

## Decomposition of Microbial Cell Components in a Semi-Arid Grassland Soil

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Received for publication 26 June 1979

Cell component fractions (<sup>14</sup>C-labeled) were prepared from bacterial and fungal cultures isolated from the Pawnee National Grassland in northeastern Colorado and tested for seasonal changes in degradability. The decomposition of cell component fractions was monitored from May to December of 1977 and during March of 1978, using soil samples taken at 2- to 3-week intervals. The release of <sup>14</sup>CO<sub>2</sub> from bacterial and fungal cell walls was inversely related ( $P < 0.01$ ) to the soil moisture content. Except for cytoplasm isolated from an *Aspergillus* sp., all other cytoplasmic and polysaccharide fractions did not demonstrate a significant relationship between soil moisture and decomposability. In general, bacterial cell walls and polysaccharides were more susceptible to decomposition than fungal cell walls, although the seasonal changes in decomposability for both fractions were similar. These patterns of cell component utilization indicate that the decomposition of cell wall material may be more closely linked, on an inverse basis, to the availability of soil moisture and release of soluble, low-molecular-weight organics resulting from primary production events.

The availability of microbial cell components and cell products in the form of cell walls, cytoplasmic components, and extracellular polysaccharides represents an organic pool which may contribute significantly to the formation of soil organic matter (8, 13). Recent investigations have shown that the more susceptible fractions of microbial cell components serve to provide a utilizable source of carbon to some members of the microbial community, while the more recalcitrant fractions such as melanin contribute to the soil humic fraction (14). Also, it is postulated that soil organic matter may contain as much as 25 to 30% polysaccharide material which is of either microbial or plant origin (11). Seasonal changes in the degradation of microbial polysaccharides may influence soil aggregation and the uptake of essential metals by plants and may also affect plant mechanisms of detoxification from heavy metal contamination (3, 16).

The mineralization of cell components as well as the contribution of microbial cell components to the stable fractions of soil organic matter have been considered, although seasonal changes in susceptibility have not been emphasized. Although the decomposition of microbial cell components has been monitored by other investigators (6, 14, 18), these studies have primarily evaluated the relative rates of microbial tissue

decomposition in different soils and with a variety of soil amendments. The time required for total decomposition varied greatly, depending on the particular organism and cell fraction. The current study was not designed to determine the amount of time required for total decomposition, but rather addressed the problem of seasonal susceptibility of bacterial and fungal cell components to decomposition processes as influenced by moisture availability in a semi-arid grassland, by using relatively short incubation test conditions.

### MATERIALS AND METHODS

**Isolation of microorganisms.** Bacterial and fungal cultures were isolated from soil cores (16.5 cm long and 10 cm in diameter) taken from plot 26 of the Central Plains Experimental Range in northeastern Colorado (administered by the U.S. Department of Agriculture Science and Education Administration-Federal Research). All soil cores were immediately placed in airtight plastic bags for 24 h and then examined for commonly occurring bacterial and fungal colonies by plating onto sodium caseinate (7) and rose bengal (10) agar, respectively. Bacterial colonies which appeared mucoid on agar plates were tested for polysaccharide production by growth in a peptone (1.0%, wt/vol)-glucose (0.5%, wt/vol) liquid medium and subjecting the culture filtrates to precipitation in ethanol (15). The appearance of a gelatinous precipitate in ethanol was taken as a positive test. Two polysaccharide-producing bacterial isolates were chosen for further study and identified as *Xanthomonas fragariae* and *Chromobacterium lividum*. The fungal cultures

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chosen for further study were identified as members of the genera *Aspergillus*, *Mucor*, and *Penicillium*.

**Preparation of  $^{14}\text{C}$ -labeled substrates.** Bacterial cultures were routinely grown in 1-liter quantities of peptone (1.0%, wt/vol)-glucose (0.5%, wt/vol) liquid medium containing 1.0  $\mu\text{Ci}$  of uniformly labeled [ $^{14}\text{C}$ ]-glucose (240  $\text{mCi}\cdot\text{mmol}^{-1}$ ) obtained from ICN (Irvine, Calif.). Cultures of *Xanthomonas* were grown at 25°C on a New Brunswick gyrotory shaker model G-25 (New Brunswick Scientific Co., New Brunswick, N.J.) operated at 150 rpm. After an incubation period of 5 days, cultures of *Xanthomonas* were centrifuged at 5,000  $\times g$  for 30 min in a Sorvall RC-5 superspeed refrigerated centrifuge (Dupont Instruments, Newtown, Conn.). The production of copious amounts of extracellular polysaccharide necessitated dilution and repeated centrifugation for complete cell removal.

