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Microbial Dynamics Associated with Multiphasic Decomposition of ^{14}C-Labeled Cellulose in Soil

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ABSTRACT

Recent emphasis on residue management in sustainable agriculture highlights the importance of elucidating the mechanisms of microbial degradation of cellulose. Cellulose decomposition and its associated microbial dynamics in soil were investigated in incubation experiments. Population dynamics of actinomycetes, bacteria, and fungi were monitored by direct counts. Populations of oligotrophic bacteria in cellulose-amended soil were determined by plate count using a low C medium containing 4 mg C liter^{-1} agar, and copiotrophs using a high C medium. Cumulative $^{14}CO_2$ evolution from ^{14}C-labeled cellulose was best described by a multiphasic curve in a 28-day incubation experiment. The initial phase of decomposition was attributed mainly to the activity of bacterial populations with a low oligotroph-to-copiotroph ratio, and the second phase mainly to fungal populations. An increase in oligotroph-to-copiotroph ratio coincided with the emergence of a rapid $^{14}CO_2$ evolution stage. Streptomycin reduced $^{14}CO_2$ evolution during the first phase and prompted earlier emergence of the second phase, compared to the control. Cycloheximide initially promoted $^{14}CO_2$ evolution but subsequently had a lasting negative effect on $^{14}CO_2$ evolution. Cycloheximide addition significantly increased bacterial biomass and resulted in substantially stronger oscillation of active bacterial populations, whereas it initially reduced, and then stimulated, active fungal biomass. The observed changes in $^{14}CO_2$ evolution could not be explained by observed shifts in fungal and bacterial biomass, probably because functional groups of fungi and bacteria could not be distinguished. However, it was suggested that oligotrophic bacteria prompted activation of cellulolytic enzumes in fungi and played an important role in leading to fungal dominance of cellulose decomposition.

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Introduction

Recent emphasis on residue management for long-term productivity of agroecosystems highlights the importance of understanding the mechanisms of microbial degradation of organic remains. Winter cover crops have been gaining increased acceptance for reducing soil erosion and nitrate leaching, and meeting the nitrogen needs of subsequent crops [10, 27]. Two short-term goals of residue management are to avoid intense competition of microbes with plants for nutrients, and synchronize nutrient release with plant demand. These are necessary since microbes act as both a source and a sink of nutrients [6]. Another goal is to prevent seedling diseases, since some pathogenic fungi can be stimulated initially by residue addition [19]. Therefore, short-term dynamics of microbial communities following residue incorporation is important for effective residue management.

Cellulose is the largest component of plant residues that enters terrestrial ecosystems [29]. Knowledge of microbial succession on cellulose is of special significance in understanding the microbial aspects of residue decomposition. A few studies have focused on the microbial dynamics of cellulose decomposition [31, 36]. Saito et al. [31] noticed that as cellulose decomposition progressed, a microbial succession on cellulose took place in a waterlogged soil, with a fungus and a few bacteria dominating in the initial stage, and various types os cellulolytic and noncellulolytic microorganisms (mainly bacteria) coexisting in the late stage in a 40-day experiment. Tribe [36] reported two main phases in cellulose decomposition, with cellulolytic fungi as the dominant components in the first phase (1 to 3 weeks), and bacteria and fauna in the second phase.

In a preliminary experiment, we noticed that $^{14}CO_2$ evolution from ^{14}C-labeled cellulose in soil was multiphasic. The multiphasic mineralization of a chemical in soil may result from a single population possessing multiple uptake systems or multiple coexiting populations capable of using the chemical [32]. A soil microbial community represents a continuum of microorganisms with various C requirements [33], with obligatory oligotrophs adapted to low C concentrations at one extreme and obligatory copiotrophs to high C concentrations at the other extreme. The appearance of bacterial colonies on agar media is also multiphasic, the first phase consisting of mostly copiotrophic bacteria and later phases of facultative and obligate oligotrophs [11]. Considering the fact that various cellulolytic microorganisms coexist in soil, we hypothesize that two mechanisms may result in multiphasic cellulose decomposition besides a time lag of microbial population increase. First, microbial community structure changes due to microbial succession from a bacteria-dominated community to a fungi-dominated community, or vice versa. Second, bacterial populations with low C requirements become dominant as decomposition progresses and C becomes scarce.

The aims of the present study were (1) to document multiphasic decomposition of ^{14}C-labeled cellulose in soil; (2) to examine the population dynamics of fungi, actinomycetes, and bacteria (including copiotrophic and oligotrophic bacteria) associated with cellulose decomposition; and (3) to assess the potential effects of antibiotics on the population dynamics of target and nontarget microorganisms and cellulose decomposition.

