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Microbial Metabolism of Carbon Monoxide in Culture and in Soil

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Nocardia salmonicolor readily oxidized CO to CO_2. Slight activity was found among species of *Actinoplanes, Agromyces, Microbispora, Mycobacterium,* and other nocardias, and no oxidation was detected in the algae, fungi, and other bacteria tested. Carbon monoxide was oxidized rapidly to CO_2 in the dark in two soils incubated in air or under flooded conditions, but little of the ^{14}C from ^{14}CO was incorporated into the organic fraction of these soils. The reaction was microbial because appreciable CO was not converted to CO_2 in autoclaved or gamma-irradiated soil. Heating the soil for 25 min at 70°C destroyed its CO-oxidizing activity. The incorporation of $^{14}CO_2$ into the cells of microorganisms in soil and soil suspension was not enhanced by incubating the samples in the presence of CO, suggesting that CO oxidation was not the result of autotrophic metabolism. The oxidation of 17 μl of CO per liter in the head space was nearly complete in 6 h in soil incubated in air or anaerobically.

Carbon monoxide is continually being added to the atmosphere in significant amounts, and the anthropogenic emissions of CO exceed all other recognized pollutants combined (8). Because the concentration of CO in the atmosphere does not appear to be increasing, the rate of destruction of this air pollutant must be large and equal to the sum of the rates of anthropogenic and natural emissions. Several mechanisms of removal of CO from the atmosphere have been demonstrated or postulated, but their relative importance is unknown.

Kummler et al. (6) suggested that transport to and destruction in the stratosphere, ground level and tropospheric chemical reactions, and biological processes were means for the removal of CO from the atmosphere. The biological mechanism appears to be mostly the result of microbial activities. Heichel (3) estimated that soil microorganisms are responsible for removing about 11% of the total global CO destroyed each year, whereas Liebl and Seiler (7) estimated that the soil microflora consumes about 50% of the total CO destroyed yearly. Tests with individual species of algae (1), fungi (4), and both aerobic and anaerobic bacteria (2, 13) have shown that these organisms can metabolize CO in axenic culture.

The present report describes investigations of the metabolism of CO by microorganisms in culture and in soil and describes potential contributions of the soil community to the maintenance of low atmospheric levels of CO.

MATERIALS AND METHODS

Labeled CO was obtained by dehydrating $H^{14}COONa$ (New England Nuclear Corp., Boston, Mass., specific activity, 58 mCi/mmol) with concentrated H_2SO_4 (2). The conversion of formic acid to CO in this process is stoichiometric (2, 11). The ethanolic solution containing 143 μg of $H^{14}COONa$ was dried at 50°C, and the vial containing the chemical was sealed with a Teflon-faced septum. The vial was then evacuated and refilled three times with high-purity N_2 by means of a needle inserted through the septum. With a syringe, 1.0 ml of concentrated H_2SO_4 was added to the vial, and the acid was removed by syringe after 3 h. The vial was then rinsed several times with distilled water by means of a syringe, and 0.1 ml of ethanolamine, a CO_2-trapping agent, was added to the final increment of 0.9 ml of distilled water. Unlabeled CO was prepared in a similar manner from $H^{12}COONa$. The gas was transferred to the samples by using a gas-tight syringe, all components being maintained at atmospheric pressure.

To prepare labeled CO_2, an aqueous solution containing 18.0 μg of [U-^{14}C]glucose (California Bionuclear, specific activity, 240 mCi/mmol) was treated with 1.0 ml of a concentrated H_2SO_4–85% H_3PO_4 (6:4) solution saturated with $K_2Cr_2O_7$. The vial containing these materials was sealed with a Teflon-faced septum to allow for removal of $^{14}CO_2$ with a gas-tight syringe.

