Carbon Mineralization in Acidic, Xeric Forest Soils: Induction of New Activities†

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Carbon mineralization was examined in Lakehurst and Atsion sands collected from the New Jersey Pinelands and in Pahokee muck from the Everglades Agricultural Area. Objectives were (i) to estimate the carbon mineralization capacities of acidic, xeric Pinelands soils in the absence of exogenously supplied carbon substrate (nonamended carbon mineralization rate) and to compare these activities with those of agriculturally developed Pahokee muck, and (ii) to measure the capacity for increased carbon mineralization in the soils after carbon amendment. In most cases, nonamended carbon mineralization rates were greater in samples of the acid- and moisture-stressed Pinelands soils than in Pahokee muck collected from a fallow (bare) field. Carbon amendment resulted in augmented catabolic activity in Pahokee muck samples, suggesting that the microbial community was carbon limited in this soil. With many of the substrates, no stimulation of the catabolic rate was detected after amendment of Pinelands soils. This was documented by the observation that amendment of Pahokee muck with an amino acid mixture, glucose, or acetate resulted in a 3.0-, 3.9-, or 10.5-fold stimulation of catabolic activity, respectively, for the added substrate. In contrast, amendment of the Pinelands soils resulted in increased amino acid and acetate catabolic rates in Lakehurst sand and increased acetate metabolism only in Atsion sand. Other activities were unchanged. The increased glucose respiration rates resulted from stimulation of existing microbial activity rather than from microbial proliferation since no change in the microbial growth rate, as estimated by the rate of incorporation of ^{14}C-labeled acetate into cell membranes, occurred after glucose amendment of the soils. A stimulation of microbial growth rate was recorded with glucose-amended Lakehurst sand collected from the B horizon.

The organic components of a forest floor, the litter layer and the soil organic matter fraction, constitute a major pool of plant nutrients for plant growth and development (7–9, 18). Mineralization of this organic matter pool, in most situations, controls the nature and quantity of above-ground biomass. Thus, in many cases, a complete understanding of the dynamics of biomass synthesis within an ecosystem requires estimates of the mineralization rates of these organic matter pools. Although litter bag and related procedures document decomposition rates of forest litter and provide a materials balance of the various pools for modeling studies, a complete understanding of the ecosystem requires direct examination of the primary mediators of mineralization, the soil microbial community. Although the microfauna play a major role in the disruption of the macrostructure of the plant debris by increasing the surface area of the debris and thereby increasing the potential sites for microbial decomposition (1, 3, 12), the primary population involved in mineralization of the organic nutrients is the soil bacterial and fungal community. Thus, environmental modifications impinging upon this community are primary in controlling forest ecosystem development.

The Pinelands of New Jersey provide an interesting ecosystem to evaluate directly the microbial contribution to nutrient mineralization in a forest soil and the limitations of this mineralization capacity. The soils of the region are primarily either highly acidic, xeric sandy soils or acidic, flooded sandy soils (11, 18). Thus, an opportunity is provided to study the adaptations of both the plant and the microbial communities to these extreme moisture and pH levels. Hence, this project was commenced with the primary

objectives of (i) examining the carbon mineralization capacity of the soil microflora, (ii) assessing changes in microbial growth and the increase in metabolic activity after amendment of the soil with a readily metabolizable energy source, and (iii) evaluating the effect of exogenously supplied fixed nitrogen on carbon mineralization. Answers were sought to such questions as (i) what are the endogenous metabolic levels in these soils, (ii) can the enzymatic activity levels be increased or are they limited by the pH and moisture extremes characteristic of the Pinelands soils, and (iii) do changes in the metabolic rates reflect increased microbial biomass or enzymatic activity? Three soils were chosen for study: Lakehurst sand and Atsion sand from the New Jersey Pinelands and Pahokee muck from the Everglades Agricultural Area. The Pinelands soils are the two major soil types of the region. These specific soils were selected for study because they provide a pH gradient, 4.34, 3.76, and 5.9, respectively, and a gradient in organic matter input and type, i.e., no litter layer, a thick litter layer, and approximately 82% humified organic matter, respectively. Also, use of the Pahokee muck allowed comparison of the data collected herein with the large body of information descriptive of microbial activity in that soil. This study is part of an ongoing project with an overall objective of evaluating nutrient cycling in the Pinelands soils and the impact of plant-microbe associations and interactions on total ecosystem nutrient balance and biomass productivity.

