

**Organic Matter Dynamics and Trophic Structure in Karst Groundwater**

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(ABSTRACT)

In this study of energy pathways in karst groundwater the first chapter examines spatial and temporal patterns of bacterial density and activity in the Dorvan-Cleyzieu karst aquifer, France. During baseflow, bacterial density and activity in the water column was similar in upper and lower zones of the aquifer. Floods apparently scoured inactive bacteria from the aquifer matrix but had little effect on respiring cells. Dissolved organic carbon was more abundant at the base of the aquifer, probably because of patchy distribution of particulate organic matter in upper aquifer zones that leached dissolved organic carbon. The temporal sequence of flooding and drying in the aquifer appears to play an important role in the maintenance of biofilms which should be important energy sources to higher trophic levels in the aquifer. The ecosystem expansion and contraction model, originally developed to describe surface streams, may be a good descriptor of spatial and temporal patterns of microbial films in karst aquifers.

The process of leaf and wood breakdown in cave streams in Organ Cave, West Virginia is examined in Chapter 2. Leaf and wood breakdown rates and microbial biomass and respiration on leaves and wood were compared between cave streams with and without coarse particulate organic matter (CPOM) input from the surface to examine the role of CPOM input in leaf and wood breakdown. Breakdown rate and pattern of microbial colonization of leaves and wood were typical of results reported for surface streams. Unlike in surface streams, CPOM input did not influence breakdown rate or microbial colonization on leaves and wood, apparently because nutrients are not limiting in cave streams. Nutrient addition had little effect on microbial films on wood in either stream type. *Gammarus minus* is an important shredder in Organ Cave streams and *G. minus* colonization accelerated leaf breakdown rates. Leaf and wood transport rates were low and, when combined with breakdown rates, suggest that CPOM will be retained and transformed to fine particles near its entry point to the subsurface.

In chapter 3 I examine cave stream food web structure and the role various organic matter sources in stream trophic dynamics. I used stable isotope ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) natural abundance analysis and a  $^{13}\text{C}$ -acetate tracer release to establish feeding relationships and to trace the use and importance of bacterial carbon in cave streams with and without CPOM input. Cave streams contained three trophic levels consisting of organic matter sources, primary consumers, and predators. Patterns of  $^{13}\text{C}$  labeling in the stream were similar to that in similar studies of surface streams.  $^{13}\text{C}$  acetate was incorporated into epilithic biofilms and fine benthic organic matter (FBOM). Some primary consumers, *Fontigens tartarea*, *Gyraulus parvus*, and *Physa* were highly labeled and showed a longitudinal labeling pattern consistent with the consumption of epilithic biofilms. An epigean caddisfly, *Dolophilodes*, was highly labeled and probably feeds on suspended organic matter. Other primary consumers, *Gammarus minus* and *Caecidotea holsingeri*, feed on FBOM and epilithon. Two amphipods, *Stygobromus emarginatus* and *S. spinatus*, and a planarian, *Macrocotyla hoffmasteri*, are predators in the streams. Leaves and wood were not major energy sources directly used by stream animals. Dissolved organic matter (DOM) originating from soils appears to be the primary energy source for stream food webs by fueling bacterial production that is then used by higher trophic levels. Because epilithon C turnover times were relatively long (12.7 - 17 days), DOM can be immobilized in cave stream biofilms, enhancing the efficiency with which the microbial loop may transfer energy to higher trophic levels.

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## General Introduction

Surface streams have been called "the gutters down which flow the ruins of continents" (Leopold *et al.* 1964). Material in streams, especially organic matter and nutrients, is not simply transported unchanged in these "gutters". Biological and physical processes in streams retain, use, and transform organic matter and nutrients as they travel downstream (Newbold *et al.* 1982). In limestone terrain, or karst, much surface water is diverted underground (White 1988) and cave streams are the "sewers" through which water and material move underground from one surface location to another. Like surface streams, these groundwater conduits are not inert pipes through which material moves unchanged. Water flowing from the surface to below-ground carries organic matter and nutrients that may be used and transformed as they are transported. Ultimately water and material arrive back at the surface through springs, emerging streams, or through hyporheic zones. The input, transport, and processing of organic matter and nutrients underground fuels groundwater communities. Also, because groundwater systems are extensive and intimately connected to surface water in karst (Gibert *et al.* 1990), ecological processes occurring below ground should be important factors in the ecology of surface systems in karst landscapes.

Our conceptual understanding of the structure and function of karst systems lags far behind advances made in the study of surface freshwaters. Models of energy cycling and community structure, such as the River Continuum Concept (Vannote *et al.* 1980), have been proposed and tested for surface streams. The concept of the karst basin as an ecosystem (Rouch 1986) has been proposed, but no unifying model of ecological structure and function in karst ecosystems exists. Energy and nutrient cycling, in particular, are almost unstudied in karst groundwater in spite of the fact that energy limitation appears to be a major factor driving the ecology and evolution of cave organisms. Food webs and energy flow in ecosystems have long been used to study ecosystem structure and function, but the complexity of energy sources and transfers in ecosystems can make food web analysis difficult. Understanding of the roles of production and consumption is central to community ecology (Polis 1994) and this may be particularly important in karst ecosystems where energy is limited because autotrophic production does not occur underground. Energy transfer through detritus is an important

alternative to direct consumption of plants in many terrestrial and aquatic ecosystems. Forested streams and groundwater systems, in particular, depend on detritus as an energy source.

Surface stream food webs are fueled by in-stream primary production by macrophytes, periphyton and phytoplankton, and by detritus produced in-stream and from riparian vegetation (Figure 0.1). In streams, detritus from aquatic and terrestrial sources is classified into different size fractions: coarse particulate (CPOM >1mm), fine particulate (1mm>FPOM>0.45µm), and dissolved (DOM<0.45µm) organic matter. Each organic matter size fraction supports a different energy pathway in the food web (Figure 0.1): shredders consume CPOM, collectors gather FPOM from the water column or the sediment, and DOM fuels a loop based on microbial production and consumption by invertebrates. Ultimately, organic matter is mineralized to CO<sub>2</sub> during respiration or exported downstream as suspended particulate organic matter (SPOM) and DOM.

Cave stream food webs should fall into a similar framework, but with one important distinction. Primary producers dominate open-canopied streams (e.g. Naiman 1976) and occur even in heavily shaded streams (e.g. Findlay and Howe 1993), but cave streams lack light and, therefore, primary production. Consequently, cave stream food webs strictly reflect the detritus-based segment of surface stream food webs (Figure 0.1). They are, in a sense, extremely shaded surface streams. Chemolithotrophy in some cave systems may function like primary production in surface streams, severing the reliance upon surface vegetation by internally producing large amounts of food for subterranean food webs (e.g. Sarbu *et al.* 1996). However, the geological requirements for this type of system do not appear to be common and dead organic matter from the surface is the sole energy source in most cave streams. Coarse, fine, and dissolved organic matter can be imported to cave streams, but geologic structure of the aquifer dictates the spatial pattern of detritus transported below ground. Some cave streams are located downstream of openings such as sinkholes, shafts, and cave entrances that permit terrestrial litter transfer from the surface to the subsurface. These "connected" streams are linked to terrestrial vegetation and receive coarse, fine, and dissolved organic matter; their food webs should emulate the detritus-based segment of forested surface streams (Figure 0.1). Streams without this link to the surface, or "disconnected" streams, are fed by water percolating through soil and small fractures in the bedrock that carries only fine and dissolved organic matter. Disconnected streams correspond to

litter-excluded surface streams (Figure 0.1). Foodwebs in disconnected streams are further reduced and contain only energy flow through FPOM and DOM-driven pathways.

Studying detrital pathways in surface streams is complicated by the presence of both autotrophic and heterotrophic pathways, complex detrital input, and diverse food webs. Cave streams may be useful surrogates for the detritus-driven portion of surface streams because cave streams: 1) have reduced food webs with only detritus as an energy source (Figure 0.1); 2) are physically similar to surface streams; 3) have similarities in community trophic structure (biofilms, collectors, shredders, scrapers, predators); 4) are relatively stable environments; and 5) have discrete inputs of organic matter. Like surface streams, cave streams are composed of riffles, runs, and pools, vary in size and discharge, can be ordered according to position in the drainage (Strahler 1957), and have substrates ranging from boulders to silt. Although community composition of surface and cave stream food webs differ, organic matter sources and consumer feeding strategies in each system are similar (Figure 0.1). Comparison of surface-connected and disconnected streams allows study of the importance of CPOM to cave stream food webs by contrasting streams with and without CPOM input. Disconnected streams should rely on FPOM and DOM-based food chains; the microbial loop and collectors should dominate the food web. CPOM input to connected streams should support shredders (obligate or facultative) which are consumed by predators (Figure 0.1). In this case, CPOM consumption reduces the importance of FPOM and DOM pathways in cave streams. On the other hand, CPOM breakdown could augment FPOM and DOM standing stocks (Figure 0.1) increasing energy flow in these pathways

In the following chapters I examine organic matter dynamics and energy flow in karst ecosystems at two scales: 1) large spatial and temporal patterns within an entire drainage basin and 2) smaller scale comparisons of headwater streams in the unsaturated zone of a karst basin. My goals were to explore organic matter cycling and trophic structure in karst ecosystems and to examine the utility of cave streams as surrogates for heterotrophic segments of surface streams. To accomplish these goals I first studied spatial and temporal patterns of microbial density and activity in the Dorvan-Cleyzieu karst aquifer, Ain, France. Following this I compared organic matter standing stocks, leaf and wood breakdown, trophic structure, and the role of DOM and bacteria in food webs of streams with and without CPOM input in streams of the Organ Cave drainage basin, West Virginia, U.S.A..

## References

- Allan, J.D. 1995. *Stream Ecology: Structure and function of running waters*. London:Chapman and Hall. 388 p.
- Findlay, S. and K. Howe. 1993. Bacterial-algal relationships in streams of the Hubbard Brook Experimental Forest. *Ecology* 74(8): 2326-2336.
- Gibert J., M.-J. Dole-Olivier, P. Marmonier and P. Vervier. 1990. Surface-groundwater ecotones. In R. J. Naiman and H. Decamps, editors. *Ecology and Management of Aquatic-Terrestrial Ecotones*. London:Parthenon Publishing. p 199-225.
- Leopold, M.G. Wolman and J.R. Miller. 1964. *Fluvial processes in geomorphology*. W.H. Freeman, San Francisco. 522 p.
- Naiman, R.J. 1976. Primary production, standing stock, and export of organic matter in a Mohave Desert thermal stream. *Limnology and Oceanography* 22(1): 60-73.
- Newbold, J.D., P.J. Mulholland, J.W. Elwood and R.V. O'Neill. 1982. Organic carbon spiralling in stream ecosystems. *Oikos* 38:266-272.
- Polis, G.A. 1994. Food webs, trophic cascades and community structure. *Australian Journal of Ecology* 72:2170-2180.
- Rouch R. 1986. Sur l'écologie des eaux souterraines dans le karst. *Stygologia* 2(4):352-398.
- Sarbu, S.M., T.C. Kane and B.K. Kinkle. 1996. A chemoautotrophically based cave ecosystem. *Science* 272:1953-1955.
- Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. *Transactions, American Geophysical Union* 38:913-920.
- Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell and C.E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130-137.
- White, W.B. 1988. *Geomorphology and hydrology of karst terrains*. Oxford University Press, New York. 464 p.

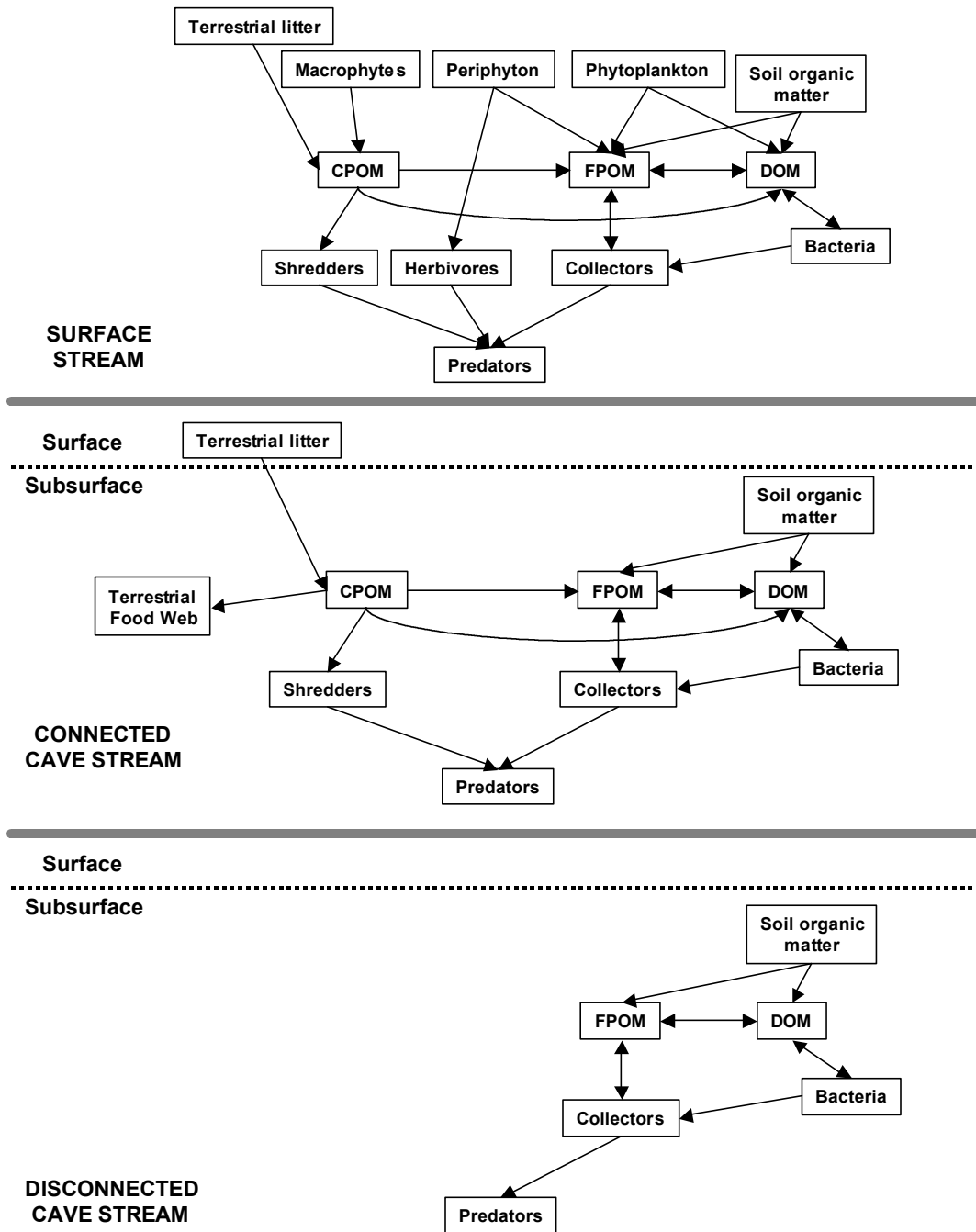


Figure 0.1. Energy flow in surface streams and cave streams connected to and disconnected from the surface. (Modified from Allan 1995)

## **Chapter 1. Spatial and temporal patterns of bacterial density and dissolved organic carbon in a karst aquifer.**

### **Abstract**

Karst aquifers are heterotrophic ecosystems fueled by organic matter imported from the surface. The temporal pattern of floods influences organic matter import and the spatial distribution of organic matter and microbial films in aquifer structural zones. We investigated spatial and temporal patterns of bacterial density and dissolved organic carbon as indicators of energy availability and microbial dynamics in a karst aquifer. During baseflow, bacterial density and microbial hydrolytic activity were similar in the upper and lower zones of the aquifer. Floods apparently scoured aquifer biofilms and transported soil bacteria into the aquifer, increasing inactive bacterial density in the water column. Respiring bacterial density did not respond to floods and changed little over time. The overall proportion of total bacteria that were respiring was very high on some dates, resulting from a reduction of inactive cell density during flood recession. Floods appear to be key events in scouring senescent microbial assemblages in karst aquifers and stimulating microbial recolonization of the aquifer matrix. We conclude that a conceptual model of karst aquifer structure and function should incorporate changes caused by alternation between flooding and drying in the aquifer.