The microbial oxidation of ^{14}CO was measured using a modification of the method of Pramer and Schmidt (9). In studies of individual cultures, 5.0 ml of inoculated medium was placed in a tube (15 by 125 mm) that was fitted with a serum stopper from which was suspended a plastic cup (1 by 7 cm; Kontes, Vineland, N.J.) containing a piece of folded glass-fiber filter paper (Whatman GF/B). The plastic cup thus was contained within the tubes. The tubes received 0.2 ml of CO-containing gas so that the final concentration of ^{14}CO in the gas phase was about 50 μl/liter with a total radioactivity of about 2.2×10^6 dpm. At the end of the incubation period at 29°C, the reaction was stopped, and the CO_2 and bicarbonates were driven into the headspace by the addition of 1.0 ml of an aqueous solution containing 95 μl of concentrated H_2SO_4 and 0.15 g of $FeSO_4 \cdot 7H_2O$. Phenethylamine (0.2 ml) (Fisher Scientific Co., scintillation grade) was then added by syringe to the filter paper to trap CO_2. After 60 min, the filter paper was placed in a vial having 11 ml of scintillation cocktail containing 5.0 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis[2]-(5-phenyloxazoyl)benzene in 1 liter of toluene. The vial was counted in a Beckman model 100 LSC scintillation counter.

To analyze for $^{14}CO_2$ formed in soil from ^{14}CO, usually 5.0 g of soil with a moisture content of 22% was placed in a 25-ml Erlenmeyer flask that was sealed as described above. Flooded soil was prepared by adding 5.0 ml of water to the soil before the flasks were sealed. Either 0.2 or 0.5 ml of CO-containing gas was injected into the flask, resulting in approximately 19 or 47 μl of CO per liter in the atmosphere, respectively. The soil was incubated at 29°C, and the reaction was terminated by the injection of 2.0 ml of the acid solution. To trap CO_2, 0.2 ml of phenethylamine was added to the filter paper, and the flasks were shaken for 60 min. The filter paper was then placed in 11 ml of scintillation cocktail, and the radioactivity was counted.

The technique used for measuring ^{14}CO incorporation into cellular material in studies of cultures and soil was a modification of a technique described by Smith et al. (11). For this purpose, the amount of ^{14}CO oxidized to CO_2 was first determined, and then the unmetabolized ^{14}CO was flushed out of the culture tube. The tube was then placed in an ice bath, and ca. 0.5 g of $K_2Cr_2O_7$ followed by 1.0 ml of an acid solution (concentrated H_2SO_4 and 85% H_3PO_4, 6:4) were added to the tube, which was then sealed with a serum stopper. The same procedure was followed for the soil samples, except that 1 to 2 g of $K_2Cr_2O_7$ and 5.0 ml of the acid were used. The samples were then removed from the ice bath, and a 25-gauge needle, connected with amber latex tubing to a 10-μl pipette, was inserted into the serum stopper after the pipette was inserted into a tube containing 2.0 ml of ethanolamine. After the internal pressure in each sample subsided, a second needle connected to a tank of CO was inserted into the stopper to force the headspace gas into the ethanolamine, and the samples were flushed in this manner for 3 to 5 h. During the flushing, the samples were brought twice to a boil for several minutes. The contents of the CO_2-trapping tube were transferred to a scintillation vial, and the tubes were rinsed three times with either 1.0 ml of scintillation cocktail (100 g of naphthalene, 5.0 g of 2,5-diphenyloxazole, and 0.5 g of 1,4-bis[2]-(5-phenyloxazoyl)benzene per liter of dioxane) for the cultures or 1.0 ml of methanol for soil, the rinses being added to the scintillation vial. The determination of ^{14}CO incorporation by this method does not distinguish between the incorporation into microbial cells of ^{14}CO or the $^{14}CO_2$ coming from ^{14}CO.

Agromyces ramosus, Actinoplanes humiferus, and *Mycobacterium phlei* were obtained from L. E. Casida, Pennsylvania State University, and were grown in heart infusion broth. Other actinomycetes and *Corynebacterium aquaticum* and *C. equi* were provided by H. Lechevalier, Rutgers University, and were grown in Trypticase soy broth. The yeast and fungi were cultured in potato dextrose broth, and the other bacteria were grown in nutrient broth. The algae, which were originally obtained from the Culture Collection of Algae at Indiana University, were grown in tubes (15 by 125 mm) with 5.0 ml of a solution containing 200 mg of $MgSO_4 \cdot 7H_2O$, 200 mg of KH_2PO_4, 100 mg of $CaCl_2 \cdot 2H_2O$, 80 mg of $NaNO_3$, 2.8 mg of H_3BO_3, 1.8 mg of $MnCl_2 \cdot 4H_2O$, 0.22 mg of $ZnSO_4 \cdot 7H_2O$, 17 μg of $Na_2MoO_4 \cdot 4H_2O$, 80 μg of $CuSO_4 \cdot 5H_2O$, and 9.2 mg of Fe (provided in the form of an ethylenediaminetetraacetate chelate) per liter of distilled water (medium C). The pH was adjusted to 7.5. The tubes were stoppered with a sterile serum stopper and cup assembly, and labeled CO was added to give ca. 50 μl/liter in the gas phase. The tubes were incubated for 5 days at 23 to 25°C under constant radiation (1.0×10^{-4} Einsteins/m^2 per s) provided by 40-W Gro-Lux fluorescent lamps suspended approximately 0.15 m under the cultures.