Materials and Methods

Soil

The soil used in this study was an organically managed soil from the Student Experimental Farm of the University of California at Davis, Calif., USA. The field plot was initiated in 1986 and had been annually planted with an oat (*Avena sativa* L.) and vetch (Lana woollypod vetch: *Vicia dasycarpa* Ten.) cover crop in winter. Synthetic fertilizers and pesticides have not been applied. Summer crops had been either tomato (*Lycopersicon esculentum*) or corn (*Zea mays* L.). Further description of this site can be found in previous publications [34, 35]. The soil is a coarse-loamy, mixed, Thermic Mollic Xerofluvent soil with 10.52 g C kg^{-1} soil, 0.78 g N kg^{-1} soil, a pH of 7.7, and water holding capacity of 22.5%. Soil cores to 20 cm depth were collected using a Dutch Auger (6 cm in diameter) 3 weeks after incorporation of the vetch-oat cover crop. Over 20 kg soil was sampled from the field of ca. 120 m^2 and composited. Soil samples were then presieved through an 8-mm sieve and stored in a 4°C cold room until used.

$^{14}CO_2$ Evolution from ^{14}C-Labeled Cellulose

Soil Incubation. Soil samples with field moisture were air dried slightly to adjust soil moisture content to approximately 10% and passed through a 2-mm mesh sieve. Soil samples equivalent to 20 g oven-dried soil (105°C) were placed in 1-liter mason jars and preincubated overnight at 20 ± 2°C. Equal amounts of ^{14}C-labeled cellulose (730700 dpm) (American Radiolabeled Chemicals, Inc., St. Louis, Mo.) mixed with unlabeled cellulose (CF11, Whatman Co., Maidstone, England) were added to each mason jar and carefully mixed with the soil. The final amount of cellulose added was exactly 10 mg g^{-1} soil, equivalent to 38% of the initial soil C content. There were four treatments: fungi inhibitor cycloheximide, bacteria inhibitor streptomycin, a combination of cycloheximide and streptomycin (Sigma Chemical Co., St. Louis, Mo.), and an untreated control, with four replicates of each treatment. Cy-

cloheximide was dissolved in ethanol and diluted into water, and streptomycin was directly dissolved in water. Both antibiotics were added to soil through a fine syringe needle right after incorporation of ^{14}C-labeled cellulose. The final concentrations of inhibitors were 3.0 mg of streptomycin and 1.0 mg of cycloheximide g^{-1} dry soil. The C input from ethanol in the cycloheximide treatment was 1.28 mg C g^{-1} dry soil. Sterile deionized water was added evely to the soil through a fine syringe needle to bring soil water content to 18%. Four other mason jars that received equal amounts of unlabeled cellulose and water only were included to allow for dilution plate-count enumeration of oligotrophic and copiotrophic bacteria associated with different decomposition stages. All the mason jars were put into plastic boxes and incubated in the dark at 22 ± 2°C.

Another 30 mason jars which received unlabeled cellulose were used to investigate the population dynamics of fungi, actinomycetes and bacteria, and total microbial biomass-C. Three treatments were used: streptomycin, cycloheximide, and water only, with ten mason jars for each treatment. Cellulose, antibiotics, and deionized water were added and all mason jars were then incubated as previously described. Two mason jars of each treatment were sampled on each sampling date (3, 6, 9, 14, and 21 days after initial incubation). Each individual sample was used for determinations of microbial biomass-C (by the fumigation-extraction method), and enumeration of total and active fungi, bacteria, and actinomycetes by direct counts [14]. Four mason jars that received ^{14}C-labeled and unlabeled cellulose mixture were included to compare $^{14}CO_2$ evolution with that in the previous experiment.

$^{14}CO_2$ Analyses. $^{14}CO_2$ was captured in 2.0 ml of 0.5 *N* NaOH contained in a beaker suspended in each mason jar. The NaOH solution was removed and replaced with fresh solution during sampling. The NaOH solution was first sampled 10 h after the incubation started and then daily for 10 days, followed by 2-day intervals and finally 4-day intervals (see details in Fig. 1). The NaOH solution containing $^{14}CO_2$ was added to 5.0 ml of ScintiVerse II scintillation cocktail (Fisher Scientific Co., Pittsburgh, Pa.) in 7.0-ml scintillation vials, and the radioactivity was counted with a liquid scintillation counter (Model LS6000SC; Beckman Institutes, Inc., Fullerton, Calif.) after 24-h storage in the dark to minimize chemiluminescence effects.

Population Dynamics of Active and Total Bacteria, Fungi and Actinomycetes

Plate Counting of Oligotrophic and Copiotrophic Bacteria. Exactly 1.50 g soil was sampled from each of four mason jars amended with only unlabeled cellulose on days 3, 6, 9, 14, 21, and 28 after initiation of incubation. Two samples each from two mason jars were composited and two pseudoreplicate samples were taken. To determine the number of oligotrophic and copiotrophic bacteria in soils at different decomposition stages of cellulose, soil extracts were plated on a high C content medium and a low C content medium. Both media contained per liter of solution: $MgSO_4 \cdot 7H_2O$, 0.5 g; KNO_3, 0.5 g; K_2HPO_4, 1.0 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.060 g; and Bacto agar, 11.0 g, and 100 ppm cycloheximide. The medium for isolating copiotrophs contained 1.0 g C l^{-1} (10% from enzymatic casein hydrolysate, and 90% from cellulose) and the medium for isolating oligotrophs contained 1.0 mg C l^{-1} (also 10% from enzymatic casein hydrolysate and 90% from cellulose), plus 2.5 ppm soluble C contained in the deionized water used. Sterile cycloheximide of 100 ppm was added after the autoclaved agar medium had cooled to 40°C. The C content of the copiotrophic medium approximately had a C concentration of root exudates [9], while the C content in the oligotrophic medium was close to the lower limit of available C in some soils [17, 21]. A subsample equivalent to 1.0 g dry soil from each composited sample was suspended in 9.0 ml of sterile distilled deionized water and vortexed at high speed for 1 min. Additional dilutions were made through serila dilution (to 10^{-6}). Aliquots (50 µl) of the 10^{-4} to 10^{-6} suspensions were plated on each medium in triplicate. Colonies of copiotrophs and oligotrophs were counted 7 and 14 days after plating, respectively.