**Key Words:** karst aquifer, groundwater, flooding, drying, bacteria, microbial activity, conceptual model

Chapter in review as: Simon, K.S., J. Gibert, R. Laurent and P. Petitot. Spatial and temporal patterns of bacterial density and dissolved organic carbon in a karst aquifer.

## Introduction

Research of karst aquifers during the past 20 years has focused primarily on hydrology and hydrochemistry (Bakalowicz and Mangin, 1980; Mangin, 1983 and 1986; Ford and Williams, 1989) as well as ecology (Rouch, 1986; Gibert, 1986; Camacho, 1992). As a result, the perception of karst aquifers has changed from a descriptive and reductionist approach to a more holistic view incorporating geology and hydrology into aquifer ecology. Structure and function of karst aquatic communities have been linked to hydrology, species interactions, pollution, and energy and nutrient availability (reviewed by Gibert *et al.*, 1994). In many cases, fluxes of water and nutrients influence spatial distribution of invertebrates and spatial heterogeneity of subterranean ecosystem function (Gibert *et al.*, 1990).

The physical structure of karst aquifers determines water and energy distribution in the ecosystem. Karst aquifers are heterogeneous at all scales (Mangin, 1986) and consist of a network of hollows in the bedrock around main drainage axes. These subterranean networks can be organized like surface hydrographic networks, but unlike surface drainages, karst aquifers are relatively inaccessible. Consequently, the study of karst aquifers has been restricted and carried out primarily at natural access points: caves and springs (e.g., Gibert, 1986; Rouch, 1982). By using access points in a variety of locations, researchers have been able to investigate specific spatial areas of karst aquifers, leading to a more detailed view of these ecosystems (e.g., Rouch, 1986). The temporal pattern of drifting invertebrates at the outlets of aquifers have been used to define some of the main biological characteristics of karst ecosystems. These biological characteristics, in conjunction with hydrologic information, have led to the distinction of three structural zones in karst aquifers: epikarst, unsaturated, and saturated zones (Mangin, 1975). These aquifer zones have different species assemblages (Gibert, 1986; Fong and Culver, 1994), species diversity (Fong and Culver, 1994), physicochemical characteristics (Gibert, 1986), and amounts of carbon (Gibert, 1986). In general, water enters the aquifer through the epikarst then flows through the unsaturated to the saturated zone before leaving the aquifer (Figure 1). The input and subsequent transfer and transformation of organic matter in each aquifer zone have implications for structure and function in "downstream" zones and for other, linked ecosystems such as springs and streams.

Because chemolithotrophic production is usually low and photoautotrophy is nonexistent in karst aquifers, the groundwater community relies on imported energy sources and consequently should be food-limited (Culver, 1985). Floods are the primary vectors for organic matter delivery to the subsurface and they strongly influence invertebrate assemblages in karst aquifers (Rouch, 1986; Gibert, 1986). Levels of organic matter have been shown to affect spatial distribution of invertebrates and energy and nutrient cycling in groundwater systems (Gibert *et al.* 1994). Some coarse organic matter, such as leaves and wood, can enter aquifers through large openings, but dissolved and fine organic matter are probably more readily available and widely distributed below ground. The importance of dissolved organic carbon (DOC) and microbial films as energy sources for higher trophic levels is well known for surface streams (Hall and Meyer, 1998; Wotton, 1990) and these energy sources should be particularly important in karst aquifer food webs where other energy sources are scarce. In spite of their apparent significance, little is known about microbial assemblages, their metabolic requirements, relationships to abiotic and biotic conditions, or the microbial role in whole-aquifer metabolism and productivity (Gounot, 1994).

We examined spatial and temporal patterns of bacterial density and dissolved organic carbon availability in order to make inferences about energy availability and distribution in a karst aquifer. Our study addressed two major questions: (1) How do the fluxes of carbon and bacteria differ between input (epikarst) and output (saturated zones) in a karst aquifer? and (2) How do carbon availability and microbial density and activity in karst aquifers relate to hydrologic change, especially to floods occurring between low and high water periods?

## **Methods**

### *Study Site*

The Dorvan-Cleyzieu aquifer is a 10 km<sup>2</sup>, low-mountain karst (average altitude 620 m and average gradient 126 m/km) situated on the southwestern range of the Southern Jura mountains of France. It lies in middle Jurassic limestone (Bathonian and Bajocian) 50 km from the city of Lyon (Figure 1). The aquifer is fed almost exclusively by rainfall. Two structural zones in the aquifer can be accessed: (1) at the top of the aquifer, the epikarst is drained by the Cormoran Cave stream (mean annual discharge 4.2 L/s) and (2) at the base of the aquifer, a main channel emerges at Pissoir Spring (mean annual discharge 76.5 L/s, range: 0 to 2,000 L/s)

and from several seeps around the spring (Gibert, 1986). The hydrologic connection between the epikarst and saturated zone has been demonstrated by a multi-tracer experiment (Gibert *et al.*, 1982). Pissoir Spring acts as an overflow for the aquifer, flowing only when the water level in the aquifer reaches a sufficient height. After exiting the aquifer, water from the spring joins the Bief Ravinet stream and flows through an alluvial plain to the Albarine River.

The hydrologic cycle consists of a high-water period during winter characterized by relatively high discharge and frequent floods. During summer, water levels are generally low and Pissoir Spring flows intermittently. Vegetation covers 80-90% of the ground surface (meadows and crops 50%, forests 40%, moors and bushes 10%). Some impact of agriculture on the groundwater quality can be detected in the Cormoran Cave stream, where chloride and nitrate concentrations are variable, ranging up to 8 and 14.7 mg/L, respectively, on some occasions (Gibert, 1986 and 1990).

### *Sampling*

We sampled in Cormoran Cave (epikarst) and Pissoir Spring (saturated zone) during 1996 and 1997 (Figure 1). This arrangement allowed us to sample water entering and exiting the aquifer. A perennial cave stream in Cormoran Cave was sampled 70 m upstream from the cave entrance. At Pissoir Spring water was collected directly at the exsurgence from the base of the aquifer. These two sites were sampled during the same periods of the annual hydrologic cycle in two years: from 1 to 29 April in 1996; and from 9 April to 28 May in 1997. Both periods included low water and flood stages in the aquifer. Rainfall data for the catchment were obtained from the National Meteorology Station of Amberieu located 5 km west of the study area. Water level recorders were installed at weirs in the cave stream and spring to continuously monitor discharge.

Single water samples for chemistry and triplicate samples for microbial study were collected approximately every 2 days in the epikarst in Cormoran Cave and from the saturated zone at Pissoir Spring and surrounding secondary seeps in 1996 (12 days) and 1997 (9 days). Temperature, conductivity, pH, and dissolved oxygen were measured *in situ* with meters. Nitrate, nitrite, chloride, and ortho-phosphate were determined by colorimetry using the HACH method (Rodier, 1978). Dissolved organic carbon (DOC) was measured only in 1997 on 0.45  $\mu\text{m}$  filtered water samples analyzed with a Dohrman DC 80 Total Carbon Analyzer.

Density of total and respiring bacteria and microbial hydrolytic activity in the water column were determined on each date from water samples collected in washed and pre-combusted (550 °C) glass bottles. Bacteria were counted using epifluorescence microscopy and DAPI staining (Porter and Feig, 1980). Fifty ml of water were filtered on 0.2 µm GTBP membranes mounted on QP20 pre-filters and stained for 10 minutes with 0.1 ml of DAPI. The membrane was then washed 3 times with 1 ml of distilled water and mounted in non-fluorescent immersion oil (Zeiss 518 C) on a microscope slide. Multiple fields of view were examined until a minimum of 400 bacteria were counted. The number of respiring bacteria were measured by direct counts of electron transport system (ETS)-active bacteria using 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC) staining (Rodriguez *et al.*, 1992). This technique was used on two dates in 1996 and all dates in 1997. Sample processing for CTC was similar to that used for DAPI staining so the numbers of total and respiring bacteria could be directly compared.

Microbial hydrolytic activity was estimated using the fluorescein diacetate (FDA) hydrolysis method (Schnurer and Rosswall 1982, Fontvieille *et al.*, 1992). One hundred ml of water were filtered through a 0.2 µm GSWP membrane, which was then placed in 3 ml of phosphate buffer (pH=7.6) with 0.1 ml of FDA solution (2 g/L) and incubated at 15 °C until a green color appeared. The assay was then stopped by freezing the sample. Fluorescein concentration was estimated from the optical density of the filtered (0.45 µm cellulose membrane) supernatant measured at 490 nm (Shimadzu U.V. spectrophotometer).

### *Statistical Analyses*

Differences in mean concentrations of nutrients and water chemistry variables between sites were analyzed with paired t-tests. Spatial and temporal differences in DOC, hydrolytic activity, and total and respiring bacterial density were analyzed using two way analysis of variance. Non-respiring bacterial density was calculated as the difference between mean total density and mean respiring density and was compared between sites using a paired t-test. Correlations between DOC, bacterial abundance (total and respiring), microbial hydrolytic activity, and discharge within and between sites were determined using Pearson Product Moment Correlation coefficients (Sokal and Rohlf, 1995).

## Results

### *Hydrologic Cycles and Water Chemistry*

Both sampling periods included floods; however, the hydrologic conditions preceding the floods differed considerably between years. The 1996 sampling period consisted of declining base flow followed by a brief flood on April 23<sup>rd</sup> (Figure 2). Over this time period, discharge in the epikarst slowly declined from the beginning of April then increased slightly after rainfall on April 23<sup>rd</sup>. Discharge from the saturated zone also slowly declined during the month. Water stopped flowing from Pissoir Spring but still emerged from secondary seeps around the spring for 12 days before responding dramatically to the April 23<sup>rd</sup> rainfall with a short flood peak (max  $Q=668$  L/s, Figure 2).

The sampling period in 1997 consisted of an extended dry period followed by multiple floods (Figure 2). Prior to sampling, water level in the aquifer was very low for >18 days and no water flowed from the springs or secondary seeps. Three storms in quick succession then caused a series of floods in late April and early May (Cormoran Cave max  $Q=0.02$ , 5 and 27 L/s, respectively, and Pissoir max  $Q=25$ , 335 and 362 L/s, respectively, Figure 3). Although total precipitation in each storm in 1997 was similar (Figure 2), discharge from Pissoir Spring was much lower during the first flood. Because of the long preceding dry period, most of the water from the first storm apparently remained in storage zones and in the saturated zone, only flowing from Pissoir spring once these areas were filled and water level in the aquifer was high enough to flow from the spring. Discharge was higher in successive floods because the water level in the aquifer was high enough to flow immediately from the spring.

Water chemistry in both years was typical for this aquifer (Gibert, 1986). Mean pH and concentrations of  $\text{NO}_3\text{-N}$  and  $\text{Cl}^-$  were significantly higher in the epikarst than in the saturated zone of the aquifer during both study years (Table 1). Water exiting the aquifer at Pissoir Spring was significantly warmer than water in the epikarst (Table 1). There were no significant differences in dissolved oxygen,  $\text{NO}_2\text{-N}$ , and  $\text{PO}_4\text{-P}$  between aquifer zones.

### *Spatial Patterns*

DOC concentration was low in both aquifer zones, but there was significantly more DOC in the saturated zone where concentrations were nearly double those recorded in the epikarst (Table 2). Mean microbial hydrolytic activity was similar in both zones in 1996 but was

significantly higher in the saturated zone in 1997 (Table 2). Similarly, there was no significant difference in total bacterial density between aquifer zones in the 1996 sampling period (Table 2). However, in 1997 total bacterial density was higher in the epikarst than in the saturated zone (Table 2). The difference in total density was due to higher numbers of non-respiring cells in the epikarst than in the saturated zone (Table 2); mean numbers of respiring bacteria were similar in both aquifer zones (Table 2).

### *Temporal Patterns*

DOC concentration varied significantly over time ( $p=0.009$ ), but there was no consistent pattern between floods or aquifer zones (Figure 3). DOC concentration was significantly correlated with discharge in the saturated zone ( $r=0.76$ ,  $p=0.02$ ,  $n=10$ ) but not in the epikarst ( $p=0.8$ ). Hydrolytic activity changed significantly over time in both years (Figure 4;  $p=0.001$ ). In 1996, hydrolytic activity was generally stable during baseflow and higher during the flood. However, hydrolytic activity increased in the saturated zone the day before the flood occurred at Pissoir Spring. Hydrolytic activity in both aquifer zones was variable in 1997, but there was no consistent trend or correlation with discharge ( $p=0.87$  in the epikarst or  $p=0.08$  in the saturated zone). Hydrolytic activity also was not significantly correlated ( $p>0.05$ ) with DOC or bacterial density in either year or aquifer zone.

