APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1993, p. 3592–3596 Vol. 59, No. 11
0099-2240/93/113592-05$02.00/0

Microbial Decomposition of Elm and Oak Leaves in a Karst Aquifer†

ANGELA C. EICHEM, WALTER K. DODDS,* CATHY M. TATE,‡ AND CHRIS EDLER
Division of Biology, Kansas State University, Manhattan, Kansas 66506

Received 10 May 1993/Accepted 9 August 1993

Dry Chinquapin oak (*Quercus macrocarpa*) and American elm (*Ulmus americana*) leaves were placed in four microcosms fed by groundwater springs to monitor changes in dry mass, ash-free dry mass, and microbial activity over a 35-day period. Oxygen microelectrodes were used to measure microbial activity and to estimate millimeter-scale heterogeneity in that activity. Oak leaves lost mass more slowly than elm leaves. Generally, there was a decrease in total dry weight over the first 14 days, after which total dry weight began to increase. However, there were consistent decreases in ash-free dry mass over the entire incubation period, suggesting that the material remaining after initial leaf decomposition trapped inorganic particles. Microbial activity was higher on elm leaves than on oak leaves, with peak activity occurring at 6 and 27 days, respectively. The level of oxygen saturation on the bottom surface of an elm leaf ranged between 0 and 75% within a 30-mm^2 area. This spatial heterogeneity in O_2 saturation disappeared when the water velocity increased from 0 to 6 cm s^{-1}. Our results suggest that as leaves enter the groundwater, they decompose and provide substrate for microorganisms. The rate of decomposition depends on leaf type, small-scale variations in microbial activity, water velocity, and the length of submersion time. During the initial stages of decomposition, anoxic microzones are formed that could potentially be important to the biogeochemistry of the otherwise oxic aquifer.

Leaf litter and other particulate materials enter the groundwater at the Konza Prairie Research Natural Area through cracks in the soil and limestone that form during dry periods. Material can then be transported rapidly into the aquifer because of the channelized nature of the flow through a Karst aquifer (20). Much work has been done on organic material decomposition in streams (18, 19) and forests (3, 17), but little research has been conducted on groundwater systems because access is difficult and because of the mistaken impression that groundwater systems are for the most part sterile. Research on factors that control microbial activity in limestone aquifers is important because Karst terrains cover about 20% of the United States (20).

We sieved water from a horizontal well that is fed by an artesian spring in the area and observed large quantities of particulate materials, including leaf parts. Presumably, the leaf litter is decomposed by groundwater microorganisms as it travels through the different layers of the aquifer. This litter input could mediate groundwater nutrient flux by providing oxidizable substrate for an active microbial assemblage. Also, a distinct invertebrate community inhabits the groundwater at the Konza Prairie, and the leaf litter or associated microorganisms may be an important food source for the invertebrates (10). The purposes of this study were to observe how leaves decompose in this groundwater habitat and how microbial activity is related to this decomposition and to describe how flow controls the decomposition rates.

MATERIALS AND METHODS

Study site. This research was conducted at the Konza Prairie Research Natural Area, a 3,487-ha area of pristine tallgrass prairie located approximately 10 km south of Manhattan, Kans. The aquifers studied are on Permian limestone in watershed N4D of the Kings Creek drainage basin in the center of the research area. The geology consists of alternating layers of shale and limestone (23), and the aquifers typically exhibit fractured flow. Groundwater was accessed through four springs (Fig. 1). This approach had less impact on the groundwater than using wells for sampling Karst aquifers (20). The dominant vegetation around the three lower sites (sites A, B, and C) is riparian prairie shrubs and trees (including Chinquapin oak [*Quercus macrocarpa*] and American elm [*Ulmus americana*]), while the upland site (site D) is surrounded by prairie grass. The exact recharge zones of these aquifers have not been well characterized but are dominated by prairie shrubs, trees, and grasses in a pristine state. The spring at site A issues from an Eiss limestone formation located next to the channel on watershed N4D. A deep (20-cm) macrophyte bed covers the site at the seep access. The springs at sites B and C flow from a Middleburg limestone layer that is exposed on a steep bank beside the stream channel. Site C is located approximately 4 m downstream from site B. The spring at site D issues from a Middleburg limestone layer located ca. 20 m uphill from the stream channel (Fig. 1). Sites B and C are on the west side of the stream channel and are probably fed by a portion of the aquifer distinct from the portion that feeds the two sites east of the stream channel (sites A and D).