Direct Microscopic Counts. Total bacterial numbers were estimatedby direct counts after staining soil smears with fluorescein isothiocyanate (FITC) [3]. Active bacteria and active or total fungal hyphal lengths were estimated by direct observation after staining with fluorescein diacetate (FDA, Sigma Chemical Co.) [13, 20]. Numbers of active bacteria were determined by counting fluorescent bacteria in three fields using epifluorescent microscopy. Active hyphal lengths (fluorescent hyphae) were estimated by direct observation of the soil-agar film by epifluorescent microscopy, with 40 fields per slide being counted. Total fungal hyphae were measured in ten fields per slide by observation of the same agar film at the same magnification but using phase-contrast microscopy [8]. Filamentous hyphae that were smaller than 1 µm in width were counted as actinomycetes. Biomass was estimated by multiplying bacterial and fungal biovolume by average bacterial density of 0.33 g cm^{-3} and average hyphal density of 0.41 g cm^{-3} [14]. All the direct counts were conducted by the Soil Microbial Biomass Service of Oregon State University (Covallis, Oreg.).

Microbial Biomass C. Microbial biomass C was determined by a chloroform fumigation-extraction method adapted from [37]. Briefly, a 5.0-g (dry wt. equivalent) moist soil sample was extracted immediately with 15.0 ml 0.5 M K_2SO_4, shaken for 30 min, and filtered (No. 42 Whatman paper) on a vacuum extraction set. A second 5.0-g sample was fumigated with chloroform for 48 h and then extracted with 15.0 ml 0.5 M K_2SO_4, as above. Extracts were immediately frozen and kept frozen until analyzed using a Shimadzu total organic carbon analyzer (Shimadzu TOC-5050, Shimadzu Scientific Instruments, Inc., Columbia, Md). Microbial biomass C was calculated from the total dissolved organic C by using a K_{ec}-factor of 0.41 [38].

Soluble Carbon and Available Nitrogen. Soluble C of the nonfumigated soil samples was determined on the Shimadzu total organic carbon analyzer. NO_3 and NH_4 concentrations of K_2SO_4 extracts of the soil samples were determined on an autoanalyzer (Department of Agricultural and Natural Resources, University of California, Davis, Calif.) and the sum of NO_3 and NH_4 contents of the extracts was taken as total extractable N.

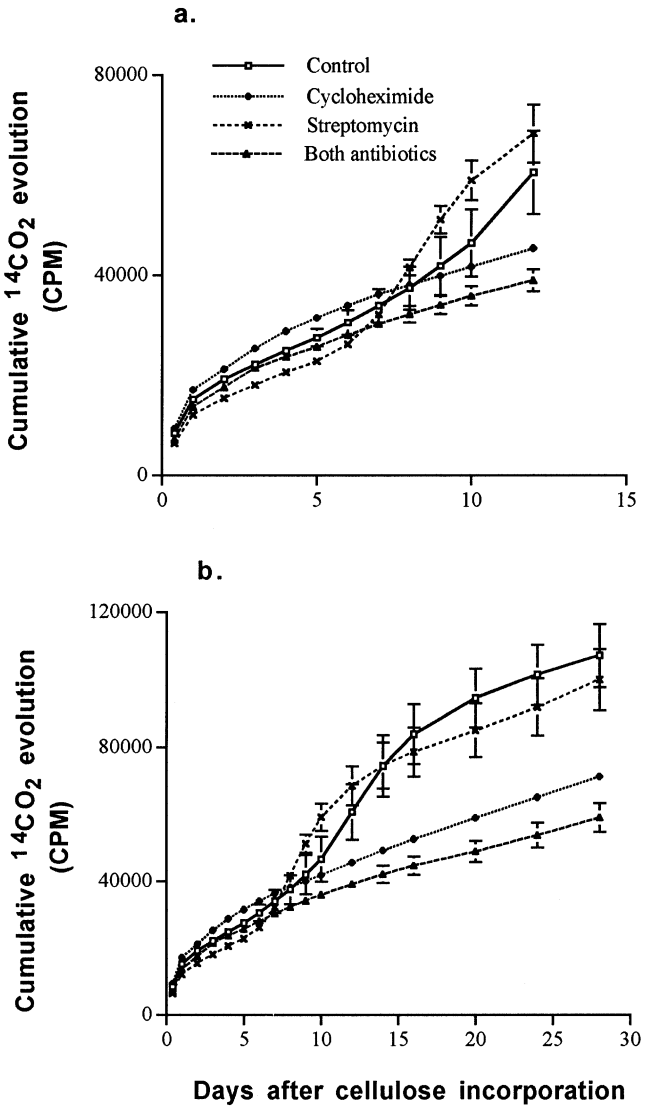

Fig. 1. Cumulative $^{14}CO_2$ evolved from ^{14}C-labeled cellulose in the presence of a procaryotic inhibitor (streptomycin), eucaryotic inhibitor (cycloheximide), or both. Values are means ± 1 SE. When the *error bars* are absent, the SE is smaller than the symbol.