For the bacteria, fungi, and actinomycetes, 5.0 ml of medium in a 15-ml culture tube was inoculated with 0.1 ml of a 7-day old culture. The tubes were sealed with sterile serum stoppers containing cups for trapping CO_2. Then ^{14}CO was injected into each tube to give about 50 μl of CO per liter or about 1.5×10^6 dpm in the gas phase. The fungi were incubated for 72 h, and the bacteria and actinomycetes were grown for 48 or 72 h at 29°C. Each culture was tested in triplicate.

Samples from the surface horizon of Dalton silt loam (pH 4.4, 5.6% organic matter) and Williamson silt loam (pH 5.8, 2.6% organic matter) were passed through a 2-mm mesh screen before use. Portions (5.0 g) of these soils were exposed to ^{14}CO added to the gas phase. The soils, which contained about 20% moisture and were in 25-ml Erlenmeyer flasks closed with serum stoppers fitted with a CO_2-trapping assembly, were incubated in the dark at 29°C.

To sterilize the soils, 5.0-g portions with a moisture content of 20% were autoclaved for 30 min at 121°C on 3 consecutive days. Between each autoclaving, the soils were incubated at 29°C in a desiccator containing water to prevent drying. In some instances, 10.0 g of soil with a similar moisture content was exposed to 2.5 to 3.0 Mrads of gamma radiation. The sterility of these samples was verified by plating suspensions on nutrient agar, Trypticase soy agar, and potato dextrose agar and by inoculating suspensions into thioglycolate medium (Difco).

For anaerobic incubation, 4.0 g of air-dried Williamson silt loam was placed in a 25-ml Erlenmeyer flask, and the soil was brought to a moisture content of 25% by adding 1.0 ml of distilled water containing 0.90 mg of glucose. The glucose was added to stimulate the growth of microorganisms and allow them to scavenge residual O_2. The flask was sealed with a serum stopper fitted with a cup and filter paper, and it was evacuated and filled twice with high-purity N_2 (Matheson Gas Products, East Rutherford, N.J.). The sample was incubated in the dark at 29°C for 2 days before adding ^{14}CO.

RESULTS

The following microorganisms failed to form significant quantities of $^{14}CO_2$ or ^{14}C-labeled cell carbon when incubated with 50 μl of ^{14}CO per liter: *Anabaena flos-aquae* 1444, *Anacystis nidulans* 625, *Chlamydomonas reinhardtii* 90, *Chlorella pyrenoidosa* 26, and *Scenedesmus quadricauda* 76 incubated for 5 days in medium C; *Saccharomyces fragilis, Penicillum* sp., and *Zygorhynchus moelleri* incubated for 72 h in potato dextrose broth; *Agrobacterium tumefaciens, Arthrobacter oxydans, Bacillus circulans, Corynebacterium fascians, Enterobacter aerogenes, Pseudomonas aeruginosa*, and *P. putida* incubated for 48 or 72 h in nutrient broth; and *C. aquaticum* LL-B2252, *C. equi* LL-C101, *Micromonospora chalcea* ATCC 12452, *Oerskovia xanthineolytica* LL-G62, *Streptomyces albus* ATCC 618, and *S. griseus* LL-FR3 grown for 48 or 72 h in Trypticase soy broth.

