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Decomposition of ^{14}C- and ^{15}N-labeled organisms in soil under anaerobic conditions

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Abstract

Carbon and nitrogen mineralized from soil under waterlogged conditions may come from the soil microbial biomass pool and potentially could be used for biomass estimations. ^{14}C and ^{15}N labeled cells added to soil were monitored for decomposition under aerobic and anaerobic conditions. Under aerobic conditions 12–42% of the added organism C was mineralized and 1–30% of the N. Under waterlogged conditions 13–33% of the C and 4–13% of the N was mineralized. The mineralized organism C as a percent of the total C evolved was consistent for both aerobic and anaerobic conditions, however the nitrogen showed extreme variations

Introduction

Microbial biomass plays a central role in nutrient cycling as both a major component of the active soil organic matter fraction and as the mediator of nutrient transformations. The most widely used method for the determination of microbial biomass C and nitrogen (N) in soils is the chloroform fumigation incubation method (CFIM) (Jenkinson and Powlson, 1976). Usually the soil sample is mixed, adjusted for moisture and preincubated prior to biomass determination. This pretreatment of soil alters the existing soil conditions and hinders attempts to follow microbial biomass fluctuations over a growing season.

A potential method for measuring microbial biomass with minimal pretreatment effects is to use an anaerobic incubation. Anaerobic incubations have previously been used for estimating soil N availability (Keeney and Bremner, 1966), however, the C evolved during the incubation has never been monitored. It is probable that some fraction of the C and N mineralized from a soil sample subjected to waterlogged conditions comes from the degradation of organisms killed by the waterlogging treatment. Recently, correlations have been made between CFIM values and anaerobic mineralized N (Hasebe *et al.*, 1985).

There would be several advantages in determining microbial biomass by the anaerobic incubation technique: firstly, the method is simple, short in duration, and can be started in the field; secondly, the method causes no pretreatment perturbation to the sample such as moisture changes and preincubation; and thirdly, the method would give both C and N values. In this preliminary study we have added live ^{14}C and ^{15}N-labeled cells to soil samples before incubation under aerobic or anaerobic conditions and determined the fraction of cell C and N that was mineralized.

Materials and methods

^{14}C and ^{15}N-labeled organisms

Two fungal and two bacterial species were isolated from soil, the bacteria were isolated on one-half strength trypticase-soy agar; and the fungi on acidified potato-dextrose agar with 100 ppm strep-

Table 1. Properties of the organisms added to soil

Designation	Organism	% C[a]	Sp. act. (kBq/mg	% N[b]	A% $^{15}N^{b}$	C/N
F1	Aspergillus	40.3	4.8	6.9	4.8	5.8
F2	Cephalosporium	44.2	4.2	8.3	4.8	4.8
B2	Bacillus	38.9	2.7	15.3	0.8	2.6
B3	Pseudomonas	41.8	3.4	17.0	1.8	2.5
mix	(equal weight)	43.2	3.8	10.2	2.6	4.2

[a] CV range, % C O.7–3.1%; Sp. act. 2.5–11.8%
[b] CV range, % N 3.3–8.7%; Atom % excess 0.7–1.6%

tomycin. Isolates from the dilution plates were purified and cultured in a glucose mineral salts medium.

The medium for cultivating labeled cells contained ^{14}C-glucose (11.5 kBq/mg-C) and $^{15}NH_4$ $^{15}NO_3$ (11 atom % (A%) ^{15}N). Organisms were grown at 25°C until late logarithmic phase, harvested by centrifugation (2,000 *g* 4°C), repeatedly washed with phosphate buffer and were suspended in solution at 10 mg organism per mL on a dry weight basis. Organism type, C and N contents are given in Table 1.

Anaerobic and aerobic incubations

The soil, a silt-loam mollic haploxeralf, was sampled at 0–10 cm depth, and has a pH of 5.9, C content of 2.6% and N content of 0.2%. The incubation flasks (50 mL) contained 10 g each of moist soil preincubated for 10 days.

At time zero 1 mg organism dry wt g^{-1} soil was pipetted into each of triplicate flasks and mixed thoroughly. The flasks to be incubated aerobically were stoppered with a rubber septum. Sixteen mL of CO_2-free water was added to the flasks to be incubated anaerobically, then flushed with N_2 gas and stoppered with a rubber septum. All flasks were incubated for 7 days at 25°C.

Carbon analysis

Organism total C and ^{14}C content was measured using a wet oxidation procedure (Snyder and Trofymow, 1984). For ^{14}C analysis a 0.1 mL aliquot of the NaOH trap was counted in a liquid scintillation spectrophotometer the rest was titrated for total carbon.

For all incubations the CO_2-C evolved was determined by G.C. analysis of headspace gas. ^{14}C was determined by injecting headspace gas into an evacuated scintillation vial containing 0.5 mL NaOH. After daily determinations the anaerobic flasks were flushed with N_2 and the aerobic flasks with CO_2 free air.

The final values of total C and ^{14}C were corrected for CO_2 dissolved in H_2O and for headspace expansion.

Nitrogen analysis

Organism total N and ^{15}N content was determined by Kjeldahl digestion (Smith *et al.*, 1980). Total N in the digested mixture was determined using automated colorimetric procedures, and ^{15}N was determined on the same solution using a ^{15}N diffusion procedure (MacKown *et al.*, 1987) with subsequent mass spectrometer analysis.

Inorganic N was extracted from all samples after 7 days using 2 *N* KCl. Analysis for inorganic-N was by automated colorimetric procedures and ^{15}N by the diffusion method.