Statistical Analyses. All data were regressed on time, treatment, and their interaction using SAS (SAS Institute Inc., Cary, N.C.). Pearson correlation coefficients were calculated between all variables and cumulative $^{14}CO_2$ evolution.

Results

$^{14}CO_2$ Evolution Rate and Cumulative $^{14}CO_2$ Production from ^{14}C-Labeled Cellulose

Kinetics of cellulose decomposition in the control and the soil receiving streptomycin only was significantly different from that in soil treated with cycloheximide or both inhibitors. There were significant time, treatment, and interaction effects ($P < 0.01$). The cumulative $^{14}CO_2$ evolution in the control and the streptomycin treatment is best described by a multiphasic curve with slow initial evolution followed by a stage of rapid release of $^{14}CO_2$ (Fig. 1). The second stage (rapid evolution stage) started about 5 days after the cellulose incorporation both in the control and the streptomycin treatment, but the evolution rate peaked 4 days earlier in the latter (Fig. 2), suggesting the direct competition between fungi and bacteria for cellulose. In both the control and streptomycin treatments, $^{14}CO_2$ production was low in the initial phase, then it peaked and decreased during the second phase. When cycloheximide was applied, the initial $^{14}CO_2$ release (Fig. 1a) increased over the control, but decreased after day 6 (Fig. 1b). In this treatment, a rapid $^{14}CO_2$ evolution phase did not emerge during the 28-day incubation experiment and the decomposition was simply first order. The $^{14}CO_2$ evolution was significantly lower in the soils treated with both cycloheximide and streptomycin than in soils treated with cycloheximide only, although both treatments produced similar cumulative $^{14}CO_2$ evolution curves (Fig. 1). The total amount of evolved ^{14}C was only about 15% of the total ^{14}C added in the control (without inhibitors). Significantly higher variability both in cumulative $^{14}CO_2$ evolution and $^{14}CO_2$ rates was observed in the control and streptomycin treatments than in the other two treatments. Variability was significantly reduced throughout the experiment when cycloheximide was applied, which suggested the effective inhibition of fungi by cycloheximide.

In the untreated control, cumulative $^{14}CO_2$ evolution was positively correlated with active fungal biomass ($r = 0.74$, $P = 0.01$), the ratio of oligotrophic to copiotrophic bacteria ($r = 0.76$, $P = 0.01$), and microbial biomass C ($r = 0.84$, $P = 0.003$). $^{14}CO_2$ evolution rates were not correlated with any of the microbial variables. Cumulative $^{14}CO_2$ evolution was negatively correlated with extractable soil N concentrations in the control ($r = -0.91$, $P = 0.0003$) and in the streptomycin treatment ($r = -0.96$, $P = 0.0001$), but a positive correlation was found in the cycloheximide treatment ($r = 0.99$, $P = 0.0001$).

Copiotrophic and Oligotrophic Bacteria

Bacterial populations (colony-forming units or CFUs), as determined using two media with different C contents from soils amended with unlabeled cellulose, increased rapidly at early stages of incubation but changed little during the later stages of the 28-day incubation experiment (Fig. 3a). The

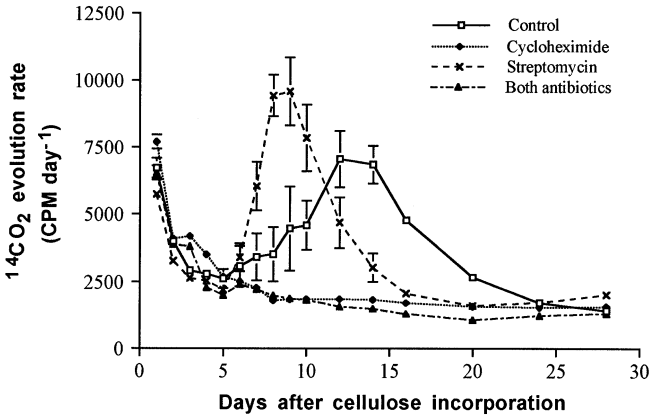

Fig. 2. $^{14}CO_2$ evolution rates from ^{14}C-labeled cellulose in the presence of streptomycin, cycloheximide or both. Values are means ± 1 SE.

ratio of oligotroph-to-copiotroph populations decreased on day 3 due to the quicker increase of copiotrophs during the early stage (Fig. 3b). This ratio increased after day 3, peaked on day 9, and stabilized thereafter. The increase in the oligotroph-to-copiotroph ratio coincided with the emergence of the second $^{14}CO_2$ release phase.