Density of total suspended bacteria changed significantly over time in both years (Figure 5;  $p<0.001$ ). In 1996, bacterial density was stable in the epikarst and in the saturated zone before the flood (Figure 5). Bacterial density then increased sharply in the saturated zone during the flood and then declined during flood recession. A similar trend occurred in both aquifer zones in 1997. Bacterial density was high during the peaks of the first and third floods and declined during flood recession (Figure 5). We do not have data for the peak of the second flood of 1997, but density during recession of the second flood was similar to density during recession of the other floods during that year.

For the two sampling dates in 1996, respiring bacterial density was similar in the epikarst and saturated zones and comprised from 6 to 24% of the total bacteria in suspension (Figure 5). In 1997, respiring bacteria density changed significantly over time ( $p<0.001$ ) but was not correlated with discharge ( $p>0.05$ ) in either the epikarst or saturated zone (Figure 5). The magnitude of change in density of respiring bacteria was smaller than the change in total

bacterial density in both the epikarst (range =  $2.9 \times 10^4/\text{mL}$  and  $9.7 \times 10^4/\text{mL}$ , respectively) and saturated zone (range =  $3.7 \times 10^4/\text{mL}$  and  $8.8 \times 10^4/\text{mL}$ , respectively). Respiring bacteria comprised from 5 to 91% of total bacteria in suspension in 1997. The proportion of total bacteria that were respiring increased in both aquifer zones during recession of the first flood (Figure 5).

## **Discussion**

### *Spatial and Temporal Patterns*

We expected higher DOC in the epikarst where water and organic matter first enter the aquifer; however, during our study, DOC was higher in the saturated zone. Water in Cormoran Cave represents only a portion of the epikarstic water draining into the saturated zone of the aquifer. The reduction of chloride concentration, a conservative solute, from our epikarst site to the saturated zone shows that water from Cormoran Cave is diluted with water from other parts of the drainage. This water must have contained higher DOC levels, possibly from leaching and breakdown of coarse organic matter, such as leaves and wood, that entered the aquifer through a few cave entrances and sinkholes outside the Cormoran Cave stream drainage. Coarse particulate organic matter (CPOM) has been shown to be a source of DOC in surface streams (Meyer *et al.*, 1998) and CPOM should act similarly when it enters karst aquifers. Because CPOM can only enter karst aquifers through openings of sufficient size, its distribution will be patchy and its effects are most easily detected at the exsurgence where water from the entire aquifer is mixed before exiting the system.

The lack of correlation between hydrolytic activity and DOC or respiring bacteria density suggests changes in substrate availability and bacteria alone were not responsible for changes in microbial hydrolytic activity in the water column. The FDA method produces a global measure of hydrolytic activity, including organic carbon consumption by organisms other than bacteria such as fungi, protozoans, and algae (Schnurer and Roswall, 1982). Increased abundance or activity of one or more of these organisms, probably protozoans, in the saturated zone may have led to the higher hydrolytic activity in the saturated zone. The variable nature of hydrolytic activity during floods also suggests that organic carbon consumption in the aquifer is not a simple relationship between bacteria, DOC, and discharge alone.

The temporal trend of high bacterial density at flood peaks and subsequent decline with flood recession seen in our study also occurs in surface streams and rivers (e.g., McDowell,

1984). Bacterial density in both aquifer zones during our study was fairly high for groundwater ( $10^4$ - $10^5$  cells/mL) and fell within the range of values reported for surface streams and rivers (range:  $10^4$ - $10^{10}$  cells/mL; Edwards, 1987; McDowell, 1984). Deep groundwater has generally been considered to have low bacterial density and has not been viewed as a major source of bacteria for surface streams and rivers. Baker and Farr (1977) reported low bacterial density in water from springs draining a chalk aquifer (<1-53 cells/mL). However, they used plate counts to enumerate bacteria, which is less efficient than direct counts (Porter and Feig, 1980). Rimes and Goulder (1986) suggested that shallow groundwater acts as a source of bacteria for streams but that deep groundwater does not. In contrast, the Dorvan-Cleyzieu aquifer appears to be a major source of bacteria for the connected surface stream, especially during floods, because bacterial density in water exiting the aquifer was fairly high and this water can account for up to 75% of the surface stream's discharge (Gibert, 1986).

Although flooding generally increased bacterial density, the hydrologic conditions preceding the floods also appear to be important by influencing the pattern of bacterial scouring during floods. Bacterial density was similar in both aquifer zones during 1996 when base-flow predominated, but during the floods in 1997, which followed a long dry period, total bacterial density was higher in the epikarst. Most of the bacteria in suspension, especially during flood peaks, were inactive, suggesting dried or senescent biofilms were scoured. In surface systems, bacteria in suspension during floods originate internally from scouring of stream and river sediments (Blenkinsopp and Lock, 1994), externally from floodplains and soils (Edwards, 1987; McDowell, 1984), or from hyporheic sediments in shallow groundwater systems (Holmes *et al.*, 1998). In the Dorvan-Cleyzieu aquifer, internal scouring of biofilms on the aquifer matrix and imported bacteria from surface soils should both contribute to density of bacteria in suspension in the aquifer. Higher total bacteria density in the epikarst during 1997 floods suggests internal and surface soil biofilms were scoured. High bacterial density only in the saturated zone during the 1996 flood suggests only internal biofilms were scoured. Because water was fairly high prior to the 1996 flood, storage zones in the upper level of the aquifer were probably filled and most of the flood water was quickly diverted through the main drainage axis resulting in a high flood peak in the saturated zone and removal of bacteria from surfaces in the unsaturated and saturated zones.

Most of our values for the proportion of respiring bacteria fell within the range of values reported for surface aquatic systems. Reported values for unpolluted systems range from <1% in groundwater samples (Marxsen, 1988) to 61% in estuary water (Tabor and Neihof, 1982). Rusterholz and Mallory (1994) found relatively high proportions (up to 58%) of respiring bacteria in a karst aquifer, which is similar to most of our values. The high values in karst groundwater may be a result of relatively high dissolved oxygen levels (>80% saturation during our study) and increased organic matter input due to the size of the fractures below ground. Two of our values were surprisingly high (80 and 91%), falling well above the highest value (61%) reported for natural systems. These values were not the result of high respiring bacterial counts, but were caused by the drastic reduction in non-respiring cells in the water column following high initial scouring during flood peaks. There appears to be differential scouring of active and inactive biofilms. Dead or inactive bacteria were scoured in large numbers by high flow during floods while active bacteria were less affected by high flow, possibly as a result of differences in biofilm architecture or the extracellular matrices that anchor bacteria to the substrate (Blenkinsopp and Lock, 1994). Active bacterial cells in suspension or those left on scoured surfaces are probably important colonizers. Continued high bacterial density in 1997 after several floods in rapid succession suggests biofilms rapidly recolonize re-wetted areas and are subject to repeated scouring.

#### *A Conceptual Framework for Karst Aquifers*

General models of ecosystem structure and function have been useful for understanding the structure and function of surface streams (e.g. Vannote *et al.*, 1980). For karst aquifers, a model should incorporate the spatial structure of the aquifer and the temporal effects of changing hydrology. Stanley *et al.* (1997) proposed an ecosystem expansion and contraction model for surface streams. In their model, flooding and drying drive stream ecosystem structure and function as patches in the drainage network are connected and disconnected. A similar process appears to occur in karst aquifers. During low water periods, portions of the epikarst and unsaturated zone are dried, resulting in "stranded" patches where water remains only in storage areas and the saturated zone (Mangin 1994). These patches would include pools, stream segments, and fractures where water is retained or drains very slowly. Ecosystem expansion occurs during flooding, resulting in the re-connection of stranded patches as the aquifer is

recharged. Flooding should also lead to greater connectivity within the aquifer between structural zones as well as connectivity between the aquifer and surface systems upstream and downstream of the aquifer (Vervier and Gibert, 1991).

During ecosystem contraction, microbial biofilms should become senescent in stranded patches as reserves of DOC or oxygen are consumed and some biofilms are completely desiccated in areas without water. Likewise, invertebrates in the aquifer will be restricted to areas that retain water. During ecosystem expansion, our results suggest that senescent and dried biofilms are heavily scoured by the first floods after dry periods and the reconnected patches are recolonized by active bacterial cells. Restoration of water flow and organic matter input should speed microbial recolonization and increase food supplies for invertebrates. During periods of expansion, invertebrates should be able to use previously dried habitats and recolonizing biofilms as food sources. Flood magnitude and length of time between floods may determine the magnitude and frequency of microbial scouring. Recent hydrologic conditions influence surface stream biofilm structure (Peterson *et al.*, 1994) and probably influences subsurface biofilm structure as well, although the specific patterns will probably be different because aquifer biofilms are bacterial dominated rather than algal dominated. Invertebrate drift, dispersal, and reproduction in karst aquifers are also strongly tied to flood timing, magnitude, and frequency (Turquin, 1981 and 1986).

Our model of karst aquifer structure and function is not complete, but it is clear that flooding and drying strongly influence microbial and invertebrate activity and distribution in karst aquifers. Ecosystem level study of karst aquifers should take into consideration both the physical structure of the aquifer and the temporal pattern of hydrologic change. The alternation between wet and dry periods with floods as key mechanisms for influencing ecosystem structure and function appears to be a major component in karst aquifer ecology as is the case with many surface aquatic systems.

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## References

- Bakalowicz M, Mangin A. 1980. L'aquifère karstique. Sa définition, ses caractéristiques et son identification. *Mem Soc Geol Fr* 11:71-79.
- Baker JH, Farr IS. 1977. Origins, characterization and dynamics of suspended bacteria in two chalk streams. *Arch Hydrobiol* 80(3):308-326.
- Blenkinsopp SA, Lock MA. 1994. The impact of storm-flow on river biofilm architecture. *J Phycol* 30(5):807-818.
- Camacho AI. 1992. The natural history of biospeleology. *Monogr Mus Nac Cienc Nat*, 7, CSIC, Madrid. 680 p.
- Culver DC. 1985. Trophic relationships in aquatic cave environments. *Stygologia* 1:43-53.
- Edwards RT. 1987. Sestonic bacteria as a food source for filtering invertebrates in two southeastern blackwater rivers. *Limnol Oceanogr* 32(1):221-234.
- Fong DW, Culver DC. 1994. Fine-scale biogeographic differences in the crustacean fauna of a cave system in West Virginia, USA. *Hydrobiologia* 287:29-37.
- Fontvieille D, Maul A. 1985. Dénombrement et mesure d'activité des bactéries. In: G. Martin, editor. *Point sur l'épuration et le traitement des effluents (eau, air)*. Paris: Lavoisier. p 79-131.
- Fontvieille D, Outaguerouine A, Thevenot DR. 1992. Fluorescein diacetate hydrolysis as a measure of microbial activity in aquatic systems: application to activated sludges. *Environ Technol* 13:531-540.
- Ford DC, Williams PW. 1989. *Karst geomorphology and hydrology*. London: Unwin Hyman. 601 p.
- Gibert J. 1986. *Ecologie d'un système karstique jurassien. Hydrogéologie, dérive animale, transits de matières, dynamique de la population de Niphargus (Crustacé Amphipode)*. *Mem Biospeol* 13:1-379.
- Gibert J. 1990. Behavior of aquifers concerning contaminants: Differential permeability and importance of the different purification processes. *Water Sci Technol* 22(6):101-108.
- Gibert J, Dole-Olivier M-J, Marmonier P, Vervier P. 1990. Surface-groundwater ecotones. In Naiman R J, Decamps H, editors. *Ecology and Management of Aquatic-Terrestrial Ecotones*. London: Parthenon Publishing. p 199-225.

- Gibert J, Guezo B, Laurent R, Marchand T. 1982. Expérience de traçage artificiel dans le Jura méridional. Mise en évidence de liaisons souterraines dans le massif de Dorvan (Torcieu, Ain, France). *Spelunca* 7:19-26.
- Gibert J, Vervier P, Malard F, Laurent R, Reygrobellet J-L. 1994. Dynamics of communities and ecology of karst ecosystems: Examples of three karsts in eastern and southern France. In: Gibert J, Danielopol DL, Stanford JA, editors. *Groundwater Ecology*. San Diego: Academic Press, Inc. p 425-450.
- Gounot AM. 1994. Microbial ecology of groundwaters. In: Gibert J, Danielopol DL, Stanford JA, editors. *Groundwater Ecology*. San Diego: Academic Press, Inc. p 189-215.
- Hall RO, Meyer JL. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79(6):1995-2012.
- Holmes RM, Fisher SG, Grimm NB, Harper BJ. 1998. The impact of flash floods on microbial distribution and biogeochemistry in the parafluvial zone of a desert stream. *Freshw Biol* 40:641-654.
- Leff LG, Leff AA, Lemke MJ. 1998. Seasonal changes in planktonic bacterial assemblages of two Ohio streams. *Freshw Biol* 39: 129-134.
- Mangin A. 1975. Contribution à l'étude hydrodynamique des aquifères karstique. (troisième partie). *Ann Speleol* 30:21-124.
- Mangin A. 1983. L'approche systémique du karst, conséquences conceptuelles et méthodologiques. *Reunion monografica sobre el karst, Larra*. p 141-157.
- Mangin A. 1986. Reflexion sur l'approche et la modélisation des aquifères karstiques. *Journées sur le karst en Euskadi 1986, San Sebastian*. p 11-31.
- Mangin A. 1994. Karst Hydrogeology. In: Gibert J, Danielopol DL, Stanford JA, editors. *Groundwater Ecology*. San Diego: Academic Press, Inc. p 43-67.
- Marxsen J. 1988. Investigations into the number of respiring bacteria in groundwater from sandy and gravelly deposits. *Microb Ecol* 16:65-72.
- McDowell WH. 1984. Temporal changes in numbers of suspended bacteria in a small woodland stream. *Verh Internat Verein Limnol* 22:1920-1925.
- Meyer JL, Wallace JB and Eggert SL. 1998. Leaf litter as a source of dissolved organic carbon in streams. *Ecosystems* 1:240-249.
- Peterson CG, Weibel AC, Grimm NB, Fisher SG. Mechanisms of benthic algal recovery following spates: comparison of simulated and natural events. *Oecologia* 98: 280-290.

