with nutrient agar, but, as a result of less spreading of the bacterial colonies, the colonies on the soil extract agar were easier to count. In all cases the microbial activity per 1,000 bacteria increased as the soil depth increased, although a statistically significant ($P = 0.05$; Duncan's multiple range test) decrease in bacteria was measured. Mean populations of 5.9×10^6 bacteria per cm^3 of muck were detected in the surface samples, compared with 4.2×10^5 bacteria per cm^3 of muck at the 60- to 70-cm level (mean values

FIG. 2. Microbial activity versus depth of soil (October 1977 to May 1978). % $^{14}\text{C}_1$, Percentage of ^{14}C added at zero time.

FIG. 3. Labeled carbon dioxide evolution per 1,000 bacteria versus depth of soil (October 1977 to May 1978).

of samples on 12 October 1977, 9 January 1978, 23 January 1978, and 1 May 1978).

Examination of the microbial activity per 1,000 bacteria assumes that the variation in the proportion of the $^{14}\text{CO}_2$ contributed by the bacterial population is minimal. To assess this possibility, the fungal population and the proportion of that population capable of cleaving aromatic rings was examined. As with the bacteria, a statistically significant decrease in the fungal propagules per cubic centimeter of muck was noted between the surface and the 60- to 70-cm depth (2.1×10^3 versus 4.0×10^2 , respectively). Similarly, the population capable of cleaving aromatic rings decreased from 1.0×10^3 to 8.8×10^1 cells per cm^3 muck. Thus, these properties of the fungal population vary similarly to those of the bacteria. These data suggest that, although it is not possible to determine the microbial source of the $^{14}\text{CO}_2$, the proportion contributed by the fungi and the bacteria likely vary similarly.

Another variable that would be expected to have an effect on the microbial activity of the soil is the crop growing upon it. Two major crops of the Florida Everglades region are pasture (St. Augustine grass) and sugarcane. Thus, soil samples were collected in October, when the metabolic activity of the fallow soil was high, and in late January, when the activity had decreased. Comparison of the catabolism of the various

substrates in grass, fallow, and cane soil reveals two patterns (Table 1). With acetate as the substrate, no significant differences in activity among the various fields was observed on either sampling date; however, with the other substrates (salicylate and succinate), although no significant differences were noted between the fallow and the cane samples, the activity in the grass samples was always significantly increased.

Except with acetate, the results were quite similar when the rate of catabolism of the substrate per 1,000 bacteria was tested (Table 2). In this case, some variations were observed between the fallow and cane, but generally the two soils were quite similar. In the case of salicylate

TABLE 1. Labeled carbon dioxide evolution from acetate, salicylate, and succinate in various soils

Isotope	Soil	$^{14}\text{CO}_2$ evolution (% of ^{14}C added at zero time per cm^3 of soil per min) on:	
		5 October 1977	31 January 1978
[1,2- ^{14}C]acetate	Grass	0.21 ^a	0.09 ^b
	Fallow	0.22 ^a	0.11 ^b
	Cane	0.21 ^a	0.11 ^b
[7- ^{14}C]salicylate	Grass	0.43 ^a	0.26 ^b
	Fallow	0.17 ^c	0.07 ^d
	Cane	0.21 ^c	0.11 ^f
[1,4- ^{14}C]succinate	Grass	1.97 ^a	0.82 ^c
	Fallow	1.18 ^b	0.56 ^c
	Cane	1.32 ^b	0.56 ^c

^{a,b,c,d,e,f} Rates for individual substrates followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

TABLE 2. $^{14}\text{CO}_2$ evolved per 1,000 bacteria in various soils

Isotope	Soil	$^{14}\text{CO}_2$ evolution (dpm $\times 10^1$) per 1,000 bacteria per min on:	
		5 October 1977	31 January 1978
[1,2- ^{14}C]acetate	Grass	ND ^a	0.13 ^b
	Fallow	0.37 ^c	0.22 ^b
	Cane	0.33 ^c	0.23 ^b
[7- ^{14}C]salicylate	Grass	ND	0.40 ^b
	Fallow	0.29 ^c	0.14 ^d
	Cane	0.33 ^c	0.21 ^c
[1,4- ^{14}C]succinate	Grass	ND	1.24 ^b
	Fallow	1.99 ^c	1.12 ^b
	Cane	2.06 ^c	1.10 ^b

^a ND, Not determined.