Leaf decomposition. Water from the springs was diverted into polyvinyl chloride pipes (internal diameter, 1.8 cm) by driving the ends of the pipes up into cracks in the limestone layers from which the springs flowed. The opposite end of each pipe was attached to a styrofoam-insulated cooler, and a series of holes were made in the downstream end of the cooler to allow water to flow through the cooler and maintain a depth of approximately 10 cm. Chinquapin oak and American elm leaves that had fallen during the previous autumn were collected from the gallery forest floor at watershed N4D in May. These species are typical of gallery forests in

* Corresponding author.
† Contribution no. 93-426-J from the Kansas Agriculture Experiment Station.
‡ Present address: Box 25046, MS415, Denver Federal Center, Denver, CO 80225.

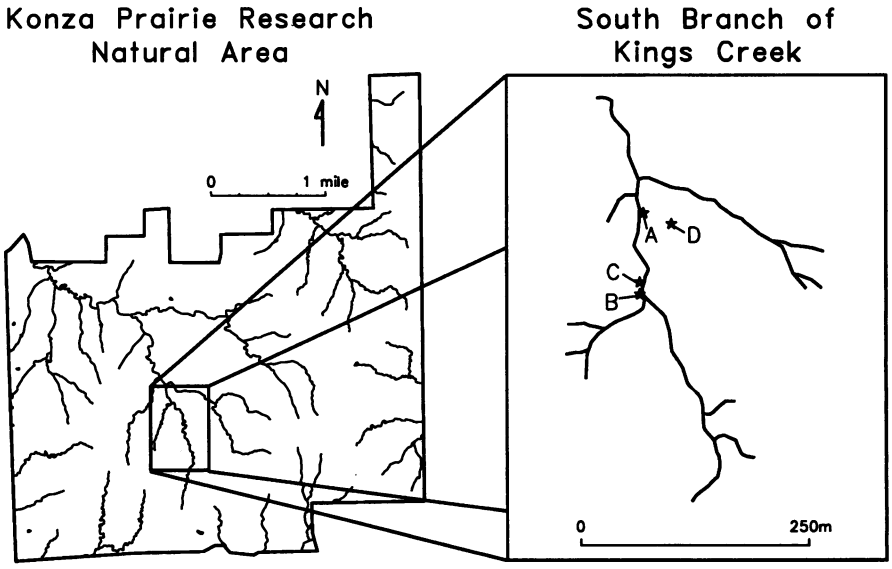

FIG. 1. Map of the study site, watershed N4D, near Kings Creek channel. See text for descriptions of sites A through D.

the area. Disks (25 mm^2) were punched from oak leaves, and 80-mm^2 pieces were broken off elm leaves; the leaf disks and pieces (air-dried mass, 0.1 g) from the two species were impaled on insect-mounting pins. Oak leaf disks and elm leaf pieces were placed in each of the four coolers by pushing the pins into the bottom of the cooler under approximately 10 cm of spring water. Lids and black plastic bags covering the coolers kept light from entering. Three replicates of each leaf sample type were removed from each of the four sites to estimate mass loss at 0, 1, 4, 7, 14, 21, 28, and 35 days. Additional pinned leaf samples were removed for O_2 microelectrode studies. Pinning leaf samples prevents some artifacts associated with leaf pack methods (5). The temperatures, dissolved oxygen concentrations (1), water velocities (16), and conductivities in the coolers were measured weekly.

The leaf samples used for mass loss determinations were collected from the coolers and placed in individual scintillation vials. The samples were dried at 68°C for 24 h, weighed to determine dry mass, and converted to ash by heating them at 490°C for 1 to 1.5 h to determine ash-free dry mass (AFDM). Mass loss was quantified by using a logarithmic decay coefficient similar to that used in stream studies (23). Inorganic mass was calculated by determining the residual mass left after conversion to ash.

Cathode type O_2 microelectrodes (21) were used to measure O_2 concentrations on the surfaces of individual leaf disks and in water adjacent to the leaves. We assumed that the O_2 concentration at a leaf sample surface was an indicator of the microbial activity (respiration) on that leaf sample. Oxygen concentrations on the top and bottom surfaces of leaf samples were measured for sites A and B after 7, 14, 21, 28, and 35 days. Heterogeneity in microbial activity was assessed by measuring O_2 concentrations on the bottom surface of an elm leaf sample from site A every millimeter over a grid (5 by 6 mm). The effect of water velocity on the O_2-free microzones was assessed in a recirculating chamber. A thermistor (16) was used to determine water velocity 1 mm from a leaf sample surface, and O_2 profiles from the surface of a leaf sample were measured at velocities of 0, 0.94, 4.6, and 6 cm s^{-1}; these velocities encompass the mean value of 5.4 cm s^{-1} measured from a nearby artesian spring during the study period (unpublished data).