Optimal polysaccharide production by *C. lividum* occurred during stationary growth for 7 to 10 days at 25°C. Because the polysaccharide produced by *C. lividum* is more closely associated with the cell surface than that produced by *Xanthomonas*, the polysaccharide was extracted with 0.1 N NaOH according to the original procedure of Martin and Richards (15).

After the removal of whole cells, polysaccharide material was precipitated from culture filtrates by the addition of 4 volumes of ethanol and kept at 4°C for 12 h. Subsequently, the precipitated polysaccharide was removed with a glass rod, redissolved in a minimum quantity of water, and dialyzed against four changes of deionized water at 4°C for 12 h. The dialyzed polysaccharide was further purified by the addition of chloroform (0.25 volume) and butyl alcohol (0.1 volume) to 1 volume of polysaccharide solution and then precipitated in 4 volumes of acetone according to the procedure of Lee and Loutit (9). The precipitated polysaccharide was then reprecipitated several times in ethanol and dissolved in deionized water.

The isolation of  $^{14}\text{C}$ -labeled bacterial cell wall and cytoplasmic fractions was accomplished by breakage of cells which had been removed during polysaccharide purification. Washed cells of the two bacterial cultures were broken by the use of sonication (Blackstone Ultrasonics, Sheffield, Pa.) for a total of 5 min, followed by two passages through a French Press (American Instrument Co., Silver Spring, Md.). Microscopic examination demonstrated cell breakage to be greater than 95% efficient. The supernatant and pellet fractions were used as the cytoplasmic and cell wall fractions, respectively. Fungal cell components ( $^{14}\text{C}$ -labeled) were isolated by growing fungal cultures in Roux flasks containing 150 ml of Sabouraud dextrose broth (Difco) in the presence of 20.0  $\mu\text{Ci}$  of uniformly labeled [ $^{14}\text{C}$ ]-glucose (240  $\text{mCi}\cdot\text{mmol}^{-1}$ ). The cultures were incubated in a hood at room temperature for 7 days, harvested, and homogenized for 5 min in a Waring blender at high speed in 0.05 M phosphate buffer (pH 7.4). The homogenates were washed repeatedly in phosphate buffer and deionized water and resuspended in sodium lauryl sulfate (1.0%, wt/vol) as described by Ballesta and Alexander (2). The fungal-detergent mixture was then incubated for 10 h at 4°C and subsequently washed again in phosphate buffer until all traces of the detergent had been removed. The fungal suspension was then sonicated for a total

of 20 to 25 min or until microscopic examination indicated that the hyphal fragments were essentially free of cytoplasm. The pellets and supernatants obtained by centrifugation at 16,000  $\times g$  were designated as the cell wall and cytoplasmic fractions, respectively. All cell wall, cytoplasmic, and polysaccharide fractions to be used for decomposition studies were stored in lyophilized form.

**Decomposition studies.** All  $^{14}\text{C}$ -labeled cell components were incubated with soil collected at the Pawnee Grassland site (taken at 2- to 3-week intervals) for a total of 7 days. The soil is a loam to sandy-loam texture and is classified as an Aridic Haplargid (20). Flasks containing 5.0 g of soil were incubated at room temperature with 5.0 mg of  $^{14}\text{C}$ -labeled cell component material which had been resuspended in a sufficient quantity of deionized water to adjust the soil water tension to one-third bar suction. The flasks were fitted with air-tight septa and flushed with  $\text{CO}_2$ -free air for 15 min at 1, 2, 4, and 7 days.  $\text{CO}_2$ -free air was obtained by passing air through successive traps of 10% NaOH, 10%  $\text{H}_2\text{SO}_4$ , and 10%  $\text{Ba}(\text{OH})_2$  before moistening by passage through water. At the end of 7 days, flasks were flushed with  $\text{CO}_2$ -free air before and after acidifying with 5 ml of 2 N  $\text{H}_2\text{SO}_4$  (to release residual  $^{14}\text{CO}_2$ ). The internal atmospheres of all flasks were flushed directly into scintillation vials containing 2-aminoethanol as a  $\text{CO}_2$  trap as recommended by Crawford and Crawford (5). All counts were adjusted to 100% efficiency using the external standard ratio method (19), and the data are reported as the percent of original activity released as  $^{14}\text{CO}_2$ .