The results in Table 1 show that some actinomycetes and related bacteria can metabolize CO. Because of the large variation among replicate tests of the same culture, the reasons for which are unknown, the significance of the data showing an apparently low activity in CO metabolism is unclear. *Nocardia salmonicolor* is noteworthy and showed an appreciable oxidation of ^{14}CO to $^{14}CO_2$ with ca. 10% of the initial ^{14}CO being recovered as $^{14}CO_2$. The amounts of $^{14}CO_2$ formed by *A. ramosus, A. humiferus*, and *M. phlei*, while small, are statistically different from the uninoculated control. The results indicate that species of *Nocardia* are capable of oxidizing CO to CO_2, although the rates are quite different among species. Oxidation of CO also seems to occur with *Actinoplanes philippinensis* and *Microbispora rosea*. With the exception of *N. opaca*, little or no radioactivity was incorporated into cellular material in these organisms.

Two soils, Dalton silt loam and Williamson silt loam, were incubated for 7 days with ca. 47 μl of ^{14}CO per liter in the headspace to determine their capacity to transform CO. The data in Table 2 suggest that there was no appreciable nonbiological oxidation of CO because the presumed conversion of ^{14}CO to $^{14}CO_2$ was not too greatly different in flasks with autoclaved or

TABLE 2. *Conversion of ^{14}CO to $^{14}CO_2$ or organic matter by two soils*

Soil	Soil treatment	cpm × 10^3 [a]	
		CO_2	Organic matter
Dalton silt loam	Field capacity	>1,000	22 (8.9)
	Field capacity, autoclaved	3.7 (0.05)	1.6 (0.48)
	Flooded	790 (240)	9.9 (4.1)
	Flooded, autoclaved	3.9 (0.24)	0.29 (0.032)
	No soil	3.3 (1.0)	
Williamson silt loam	Field capacity	>1,000	41 (22)
	Field capacity, autoclaved	2.6 (0.9)	0.35 (0.17)
	Field capacity, gamma irradiated	3.3 (2.7)	0.66 (0.51)
	Flooded	>1,000	27 (5.7)
	No soil	5.5 (0.3)	

[a] Numbers reported are means of at least three replicates. Values in parentheses represent one standard deviation. The gas phase originally contained ^{14}CO equivalent to ca. 3.5×10^6 dpm.

TABLE 1. *Metabolism of ^{14}CO by actinomycetes and related bacteria*

Microorganism	Medium[a]	Incubation (h)	cpm[b]	
			CO_2	Cell C
None[c]	NB	48	274 (180)	286 (207)
Nocardia salmonicolor	NB	48	160,000 (65,000)[d]	10,000 (12,000)
Streptomyces griseus LL-FR3	NB	48	597 (460)	102 (43)
None[c]	HIB	72	486 (88)	478 (71)
Agromyces ramosus	HIB	72	944 (326)[d]	314 (265)
Actinoplanes humiferus	HIB	72	969 (343)[d]	338 (62)
Mycobacterium phlei	HIB	72	1,630 (964)[d]	213 (205)
Actinoplanes philippinensis LL-P15	TSB	72	822 (200)[d]	68 (41)
Microbispora rosea IMRU37485	TSB	72	928 (145)[d]	71 (38)
Nocardia asteroides LL-278	TSB	72	1,290 (921)	87 (25)
Nocardia autotrophica ATCC19727	TSB	72	13,000 (11,000)[d]	306 (445)
Nocardia opaca ATCC4276	TSB	48	70,000 (35,000)[d]	3,700 (2,600)[d]

[a] NB, nutrient broth; HIB, heart infusion broth; TSB, trypticase soy broth.
[b] Values are the means of at least three replicates. Values in parentheses represent one standard deviation.
[c] Sterile medium.
[d] Significantly different (at 5% confidence level) from sterile medium.

irradiated soil than in flasks incubated with no soil. With nonsterile soil at field capacity (a moisture content of about 25% for the Dalton soil and 22% for Williamson silt loam), appreciable oxidation of ^{14}CO to $^{14}CO_2$ occurred. Radioactivity in the organic matter was several orders of magnitude less than in the CO_2, and the determination of radioactivity in the organic matter did not distinguish between ^{14}CO fixation and $^{14}CO_2$ fixation. Appreciable activities were also observed with the flooded soil. No viable organisms were detected in the irradiated and autoclaved soils.

The rate of microbial metabolism of CO was determined in Williamson silt loam maintained at field capacity in the presence of air and under anaerobiosis. To each flask was added 4.0 g of air-dried soil, and the sample was moistened with 1.0 ml of distilled water before the addition of ^{14}CO to a concentration of ca. 17 μl/liter (1.5 $\times 10^6$ dpm). After 3 h, the amount of $^{14}CO_2$ produced from ^{14}CO in the soil incubated in air was about half of the amount at 24 h, and the reaction was complete at 12 h (Table 3). Approximately 50% of the ^{14}CO had been recovered as $^{14}CO_2$ at 12 h. The amount of radioactivity incorporated into organic matter was consistently low.