- Porter KG, Feig, YS. 1980. Use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25:943-948.
- Rimes CA, Goulder R. 1986. Suspended bacteria in calcareous and acid headstreams: abundance, heterotrophic activity and downstream change. *Freshw Biol* 16:633-651.
- Rodier, J. 1978. *L'analyse de l'eau*. Paris:Dunod. 1136 p.
- Rodriguez GG, Phipps D, Ishiguro K, Ridgway HF. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl Environ Microbiol* 58(6):1801-1808.
- Rouch R. 1982. Le système karstique du Baget. XIII. Comparaison de la dérive des Harpacticides à l'entrée et à la sortie de l'aquifère. *Ann Limnol* 18(2):133-150.
- Rouch R. 1986. Sur l'écologie des eaux souterraines dans le karst. *Stygologia* 2(4):352-398.
- Rusterholtz KJ, Mallory LM. 1994. Density, activity and diversity of bacteria indigenous to a karstic aquifer. *Microb Ecol* 28:79-99.
- Sokal RR, Rohlf FJ. 1995. *Biometry. The principles and practice of statistics in biological research*. New York:W. H. Freeman and Company. p 887.
- Schnurer J, Rosswall T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl Environ Microbiol* 43:1256-1261.
- Stanley EH, Fisher SG and Grimm NB. 1997. Ecosystem expansion and contraction in streams. *BioScience* 47(7):427-435.
- Tabor PS, Neihof RA.. 1982. Improved method for determination of respiring individual microorganisms in natural waters. *Appl Environ Microbiol* 43(6):1249-1255.
- Turquin MJ. 1981. The tactics of dispersal of two species of *Niphargus* (Perennial, troglotic Amphipoda). *Proc Int Congr Speleol*, 8<sup>th</sup>, BowlingGreen. p 353-354.
- Turquin MJ. 1986. Mortalité et stock de l'Amphipode *Niphargus virei* dans le système karstique de Drom. *Bull Soc Linn Lyon* 55:293-304.
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. *Can J Fish Aquat Sci* 37:130-137.
- Vervier P, Gibert J. 1991. Dynamics of surface water/groundwater ecotones in a karstic aquifer. *Freshw Biol* 26:241-250.
- Wotton RS. 1990. Particulate and dissolved organic material as food. In Wotton RS, editor. *The Biology of Particles in Aquatic Systems*. Boca Raton:CRC Press. p 213-261.

Table 1.1. Water chemistry of the epikarst and saturated zone (SZ) during each year.

Variable	1996		1997	
	Epikarst	SZ	Epikarst	SZ
NO <sub>3</sub> -N (mg/L)	5.1 (0.9)	0.7* (0.3)	7.8 (1.8)	1.6* (2.0)
NO <sub>2</sub> -N (µg/L)	1.7 (1.0)	1.6 (2.3)	2.0 (1.8)	1.4 (1.1)
PO <sub>4</sub> -P (mg/L)	0.02 (0.01)	0.03 (0.02)	0.03 (0.05)	0.04 (0.02)
Cl <sup>-</sup> (mg/L)	nd	nd	0.85 (0.06)	0.48* (0.11)
Cond. (µS/cm)	289 (23)	410* (8)	338 (27)	404* (14)
Temp. (°C)	9.6 (0.1)	10.9* (0.2)	9.7 (0.3)	11.0* (0.2)
pH	8.1 (0.2)	7.4* (0.1)	8.0 (0.3)	7.5* (0.07)

Values are means (1996 n=12, 1997 n=9) with standard error in parentheses.

\* p<0.05, epikarst vs. SZ; nd = no data

Table 1.2. Spatial means for DOC and microbial parameters in the epikarst and saturated zone (SZ) with results of 2 way ANOVA indicating spatial and temporal differences.

Variable	Year	Spatial Mean		P-value		
		Epikarst	SZ	Site	Date	Site x Date
DOC (mg/L)	1997	1.0 (0.1)	1.7 (0.1)	0.001	0.009	0.004
Hydrolytic Activity (nmol FDA/L/hr)	1996	0.35 (0.04)	0.39 (0.09)	0.29	<0.001	0.003
	1997	0.80 (0.22)	1.85 (0.33)	<0.001	<0.001	<0.001
Bacterial Density (10 <sup>4</sup> bacteria/mL)						
Total	1996	8.31 (0.79)	9.76 (2.08)	0.06	<0.001	<0.001
Total	1997	8.29 (1.07)	6.45 (0.82)	<0.001	<0.001	<0.001
Respiring		2.10 (0.45)	2.80 (0.65)	0.002	<0.001	0.006
Non-respiring		6.35 (1.16)	4.35 (1.01)	0.03*		

Spatial data are means with one standard error in parentheses. P-values are results of 2 way ANOVA with 1996 df = 1,12,12; 1997 df = 1,9,9. \* result of paired t-test (n=9).

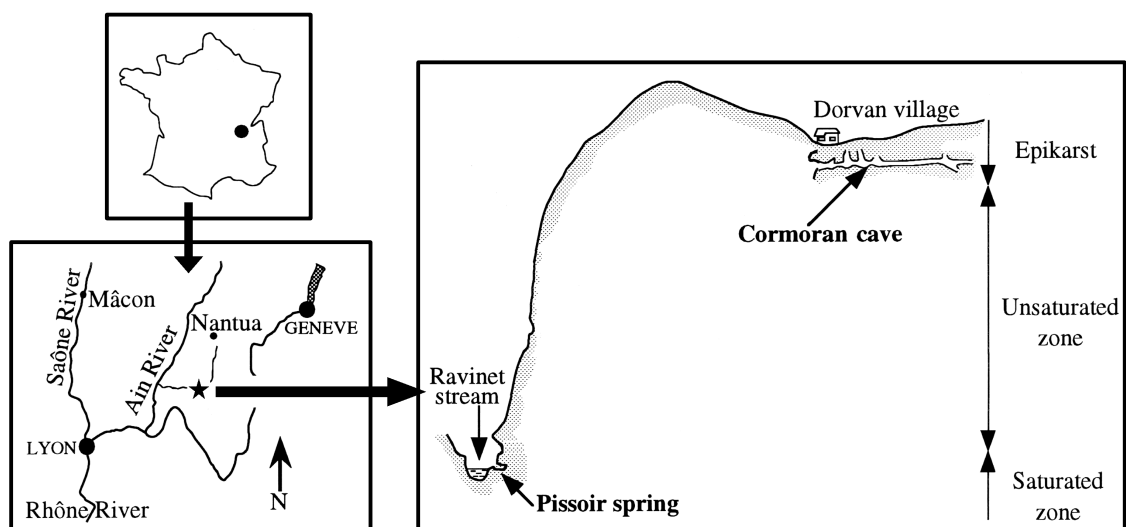


Figure 1.1. Diagram indicating the location of the Dorvan-Cleyzieu karst aquifer and sampling sites within the aquifer.

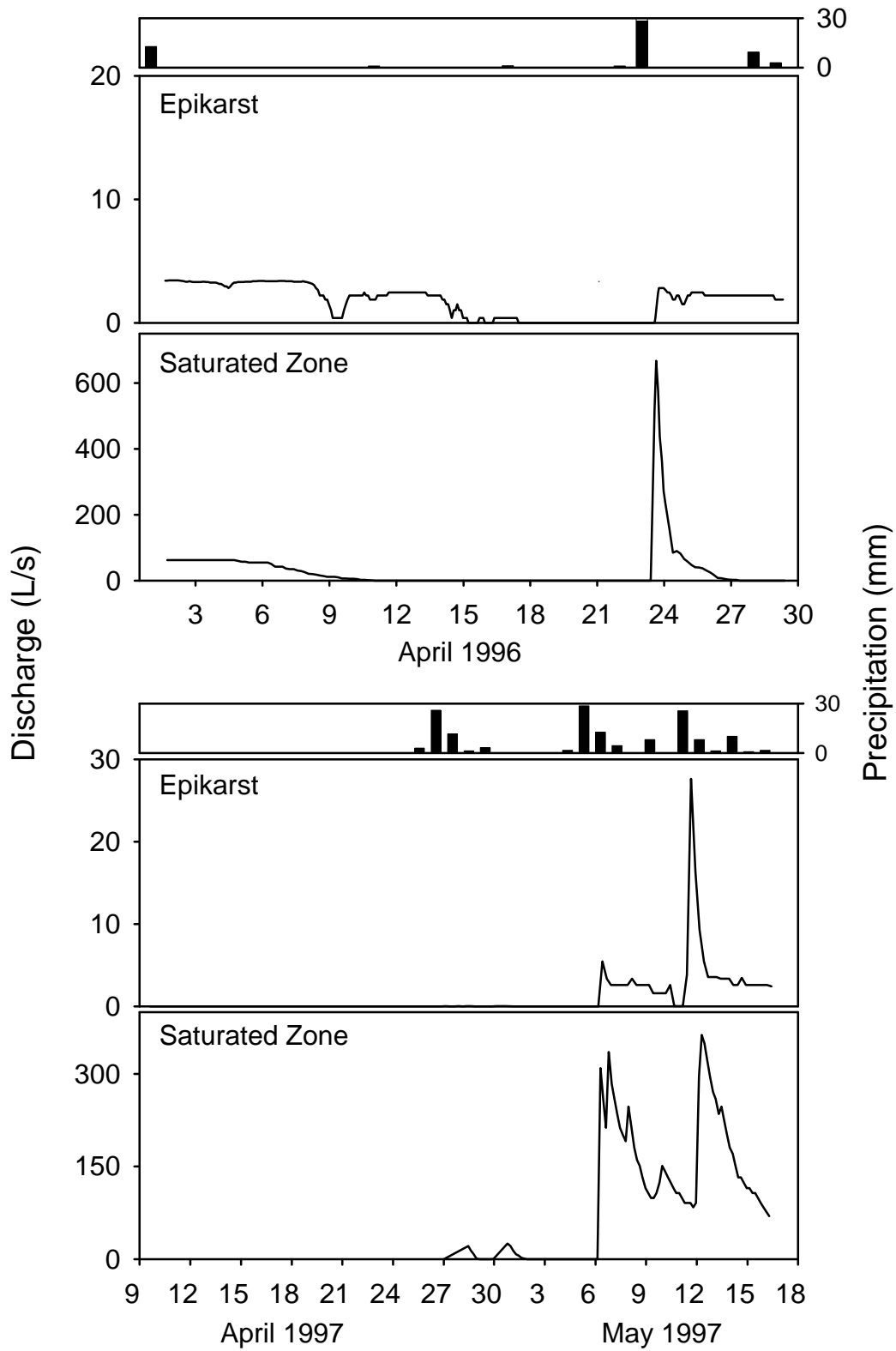


Figure 1.2. Precipitation and discharge in the epikarst and saturated zone in 1996 and 1997.

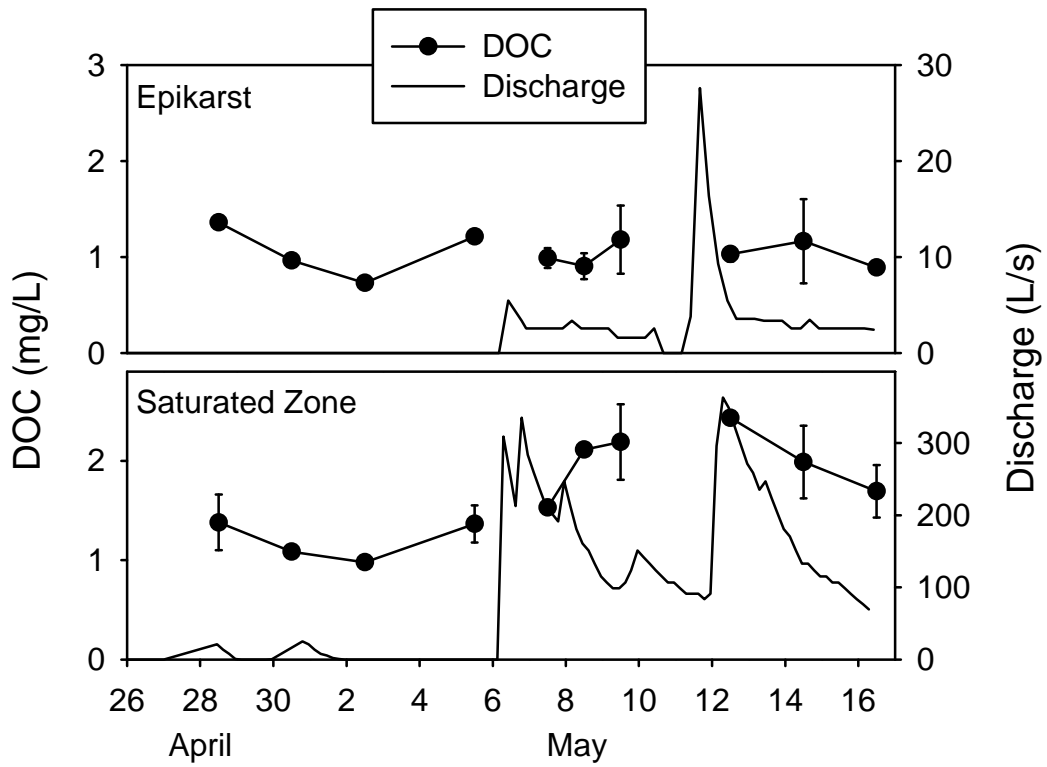


Figure 1.3. Dissolved organic carbon and discharge in the epikarst and saturated zone in 1997. Bars are + and - 1 SE.