The specific activities of the cell components used in this study were determined by internal standardization (19), and the following values were obtained: 2,080 to 2,360  $\text{dpm}\cdot\text{mg}^{-1}$  for bacterial and fungal cell walls; 3,020 to 3,680  $\text{dpm}\cdot\text{mg}^{-1}$  for bacterial polysaccharides; 500 to 1,600  $\text{dpm}\cdot\text{mg}^{-1}$  for fungal cytoplasm; and 2,600 to 2,840  $\text{dpm}\cdot\text{mg}^{-1}$  for bacterial cytoplasm.

**Soil core analyses.** Soil water content was determined by weighing soil plugs taken through the entire depth of a sample core (approximately 250 g), using a sharpened, 12-mm-diameter cork borer, before and after drying at 110°C for 24 h. The procedures used to determine oxygen utilization in the presence of various substrates and soil organic matter content were performed as previously described (7).

## RESULTS

**Cell wall decomposition-soil moisture relationships.** The decomposition of bacterial and fungal cell walls demonstrated an inverse relationship with the soil water content (Fig. 1 and 2). This was especially evident in the spring and early summer of 1977, during which time reduced moisture conditions prevailed (Table 1). As the moisture content of the soil decreased from April through July, the mineralization of bacterial and fungal cell walls gradually increased until mid-July, when a major precipitation event (15.2 cm in 3 days) increased the soil water content to 15.5% (wt/wt). This resulted in a significant decrease in the decomposition of

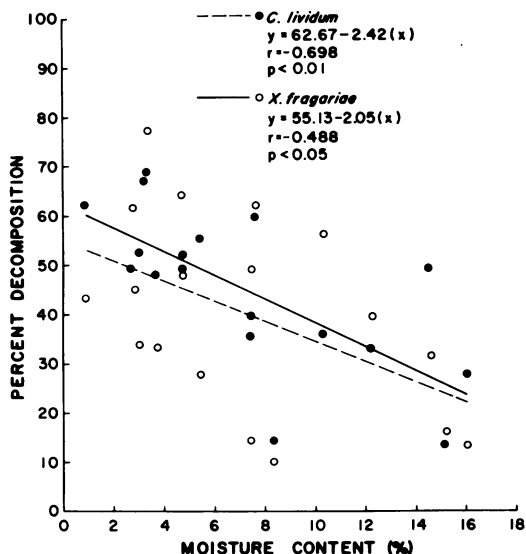


FIG. 1. Decomposition of bacterial cell walls as related to the soil water content. The cell wall fractions were isolated from *C. lividum* and *X. fragariae*.

bacterial and fungal cell walls until the soil system was again stressed for moisture availability. The decomposition of bacterial cell walls varied from 10 to 80% during this entire sampling period depending on available moisture, which fluctuated between 0.9% (wt/wt) and 15.5% (wt/wt). Statistical analyses by a least-squares-fit method revealed that the decomposition of cell walls isolated from *C. lividum* and *X. fragariae* as well as from the three fungal isolates was inversely related to the moisture content of the soil at  $P < 0.01$  and  $P < 0.05$  levels of significance.

**Decomposition of microbial cytoplasm.** Although the decomposition of all four cytoplasmic fractions tested resulted in a negative slope when graphed against soil moisture content, only the decomposition of cytoplasm isolated from an *Aspergillus* sp. demonstrated a statistically significant inverse relationship at  $P < 0.05$  level of significance (Fig. 3 and 4). However, cytoplasmic fractions from *Penicillium* and *Trichoderma* exhibited an increased susceptibility to mineralization as compared to cytoplasmic fractions from *Aspergillus*, regardless of soil water availability. Also, the mineralization of cytoplasm from *X. fragariae* remained relatively unchanged (15 to 30%) during this entire time period and showed no discernible relationship to the soil water content.

**Decomposition of extracellular polysaccharides.** Although extracellular polysaccharides were degraded more extensively than other cell components during the most intense mois-

ture stress conditions (Table 1), statistical analyses for the entire year did not reveal significant relationships between polysaccharide decomposition and soil moisture content (Fig. 5).