Organic particle output. Particles were collected from a nearby artesian spring (10) with a 250-μm-mesh sieve over the period of the study. This particulate material was separated into three size classes (250 to 516, 516 to 700, and >700 μm). The material was dried and weighed, and the AFDM of the particulate material was determined.

RESULTS

All sites were similar with respect to temperature, O_2 concentration, alkalinity, and conductivity, and the variance within each site was low throughout the study (Table 1). Site D became dry on day 4, and site C became dry on day 25; sites A and B continued to flow throughout the experiment.

There were initial increases in AFDM for both elm and oak leaf samples at sites A, C, and D (Fig. 2); these increases were followed by decreases in the mass of elm leaf samples and slight decreases in oak leaf sample mass. There was a net decrease in the AFDM over a period of 35 days for both leaf types, yet over time elm leaf samples lost a greater percentage of AFDM than oak leaf samples. Inorganic mass increased with incubation time for both leaf types (Fig. 2). The gradual increases in AFDM after 15 days observed with elm leaf samples at sites A and B indicate that exogenous organic materials accumulated on the surfaces of the sam-

TABLE 1. Characteristics of four spring sites[a]

Site	Temp (°C)	Dissolved O_2 concn (mg $liter^{-1}$)	Alkalinity (mg of $CaCO_3$ $liter^{-1}$)	Conductivity (mS cm^{-1})
A	14.8 (0.5)	6.4 (2.7)	293 (11)	0.46 (0.10)
B	17.5 (1.4)	6.7 (2.8)	307 (11)	0.48 (0.10)
C	17.3 (2.2)	7.3 (0.9)	303 (15)	0.44 (0.07)
D	15.5 (0.6)	7.2 (0.4)	279 (4)	0.41 (0.06)

[a] The means of values from weekly data collection from 12 June 1991 to 6 August 1991 are given. Site D became dry on 2 July, and site C became dry on 23 July. The values in parentheses are standard deviations.

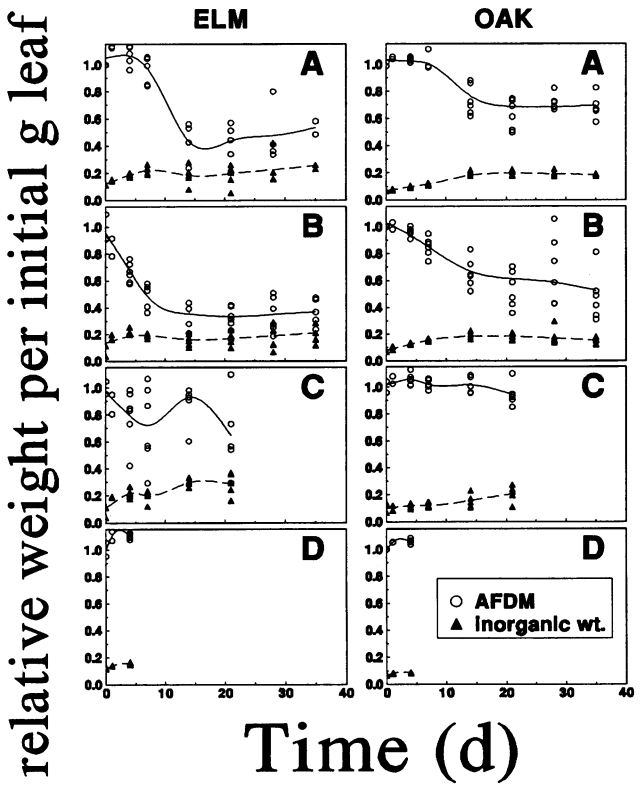

FIG. 2. Changes in mass per initial gram of AFDM and inorganic mass over 35 days at the four sites. Curves were estimated by using locally fitted regression (lowess).

ples. The rates of decomposition (k values) were calculated for both elm and oak leaf samples (Table 2). Because there was an initial increase in mass followed by a decrease and finally another increase, only the degradative portions of the data plotted in Fig. 2 were used to calculate k values (days 4 to 17 for elm leaf samples and days 4 to 21 for oak leaf samples). The k values for elm leaf samples were always higher than the k values for oak leaf samples.