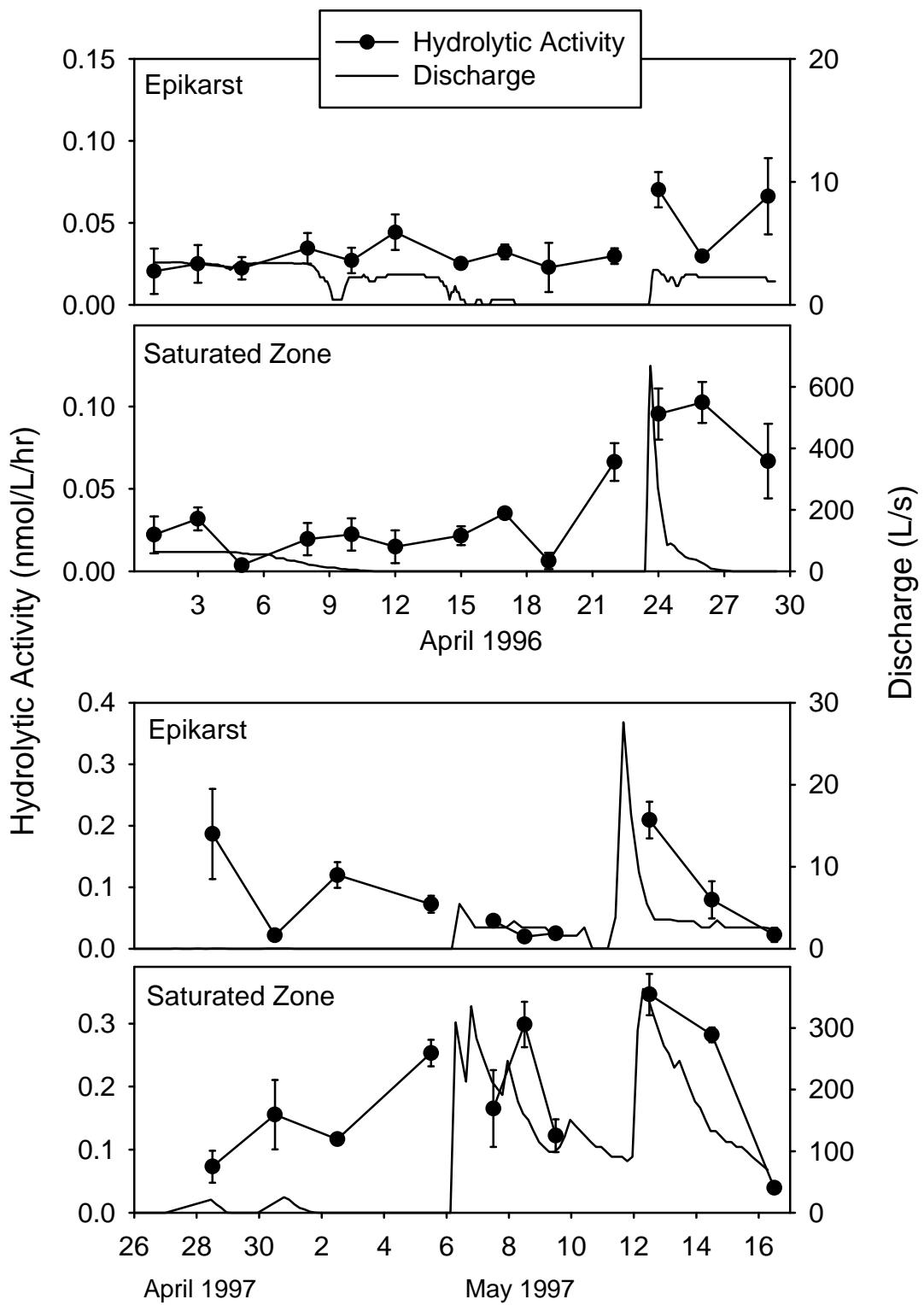


Figure 1.4. Microbial hydrolytic activity and discharge in the epikarst and saturated zones in 1996 and 1997. Bars are + and - 1 SE.

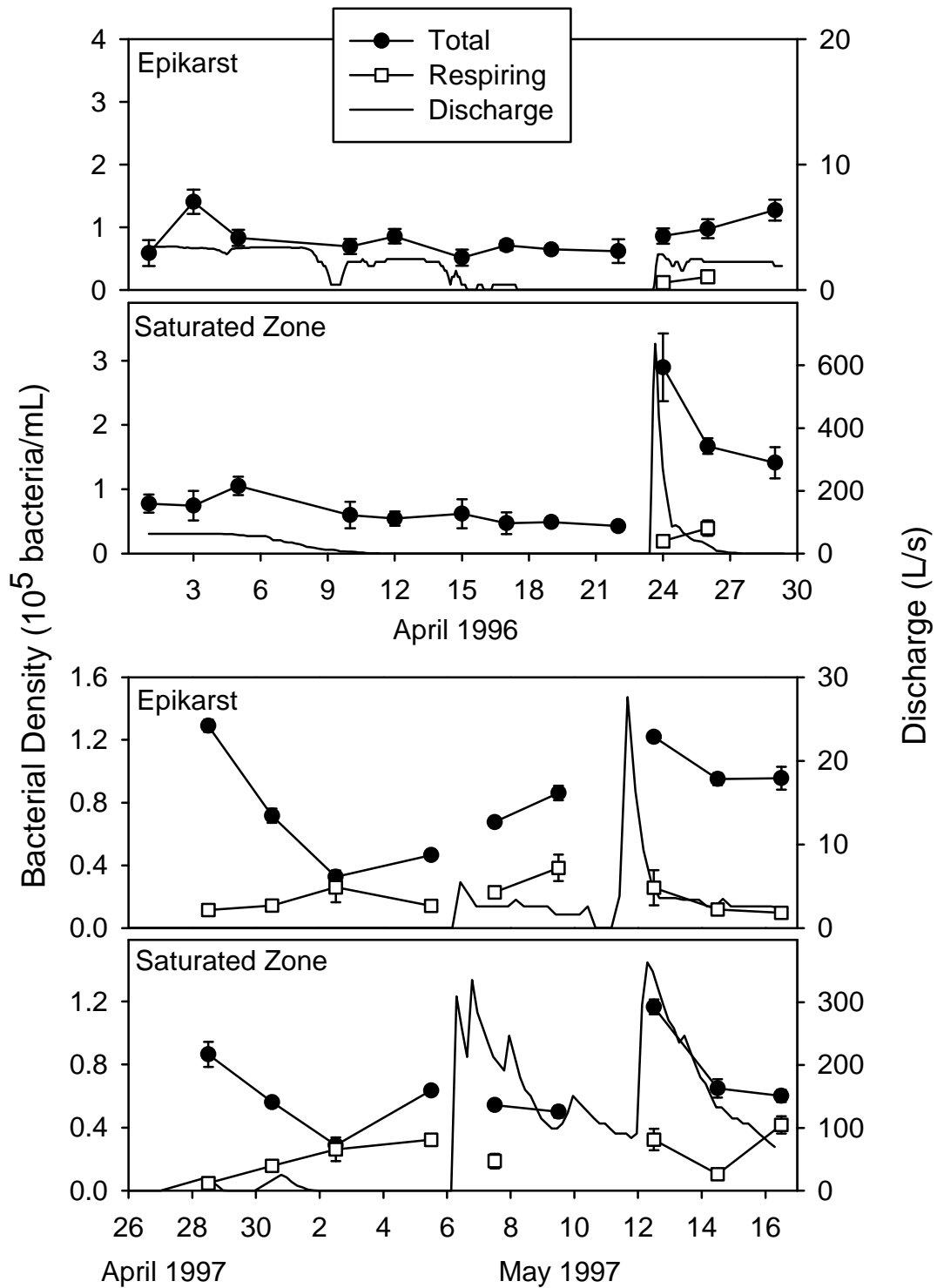


Figure 1.5. Bacterial transport (total and respiring) and discharge in the epikarst and saturated zones in 1996 and 1997. Bars are + and - 1 SE.

## Chapter 2. Leaf and wood breakdown in cave streams

### Abstract

We examined leaf and wood breakdown in 3 cave streams with and 3 without litter input from the surface. White oak (*Quercus alba*) leaf breakdown rates in cave streams spanned the range of values reported for surface streams. Leaf breakdown was faster in streams with invertebrate shredders, and leaf breakdown rates were positively correlated with shredder and *Gammarus minus* density in leaf packs. Wood breakdown rates were high, partly a result of the type of wood we used, and did not differ among streams. Microbial colonization of leaves and wood followed a pattern typical of that seen on the surface: microbial biomass and respiration peaked within 62 days then declined on leaves, but biomass and respiration on wood gradually increased to a steady plateau on wood. CPOM breakdown and microbial colonization in cave streams were not influenced by litter input, unlike surface streams, apparently because nutrients were not limiting in cave streams. Nutrient levels were high and nutrient enrichment had little effect on microbial biomass and respiration on wood in cave streams. Leaf and stick transport distances were short and, when combined with breakdown rates, suggest that CPOM is efficiently retained near its entry point to the subsurface drainage network. Similarities in CPOM processing between surface and cave streams suggest cave streams may be useful models for studying energy flow through detritus in streams.

**Key Words:** organic matter, breakdown, biofilm, litter exclusion

## Introduction

Most energy in forested surface streams comes from detritus rather than in-stream photosynthesis (Fisher and Likens 1973, Minshall et al. 1983). Leaves and wood from riparian vegetation enter streams and are then transformed by abiotic and biotic processes while being continually displaced downstream. As a result, streams are open systems with strong linkages to the surrounding terrestrial environment and downstream systems. Similarly, cave streams are also open, detritus-driven systems linked to terrestrial vegetation. In caves, where primary production is absent and chemoautotrophy is rare, detritus from the surface fuels stream food webs (Culver 1982). Because there is no riparian vegetation to feed streams, coarse particulate organic matter (CPOM) from the surface must enter caves through discrete openings such as cave entrances. After entering cave streams, detritus can be used, transformed and transported downstream, and eventually exported to the surface through springs, emerging streams, and hyporheic zones. In both surface and cave streams, factors that govern distribution and transformation of detritus have implications for structure and function of stream communities. This should be particularly important in caves where energy sources are scarce. Subsurface drainage networks dominate karst landscapes (White 1988) and these groundwater systems are strongly linked to surface ecosystems such as springs, streams and wetlands (Gibert et al. 1990). Consequently, organic matter dynamics in caves should influence the quantity and quality of matter delivered to surface aquatic ecosystems in karst.

Transformation of CPOM into smaller particles and mineralization to CO<sub>2</sub>, or breakdown, influences energy availability in streams and is an ecosystem-level process that integrates physical and biological factors (Benfield 1996). The process of CPOM breakdown in surface streams follows a predictable pattern and breakdown rates calculated from mass loss are commonly used to assess CPOM quality and energy processing in streams (Webster and Benfield 1986). CPOM breakdown in streams has been attributed to 4 major processes: soluble organic matter leaching; physical abrasion and fragmentation; microbial decomposition; and invertebrate feeding activity (Suberkropp and Klug 1980, Wallace et al. 1982, Petersen et al. 1989). Interaction among these processes, such as microbial influence on invertebrate feeding (Arsuffi and Suberkropp 1989), can regulate CPOM breakdown rate. Furthermore, external

variables such as temperature and nutrient availability can speed or slow breakdown by affecting the activity of the 4 major processes (Webster and Benfield 1986).

Factors important in CPOM processing in cave streams are not known, but physical and microbial action may dominate because invertebrate density is relatively low in cave streams. On the other hand, because organic matter is scarce CPOM may attract invertebrates, increasing local invertebrate density, which may accelerate breakdown (Webster and Waide 1982). Theoretically, CPOM breakdown should follow a pattern similar to that on the surface because cave streams are physically and biologically similar to surface streams. Physically, cave streams have unidirectional flow, are composed of riffles, runs and pools, and have substrates typical of surface streams. Biologically, cave streams are dominated by crustaceans, as opposed to insects as in many surface streams, but crustaceans use the same functional feeding strategies used by surface-stream insects, including CPOM shredding.

By excluding litter input to a stream, Wallace et al. (1997) demonstrated that leaf input strongly influences stream food webs. In these same streams, litter exclusion led to accelerated breakdown of wood veneer discs by reducing nutrient limitation in the stream (Tank and Webster 1998). Two differences between surface and cave streams make cave streams potentially useful models for studying the effect of CPOM input to lotic systems. First, responses of cave stream communities to litter input represent only changes in detrital pathways because cave streams lack energy pathways based on primary production. Chemoautotrophy is important in some caves (Sarbu et al. 1996), but most cave stream communities only use coarse, fine, and dissolved organic matter as energy sources. Second, because detritus input to cave streams is controlled by geology and hydrology, streams with and without CPOM sources can be identified and studied. In caves, CPOM is found in areas linked to the surface by openings and cave streams connected to these openings receive litter input. In other areas, cave streams fed by water that must percolate through the soil and bedrock, i.e. disconnected streams, receive only fine and dissolved organic matter that can move through small fractures in the rock.

In caves, leaves and wood could be processed more efficiently in streams that receive CPOM as a result of higher abundance of microbial or invertebrate consumers. On the other hand, detritus may be more rapidly depleted in streams without litter input because nutrient limitation of microbial films is released or because CPOM is a highly limited and desirable resource rapidly used by invertebrates.

Our objectives were to: 1) determine the effect of litter input on organic matter processing in cave streams by comparing streams connected to and disconnected from the surface and 2) identify the factors responsible for leaf and wood breakdown and distribution in cave streams.

## **Methods**

### *Study Site*

We studied six first-order and one second order stream in Organ Cave, Greenbrier County, West Virginia. This extensive cave (>60 km length) contains a network of streams ranging from 1<sup>st</sup> to 3<sup>rd</sup> order, which account for nearly all the drainage in the 8.1 km<sup>2</sup> basin (Culver et al. 1994). The 1<sup>st</sup> order streams lie near the surface and can be classified into two types depending on linkage to the surface. "Disconnected streams", Jones Canyon (D1), Skid Row Side (D2), and Sively 3 (D3), receive little CPOM input because they are fed exclusively by water percolating through the overlying soil and bedrock. "Connected streams", Lipps Entrance (C1), Sively 2 (C2), and 1812 (C3), are linked at their upstream ends to the surface by openings that allow CPOM to enter the streams during floods. We also included a second order stream, Organ Main (SO), which is fed by D3, C2, and C3 in some of our analyses for comparison to larger order surface streams. The 1<sup>st</sup> order streams are all small and sometimes flow intermittently during very dry years. During this study, leaves and wood in one site, C3, were stranded when flow ceased for approximately one month. Aquatic fauna in these streams are dominated by amphipods (*Gammarus minus*, *Stygobromus emarginatus*, and *S. spinatus*) and the isopod *Caecidotea holsingeri*. Water temperature is relatively stable in these streams (coefficient of variation ranges 0.2-15.8) and nutrient levels are high (Table 2.1).

### *Benthic organic matter*

Standing stocks of coarse and fine benthic organic matter (CBOM and FBOM, respectively) were measured from samples collected at 5-m intervals along a 50-m reach of each stream on 24 March 1998. CBOM was collected by placing a Surber sampler (0.09 m<sup>2</sup>, 1-mm mesh) on the stream bottom and stirring the substrate by hand to wash CBOM into a net. FBOM was collected using a pump and a plastic cylinder (10-cm diam). The pipe was pushed 5 cm into the substrate and sealed around the outside with foam insulation. Sediment and water in the pipe

were stirred and the slurry was removed using a hand pump. CBOM was air dried, sorted in the laboratory into leaves, wood, and other particles, then ground in a Wiley mill. Triplicate subsamples of the FBOM slurry were filtered onto pre-ashed Gelman AE filters. FBOM and ground CBOM samples were then dried at 60°C, weighed, ashed at 550°C, and re-weighed to calculate ash-free dry mass (AFDM).