**Seasonal fluctuation.** The decomposition of 11 different cell component substrates was monitored from May of 1977 through March of 1978 (Table 1). It was observed that the bacterial cell walls and polysaccharides were degraded to a greater extent than either fungal cell walls or fungal cytoplasm. However, at no time was the bacterial cytoplasmic fraction degraded to a greater extent than other bacterial and fungal cell components. The degradation of bacterial cell walls was greatest from August through December, whereas bacterial polysaccharide decomposition was dominant in June and July. Only 11% of the samples demonstrated greater fungal cell wall decomposition than that shown for the bacterial cell walls. The decomposition of bacterial and fungal cytoplasmic fractions displayed similar trends, but neither substrate group was ever degraded by more than 50% under these test conditions. The degradation of bacterial polysaccharides exceeded the decomposition of bacterial cytoplasmic fractions but was less extensive than that demonstrated by fungal cytoplasmic fractions.

**Relationship to other parameters.** The relationships between cell component mineralization and other soil and microbial activity indicators are presented in Fig. 6. This correlation

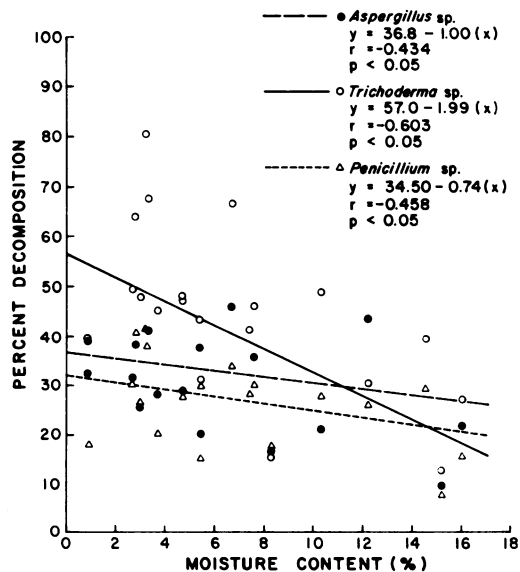


FIG. 2. Decomposition of fungal cell walls isolated from *Aspergillus*, *Trichoderma*, and *Penicillium* sp. as related to soil water content.

TABLE 1. Seasonal changes in percent total decomposition of bacterial and fungal cell components<sup>a</sup>

Sampling date (mo./day/yr.)	Bacterial cell walls	Bacterial polysaccharides	Bacterial cytoplasm	Fungal cell walls	Fungal cytoplasm	Soil water content (%)
5/2/77	14.8	6.5	— <sup>b</sup>	9.0	—	15.2
5/16/77	12.3	16.1	—	16.0	—	8.3
5/31/77	25.0	22.6	—	27.2	—	7.4
6/13/77	43.2	20.1	—	33.3	—	3.0
6/28/77	40.5	44.0	—	31.2	—	3.7
7/22/77	67.8	—	29.2	48.0	—	2.8
7/25/77	20.6	23.9	12.7	21.4	—	15.5
8/9/77	55.5	30.2	19.6	36.7	54.4	2.7
8/22/77	71.1	40.4	49.4	49.1	27.3	3.3
9/6/77	—	36.0	26.9	70.0	63.4	3.3
9/19/77	52.7	43.7	17.2	29.9	55.4	0.9
10/1/77	41.6	13.6	26.8	32.1	57.5	5.4
10/17/77	—	38.5	23.3	28.9	42.3	5.7
11/2/77	58.2	44.7	18.5	35.3	26.9	4.7
11/29/77	46.1	42.5	26.5	32.6	28.5	10.3
12/18/77	60.8	49.7	19.5	37.4	31.3	7.6
3/1/78	40.5	41.6	19.3	48.8	32.5	14.5
3/14/78	78.3	77.9	36.9	58.7	52.8	13.7

<sup>a</sup> Each value represents the average percent decomposition for a specified sampling date, using soil samples run in duplicate.

<sup>b</sup> —, Not tested.