MATERIALS AND METHODS

Pinelands sites were selected within the McDonald's Branch Watershed of the Lebanon State Forest, N. J. The specific sites include the following types, based on vegetation classifications and descriptions by Forman (6): (i) pine upland, canopy dominantly or exclusively of pitch pine (*Pinus*

rigida), with an understory of primarily scrub oak (*Quercus ilicifolia*), low bush blueberry (*Vaccinium vacillans*), and black huckleberry (*Gaylussacia baccata*); and (ii) pine lowland, a canopy of *Pinus rigida* with an understory of sheep laurel (*Kalmia angustifolia*), dangleberry (*Gaylussacia frondosa*), inkberry (*Ilex glabra*), and other shrubs of this type (4). For comparison, soil samples were also collected from a fallow (bare) field in the Everglades Agricultural Area. The latter soil was Pahokee muck (a Lithic medisaprist).

Soils used in this study are described in Table 1. Soils from the Pinelands were Lakehurst sand, A and B horizons (a mesic, coated Haplaquodic Quartzipsamments), from the upland site and the A horizon of the soil from the Pine lowland site (Atsion series, a mesic Aeric Haplaquod). The Atsion sand horizon sampled consisted of the first 12 cm of the soil profile. This was primarily partially humified plant debris intermixed with plant roots (the rhizosphere). For all the soils, undecomposed surface litter was removed before the soil samples were collected. Characteristics of the Pahokee muck have been described previously (19). The Pinelands soils have been previously described by Tedrow (18). Composited soil samples were collected, transported to the laboratory (a trip of approximately 2 h), sieved to pass 2 mm, and stored at 4°C for no longer than 14 days. Before the initiation of any studies, the soils were dispensed as described below, the moisture was adjusted to approximately field capacity, and the soils were incubated for 16 h at room temperature (25 to 27°C).

Total lipids and protein levels in the soil were analyzed by the methods of Federle et al. (5) and Stevenson (13), respectively. Essentially, lipids were extracted with a mixture of phosphate buffer, methanol, and chloroform. The chloroform layer was collected and evaporated. The weight of the residual material was used as an estimate of the total lipid content of the soil. Proteins were estimated by ninhydrin colorimetric analysis of 6 N HCl hydrolysates of soil samples.

Metabolic rates were estimated by the method of Tate (14) except that the ^{14}C-labeled substrates contained unlabeled carrier (0.5% [wt/vol] for glucose and sodium acetate and 0.2% [wt/vol] for the Casamino Acids). For these studies, soil samples were dispensed at 5 g per test tube (20 by 200 mm), preincubated as described above, and then amended with the isotope solution (1 ml of labeled substrate plus carrier per 10 g of soil). Final substrate concentrations were therefore 0.1% (wt/wt) for glucose and sodium acetate and 0.02% (wt/wt) for the amino acids. $^{14}CO_2$ was collected in Oxifluor-CO_2 (New England Nuclear Corp.) and quantified in a scintillation counter. Labeled substrates used in this study were sodium [1-^{14}C]acetate, a U-^{14}C-amino acid mixture (NEC-445E, a mixture of 15 purified amino acids), and D-[1-^{14}C]glucose. All labeled substrates were purchased from New England Nuclear Corp. Incubation conditions and substrate levels were such that aerobic carbon metabolism was measured (i.e., the soils did not become anoxic).

The lipid synthesis rate was determined in soils dispensed (1 g of wet soil per test tube [16 by 150 mm]) and preincubated as described above. After the preincubation period, soils were amended with 1.0 ml of 0.1 μCi of [1-^{14}C]acetate per ml per g of wet soil. After 1 h of incubation, the unreacted substrate was removed from the soil by washing twice with 0.2 M citrate buffer, (pH 2.2). The lipids were then extracted by the method of Federle et al. (5). For this study, the chloroform layer containing the newly synthesized ^{14}C-labeled lipids was evaporated to dryness in a 20-ml scintillation vial, 10 ml of aquasol II was added, and the

TABLE 1. Properties of soils used in this study

Soil	Depth (cm)	pH	Organic content (%)
Lakehurst sand	0–15	4.34	2.26
	15–30	4.50	ND[a]
Atsion sand	0–12	3.76	88.5
Pahokee muck	0–20	5.9	82

[a] ND, Not determined.

radioactivity was quantified by scintillation counting. Counts per minute were converted to disintegrations per minute by the internal standard method.