#### *Leaf and wood breakdown rates*

White oak (*Quercus alba*) leaves were collected prior to abscission, dried to a constant mass at room temperature, then placed into mesh bags (3 g per pack, mesh size = 5 mm). Untreated wood veneer strips (2.5 cm × 15 cm × 1 mm) were attached to plastic mesh holders (4 per holder) with cable-ties. Leaf bags and veneer holders were anchored in the center of each stream channel with gutter nails on 9 January 1998 and 4 bags and 1 holder were collected from each stream after 31, 62, 105, 172, and 261 days. Leaves and wood were transported in cold stream water directly to the laboratory and processed. For each site, three bags and one holder were carried in the field and returned to the laboratory on day 0 and then processed to determine mass loss due to handling and initial % AFDM, fungal biomass, and microbial respiration.

#### *Microbial assays*

In the laboratory, two discs (2 cm diameter) were punched from a leaf randomly chosen from each bag and two strips (2.5 cm × 3 cm) were cut from each veneer strip for measuring fungal biomass and microbial respiration. Fungal biomass was measured from ergosterol content of biofilms on leaves and wood (Newell et al. 1988). Leaf discs and wood strips were refluxed in 5 mL of methanol for 2 h at 65 °C. Samples were cooled, saponified by adding 1 mL of 4% KOH in methanol, and then refluxed for 0.5 h at 65 °C. Samples were cooled, centrifuged, and the supernatants were decanted into clean tubes. The pellets were re-suspended in 2 mL of methanol, centrifuged, and the supernatant was added to that from the first wash. The samples were then extracted twice with 2-mL aliquots of pentane. The pentane extracts were evaporated under a fume hood and the residue was re-dissolved in 1 mL of methanol and filtered through a 0.45-µm filter. Ergosterol was quantified using a Waters reverse-phase HPLC system (solvent = methanol; column = Nova-Pak ODS C18; flow rate = 2 mL/min; absorbance = 282 nm).

Ergosterol concentration was converted to fungal biomass using a conversion factor of 6 mg ergosterol/g fungal biomass (Newell et al. 1988).

Respiration was measured using a Gilson differential respirometer. Four leaf and 4 wood replicates and one control (water only) from each stream were incubated in 15-mL Warburg vials containing 5 mL filtered (Gelman AE) site water at 15°C. Samples were incubated 0.5-5 hours, depending on respiration rate, in order to obtain sufficient volume change for accurate readings. Respiration rates were calculated using the formula (Umbreit et al. 1964):

$$X = (\Delta V_g) \frac{P - P_w}{P_1} \frac{T_1}{T}$$

where X= oxygen uptake ( $\mu\text{L}$ ),  $\Delta V_g$ = volume change in respirometer ( $\mu\text{L}$ ), P=total gas pressure within the respirometer (mmHg),  $P_w$ =vapor pressure of water at temperature (T) in mm Hg,  $P_1$ =standard pressure=760 mm Hg,  $T_1$ =standard temperature=273.15 °K, and T= incubation temperature (°K).

#### *Invertebrate colonization*

After leaf packs were sampled for microbial assays, the contents of the packs were rinsed over a 250- $\mu\text{m}$  mesh sieve. Contents of the sieve were preserved in 80% ethanol and sorted for invertebrates. Surface (epigeal) invertebrates found in the cave streams were identified and assigned functional feeding groups according to Merritt and Cummins (1996). We assigned *Gammarus minus* as a shredder based on observations of feeding by animals kept in the laboratory with leaf material and published information (Kostalos and Seymour 1976).

#### *Nutrient releasing substrates*

Nutrient releasing substrates were used to measure the effect of  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  addition on wood respiration and fungal biomass accumulation (Tank and Webster 1998). Discs (12.5  $\text{cm}^2$ ) of white oak wood were attached to the top of 60-mL plastic cups containing 2% agar. Four groups were used: control (agar only), N addition (0.66M  $\text{NaNO}_3$ ), P addition (0.51M  $\text{KH}_2\text{PO}_4$ ), and N+P addition (0.66M  $\text{NaNO}_3$  + 0.51M  $\text{KH}_2\text{PO}_4$ ). Five replicates of each treatment were placed in each stream on 17 July 1998 and recovered after 30 days. Wood discs from each substrate were analyzed for fungal biomass and microbial respiration using the procedures described above.

### *Leaf and wood transport*

We measured leaf and wood transport by following the movement of marked leaves (*Ginkgo biloba*) and wooden dowels (25 cm length  $\times$  1 cm diameter, referred to as sticks) in each stream. Leaves and sticks were marked with spray paint, soaked in distilled water, and then placed individually in each stream on 17 July 1998. We monitored the distance 100 leaves and 25 sticks traveled downstream from the release point approximately monthly over 252 days. Sticks were removed from C3 by vandals so a wood transport estimate was not made for that stream.

### *Data Analyses*

Leaf and wood breakdown rates ( $k$ ) were calculated by regressing the natural log of mean % AFDM remaining on time (Webster and Benfield 1986). Leaf and wood breakdown rates in connected and disconnected streams were compared using t-tests with streams as replicates. Breakdown rates also were compared among all streams using analysis of covariance (ANCOVA). Microbial respiration and fungal biomass were compared between stream types and over time using a two-way repeated measures analysis of variance (ANOVA). Microbial respiration and fungal biomass on wood used in the nutrient releasing substrates were compared for each stream using a one-way ANOVA followed by Dunn's comparisons of treatments versus control. Differences in leaf and wood transport distances among streams were examined using a Kruskal-Wallis one-way ANOVA on ranks, because transport distances were not normally distributed, followed by Dunn's post-hoc test. We predicted the distance 10% of the initial CPOM entering each stream would travel, referred to as penetration distance or  $P_{10}$ , by multiplying time to 90% mass loss, calculated from breakdown regressions, by transport rate for both leaves and wood.

## Results

### *Breakdown*

Mean wood breakdown rates were not different between connected and disconnected streams (0.0051 and 0.0044, respectively;  $p = 0.37$ , t-test) and individual breakdown rates were similar in all streams ( $p > 0.05$ , ANCOVA; Table 2.2). Wood breakdown rates in our streams were faster than most reported values for wood in surface streams (Table 2.3). Mean leaf breakdown rate was not significantly different between connected and disconnected streams (0.0121 and 0.0075, respectively;  $p = 0.45$ , t-test). Leaf breakdown rates varied significantly ( $p < 0.05$ , ANCOVA) among individual streams (Table 2.2). Leaves in D1 and D2 lost mass slowly, breaking down at roughly the same rate as wood. Leaves lost mass faster in the remaining sites (Table 2.2). Leaf breakdown rates in the cave streams spanned the range of values reported for surface systems (Table 2.4): breakdown was relatively slow in D1 and D2, intermediate in D3, C1, and C2, and fast in C3 and SO, which exceeded the highest reported  $k$  for white oak in surface streams and lakes (Table 2.4).

### *Microbial Colonization*

Fungal biomass was significantly higher on leaves in connected streams than in disconnected streams ( $p = 0.03$ ) but there was no difference in fungal biomass on wood between stream types ( $p = 0.26$ ). Fungal biomass on leaves and wood changed significantly over time ( $p = 0.001$  and  $0.003$ , respectively) but in different patterns (Figure 2.1). On leaves, fungal biomass reached a maximum by day 62 then sharply declined. Fungal biomass on wood increased ( $p = 0.003$ ) until day 105 then maintained a relatively steady plateau for the duration of the study.

Microbial respiration did not differ between stream types on either leaves or wood ( $p = 0.9$  and  $0.3$ , respectively) and it followed a pattern similar to that of fungal biomass (Figure 2.2). Respiration on leaves rapidly increased and peaked by day 62 then declined. On wood, respiration slowly climbed to a plateau by day 105. Although fungal biomass and respiration generally followed similar patterns, there was only a weak positive relationship between fungal biomass and respiration on leaves ( $p = 0.05$ ,  $r^2 = 0.19$ ) and wood ( $p < 0.001$ ,  $r^2 = 0.50$ ). This was due primarily to a high respiration:fungal biomass ratio early in the study (Figure 2.3). On both leaves and wood, respiration per unit fungal biomass varied significantly over time ( $p < 0.001$ ) but not between stream types ( $p = 0.82$  and  $0.17$ , respectively).

### *Invertebrate and Nutrient Influences*

Leaf packs were colonized by both epigeal and hypogean fauna, but leaf packs in connected streams generally contained more epigeal taxa than packs in disconnected streams (Table 2.5). Mean invertebrate density in leaf packs was not different among streams ( $p = 0.4$ ) and there was no significant relationship between total invertebrate density in packs and leaf breakdown rate ( $p = 0.17$ ,  $r^2 = 0.34$ ). However, there was a strong positive relationship between leaf breakdown rate and both total shredder density ( $p < 0.001$ ,  $r^2 = 0.93$ ) and *Gammarus minus* density alone ( $p = 0.002$ ,  $r^2 = 0.88$ ) in the leaf packs (Figure 2.4).

Nutrient enrichment had little effect on microbial respiration or fungal biomass on wood (Figure 2.5). Fungal biomass was nutrient limited in only one stream, D2, which was co-limited by nitrogen and phosphorus ( $p < 0.05$ ). Nitrogen enrichment significantly increased ( $p < 0.05$ ) microbial respiration on wood in one stream, C1. Respiration was significantly lower on N+P treated wood than on controls in S3 ( $p < 0.05$ ).

### *Leaf and wood transport*

Leaves and wood moved during two floods but otherwise remained stationary. Leaf and wood transport rates were low, and leaf transport varied significantly among streams (Table 2.6). However, differences in leaf transport did not strictly fall between connected and disconnected streams (Table 2.6). Transport was very high in one stream, C3, in which leaves were transported out of our study reach and into the second order stream during the second flood. For this stream we used a 100m transport distance (the length of the stream) to calculate a minimum transport rate and penetration distance. Penetration distance was short in all streams except C3 (Table 2.6).

## **Discussion**

### *CPOM breakdown in cave streams*

Patterns of leaf and wood breakdown in cave streams appear to be similar to that occurring in surface streams. Leaf breakdown in our study generally fell into two groups. Leaves at sites D1 and D2 broke down at slow rates generally associated with lakes and low order streams. Griffith et al. (1995) reported a  $k$  value in a similar range for white oak leaves in a

surface stream also in the Greenbrier limestone of West Virginia. Leaves in our remaining sites lost mass faster, falling at the high range of breakdown reported for larger surface streams. Rapid oak leaf breakdown has been reported in other karst systems (Brown and Schram 1982, Eichen et al. 1993). Eichen et al. (1993) reported very high breakdown rates for *Q. macrocarpa* in a karst aquifer. However, they used small leaf discs which probably decayed faster than leaf packs (D'Angelo and Webster 1992). Faster leaf breakdown in the 2<sup>nd</sup> order stream, SO, than in the 1<sup>st</sup> order streams in this study is typical of results in surface drainages (Benfield and Webster 1985). The rapid mass loss in SO was probably due to a combination of leaf shredding by *Gammarus minus*, which were abundant in the leaf packs, and physical fragmentation during a few large floods.

Our wood breakdown rates are high relative to most studies in surface streams. The high surface area:volume ratio of our wood veneers contributed to this, and we have certainly overestimated natural wood breakdown rate in cave streams. However, our wood breakdown rates are 2.5-4.3 times higher than that measured by Tank and Webster (1998) who used similar oak veneers in 1<sup>st</sup> order surface streams.

The pattern of respiration and fungal biomass accumulation on leaves and wood in our study was typical of biofilm colonization on CPOM in surface streams (Golladay and Sinsabaugh 1991, Suberkropp 1995, Weyers and Suberkropp 1996). Rapid increases in microbial biomass and respiration followed by a sharp decline on leaves relative to wood have been attributed to higher substrate quality and rapid deterioration of stability of leaves in surface streams (Golladay and Sinsabaugh 1991). The initial high respiration:biomass ratio, particularly on leaves, could be the result of bacterial respiration early in the study or high fungal respiration without accompanying rapid accumulation of biomass. High bacterial respiration is unlikely because bacteria usually dominate late stages of breakdown (Weyers and Suberkropp 1996). A more likely explanation is the allocation of fungal production to reproduction early in the study. Conidia produced and released into the water column are not accounted for by our ergosterol extraction method, possibly leading to underestimation of fungal biomass production early in the study (Baldy et al. 1995, Tank and Webster 1998).

Unlike in surface streams, litter exclusion did not affect wood breakdown in cave streams. Tank and Webster (1998) found litter accelerated wood breakdown and increased microbial respiration and fungal biomass on wood in surface streams. Increased nutrient

availability, resulting from the lower nutrient demand of smaller CBOM standing stocks in litter excluded streams, apparently stimulated microbial activity and accelerated wood decomposition in litter excluded streams. Higher fungal biomass and activity in streams with higher nutrient levels has been demonstrated in other streams as well (Suberkropp 1995). Wood did not lose mass faster in our litter-less cave streams apparently because nutrients were not limiting. Microbial biomass and respiration on wood were similar in connected and disconnected streams, and microbial films on wood generally were not stimulated by nutrient addition in our study. On a unit area basis, maximum fungal biomass ( $\sim 2 \text{ mg/cm}^2$ ) and respiration ( $\sim 7 \text{ } \mu\text{l/cm}^2/\text{h}$ ) on wood in our streams were roughly 2-3 times higher than peak values on wood veneers in surface streams ( $\sim 0.6 \text{ mg/cm}^2$  and  $\sim 4 \text{ } \mu\text{l/cm}^2/\text{h}$ , respectively, Tank and Webster 1998). Higher microbial activity on our wood could have led to the faster breakdown of our wood veneers than in surface streams. Because standing stocks of CBOM in our streams were relatively low compared to surface streams (see reviews in Webster and Meyer 1997) and nutrient levels were high and similar among streams, microbial activity on CBOM probably was not nutrient limited or affected by litter input to the cave streams.

Microbial decomposition was apparently the primary cause of wood breakdown: there was little evidence of physical fragmentation or invertebrate gouging of the veneers. Leaf breakdown, however, was accelerated by the presence of shredding invertebrates, particularly *Gammarus minus*. Shredders, usually insects rather than crustaceans, speed leaf breakdown in surface streams (Wallace et al. 1982) and *Gammarus minus* is an effective leaf shredder (Kostalos and Seymour 1976). The biogeographic history of *Gammarus minus* in the Organ Cave drainage and connection to the surface both influence the pattern of organic matter breakdown in the subsurface drainage network. *Gammarus minus* occurs only in one portion of the Organ Cave drainage (sites D3, C2, C3 and SO in our study) probably as a result of its invasion history into the subsurface (Culver et al. 1994). *Gammarus minus* likely colonized Organ Cave from the downstream end of the drainage network through the resurgence spring, but never reached one sub-basin of the drainage network. Leaf breakdown was much faster in D3, which contains *Gammarus minus*, than in the other disconnected streams, D1 and D2, from which *G. minus* is absent. Leaves in D3 were skeletonized and *Gammarus minus* was abundant in the leaf packs in D3. In D1 and D2, chironomids, cave amphipods (*Stygobromus spinatus*), and cave snails (*Fontigens tartarea*) which were the primary colonizers of leaf packs had little

effect on leaf breakdown. These animals are very small (<5 mm) and apparently feed on microbial films on the leaves with little effect on the leaf itself; there was no indication of leaf skeletonization.