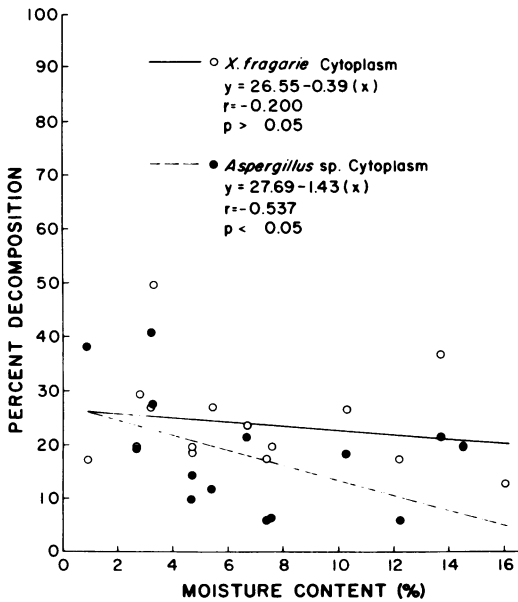


FIG. 3. Decomposition of microbial cytoplasm isolated from *X. fragariae* and an *Aspergillus* sp. as related to soil water content.

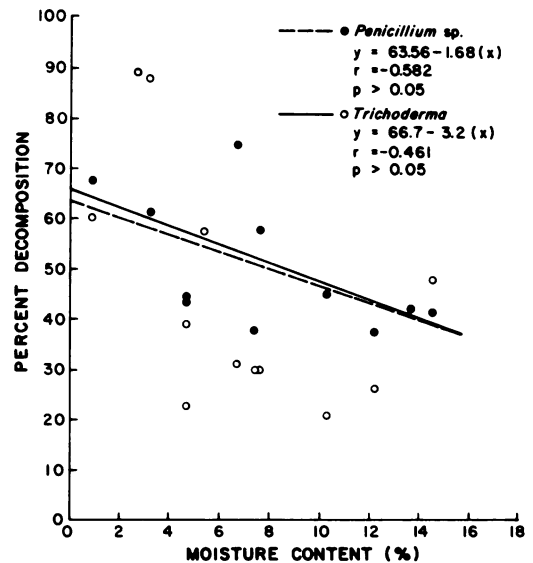


FIG. 4. Decomposition of microbial cytoplasm isolated from *Penicillium* and *Trichoderma* sp. as related to soil water content.

matrix depicts some of the inherent differences between cell component mineralization versus the utilization of materials considered as representative of plant-derived substrates (glucose, amino acids, starch, and cellulose). The mineralization of microbial cell wall components dem-

onstrated a number of inverse relationships with the utilization of plant-derived substrates as measured by the amount of O<sub>2</sub> utilized. In total, 19 of 22 possible relationships between cell component and plant-derived substrate utilization were inverse at the  $P < 0.01$  or  $P < 0.05$  level of significance.

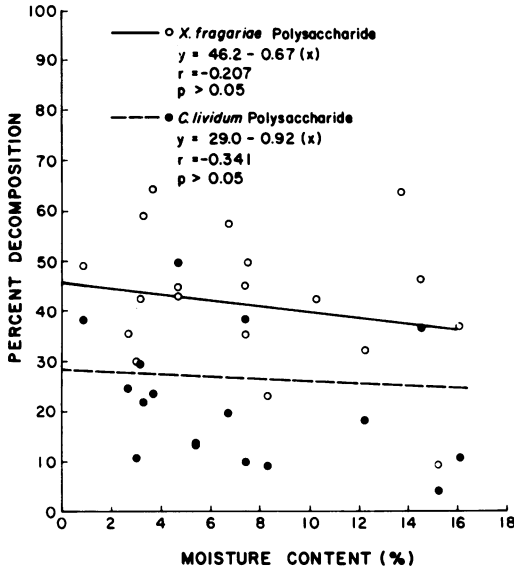


FIG. 5. Decomposition of extracellular polysaccharides isolated from *X. fragariae* and *C. lividum* as related to soil water content.

The degradation of each cell wall fraction exhibited a close correlation ( $P < 0.01$ ) with the degradation of all other cell wall fractions, indicating that these materials comprise a distinct substrate class whose decomposition is inversely correlated with moisture content (Table 1). Also, the degradation of polysaccharide material from *C. lividum* was correlated ( $P < 0.05$ ) with the decomposition of extracellular polysaccharide from *X. fragariae*. However, the mineralization of microbial cytoplasmic fractions demonstrated only one positive relationship to the mineralization of other cytoplasmic fractions, suggesting fluctuations in the utilization of the many compounds in this substrate class. The seasonal decomposability of cell walls isolated from *Trichoderma*, *Aspergillus*, and *Xanthomonas* also was inversely related to surface soil organic matter changes, suggesting that these substrates may be subject to a faster turnover rate during periods of decreased root exudate release.