Bacterial Biomass

Total bacterial biomass estimated by direct counts was significantly higher in the cycloheximide treatment than in the streptomycin and control treatments for the first 14 days, and then decreased (Fig. 4a). Total bacterial biomass was essentially stable in the control soil after day 3. Active bacterial biomass was also significantly higher in the cycloheximide and control treatments than in the streptomycin treatment, except on days 3 and 6 when no significant difference in total biomass was observed between the control and the streptomycin treatments (Fig. 4b). Active bacterial biomass increased rapidly and oscillated in the cycloheximide treatment, an effect that was not observed in the other treatments (Fig. 4b). There was no significant difference in the total actinomycetal biomass among the three treatments, but active biomass was significantly higher in the cycloheximide treatment than in the streptomycin treatment (data not shown). Compared with fungi and bacteria, the biomass of actinomycetes (both active and total) was significantly lower, and, therefore, consisted only of a small percentage of the total biomass.

Fungal Biomass

Total fungal biomass determined by direct counts was high in the original soil (107 μg biomass g^{-1} dry soil) and remained fairly high in all the treatments for the first 6 days, then rapidly decreased between day 6 and day 9 after the incubation (Fig. 5a). After day 9, total fungal biomass increased significantly in the streptomycin treatment. Active fungal biomass values were low, ranging from 2 in the streptomycin treatment to 10 μg g^{-1} in the control, for the first 14 days in all the treatments, but significantly increased on day 21 (Fig. 5b). The streptomycin treatment had the lowest active fungal biomass throughout the experiment, and the control had the highest on most sampling dates. On the last sampling date, active fungal biomass was the highest in the cycloheximide treatment. This biomass may have consisted primarily of sugar fungi recovering from the cycloheximide treatment rather than cellulolytic fungi. In the streptomycin treatment, total fungal biomass increased rapidly from day 9 to day 14 while active fungal biomass was low (Fig. 5a, b) and $^{14}CO_2$ evolution rates were high during this period (Figs. 1 and 2), suggesting that the death rate of fungi could have been high.

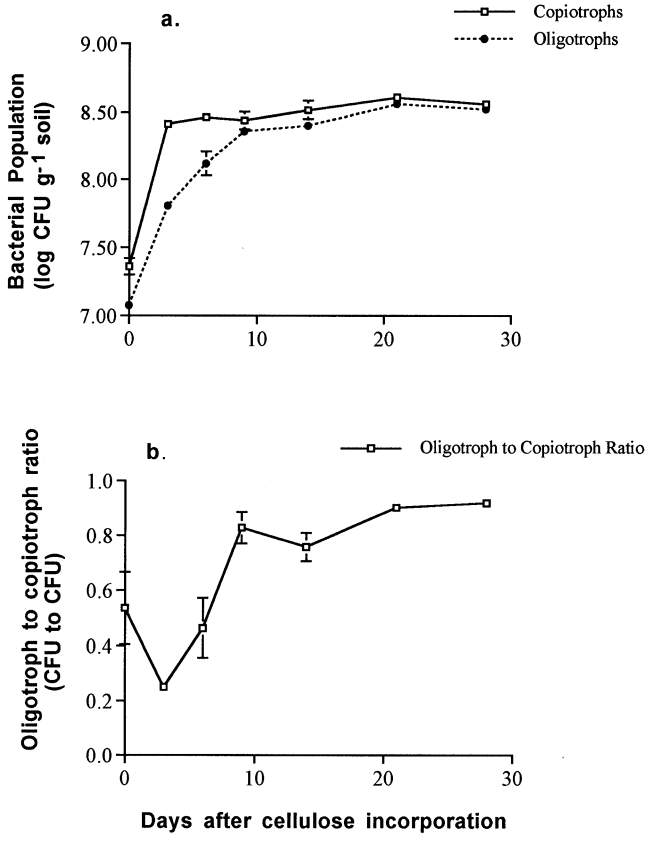

Fig. 3. Populations of copiotrophic and oligotrophic bacteria (**a**) and oligotroph to copiotroph ratios (**b**) in cellulose-amended soils estimated by plate counts over time during cellulose decomposition. Values are means ± 1 SE.