To determine whether CO oxidation is significant in the absence of O_2, 5.0 g of Williamson silt loam at field capacity was provided with a gas phase containing ^{14}CO (about 17 μl/liter) under anaerobic conditions and then analyzed for the oxidation to $^{14}CO_2$. The data show a rapid oxidation of ^{14}CO under anaerobic conditions (Table 3), the rate of anaerobic oxidation being greater than the aerobic rate. This greater rate of oxidation may be a result of the larger microbial numbers in the anaerobic soil, because 0.90 mg of glucose was added before the introduction of ^{14}CO to allow the indigenous microflora to scavenge residual O_2 in the soil. The extent of incorporation of radioactivity into organic matter again was small. These data show that free O_2 is not necessary for the microbial removal of CO from the gas phase.

TABLE 3. *Conversion of ^{14}CO to CO_2 or organic matter by Williamson silt loam in air or under anaerobiosis*

Incubation time (h)	cpm $\times 10^3$ [a]			
	In air		Anaerobiosis	
	CO_2	Organic matter	CO_2	Organic matter
0[b]	22 (3.6)	0.3 (0.3)	14 (4.0)	0.1 (0.07)
3	350 (92)	0.3 (0.1)	1,000 (130)	0.3 (0.07)
6	610 (67)	0.8 (0.5)	710 (55)	0.6 (0.3)
12	750 (110)	3.0 (2.4)	740 (180)	1.6 (0.1)
24	730 (130)	4.4 (1.8)	720 (100)	1.7 (0.4)

[a] Numbers reported are means of at least three replicates and are not corrected for efficiency of counting. Numbers in parentheses represent one standard deviation.

[b] The zero-time sample actually represents a 10- to 15-min incubation period because that was the time before the reaction was stopped.

To determine whether clostridia might participate in the anaerobic oxidation, the samples of Williamson silt loam were heated to 70°C for 25 min under anaerobic conditions. After pasteurization, the soil samples were incubated under anaerobiosis for 24 h before ^{14}CO (17 μl/liter) was added. Analysis after 24 h of incubation with ^{14}CO showed that the radioactivity as $^{14}CO_2$ was 5,400 and 3,300 cpm (standard deviations of 2,600 and 2,700, respectively) for the pasteurized and gamma-irradiated soil. Because the gamma-irradiated (2.5 to 3.0 Mrads) anaerobic soil, which was sterile, had essentially the same amount of $^{14}CO_2$ as the pasteurized soil, clostridia did not appear to be responsible for the anaerobic oxidation of CO. When similarly treated samples were incubated for 4 days to allow the clostridia to increase before the addition of ^{14}CO, the yield of $^{14}CO_2$ was still only 2,700 cpm. No appreciable formation of organic matter from the ^{14}CO occurred since the organic fraction only contained 500 cpm after 24 h of incubation with ^{14}CO.

The incorporation of CO_2 into cellular material during the microbial oxidation of CO was measured by using Williamson silt loam. In one test, 5.0-g portions of Williamson silt loam at field capacity (22% moisture) in 25-ml Erlenmeyer flasks were amended with $^{14}CO_2$ (ca. 2.2 μl/liter or 3 $\times 10^5$ dpm) and various levels of ^{12}CO. Because $^{12}CO_2$ in each sample could compete with $^{14}CO_2$ for incorporation into organic matter, a suspension of 10 g of Williamson silt loam in 90 ml of distilled water was bubbled for 4 h with CO_2-free air. Then, 5.0-ml portions of this suspension were added to 25-ml Erlenmeyer flasks. The samples were incubated for 24 h at 29°C, and then $^{14}CO_2$ incorporation into microbial cells was determined by the procedure used for estimating ^{14}CO incorporation. The data in Table 4 show that the assimilation of $^{14}CO_2$ was not enhanced if CO was added to the headspace at any of the test levels, the differences not being statistically significant at the 5% confidence level. These data suggest that autotrophs are not responsible for CO oxidation.