For studies of flooded Pahokee muck, soil was dispensed at 10 g per serum bottle (60 ml). Sufficient water was added to give a depth of 1 cm above the soil surface. The bottles containing the soil were then plugged with serum stoppers and incubated at 25°C. After this incubation period, soil carbon catabolic rates were assayed as described above.

Data were analyzed by standard statistical procedures (10). Metabolic rates were calculated as micrograms of substrate metabolized from measurement of the quantities of $^{14}CO_2$ evolved during the incubation. Significance of differences between these rates was determined by linear regression analysis with rate as the independent variable and time as the dependent variable. Significance ($P < 0.05$) was indicated by slopes that were significantly different from zero. The F test and Duncan's New Multiple Range Test were used to determine significance of differences in rates between soils at a single sample time.

RESULTS

Carbon metabolism capacity. Nonamended glucose catabolism rates (i.e., catabolic rates associated with metabolism of organic components contained in the soil sample when collected) in Pahokee muck, Lakehurst sand, and the Atsion sand were 1.4, 3.5, and 4.9 μg/cm³ per h, respectively (Fig. 1). The rates were significantly different ($P < 0.05$; Duncan's New Multiple Range Test). Over a 45-h incubation period, rates declined with the Pinelands soils, whereas activity increased approximately 3.6-fold in the Pahokee muck before gradually declining. After 45 h of incubation, glucose-oxidizing activity in the muck was still approximately twice that initially observed. The decline in activity in the Atsion sand may reflect, in part, substrate depletion in that 40% of the added glucose had been metabolized into CO_2 after 45 h of incubation. After comparable incubation, 8.8 and 22.0% of the substrate had been metabolized in the Lakehurst fine sand and Pahokee muck soils, respectively.

Less variation in initial amino acid catabolic rates was observed among the three soils than was noted with glucose metabolism. Linear regression analysis of the first 7 and 23.3 h of incubation data for Pahokee muck and Lakehurst sand, respectively, indicated significant ($P < 0.05$) increases in $^{14}CO_2$ evolution rates in both soils (Fig. 1). Amino acid catabolism in the Atsion sand declined during the same period. Activities for both Atsion sand and Pahokee muck were well below initial activities after nearly 24 h of incubation.

In the above studies, different batches of preincubated soil were used for each substrate. To negate any variation in data resulting from batch differences, a series of studies was conducted in which carbon mineralization and induction of new metabolic activities in soil samples in parallel incuba-
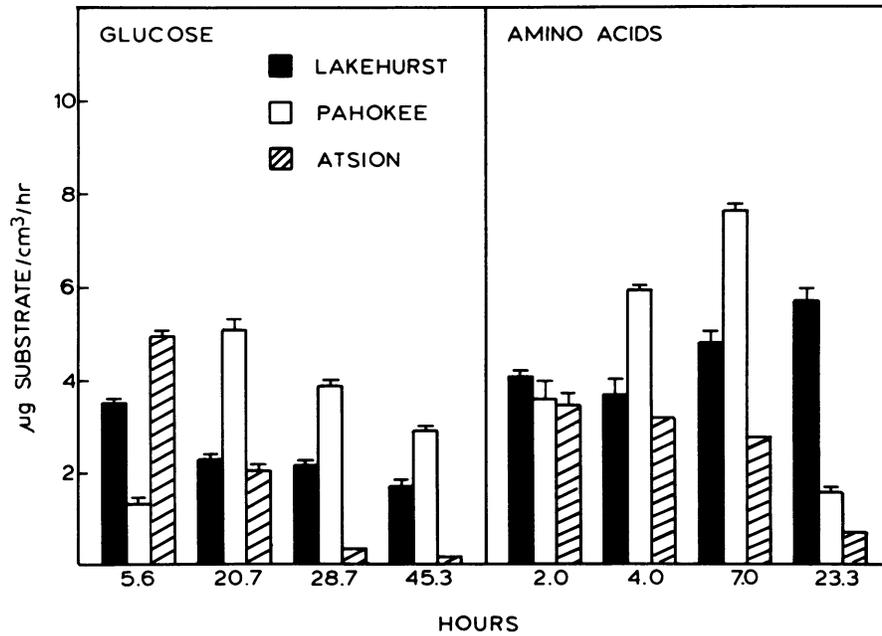
FIG. 1. Glucose and amino acid metabolism in Lakehurst and Atsion sands and Pahokee muck. Error bars represent standard error ($n =$ 3). Standard errors of <0.05 μg of substrate per cm^3 per h are not shown.