The total amount of organic material that was washed from the nearby artesian spring ranged from 0.26 to 62 μg of AFDM per liter (mean, 18.5 μg/liter) over the study period. From 66 to 99% of this material did not pass through a 700-μm-pore-size mesh. Considering that the material had been degrading in the aquifer, this measurement of particulate material provided a minimum particulate organic material estimate for the aquifer. Furthermore, the preponderance of large particles suggests that the sizes of the leaf pieces which we used were reasonable.

TABLE 2. k values for elm leaf samples from days 4 to 14 and for oak leaf samples from days 4 to 21 at sites A, B, and C

Site	Elm leaf samples		Oak leaf samples	
	k value[a]	r^2	k value[a]	r^2
A	0.069 (0.019)[b]	0.75	0.026 (0.003)	0.90
B	0.120 (0.005)	0.97	0.037 (0.001)	0.99
C	0.026 (0.018)	0	0.009 (0.001)	0.70

[a] k values are expressed in the following units: ln (g of AFDM) (g of initial mass)$^{-1}$ day^{-1}.
[b] Values in parentheses are standard errors.

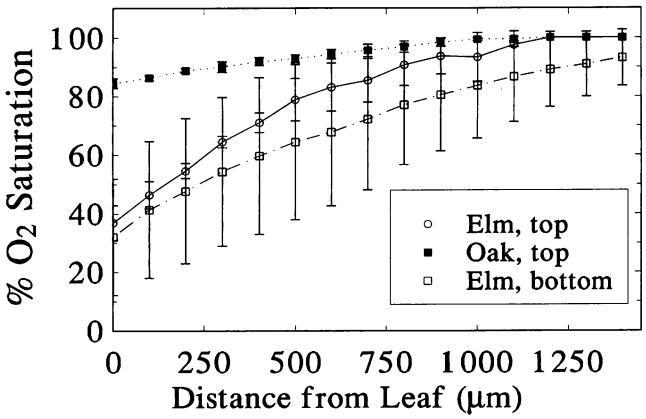

FIG. 3. Profiles of levels of O_2 saturation approaching the top and bottom surfaces of an elm leaf sample and the top of an oak leaf sample collected from site A after 7 days of submersion. Error bars indicate ± 1 standard deviation (n = 3; 0 distance = leaf surface).

There were no differences in O_2 saturation profiles between the tops and bottoms of the elm leaf samples, but there was more variation in the O_2 saturation profiles on the bottoms of the elm leaf samples (Fig. 3). The oak leaf samples had a shallower gradient (i.e., little change in the percentage of O_2 saturation with distance from leaf surface) than the elm leaf samples. This shallower gradient indicates that there was less microbial activity on the oak leaf samples and greater microbial activity on the elm leaf samples (Fig. 3). In separate experiments, inorganic materials (silt, sand, gravel) were incubated in groundwater for 8 weeks. None of these materials exhibited O_2 gradients as steep as those seen near leaf surfaces (unpublished data), suggesting that the steep gradients observed near the leaf samples were a result of microbial activity stimulated by the organic content of the leaf samples.

The elm leaf samples had greater microbial activity (lower O_2 tension at the leaf surface) than the oak leaf samples for up to 20 days at sites A and B (Fig. 4). The rates of microbial activity on elm leaf samples peaked on day 6 at site A and were not measured after day 21 because the leaf samples had degraded to the point that they could not be kept intact during transport to the laboratory (Fig. 4). The value for oxygen saturation on the surfaces of oak leaf samples varied from an initial level of approximately 90% to approximately 62% at site A, showing that there was an increase in microbial activity over 27 days.

The level of oxygen saturation on elm leaf samples varied from an initial value of approximately 25% to nearly 75% at site B. Oak leaf samples at site B exhibited roughly constant rates of microbial activity for 35 days. The level of oxygen saturation varied from 0 to 75% on the bottom surface of an elm leaf sample (Fig. 5). Low rates of activity occurred on the leaf veins, and high rates occurred on the leaf mesophyll. These data also indicate that distinct anoxic zones occur at the surface of a leaf. These oxygen-free zones were evident even though activity was maximal 9 days earlier. When the electrode was placed inside the leaf, O_2 tensions were often below the limit of detection.

As water velocity increased from 0 to 6 cm s^{-1}, the level of O_2 saturation at the leaf surface approached 100% (Fig. 6).