DISCUSSION

Although the cultures of algae in this investigation showed no oxidation or incorporation of

TABLE 4. *Incorporation of $^{14}CO_2$ into organic matter by Williamson silt loam or a soil suspension exposed to various concentrations of ^{12}CO*

CO level (μl/liter)	Radioactivity in organic matter (cpm[a])	
	Soil at field capacity	Soil suspension
0	143 (73)	86 (13)
20	109 (33)	99 (12)
50	130 (71)	67 (21)
100	203 (59)	117 (21)

[a] Numbers reported are means of at least three replicates. Values in parentheses represent one standard deviation.

CO, Chappelle (1) has reported the oxidation of CO by several species of algae, including several used in the present study. It is possible that the difference in results arises from the different CO concentrations provided to the organisms. Chappelle used 20% CO in the gas phase above the cultures, while only 0.005% CO was used in these experiments, a concentration more closely approximating those in the atmosphere (12), albeit still somewhat higher. It has already been shown that microorganisms in culture and in natural waters may be unable to metabolize an organic compound at low concentrations, although able to do so at high levels (R. S. Boethling and M. Alexander, manuscript submitted). These findings are noteworthy in attempts to assess the microorganisms responsible for the transformation of air pollutants whose concentrations in air are usually far lower than those provided to microbial cultures in the laboratory.

The microbial destruction of CO could be a result of its metabolism and conversion into cellular material, its oxidation to CO_2 by a cometabolic process, or its conversion to CO_2 by autotrophs. Because the radioactivity in the organic matter was several orders of magnitude less than in the CO_2 in soil samples amended with ^{14}CO, it appears unlikely that direct incorporation of CO into cell carbon is significant in the soils tested. Moreover, although it has been claimed that CO-oxidizing autotrophs are largely responsible for the removal of CO in soil (13), the results presented here indicate that autotrophs are not important in soil in the removal of atmospheric concentrations of CO. Thus, cometabolic oxidation of CO appears to be the major microbial removal mechanism.

Nevertheless, it is not clear which microorganisms are responsible for the oxidation. The only organisms found in this study to have markedly high activity were species of *Nocardia*, and it is possible that nocardias and related microorganisms bring about the conversion, but too few organisms have been tested to designate or exclude a particular group. Populations able to function under anaerobiosis must be involved in view of the rapid conversion in O_2-free soil, but the present data appear to exclude the clostridia because of the abolition of CO oxidation in pasteurized soil incubated anaerobically.

The slope of a regression line fitted to the essentially linear CO oxidation rate in the 0- to 6-h period in Williamson silt loam under aerobic conditions yields a value of 1.1×10^5 dpm/h, assuming a counting efficiency of 90%. This is equivalent to 0.81 nmol of CO oxidized per 5.0 g of soil per h or, in terms of surface area of soil exposed to the gas phase, the rate of oxidation was approximately 19 μg of CO/m^2 per h. Other reported CO uptake rates by soil are 8.2 mg (5), 0.54 mg (10), and 80 μg (3) of CO/m^2 per h. The low rate of oxidation reported in this study as compared to others might be the result of a lack of mixing of CO in the gas phase, the shallow depth of soil in each flask, or calculations based solely on the amount of ^{14}CO oxidized, thus ignoring ^{12}CO oxidation. The particularly high rates of Inman et al. (5) may be misleading because they were obtained using 100 μl of CO per liter in the gas phase, and the rate of microbial activity is markedly lower when tested with substrates at concentrations typical of those in nature rather than the high levels commonly used in laboratory trials (Boethling and Alexander, manuscript submitted).

The finding of anaerobic oxidation of CO in Williamson silt loam is in contrast with the report of Inman and Ingersoll (4), who detected no uptake of CO by soil under anaerobic conditions. The explanation for the apparent contradiction may be the fact that Inman and Ingersoll (4) tested for CO uptake by determining the total amount of CO in the gas phase above the soil after a period of time, and the existence of equal rates of production and destruction would have led to the conclusion that CO is not utilized anaerobically. In this connection, it has been proposed that CO is produced largely in anaerobic sites in the soil (13). Nevertheless, the body of information is too small to make generalizations, and further studies with other soils, additional environmental factors, and CO levels more characteristic of natural ecosystems are required before a valid quantitative assessment of the role of soil microorganisms in CO destruction is possible.

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