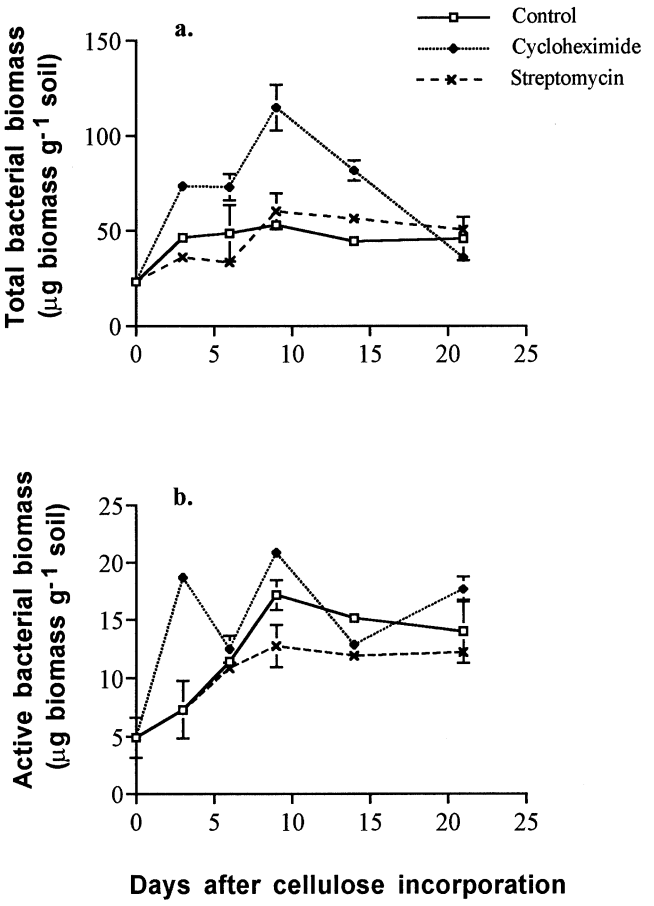

Fig. 4. Total (**a**) and active (**b**) bacterial biomass estimated by direct counts in the presence of streptomycin or cycloheximide over time during cellulose decomposition. Biomass can be converted to biomass-C by multiplying with a factor of 0.50. Values are means ± 1 SE.

Total Microbial Biomass-C

In the control, microbial biomass C estimated by the fumigation-extraction method increased gradually and peaked on day 21, while it peaked earlier in both antibiotic treatments (Fig. 6). Compared with the control, microbial biomass C increased rapidly from day 9 to day 14 and was significantly lower only for the first 3 days in the streptomycin treatment, probably due to the transient nature of the streptomycin effects on soil bacteria. In the cycloheximide treatment, microbial biomass C increased significantly over the first 3 days due to an increase in active bacteria, possibly stimulated by the increased available C from ethanol used to dissolve cycloheximide (Fig. 4b), and stabilized thereafter, suggesting that rapid bacterial growth may compensate for the reduction due to the inhibitory effects of cycloheximide. Microbial biomass C determined by the fumigation-extraction method was negatively correlated with soil extractable N in the control ($r = -0.94$, $P = 0.0001$), but not in the antibiotic treatments.

Soluble Carbon in the Control Treatment

Soluble C of the original soil (day 0) was low at 34.2 ppm but increased to 82.4 ppm 3 days after cellulose incorporation (Fig. 7). Soluble C decreased after day 3, coinciding with a rapid $^{14}CO_2$ evolution stage. There was a negative correlation between soluble C and the ratio of oligotroph-to-copiotroph populations ($r = -0.60$, $P = 0.06$), indicating that oligotrophs became dominant as soluble C decreased.

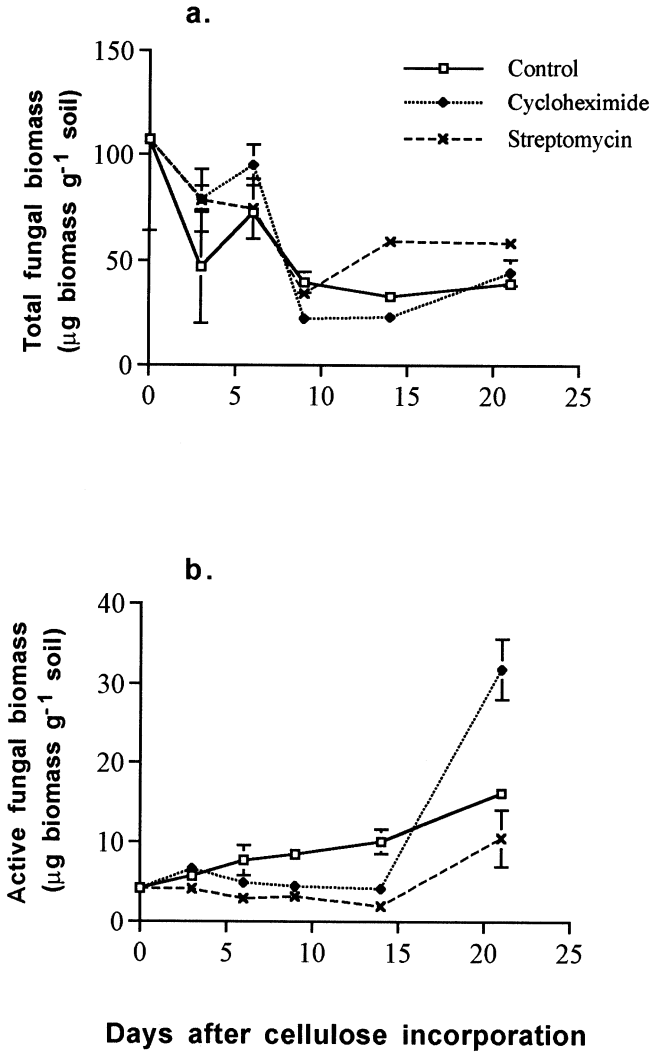

Fig. 5. Total (**a**) and active (**b**) fungal biomass estimated by direct counts in the presence of streptomycin or cycloheximide over time during cellulose decomposition. Values are means ± 1 SE.