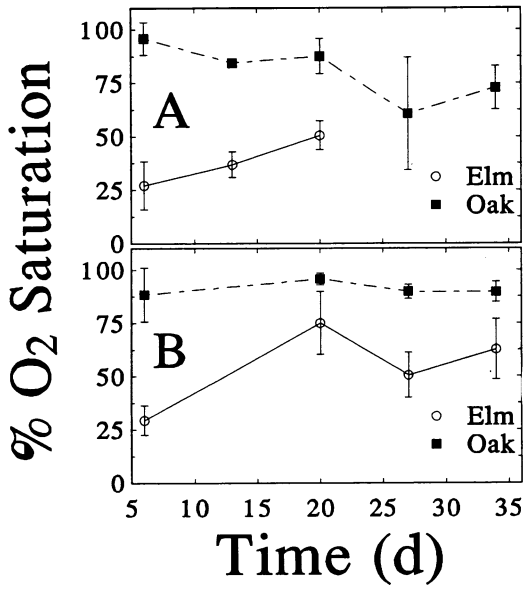

FIG. 4. Microbial activity expressed as the levels of O_2 saturation at the top surfaces of elm and oak leaf samples as a function of number of days of submersion at sites A and B. Error bars indicate ± 1 standard deviation (for each point, $n = 3$).

With increased water velocity, anoxic microzones are much less likely to occur because of increased inward transport of O_2. If one assumes that (i) Fickian diffusion occurs (2), (ii) molecular diffusion dominates O_2 transport within the diffusion boundary, and (iii) the diffusion constant is 2.35×10^{-5} $cm^2\ s^{-1}$, then the steepness of the diffusion boundary can be used to calculate inward O_2 flux (14). This inward flux is the respiration rate. Respiration rates were calculated for flow rates of 0, 0.94, and 4.6 cm s^{-1} and were 43, 108, and 126 pmol of O_2 $cm^{-2}\ s^{-1}$, respectively. Therefore, respiration increased with an increase in velocity.

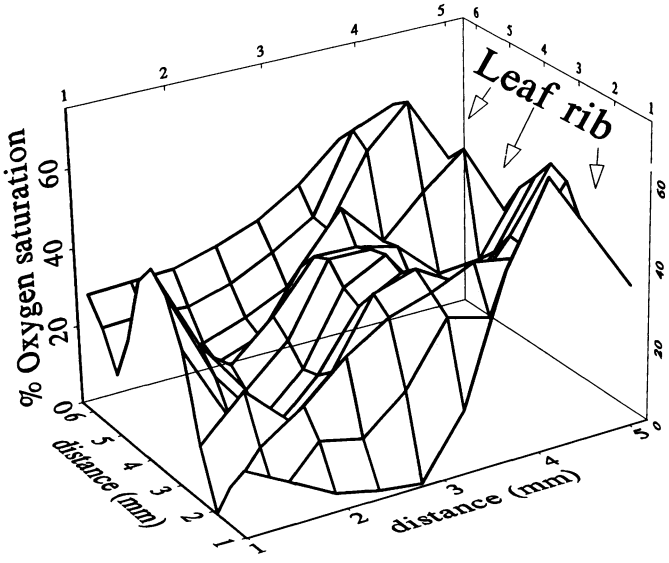

FIG. 5. Spatial heterogeneity of levels of O_2 saturation at the bottom surface of an elm leaf sample collected from site A after 14 days of submersion.

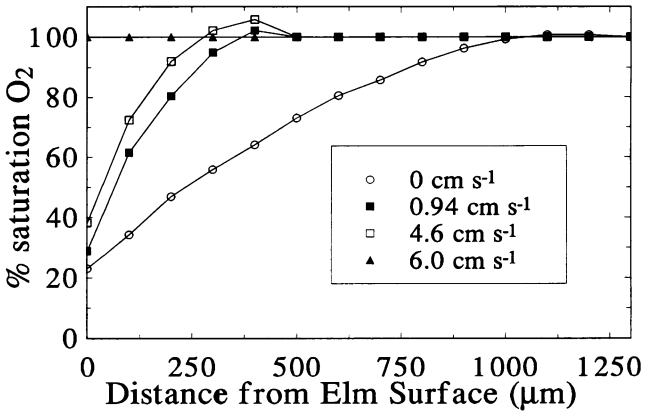

FIG. 6. Effect of water velocity on O_2 saturation profiles from the top of an elm leaf sample collected from site A after 14 days of submersion.