DISCUSSION

In this study, the susceptibility of bacterial and fungal cell walls to decomposition appears

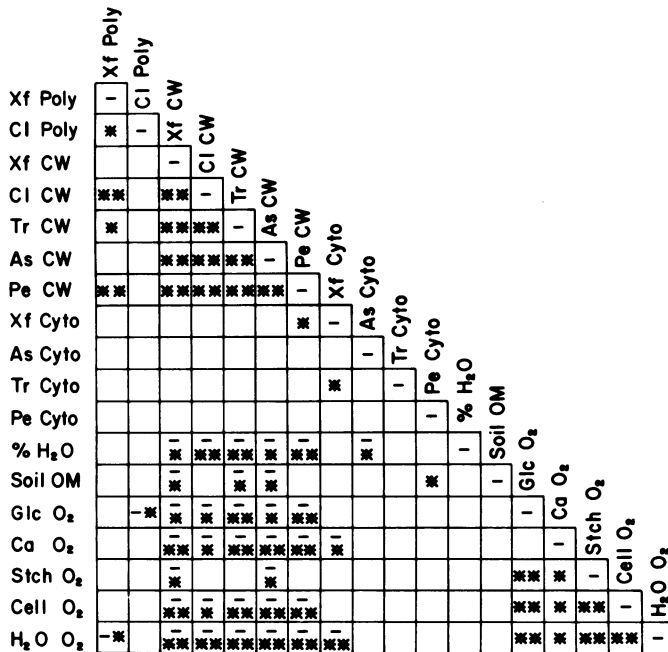


FIG. 6. Correlation matrix analysis for cell component mineralization versus plant-related substrate utilization from May 1977 to March 1978. List of abbreviations: Xf, *X. fragariae*; Cl, *C. lividum*; Tr, *Trichoderma* sp.; As, *Aspergillus* sp.; Pe, *Penicillium* sp.; OM, organic matter; Glc, glucose; Ca, Casamino Acids; Stch, starch; Cell, cellulose; H<sub>2</sub>O, control soil with no substrate amendment; Poly, polysaccharide; CW, cell wall; Cyto, cytoplasm; \*, significant at  $P < 0.05$ ; \*\*, significant at  $P < 0.01$ .

to show an inverse relationship with the soil water content (Fig. 1 and 2). In contrast, with the exception of cytoplasm isolated from an *Aspergillus* sp., the decomposition of microbial cytoplasmic fractions and extracellular polysaccharides did not exhibit a significant correlation to the field soil water content. When compared using a correlation matrix, the decomposition of each cell wall fraction exhibited a high degree of correlation with all other cell wall fractions, thereby indicating a strong similarity in the seasonal rates of decomposition. Although the bacterial cell walls were usually degraded to a greater extent than fungal cell walls (Table 1), the general pattern of decomposition for both substrate groups was similar. Using prolonged incubation times, Hurst and Wagner (6) demonstrated greater decomposition of cell walls than cytoplasmic fractions isolated from *Aspergillus niger*, but found greater decomposition of cytoplasmic fractions from an unidentified melanin-containing fungal isolate. These investigators also demonstrated that all fungal fractions were decomposed at maximal rates within 7 days. Preliminary experiments carried out for this study revealed 20 to 70% decomposition within 1 week, and for this reason all experiments were terminated after 7 days of incubation.

Verma and Martin (17) investigated the decomposition of cell components isolated from six blue-green bacteria (cyanobacteria) and one green alga, and they demonstrated greater decomposition of the cytoplasmic fraction as compared to the cell wall fraction for every culture studied. The same pattern of cell component degradation was also demonstrated when these materials were introduced into the reaction mixture directly or complexed with model phenolase polymers. In our studies, the cytoplasmic fractions isolated from fungi were extensively degraded as compared to cytoplasmic fractions isolated from bacteria. As shown in Table 1, the cytoplasmic fractions isolated from fungi were degraded by more than 50% on 5 of 11 sampling dates, whereas the bacterial cytoplasmic fractions were never degraded by more than 50%.