In the connected streams, leaf breakdown was not slower in the stream without *Gammarus minus* (C1) than in the streams with *G. minus* (C2 and C3). Shredding stoneflies (*Leuctra*, *Allocapnia*, and *Amphinemura*) and caddisflies (*Isonychia*) were present in leaf packs in all connected streams. These epigeal insects apparently washed into the cave stream and probably shredded leaves, leaves in all three connected streams were skeletonized, causing the relatively fast leaf breakdown in C1. Connection to the surface influences organic matter breakdown by permitting epigeal animals to enter the cave rather than by simply adding CPOM to the streams.

We were surprised by the similarity in leaf and wood breakdown rates in two streams: D1 and D2. The absence of shredders and lack of large floods in these streams left leaching and microbial activity as the only mechanisms of leaf mass loss as was the case for wood. The thin shape of our wood veneers caused high estimates of wood breakdown by increasing oxygen diffusion to the substrate and by exposing more wood surface area for microbial colonization than is typical of natural wood in streams (Golladay and Sinsabaugh 1991). Decomposition of the veneers in our study probably simulated only the outer surface of wood, such as small sticks, usually found in cave streams. However, because of the similar shape of leaves and wood veneer, comparison of leaf and wood mass loss without the complication of differing surface-area:volume ratios of sticks and leaves can be made. The similar breakdown rates of leaves and wood suggest that, from a microbial perspective, white oak leaf and wood tissue were of roughly the same nutritional quality. For microbes, wood is generally considered to be of lesser nutritional quality than leaves (Golladay and Sinsabaugh 1991). However, oak leaves are not high quality compared to other leaf species (Webster and Benfield 1986) and may be more nutritionally similar to wood than most leaves.

#### *CPOM distribution in caves*

Forested headwater streams are relatively efficient at retaining CPOM (Webster et al. 1999) as leaves and wood generally move only during floods and, at base flow, do not travel far downstream from their entry point ( Webster et al. 1994, Wallace et al. 1995). CPOM transport

and retention behave similarly in cave streams. Leaves and sticks in our study only moved during two floods and our calculated  $P_{10}$  values suggest that, in most instances, CPOM will not penetrate far into caves before being metabolized or transformed into smaller particles. Because CPOM enters at discrete points in cave streams, rather than along entire stream reaches, CPOM use and transport will dictate energy distribution downstream in the drainage. This has unique consequences in caves because cave streams do not shift towards autotrophic input with increasing stream order as typically seen in surface drainages (Vannote et al. 1980). Our results suggest that CPOM generally will not be an available energy source very far from its entry point to Organ Cave. Downstream reaches must therefore rely on dissolved organic carbon and FPOM for food. An important exception to this was stream C3, which had a minimum  $P_{10}$  estimate of 134 m. This stream is located near a large entrance, is fed by an intermittent surface stream, and experiences periodic severe flooding. Our study period was a relatively dry year with only two large floods. More frequent floods probably would have increased CPOM input and penetration into the cave. Because of the high CPOM transport and probably high total CPOM input from the surface at this location, this stream likely acts as a hot spot for energy input to the subsurface drainage.

### *Summary*

In spite of the differences in primary production and community composition between surface and cave streams, organic matter processing is similar in the two systems. Leaf and wood breakdown proceed at rates similar to rates in surface streams and the pattern of mass loss and microbial colonization is similar in both systems. Because of these similarities, we believe cave streams can be useful models for studying organic matter processes and energy flow through detritus in streams. The apparent organic carbon, rather than nutrient, limitation in cave streams and importance of shredders suggests cave streams will be most useful for studying detritus limitation and its role in food web structure in streams.

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## References

- Arsuffi, T. L. and K. Suberkropp. 1989. Selective feeding by shredders on leaf-colonizing stream fungi: Comparison of macroinvertebrate taxa. *Oecologia* 79:30-37.
- Baldy, V., M. O. Gessner and E. Chauvet. 1995. Bacteria, fungi, and the breakdown of leaf litter in a large river. *Oikos* 74:93-102.
- Barnes, J. R., R. Ovink and K. W. Cummins. 1978. Leaf litter processing in Gull Lake, Michigan, USA. *Verhandlung der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie* 20:475-479.
- Benfield, E. F. 1996. Leaf breakdown in stream ecosystems. 579-589 in F. R. Hauer and G. A. Lamberti. *Methods in stream ecology*. Academic Press, San Diego.
- Benfield, E. F. and J. R. Webster. 1985. Shredder abundance and leaf breakdown in an Appalachian Mountain stream. *Freshwater Biology* 15:113-120.
- Bott, T. L., J. Preslan, J. Finlay and R. L. Brunker. 1977. The use of flowing water microcosm and ecosystem streams to study microbial degradation of leaf litter and nitrilotriacetic acid (NTA). *Developments in Industrial Microbiology*. 18:171-184.
- Brown, A. V. and M. D. Schram. 1982. Leaf detritus processing in an Ozark cave stream. *Arkansas Academy of Science Proceedings* 36:14-16.
- Brown, A. V. and M. D. Schram. 1982. Leaf detritus processing in an Ozark cave stream. *Proc. Ark. Acad. Sci.* 36:14-16.
- Brussock, P. P., L. D. Willis and A. V. Brown. 1988. Leaf decomposition in an Ozark cave and spring. *Journal of Freshwater Ecology* 4:263-269.
- Carpenter, J., W. E. Odum and A. E. Mills. 1983. Leaf litter decomposition in a reservoir affected by acid mine drainage. *Oikos* 41:165-172.
- Culver, D. C. 1982. *Cave Life: Evolution and Ecology*. Harvard University Press, Cambridge, Massachusetts.
- Culver, D. C., W. K. Jones, D. W. Fong and T. C. Kane. 1994. Organ Cave karst basin. 451-473 in J. Gibert, D. L. Danielopol and J. A. Stanford. *Groundwater Ecology*. Academic Press, San Diego.
- D'Angelo, D. J. and J. R. Webster. 1992. Natural and constraint-induced factors influencing the breakdown of dogwood and oak leaves. *Hydrobiologia* 237:39-46.

- Eichem, A. C., W. K. Dodds, C. M. Tate and C. Edler. 1993. Microbial decomposition of elm and oak leaves in a karst aquifer. *Applied and Environmental Microbiology* 59:3592-3596.
- Fisher, S. G. and G. E. Likens. 1972. Stream ecosystem: organic energy budget. *BioScience* 22:33-35.
- Fisher, S. G. and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecological Monographs* 43:421-439.
- Gasith, A. and W. Lawacz. 1976. Breakdown of leaf litter in the littoral zone of an eutrophic lake. *Ekol. Pol.* 24:421-430.
- Gibert J., M.-J. Dole-Olivier, P. Marmonier, and P. Vervier. 1990. Surface-groundwater ecotones. In R. J. Naiman and H. Decamps, editors. *Ecology and Management of Aquatic-Terrestrial Ecotones*. London:Parthenon Publishing. p. 199-225.
- Golladay, S. W. and R. L. Sinsabaugh. 1991. Biofilm development on leaf and wood surfaces in a boreal river. *Freshwater Biology* 25:437-450.
- Golladay, S. W. and J. R. Webster. 1988. Effects of clearcut logging on wood breakdown in Appalachian mountain streams. *American Midland Naturalist* 119:143-155.
- Griffith, M. B., S. A. Perry and W. B. Perry. 1995. Leaf litter processing and exoenzyme production on leaves in streams of different pH. *Oecologia* 102:460-466.
- Hanson, B. J., K. W. Cummins, J. R. Barnes and M. W. Carter. 1984. Leaf litter processing in aquatic systems: a two variable model. *Hydrobiologia* 111:21-31.
- Kaushik, N. K. and H. B. N. Hynes. 1971. The fate of the dead leaves that fall into streams. *Archiv fur Hydrobiologie* 68:465-515.
- Kostalos, M. and L. R. Seymour. 1976. Role of microbial enriched detritus in the nutrition of *Gammarus minus*. *Oikos* 27:512-516.
- Melillo, J. M., R. J. Naiman, J. D. Aber and K. N. Eshleman. 1983. The influence of substrate quality and stream size on wood decomposition dynamics. *Oecologia* 58:281-285.
- Merritt, R. W. and K. W. Cummins. 1996. An introduction to the aquatic insects of North America, 3rd edition. Kendall-Hunt Publishing Company, Dubuque, Iowa.
- Minshall, G. W., R. C. Petersen, K. W. Cummins, T. L. Bott, J. R. Sedell, C. E. Cushing and R. L. Vannote. 1983. Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs* 53:1-25.

- Newell, S. Y., T. L. Arsuffi and R. D. Fallon. 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. *Applied and Environmental Microbiology* 54:1876-1879.
- Petersen, R. C. and K. W. Cummins. 1974. Leaf processing in a woodland stream. *Freshwater Biology* 4:345-368.
- Petersen, R. C., Jr., K. W. Cummins and G. M. Ward. 1989. Microbial and animal processing of detritus in a woodland stream. *Ecological Monographs* 59:21-39.
- Sarbu, S. M., T. C. Kane and B. K. Kinkle. 1996. A chemoautotrophically based cave ecosystem. *Science* 272:1953-1955.
- Stout III, B. M. and C.B. Coburn. 1989. Impact of highway construction on leaf processing in aquatic habitats of eastern Tennessee. *Hydrobiologia* 178:233-242.
- Suberkropp, K. 1995. The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. *Canadian Journal of Botany* 73:1361-1369.
- Suberkropp, K., G. L. Godshalk and M. J. Klug. 1976. Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology* 57:720-727.
- Suberkropp, K. and M. J. Klug. 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. *Canadian Journal of Botany*. 58:1025-1031.
- Suberkropp, K., M. J. Klug and K. W. Cummins. 1975. Community processing of leaf litter in woodland streams. *Verhandlung der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie* 19:1653-1658.
- Tank, J. T. and J. W. Webster. 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology* 79:2168-2179.
- Thomas, W. A. 1970. Weight and calcium losses from decomposing tree leaves on land and in water. *The Journal of Applied Ecology*. 7:237-242.
- Umbreit, W. W., R. H. Burris and J. F. Stauffer. 1964. *Manometric Techniques*. Burgess, Minneapolis, Minnesota.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130-137.
- Wallace, J. B., S. L. Eggert, J. L. Meyer and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 277:102-104.

- Wallace, J.B., D.S. Vogel and T.F. Cuffney. 1986. Recovery of a headwater stream from an insecticide-induced community disturbance. *Journal of the North American Benthological Society* 5: 115-126.
- Wallace, J. B., J. R. Webster and T. F. Cuffney. 1982. Stream detritus dynamics: regulation by invertebrate consumers. *Oecologia* 53:197-200.
- Wallace, J. B., M. R. Whiles, S. Eggert, T. F. Cuffney, G. J. Lugthart and K. Chung. 1995. Long-term dynamics of coarse particulate organic matter in three Appalachian Mountain streams. *Journal of the North American Benthological Society* 14:217-232.
- Webster, J. R. and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* 17:567-594.
- Webster, J. R., E. F. Benfield, T. P. Ehrman, M. A. Schaeffer, J. L. Tank, J. J. Hutchens and D. J. D'Angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology* 41:687-705.
- Webster, J. R., A. P. Covich, J. L. Tank and T. V. Crockett. 1994. Retention of coarse organic particles in streams in the southern Appalachian Mountains. *Journal of the North American Benthological Society* 13:140-150.
- Webster, J. R. and J. L. Meyer. 1997. Organic matter budgets for streams: a synthesis. 141-161 in J. R. Webster and J. L. Meyer. *Stream organic matter budgets*. *Journal of the North American Benthological Society* 16:3-161,
- Webster, J. R. and G. M. Simmons, Jr. 1978. Leaf breakdown and invertebrate colonization on a reservoir bottom. *Verhandlung der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie* 20:1587-1596.
- Webster, J. R. and J. B. Waide. 1982. Effects of forest clearcutting on leaf breakdown in a southern Appalachian stream. *Freshwater Biology* 12:331-344.
- Weyers, H. S. and K. Suberkropp. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. *Journal of the North American Benthological Society* 15(4):408-420.
- White, W.B. 1988. *Geomorphology and hydrology of karst terrains*. Oxford University Press, New York. 464 p.
- Witkamp, M. and M. L. Frank. 1969. Loss of weight, <sup>60</sup>Co and <sup>137</sup>Cs from tree litter in three subsystems of a watershed. *Environmental Science and Technology*. 3:1195-1198.

Table 2.1. Mean temperature, nutrients (n ranges 2-5), and benthic organic matter standing stocks (n=10) at each study site.

<b>Variable</b>	<b>D1</b>	<b>D2</b>	<b>D3</b>	<b>C1</b>	<b>C2</b>	<b>C3</b>
Temperature	11.6	11.3	10.6	11.2	10.5	9.3
NO <sub>3</sub> -N (mg/L)	2.5	2.7	1.3	2.9	1.6	0.9
SRP (mg/L)	0.015	0.020	0.015	0.031	0.018	0.019
FBOM (g/m <sup>2</sup> )	37.4 <sup>a</sup>	44.3 <sup>a</sup>	28.2 <sup>a</sup>	48.6 <sup>a</sup>	33.5 <sup>a</sup>	19.8 <sup>a</sup>
CBOM (g/m <sup>2</sup> )						
Total	0 <sup>a</sup>	0 <sup>a</sup>	0.09 <sup>ab</sup>	41.4 <sup>b</sup>	17.8 <sup>b</sup>	6.9 <sup>b</sup>
Leaves	0 <sup>a</sup>	0 <sup>a</sup>	0.05 <sup>ab</sup>	15.5 <sup>b</sup>	5.9 <sup>b</sup>	4.4 <sup>b</sup>
Wood	0 <sup>a</sup>	0 <sup>a</sup>	0.04 <sup>a</sup>	23.4 <sup>ab</sup>	7.5 <sup>b</sup>	2.5 <sup>b</sup>

Means with the same letter within each row are not significantly different (p>0.05, Tukey's HSD)

Table 2.2. Breakdown rates ( $k$ ) of leaves and wood.