tions were examined (Fig. 2 and 3). As compared with the situation in Pahokee muck, in which amino acid, glucose, and acetate catabolic activities increased 3.0-, 3.9-, and 10.5-fold, respectively, little change in the catabolic activity of the microbial community in the Atsion sand was observed (Fig. 2). With the latter soil, the only increase in activity followed acetate amendment. Note that whereas the glucose oxidation rates and, frequently, the amino acid oxidation

rates declined in the later time samples, acetate catabolism rates increased. No increase in glucose metabolism rate after amendment was found with the Lakehurst sand, but both amino acid and acetate metabolic rates increased approximately 3- and 15-fold, respectively (Fig. 3). Comparison of these data with those in Fig. 1 indicates that, as expected, some variation in the absolute rates between batches of soil did occur. For example, with the studies of Lakehurst sand
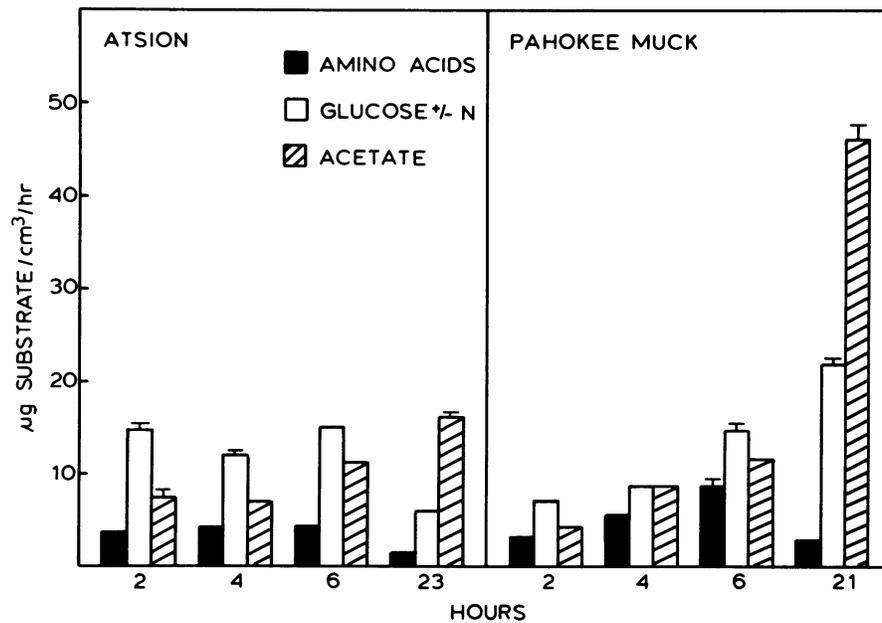
FIG. 2. Amino acid, glucose, and acetate metabolism in Atsion sand and Pahokee muck. Glucose metabolism was examined in the presence and absence of ammonium sulfate (21 μg of ammonium N). Since no significant effect of ammonium sulfate amendment was observed, data plotted are mean values of the two treatments. Error bars represent standard error ($n = 3$). Standard errors of <0.05 μg of substrate per cm^3 per h are not shown.
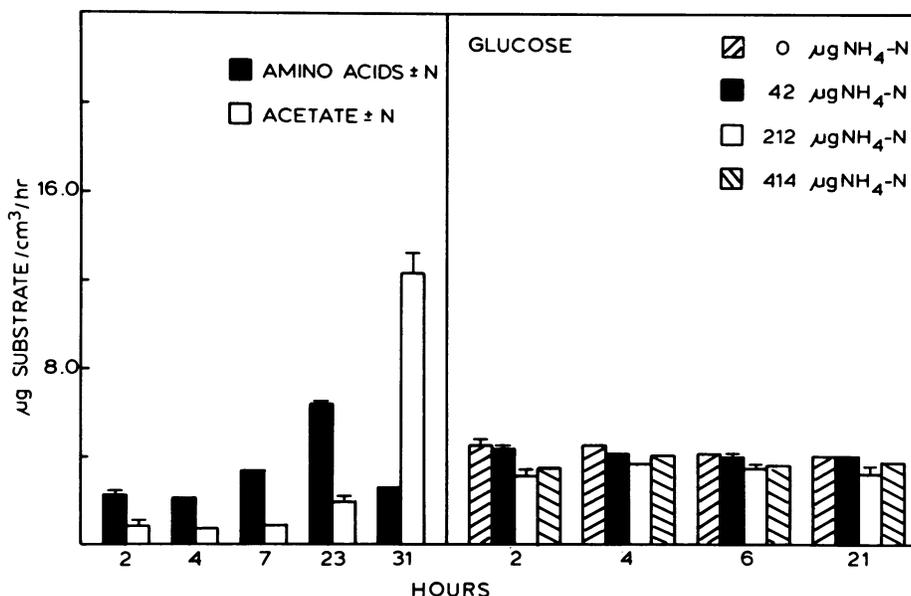
FIG. 3. Amino acid, acetate, and glucose metabolism in Lakehurst sand. Acetate and amino acid metabolism were examined in the presence and absence of 21 μg of ammonium N (as ammonium sulfate). Glucose metabolism was evaluated in the presence of 0 to 414 μg of ammonium N (as ammonium sulfate). Since no significant effect of ammonium sulfate amendment was observed, data plotted are mean values of the two treatments. Error bars represent standard error (n = 3). Standard errors of <0.10 μg of substrate per cm^3 per h are not shown.