DISCUSSION

We compared groundwater data with stream data because leaves are saturated with water in both habitats. The two systems have the following similarities: (i) elm leaves degrade faster than oak leaves (11, 22), (ii) increased degradation rates occur with increased flow rates (5), (iii) exogenous materials deposit or grow on leaf surfaces (23), and (iv) microbes take an active part in the degradation of organic materials (11, 19). We saw some groundwater invertebrates (the isopod *Caecidotea tridentata* and the amphipod *Bactrurus hubrichti*) in our enclosures. We observed little effect on degradation rates related to invertebrate densities. Thus, we suspect that microbial degradation was the most important factor.

The *k* values for elm leaves in groundwater (Table 2) were several times higher than the *k* values observed in streams on the Konza Prairie (12, 23) and were on the high end of the values observed in other prairie streams (11), rivers (13), and streams and forests in general (18). The rates of oak leaf degradation were within the ranges reported for Shummard oak (*Quercus shumardii*) for prairie streams (11), but were high compared with the rates observed for oak leaves in forests and some other stream studies (18). This may have been because our analysis of *k* values concentrated on the days of the experiment when decomposition predominated, because we used pieces of leaves, or because we did not use mesh bags (leaf packs) that impeded flow and transport of broken leaf parts. However, if leaf parts were being transported, the consistent increase in the inorganic weight shown in Fig. 2 would not be expected. These problems and the fact that a logarithmic decay model does not always fit the data suggest that caution should be used when decay rates are interpreted with *k* values.

As in streams, elm leaves were processed faster than oak leaves in groundwater. Oak leaves may last longer and may be more likely to enter the groundwater whole because they have more recalcitrant compounds, such as tannins, and lower N contents (15) and thus may move further into the aquifers and provide more long-term nutrition for consumers (6).

Microbial activity on leaves was a function of water

velocity. Increased flow increased the inward transport of O_2 (and possibly the transport of other required substances and export of deleterious waste products) and thus promoted higher rates of metabolism and processing. The metabolic rates of microbial assemblages from streams have been related to water velocity; increases in water velocity on the order of several centimeters per second can have significant effects (8, 9). The water velocities in main flow channels of limestone aquifers in general can range from 0.3 to 14 cm s^{-1} (20), and our data from a nearby artesian spring (unpublished data) fall within this range, so the range used in our study is reasonable. Our data suggest that faster flowing groundwater results in faster degradation rates than more slowly moving groundwater. This concept is also true for streams when riffles are compared with pools (22).

The water in the aquifer is oxygenated (Table 1), yet we observed anoxic zones on leaf samples. Such zones could be maintained if leaves reach areas of the aquifer that have low water velocities. Chemical transformations such as denitrification and methanogenesis could occur in these zones. The importance of microscale anoxic zones in stream nutrient cycling is beginning to be recognized (7). Data on the overall importance of such zones in the study aquifer are lacking, so estimation of the system-wide significance of the zones is difficult (4). We do have data from a nearby artesian spring that show an approximately 3-week lag between precipitation events and discharge response (10), suggesting that an estimate of at least several weeks for the residence time of particulates in the aquifer is reasonable.

The organic material could also determine small-scale flow patterns in the aquifer by causing localized accumulation of inorganic material on the decomposing leaves. Such leaves have a fuzzy appearance, and the biofilm containing inorganic material extends several millimeters from the surface of the leaf at times. Mucilage production by the microorganisms associated with the leaf surface may facilitate sediment accumulation, as shown by increases in the inorganic weight of leaves.

We are not able to make strong predictions about the overall importance of large organic particles in the Karst aquifers underlying the Konza Prairie, but it is certain that such particles enter the groundwater. These particles probably start degrading as soon as they become wet, and the most active decomposition probably occurs at the very top of the groundwater (in the saturated vadose zone). Elm leaves degrade more rapidly and are less likely to be transported through the aquifer than oak leaves. Other recalcitrant plant parts, such as roots and twigs, may break down even more slowly and be transported further into the aquifer. In unconsolidated aquifers, transport of such particles is obviously minimal. With flow through cracks and channels in limestone aquifers, millimeter size organic particles can be moved relatively rapidly over long distances. Thus, the dynamics of microbial degradation of these particles may have ecological significance in Karst aquifers.

ACKNOWLEDGMENTS

We thank Dolly Gudder for technical assistance and John Blair for comments.

This research was supported by a Research Experience for Undergraduates supplement to National Science Foundation grant BSR-9011662 for long-term ecological research at Konza Prairie.

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