The decomposition of extracellular polysaccharides from *X. fragariae* and *C. lividum* did not demonstrate any relationships to the seasonal changes in the water content of the soil. These two substrates were degraded to a lesser extent than the cell wall fractions from the same organisms, suggesting that these compounds are stabilized to a certain degree in the more resistant humus fraction of soil. Although extensive decomposition of these extracellular polysaccharides did not occur under our experimental con-

ditions, the data obtained compare favorably with results published by Martin et al. (14) and Verma and Martin (17), in which algal polysaccharides as well as polysaccharides isolated from *Hansenula*, *Leuconostoc*, and *Azotobacter* were used. In general, 20 to 50% of the introduced polysaccharides were degraded within 1 week. These investigators also observed that the amount of  $^{14}\text{C}$  activity detected in the humic acid, fulvic acid, and extracted soil fractions after  $^{14}\text{C}$ -labeled polysaccharides had been incubated in soil was a function of the individual polysaccharides applied.

The increased utilization of bacterial and fungal cell wall components during periods of decreased water availability suggests that these cell fractions may constitute an important nutrient source during times of relative moisture stress. These data indicate that mineralization of microbial cell walls is subject to a different set of regulatory mechanisms than other cell components and plant-derived substrates. These mechanisms may incorporate substrate availability, water solubility, and structural susceptibility to microbial decomposition. Soluble components such as cell cytoplasm and polysaccharide material may provide a more readily available carbon source during periods of normal precipitation. Although soil temperature was not monitored during this study, it is obvious that temperature fluctuations would play a substantial role regulating decomposition rates. In these experiments the effects of temperature were minimized by incubating the field-derived samples at a controlled temperature in the laboratory. In this way, the effects of varied soil water contents, resident soil organic matter, and possible microbiological changes in cell component degradation could be evaluated.

The role of microorganisms in the chemical and physical stabilization of soil aggregates has been well established (1, 14, 18), and the available data seem to indicate that microbial polysaccharides provide the greatest degree of soil stabilization as compared to other cell components. In this study, no attempt was made to incorporate bacterial extracellular polysaccharides into the soil humic fraction by complexing with model phenolase phenolic polymers. Polysaccharides present in concentrations as low as 0.1% (wt/wt) have been shown to increase the aggregation of silt and clay particles from 35 to 75% (12). It has been suggested by Martin (11) that the polysaccharide content of soil humus consists primarily of microbial and plant polysaccharides in various stages of decomposition, which become an integral part of the soil humic fraction. The association is achieved by combin-

ing with clays, metal ions, or soil colloids which also impart enhanced resistance to microbial decomposition.

The decomposition of bacterial and fungal cell walls was observed to contribute more significantly to the available carbon pool than either the cytoplasmic or polysaccharide fractions (Table 1). Verma and Martin (17) obtained similar results after complexing microbial cytoplasmic and cell wall fractions with model phenolase phenolic polymers. They attributed the greater cell wall decomposition to decreased solubility and fewer available amino groups, resulting in reduced linkage of cell wall material to quinones formed during polymerization reactions.

The pattern of cell component mineralization established in this study may assist in the development of model systems to more accurately interpret the seasonal contribution of microbial cell components to the biologically utilizable carbon pool. It can be expected that, during active microbial growth in the presence of soluble and easily utilizable carbon sources, the activity of autolytic enzymes would be repressed. Under these conditions, the mineralization of the insoluble cell component polymers would decrease during periods of higher soil water availability, and these compounds would accumulate in the soil. However, during periods of lesser water availability and with lower rates of organic matter release from plant roots, the standing microbial biomass would enter a senescent period of greatly decreased growth, causing a derepression of autolytic enzymes concomitant with an increase in the mineralization of insoluble and generally more recalcitrant biopolymers.

The results obtained in this study demonstrate that it may be inaccurate to judge the susceptibility of cell components to decomposition processes based solely on variation in the time required for total decomposition under optimal laboratory conditions. The decomposition of microbial cell components represents only one aspect of the total biological nutrient cycle, and additional studies are needed which will encompass invertebrate as well as protozoan effects on the turnover of active versus inactive microbial populations, as has been suggested by Coleman et al. (4).

#### ACKNOWLEDGMENTS

We are especially thankful to Nicholas J. Nagle for technical assistance throughout this study.

This work was supported by National Science Foundation grant DEB 75-18765.

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