after approximately 21 h of incubation, the rate had declined approximately 20% in one study, whereas no decline was noted in the other. Although the overall conclusions between the various experiments remained the same, the absolute values measured frequently differed. This would be expected since each sample constituted a single preincubated microcosm.

Nitrogen amendment effects. The effect of nitrogen amendment on glucose metabolism was evaluated. Amendment of Pahokee muck and Atsion sand with 21 μg of NH_4^+ N [as $(NH_4)_2SO_4$] per g of wet soil had no significant effect on glucose oxidation rates or the ability to induce new glucose oxidation activity in these soils (Fig. 2). Similarly, comparable ammonium amendments of Lakehurst sand had no effect on amino acid metabolism or acetate oxidation. Variation of the ammonium level from 0 to 414 μg of NH_4^+ N per g of wet soil had no effect on the metabolic rate or glucose metabolism induction over a 21-h incubation period (Fig. 3).

Microbial growth rate. For those soils in which new metabolic activities were detected, we wished to determine whether the increased carbon mineralization resulted from microbial growth or simply from enzyme synthesis within existing microbial populations. A technique useful for such determinations is the measurement of labeled acetate incorporation into the soil lipid fraction (2). Newly synthesized lipids are considered to be integrated into living microbial membranes. To evaluate this parameter in the soils used in this study, acetate incorporated into the soil lipid fraction was measured in soil samples incubated in the presence or absence of glucose (Table 2). Glucose was selected for this study in that (i) since both positive and negative effects were found with the soils, a negative control was included should significant microbial growth be detected and, alternatively, (ii) any indirect effects on microbial growth could be observed in soils in which stimulation of glucose catabolism was not found. In the latter situation, although a stimulation of growth rate could be detected, it would not result from

augmented glucose catabolism. Although glucose metabolism was detected in Pahokee muck within 24 h of amendment, no increase in microbial growth rate was found over an incubation period of approximately 3 days (note that the growth rate was measured at hours 16, 40, and 64 of incubation). Thus, it appears that with Pahokee muck, the increased glucose metabolic capacity resulted from enzyme synthesis in previously inactive or less active microbial populations. Similar situations likely exist with other metabolic activities in this soil (17). To confirm this observation, total soil lipids and proteins were measured in unamended Pahokee muck or muck amended with glucose at the same level as in the above studies and incubated at 25°C. Protein and lipid levels in the soil were estimated at intervals over an 11-day incubation period. No significant variation (P < 0.05) in these values was detected over the incubation period. Protein and lipid levels remained at 0.81 and 3.30 mg/cm^3,

TABLE 2. Incorporation of [^{14}C]acetate into microbial biomass lipids: effect of glucose amendment and time

h (treatment)	[^{14}C]acetate incorporation (dpm/cm^3) in the following soil:			
	Pahokee muck	Lakehurst A horizon	Lakehurst B horizon	Atsion
16				
None	6,206	14,821	9,108	2,328
Glucose	8,417	25,410	77,666[a]	2,727
40				
None	6,156	14,599	7,756	1,859
Glucose	7,811	18,015	22,275[a]	2,504
64				
None	4,688	11,362	6,415	1,327
Glucose	5,520	12,495	12,309	1,688