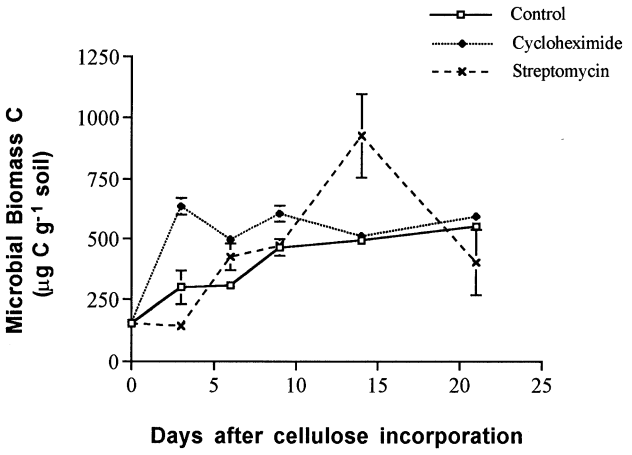

Fig. 6. Microbial biomass C in the presence of streptomycin or cycloheximide determined by fumigation-extraction over time during cellulose decomposition. Values are means ± 1 SE.

K_2SO_4 Extractable Nitrogen in Nonfumigated Soils

Dynamics of K_2SO_4 extractable N (NO_3-N + NH_4-N) in the control and streptomycin treatments followed a pattern distinct from that of the cycloheximide treatment (Fig. 8). Changes in NO_3-N concentrations of the extracts followed a similar pattern as total extractable N since NH_4-N represents a very low percentage of the total extractable N. In the control and streptomycin treatments, total extractable N increased initially due to the mineralization of dead microbes and some organic debris without a corresponding increase in microbial biomass (Fig. 8), suggesting that the soil originally contained low available C as suggested by low soluble C (Fig. 7). Extractable N decreased rapidly when rapid $^{14}CO_2$ evolution rates were recorded, indicating that N was incorporated into microbial biomass. When extractable N decreased to about 5 ppm, the $^{14}CO_2$ evolution rates in the control and the streptomycin treatment were no longer significantly higher than those in the other two treatments (Fig. 2). In the cycloheximide treatment, N concentration rapidly decreased over the first 3 days, coinciding with an increase in active bacterial biomass and populations of viable bacteria, and then gradually increased (Fig. 8).

Discussion

Results from the present experiments indicated that the microbial community, and C and N availability interactively control cellulose decomposition in soil. The cumulative $^{14}CO_2$ evolution in the control is best described by a multiphasic curve with slow initial evolution followed by a stage of rapid release of $^{14}CO_2$ (Fig. 1). Streptomycin and cycloheximide reduced the initial and second stages of $^{14}CO_2$ evolution, respectively (Fig. 2), suggesting that the initial phase of decomposition was due to the activity of bacterial populations and the second phase to fungal populations. Microbial succession during the cellulose decomposition has been previously reported [31, 36], but mechanisms underlying the succession are not clear. We postulate that oligotrophic bacteria play an important role in the succession since an increase in oligotrophic bacteria coincided with the emergence of a rapid $^{14}CO_2$ evolution stage (Figs. 2 and 3). Oligotrophic bacteria have high substrate affinity as evidenced by the low K_m values of their transport systems and, therefore, have competitive advantages over copiotrophs when substrate concentrations are low [17, 33], although they may have only a limited contribution to total respiratory activity when substrate is abundant [15]. When cellulose is added to soil, copiotrophic bacteria populations increase because of their high growth rates [22, 33]. As available C decreases, an increase in oligotrophs can deplete simple C compounds to such low levels that they become inaccessible to copiotrophic bacteria [33]. This may result in activation of cellulolytic enzymes in fungi and lead to fungal dominance of cellulose decomposition. The magnitude of the reduction in $^{14}CO_2$ evolution by cycloheximide observed in the present experiment suggests that fungi indeed play an

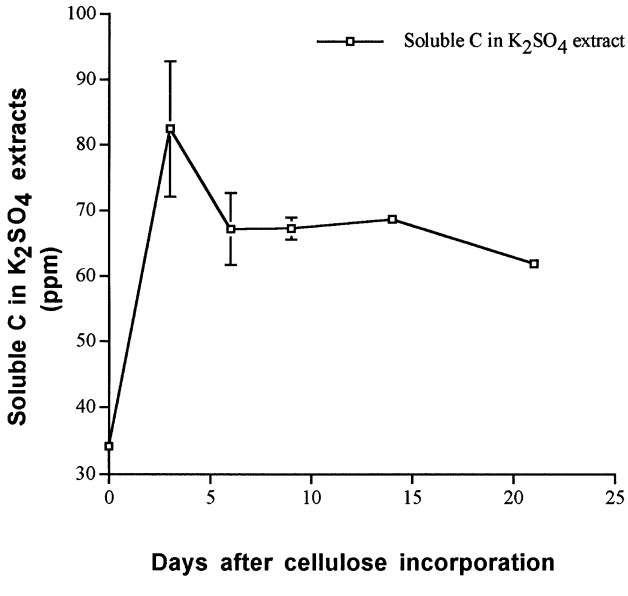

Fig. 7. Soluble carbon in K_2SO_4 extracts from cellulose-amended soils over time during cellulose decomposition. Values are means ± 1 SE.