Results

Organisms

Two fungal and two bacterial species were chosen based on their common occurrence on agar plates and identified by standard morphological and physiological test (Table 1). The Cephalosporium fungus is a prolific spore former when cultured in nutrient media, however, by using this organism we could observe ^{14}C and ^{15}N labeled spore decomposition under aerobic and anaerobic

conditions. *Pseudomonas* sp. are capable of growth and metabolism under anaerobic conditions. The diversity of organisms and structures provides a broad range of characteristics relating to cell decomposition in soil.

The fungi were cultured for 8 days and the bacteria for 2 days before harvesting. The specific activity (Sp. Act.) of all the organisms were similar excepting the *Bacillus* sp. which also had the lowest dry weight yield. The percent N for the fungi averaged 7.5% with an A% ^{15}N of 5%, the resultant C/N ratio was 6. For the bacteria nutrient broth was added which increased the available nitrogen resulting in a high percent N in the organisms and a concurrent decrease in the A% ^{15}N.

The total amount of organism C added to the soil was 450 $\mu g\, C\, g^{-1}$ soil with the quantity of microbial biomass initially estimated to be 1752 $\mu g\, C\, g^{-1}$ soil.

Recovery of ^{14}C and ^{15}N from labeled organisms

The percent of the added ^{14}C and ^{15}N labeled organisms recovered as CO_2-C and inorganic N is shown in Table 2. Under anaerobic conditions variable amounts of both C and N were mineralized during the 7-day incubation. Cell C mineralization from the F1 and B2 organisms was about twice that of the F2 and B3 organisms. The mix of organisms decomposed at about the mean of the individual organisms. The ^{15}N recovered as inorganic N ranged from 4 to 13.5% for the anaerobic incubated organisms with the mix being nearly the mean of the individual percentages.

The C mineralized from the added organisms during aerobic incubation ranged from 11.7 to 42.1%. The percent C from the mixture of organisms was 34.9%, similar to the average of the individual organisms when the F2 sporulating fungi was excluded. Labeled N recovered from the aerobically incubated cells varied widely between 2–31% and was generally lower than the corresponding organism incubated under anaerobic conditions.

Table 2. Percent of added ^{14}C and ^{15}N labeled organisms recovered as CO_2-C and inorganic N

Added organism	Anaerobic		Aerobic	
	C	N	C	N
F1	22.7	8.6	42.1	2.1
F2	12.7	4.0	11.7	0.8
B2	32.9	13.5	25.2	11.3
B3	16.9	8.5	35.8	30.7
mix	24.1	7.3	34.9	2.2

Table 3. Mineralized added organism C and N as a percent of the total C and N mineralized during the 7-day incubation

Added organism	Anaerobic		Aerobic	
	C	N	C	N
F1	35	28	40	3
F2	27	7	26	1
B2	46	36	29	12
B3	36	32	41	26
mix	41	27	38	4

The relationship between organism C and N mineralized and total C and N mineralized, expressed as a percentage, is shown in Table 3. The C mineralized from the labeled cells under anaerobic conditions ranged from 27 to 46% of the total C mineralized, averaging 37 $\pm$ 7% for all treatments. The cell C mineralized aerobically ranged from 26–41% with an overall treatment average of 35 $\pm$ 7%. A linear relationship between organism C mineralized and total C mineralized was found with either treatment over time. The r^2 value for this time dependent correlation ranged from 0.97 to 1.00, indicating that a consistent proportion of the C mineralized over time was from the added microbial cells.

The N mineralized from the added organisms as a percentage of the total N mineralized was consistent for the anaerobic samples except for the F2 treatment. The values ranged from 27 to 36% with an average of 31 $\pm$ 4%. The aerobic samples showed no consistent relationship between organism N mineralization and total N mineralization, which is probably due to varing immobilization under aerobic conditions.

Discussion

The objective of this experiment was to evaluate an anaerobic incubation method for potential in measuring soil microbial biomass C and N values. If a consistent fraction of added cells was mineralized a factor could be calculated to estimate

total microbial biomass as in the CFIM procedure. The potential benefit of the anaerobic incubation is the uniformity in sample preparation and the expediency of the analyses.

In studies with chloroform fumigated soil and added labeled live organisms (Anderson and Domsch, 1978) the percent of fungal carbon mineralized ranged between 32 and 52% and bacteria between 7 and 50%. Our study, even though limited in number of organisms, shows similar variations. Over time there was a consistent amount of carbon mineralized from organisms in relation to the total carbon mineralized. This relationship was evident under both aerobic and anaerobic conditions.

That the anaerobic control mineralized greater amounts of C than the aerobic control (data not shown) form the basis of the hypothesis that waterlogging a soil may kill and subsequently cause mineralization of microbial biomass C. However, for added labeled cells we do not see an increase in mineralization under anaerobic conditions as compared to aerobic conditions. This apparent anomaly may be due to the partial mineralization of labeled cells under anaerobic conditions to organic acids *via* fermentative metabolism rather than largely to CO_2 as in an aerobic system.

The nitrogen mineralized from labeled cells under anaerobic conditions is more consistent than under aerobic conditions. The mineralized N may be rapidly immobilized in aerobic growing systems. The proportion of labeled N mineralized to the total N mineralized in the anaerobic soil was fairly constant in contrast to the aerobic incubated soil (Table 3).

More investigation of carbon and nitrogen mineralization under anaerobic conditions is needed. This information would be useful for biomass methodology development and submerged soil studies.

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