^{b,c,d,e} Rates for individual substrates followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

and succinate, the rate with the grass was again elevated.

DISCUSSION

Past methods for the estimation of the rate of soil subsidence have involved taking sightings of the soil elevation (10) or the measurement of the carbon dioxide evolved from the soil surface (7). These techniques are useful in determining the gross changes involved with the oxidation of the soil organic matter, but lack the sensitivity necessary for the evaluation of the microbial activities at various points in the soil profile under different environmental conditions. High-specific-activity radioisotopes have been used successfully in soil samples (5) and sediments (3, 4) to estimate microbial activity. Ideally, the best numbers could be obtained if these chemicals could be added to the soil *in situ* without disturbing it and the labeled carbon dioxide evolved analyzed. Obviously, this was not possible for the present study, because of the depths of the soil profile examined. Thus, soil was collected and transported aseptically to the laboratory, where the catabolic activity for several compounds was examined. Since the soil was collected and immediately transported to the laboratory (transit time, less than 5 min), the changes would be expected to be minimal. Thus, it is reasonable to assume that the values for the microbial activity measured in this study are good approximations of the activity *in situ*.

Utilization of the radioisotope method along with classical microbiological techniques reveals that the capacity for microbial oxidation of the soil organic matter in Pahokee muck occurs throughout the soil profile to the limits of the depths of the soil columns examined. Variation in the rate of oxidation of the various substrates was noted to occur as the depth of soil increased and as the dry season developed. Comparison of microbial numbers and catabolic activity per 1,000 bacteria suggests that many of the bacteria found in the surface samples were inactive.

An interesting extrapolation that could be made from the data involves determination of the amount of soil carbon that would be evolved from the soil if the isotopically labeled compounds are catabolized at rates representative of the carbon evolved from the field. This would provide an indication of the amount of soil organic matter oxidized at various depths of soil. A set of these extrapolations of the rate data are presented in Table 3. Several assumptions were made in deriving these values. (i) Subsidence is occurring at a rate of 3 cm/year. (This assumption is immaterial in that changes in the rate would merely affect the gross value reported and

TABLE 3. *Relative carbon evolution and subsidence at various soil depths*

Substrate	Depth (cm)	Carbon evolution (kg of C per hectare per year)	Relative subsidence (cm) ^a
Acetate	0-10	3,203 ^b	1.0 ^c
	30-40	2,263 ^c	1.3 ^b
	60-70	1,593 ^d	0.8 ^d
Succinate	0-10	2,369 ^b	1.0 ^c
	30-40	2,179 ^b	1.5 ^b
	60-70	1,268 ^c	0.9 ^c

^a Averages calculated from October and January ¹⁴CO₂ evolution rates.

^{b,c,d} Values within a column for individual substrates followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

not the relationship between the various samples.) (ii) The soil is composed of 40% carbon. (iii) All carbon is lost from the soil ecosystem as carbon dioxide. (iv) For each substrate examined, the rate of carbon dioxide evolved was assumed to be representative of all of the carbon evolved; that is, the rate of carbon evolution from acetate would represent the rate of carbon evolution from the various other compounds composing the soil organic matter. Since this is not the case, the gross values for carbon evolved listed in Table 3 vary considerably for each of the substrates tested. To minimize the variation, carbon evolution was calculated for acetate and succinate, two substrates more likely to represent the overall aerobic metabolic rate of the soil microflora. These data indicate that the CO₂ evolution decreases with sample depth.

As indicated above, the subsidence rate varies linearly with changes in the depth to the water table (8, 10). The above calculations reflect the absolute amount of carbon lost from each level of the soil profile. The bulk density of organic soils varies about threefold throughout the soil profile. Thus, 1 kg of carbon at the surface would occupy one-third the volume of the same weight of carbon at the 60- to 70- cm level. Correction of the above data for bulk density changes and conversion of the rate to relative values (assuming a rate of one for the surface samples) indicates that the effect of loss of soil carbon on soil elevation (subsidence) remained nearly the same throughout the soil profile.

It is interesting that the rate of subsidence appeared to decrease about 50% during the latter part of the dry season. The total number of bacteria did not significantly decrease in these samples, and the moisture and temperature were well within the range noted by Waksman and

Purvis (14) to be conducive to bacterial oxidation of organic soils. Whether this is a phenomenon peculiar to 1978 or will be repeated in subsequent years requires further study.

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