[a] Differences between unamended and glucose-amended samples were significantly different (P < 0.05). All other differences were not significant.

respectively. This stability in levels could result either from there being no change in the soil components examined or in there being a significant change in that portion of the soil protein or lipid fractions involved in microbial growth, but the amount of material involved in these fractions was too small to result in measurable changes in the total lipid and protein levels in the soil. To test these hypotheses, differences between protein and lipid in flooded soil and soil incubated at field moisture levels were examined. Previous work demonstrated significant changes in microbial activity of Pahokee muck incubated under flooded conditions (15). After 11 days of flooding, no change in the soil proteins was detected but lipid levels were increased 23.9%. Thus, the failure to increase soil lipids observed in the glucose-induced soils most probably resulted from synthesis of new enzymes in previously existing cells. Failure to observe changes in the protein levels most probably resulted from the fact that both humified and microbial biomass proteins were extracted by the procedure. Thus, the humified protein concentrations are sufficiently high in these soils to mask any changes in biomass protein levels due to microbial growth.

No significant effect ($P < 0.05$) of glucose amendment on microbial growth rate was found in the Lakehurst A horizon and the Atsion sand. Also, no indirect effects of glucose on the growth rate in these soils was recorded. The capability of this procedure to demonstrate increased microbial growth in mineral soils was demonstrated with the Lakehurst B horizon soil in which an approximate eightfold increase in growth rate was detected.

DISCUSSION

A variety of microbial reactions to inputs of exogenous carbon into the ecosystem were demonstrated in this study. That is, with some substrates, increased catabolic activity was detected with all carbon-amended soils examined, whereas with other substrates, the reaction of the microbial community to substrate input varied with soil source. The failure of the microbial community to increase its mineralization capacity for the amended substrate could result from the fact that the substrate may already be present in concentrations which cause the requisite metabolic enzymes to be fully induced. In this case, any new enzymatic activity would result from population growth, which could be limited by the prevailing environmental conditions. Interestingly, two different effects of carbon amendment were observed with the two Pinelands soils. With the Lakehurst soil, increased mineralization resulted from inputs of both amino acids and acetate but not from glucose amendment, whereas with the Atsion sand, only amendment with acetate caused a stimulation of mineralization activity. Although it is difficult and perhaps dangerous to extrapolate such laboratory studies to the field, these data can best be understood when the nature of the total ecosystem from which the soils were collected is considered. The microbial community and the properties and types of native soil organic matter are directly affected and, to some degree, controlled by the ecosystem plant community. In that no litter layer accumulates on the Lakehurst sand site, the rhizosphere is the major source of exogenous carbonaceous substrates. The data suggest that glucose is supplied by this source at concentrations which saturate its mineralization potential. Alternatively, the population may have a limited capacity to catabolize glucose. This possibility is considered to be unlikely in that it would be anticipated a priori that the soil inputs of glucose containing polysaccharides as plant residues would select for microbial populations capable of using the glucose as a carbon

source. The effects of carbon amendment are perhaps easier to explain with the Atsion sand. The Atsion sand contains large quantities of partially decayed plant material which most likely saturated the microbial metabolic capacity; that is, carbon is not the primary factor limiting microbial respiration. This can be contrasted with the results from Pahokee muck, in which all substrate additions resulted in increased microbial activity. Pahokee muck is a well-humified Histosol (16). Thus, the pool of readily oxidizable nutrients has been essentially depleted. These data confirm the previous hypothesis that the Pahokee muck is carbon limited (19). In this situation, addition of any metabolizable exogenous carbon source results in synthesis of new catabolic activity.

The labeling rate of cellular lipids was shown to be a useful characteristic for differentiating between induction of enzyme synthesis and growth of specific populations capable of metabolizing the amended substrate in Pahokee muck. From these data, it is clear that with glucose amendment, the new enzymatic activity resulted from synthesis of enzyme in existing cells rather than from augmentation of the glucose-oxidizing soil microbial community. Measurement of the labeling rate of cellular lipids will prove useful in future studies to determine the adaptability of the ecosystem to a variety of perturbations and the speed at which a new steady state is established. Needless to say, for those functions in which the microbial community reacts via enzyme synthesis, reestablishment of the equilibrium state will be more rapid than for those in which cell proliferation is required.

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