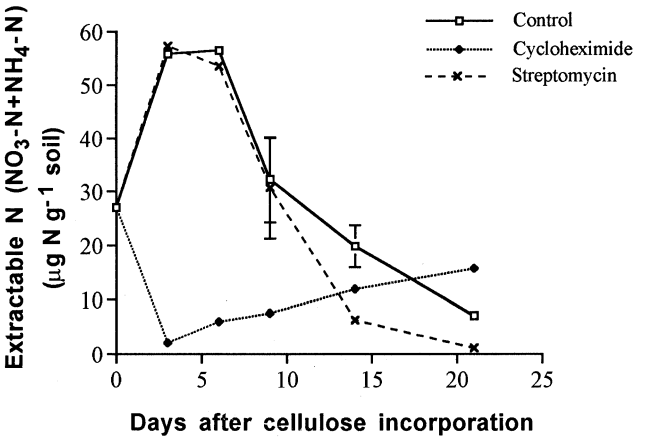

Fig. 8. K_2SO_4 extractable soil nitrogen (NO_3-N + NH_4-N) in the presence of streptomycin or cycloheximide over time during cellulose decomposition. Values are means ± 1 SE.

important role in cellulose decomposition, although bacteria are of significance in cellulose degradation at early stages. The dominant role of fungi was also suggested by the high variability of $^{14}CO_2$ evolution in the control and streptomycin treatments. High variability in those treatments may be attributed to the non-uniform growth of fungal hyphae at heterogeneous locations [30].

The first order decrease in $^{14}CO_2$ production after it peaked both in the control and streptomycin treatments was due to N limitations, which was also supported by the negative correlations between cumulative $^{14}CO_2$ evolution and extractable N. Rapid $^{14}CO_2$ evolution coincided with a decrease in extractable N until insufficient N was available for cellulose decomposition. Results from previous studies indicate that the decomposition of cellulose both in wheat straw and in agar media can be significantly limited by N availability [16, 24, 28], suggesting that there is an N threshold concentration for fungal production of cellulases. The threshold for fungal species tested was reported to be around 40 ppm l^{-1} agar [24]. In our experiment, significant cellulose decomposition was observed when soil extractable N was as low as 10 ppm, which may be attributed to fungal N recycling within the mycelium via cytoplasm translocation or self-lysis [7, 18, 23, 25, 26]. Subsidizing growth through translocation is supported by comparing our data on respiration (Figs. 1 and 2) and biomass production (Figs. 5 and 6) in the streptomycin treatment, where rapid increase in total biomass and high $^{14}CO_2$ evolution occurred during day 9 to day 14 while active biomass was low. The high N use efficiency and the relatively high C/N ratio of fungal biomass may contribute to the competitive advantage of fungal populations over bacterial populations for cellulose decomposition when N is limited.

Antibiotics not only have direct effects on the populations of target microorganisms, but also exert substantial indirect effects on nontarget organisms by changing competition and nutrient supply [8]. Cycloheximide and streptomycin have been used previously to estimate the contribution of bacteria and fungi to total soil respiration by studying changes in the rate of glucose utilization in antibiotic-treated soils or residues [1, 2, 5]. Results from those studies indicated that the inhibitory effects of antibiotics, as measured by glucose-induced respiration, were selective and effective over short incubation periods. Rapid increase and stabilization of microbial biomass C in the cycloheximide treatment suggeststhat nontarget organisms can compensate for the reduction in the biomass of the target organisms (Fig. 6). Nevertheless, consistent and significant inhibitory effects of cycloheximide on cellulose decomposition were recorded throughout the 28-day period in the present experiments. The prolonged effects of cycloheximide have also been reported in previous studies where the microbial C:N ratio was significantly lower throughout a 7-day incubation experiment [4] and fungal biomass was lower 22 days after the cycloheximide treatment [12]. Our results indicate that cycloheximide has a more profound effect on cellulose degradation than on glucose utilization. We acknowledge the limitations of selective inhibition by antibiotics in quantifying the contribution of target organisms to a specific function because of incomplete inhibition of the target microorganisms, indirect effects on nontarget microorganisms, and antibiotic degradation. However, our results did illustrate that combined with ^{14}C-labeled substrate and direct microbial quantification, selective inhibition of bacteria by streptomycin or fungi by cycloheximide can be very useful to reveal distinct patterns of substrate utilization by these two groups and estimate the relative contributions to substrate utilization.

In summary, decomposition of ^{14}C-labeled cellulose was best described by a multiphasic curve, which resulted from the changes in substrate utilization by bacterial and fungal populations and N availability. Bacteria and fungi play a dominant role during the initial and secondary stages of cellulose decomposition, respectively. Fungal decomposition of cellulose became dominant during the second phase, possibly, as a result of release of catabolite repression and subsequent cellulase production after depletion of available C by oligotrophic bacteria, and fungal competitive advantages over bacteria with respect to N acquisition. The role of oli-

gotrophic bacteria in promoting microbial succession requires further investigation.

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