Site	Leaves		Wood	
	$k$ (day <sup>-1</sup> )	$r^2$	$k$ (day <sup>-1</sup> )	$r^2$
D1	0.0033 <sup>a</sup>	0.99	0.0040 <sup>a</sup>	0.94
D2	0.0033 <sup>ab</sup>	0.95	0.0042 <sup>a</sup>	0.90
D3	0.0158 <sup>cd</sup>	0.80	0.0049 <sup>a</sup>	0.96
C1	0.0102 <sup>c</sup>	0.94	0.0041 <sup>a</sup>	0.97
C2	0.0191 <sup>de</sup>	0.92	0.0065 <sup>a</sup>	0.89
C3	0.0070 <sup>bcd</sup>	0.90	0.0048 <sup>a</sup>	0.93
SO	0.0259 <sup>e</sup>	0.85	0.0053 <sup>a</sup>	0.96

$k$  values with the same letter are not significantly different (ANCOVA,  $p > 0.05$ )

Table 2.3. Wood breakdown rates reported in the literature and from this study.

Reference	$k$ (day <sup>-1</sup> )	Site	Notes
Webster et al. 1999	0.0003		<i>Pinus strobus</i> ; sticks
Golladay and Webster 1988	0.0004	2 <sup>nd</sup> order	<i>Quercus rubra</i> ; sticks
Webster et al. 1999	0.0005		<i>Liriodendron tulipifera</i> ; sticks
Melillo et al. 1983	0.0007	1 <sup>st</sup> -2 <sup>nd</sup> order	<i>Picea mariana</i> ; chips
Golladay and Webster 1988	0.0008	2 <sup>nd</sup> order	<i>Q. rubra</i> ; sticks
Webster et al. 1999	0.0010		<i>L. tulipifera</i> ; sticks
Tank and Webster 1998	0.0015	1 <sup>st</sup> order	<i>Q. alba</i> ; veneers; litter excluded
Golladay and Sinsabaugh 1991	0.0016	4 <sup>th</sup> order	<i>Betula papyrifera</i> ; ice cream sticks
Golladay and Sinsabaugh 1991	0.0019	4 <sup>th</sup> order	<i>B. papyrifera</i> ; ice cream sticks
Melillo et al. 1983	0.0033	1 <sup>st</sup> -2 <sup>nd</sup> order	<i>Alnus rugosa</i> ; chips
Tank and Webster 1998	0.0040	1 <sup>st</sup> order	<i>L. tulipifera</i> ; litter excluded
<b>D1</b>	<b>0.0040</b>		<i>Q. alba</i>
<b>C2</b>	<b>0.0041</b>		<i>Q. alba</i>
<b>D2</b>	<b>0.0042</b>		<i>Q. alba</i>
Tank and Webster 1998	0.0048	1 <sup>st</sup> order	<i>L. tulipifera</i> ; reference
<b>C1</b>	<b>0.0048</b>		<i>Q. alba</i>
<b>D3</b>	<b>0.0049</b>		<i>Q. alba</i>
<b>SO</b>	<b>0.0053</b>		<i>Q. alba</i>
<b>C3</b>	<b>0.0065</b>		<i>Q. alba</i>
Tank and Webster 1998	0.0085	1 <sup>st</sup> order	<i>L. tulipifera</i> ; litter excluded

Table 2.4. Leaf breakdown rates reported in the literature and from this study.

Reference	k (day <sup>-1</sup> )	Site	Species
Stout and Coburn 1989	0.0010-0.0029	pond	<i>Q. alba</i>
Kaushik and Hynes 1971	0.0012-0.0013	? order	<i>Q. alba</i>
Stout 1982	0.0018-0.0022	5 <sup>th</sup> order	<i>Q. alba</i>
Griffith et al. 1995	0.0020	2 <sup>nd</sup> order	<i>Q. alba</i>
Benfield and Webster 1985	0.0021	1 <sup>st</sup> order	<i>Q. alba</i>
Gasith and Lawacz 1976	0.0021	lake	<i>Q. alba</i>
Stout and Coburn 1989	0.0026	3 <sup>rd</sup> order	<i>Q. alba</i>
<b>D1</b>	<b>0.0033</b>		<i>Q. alba</i>
<b>D2</b>	<b>0.0033</b>		<i>Q. alba</i>
Witkamp and Frank 1969	0.0036	lake	<i>Q. alba</i>
Webster and Waide 1982	0.0038	2 <sup>nd</sup> order	<i>Q. alba</i>
Griffith et al. 1995	0.0038	2 <sup>nd</sup> order	<i>Q. alba</i>
Benfield and Webster 1985	0.0039	1 <sup>st</sup> order	<i>Q. alba</i>
Wallace et al. 1982	0.0040	1 <sup>st</sup> order	<i>Q. alba</i>
Barnes et al. 1978	0.0041	lake	<i>Q. alba</i>
Petersen and Cummins 1974	0.0045	2 <sup>nd</sup> order	<i>Q. alba</i>
Hanson et al. 1984	0.0047	3 <sup>rd</sup> order	<i>Q. alba</i>
Suberkropp et al. 1975	0.0047	3 <sup>rd</sup> order	<i>Q. alba</i>
Carpenter et al. 1983	0.0050	lake	<i>Q. alba</i>
Webster and Simmons 1978	0.0052	lake	<i>Q. alba</i>
Golladay and Webster 1988	0.0056	2 <sup>nd</sup> order	<i>Q. alba</i>
Griffith et al. 1995	0.0059	3 <sup>rd</sup> order	<i>Q. alba</i>
Petersen and Cummins 1974	0.0059	1 <sup>st</sup> order	<i>Q. alba</i>
Webster and Waide 1982	0.0064	2 <sup>nd</sup> order	<i>Q. alba</i>
Stout and Coburn 1989	0.0072	3 <sup>rd</sup> order	<i>Q. alba</i>
Suberkropp et al. 1976	0.0074	3 <sup>rd</sup> order	<i>Q. alba</i>
Brussock <i>et al.</i> , 1988	0.0075	cave stream	<i>Q. alba</i>
Barnes et al. 1978	0.0080	lake	<i>Q. alba</i>
Brussock et al. 1988	0.0085	cave stream	<i>Q. alba</i>
<b>C1</b>	<b>0.0085</b>		<i>Q. alba</i>
Webster and Waide 1982	0.0090	2 <sup>nd</sup> order	<i>Q. alba</i>
Thomas 1970	0.0092	1 <sup>st</sup> order	<i>Q. alba</i>
Benfield and Webster 1985	0.0094	2 <sup>nd</sup> order	<i>Q. alba</i>
<b>C2</b>	<b>0.0102</b>		<i>Q. alba</i>
Wallace et al. 1986	0.0105	1 <sup>st</sup> order	<i>Q. alba</i>
Wallace et al. 1982	0.0108	1 <sup>st</sup> order	<i>Q. alba</i>
Golladay and Webster 1988	0.0116	2 <sup>nd</sup> order	<i>Q. alba</i>
Witkamp and Frank 1969	0.0121	2 <sup>nd</sup> order	<i>Q. alba</i>
<b>D3</b>	<b>0.0158</b>		<i>Q. alba</i>
Bott et al. 1977	0.0169	3 <sup>rd</sup> order	<i>Q. alba</i>
Benfield and Webster 1985	0.0182	2 <sup>nd</sup> order	<i>Q. alba</i>
Brussock et al. 1988	0.0190	spring brook	<i>Q. alba</i>
<b>C3</b>	<b>0.0191</b>		<i>Q. alba</i>
Brussock et al. 1988	0.0240	spring brook	<i>Q. alba</i>
<b>SO</b>	<b>0.0259</b>		<i>Q. alba</i>
Eichem et al. 1993	0.0260-0.1200	karst aquifer	<i>Q. macrocarpa</i>
Brown and Schram 1982	0.0498	cave stream	<i>Q. stellata</i>

Table 2.5. Mean % relative abundance of the major taxa colonizing leaf packs in each stream, mean density, and number of total, hypogean and epigeal taxa found in packs from each stream.

Taxa	D1	D2	D3	C1	C2	C3	SO
Crustacea							
<i>Caecidotea holsingeri</i> *	1.7	1.1	1.7	22.7	-	-	1.9
<i>Gammarus minus</i> *	-	-	46.9	-	66.2	14.0	53.3
<i>Stygobromus emarginatus</i> *	-	-	1.0	-	-	-	-
<i>Stygobromus spinatus</i> *	9.4	14.5	-	0.5	-	-	-
Terrestrial isopod	-	-	-	2.2	1.5	-	-
Insecta							
Collembola*	-	3.7	3.3	4.0	3.3	0.6	1.3
Coleoptera	-	-	-	-	-	0.4	-
Diptera							
Chironomidae	28.2	25.1	25.9	43.3	9.9	15.5	1.7
Other	-	-	1.5	-	1.4	23.0	31.1
Megaloptera	-	-	-	-	-	0.9	-
Plecoptera	-	-	-	2.6	2.3	6.6	-
Trichoptera	-	-	-	1.3	1.3	-	1.3
Gastropoda							
<i>Fontigens tartarea</i> *	55.1	46.1	3.0	1.3	1.7	-	-
Other	1.6	4.9	-	1.8	-	-	-
Acarina	-	-	-	-	-	-	1.6
Diplopoda							
<i>Pseudotremia</i> sp.*	4.0	4.7	12.7	18.0	3.0	0.4	-
Oligochaeta	-	-	3.0	2.1	10.8	39.1	7.8
Turbellaria							
<i>Macrocotyla hoffmasteri</i> *	-	-	1.0	-	-	-	-
Mean density (#/g AFDM)	10.2	8.2	11.0	11.7	7.2	4.7	9.7
Total # of taxa	6	7	11	12	10	10	8
# of Hypogean taxa	4	5	7	5	5	3	4
# of Epigeal taxa	2	2	4	7	5	7	4

\* indicates hypogean taxon

Table 2.6. Mean leaf and wood transport rates, time to 10% mass remaining ( $T_{10}$ ), and penetration distance ( $P_{10}$ ).

Site	Leaves			Wood		
	Transport (m/y)	$T_{10}$ (y)	$P_{10}$ (m)	Transport (m/y)	$T_{10}$ (y)	$P_{10}$ (m)
D1	8.7 <sup>a</sup> (0.6)	1.88	16.4	0 (0)	1.67	0
D2	1.7 <sup>b</sup> (0.2)	1.88	3.2	2.4 (1.5)	1.64	3.9
D3	13.2 <sup>a</sup> (4.3)	0.51	6.7	1.0 (0.9)	1.39	1.4
C1	0 <sup>c</sup> (0)	0.69	0.0	0 (0)	1.57	0
C2	1.9 <sup>b</sup> (0.1)	0.40	0.8	1.2 (0.04)	1.04	1.2
C3	>144* (4.0)	0.93	>134*	-	-	-

Standard errors in parentheses. Transport rates with same letter are not significantly different (Kruskal-Wallis ANOVA on ranks, Dunn's Procedure). \* indicates a minimum estimate as explained in text.

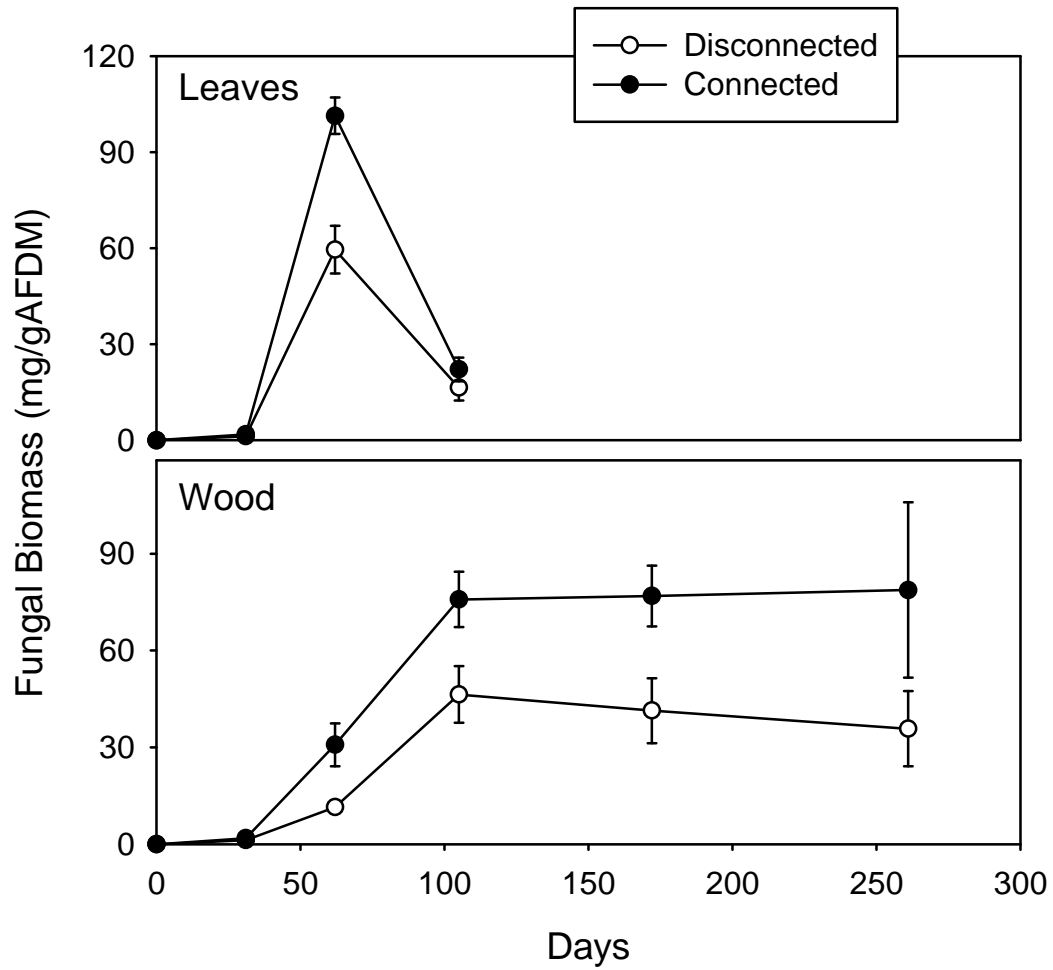


Figure 2.1. Fungal biomass on leaves and wood over time.

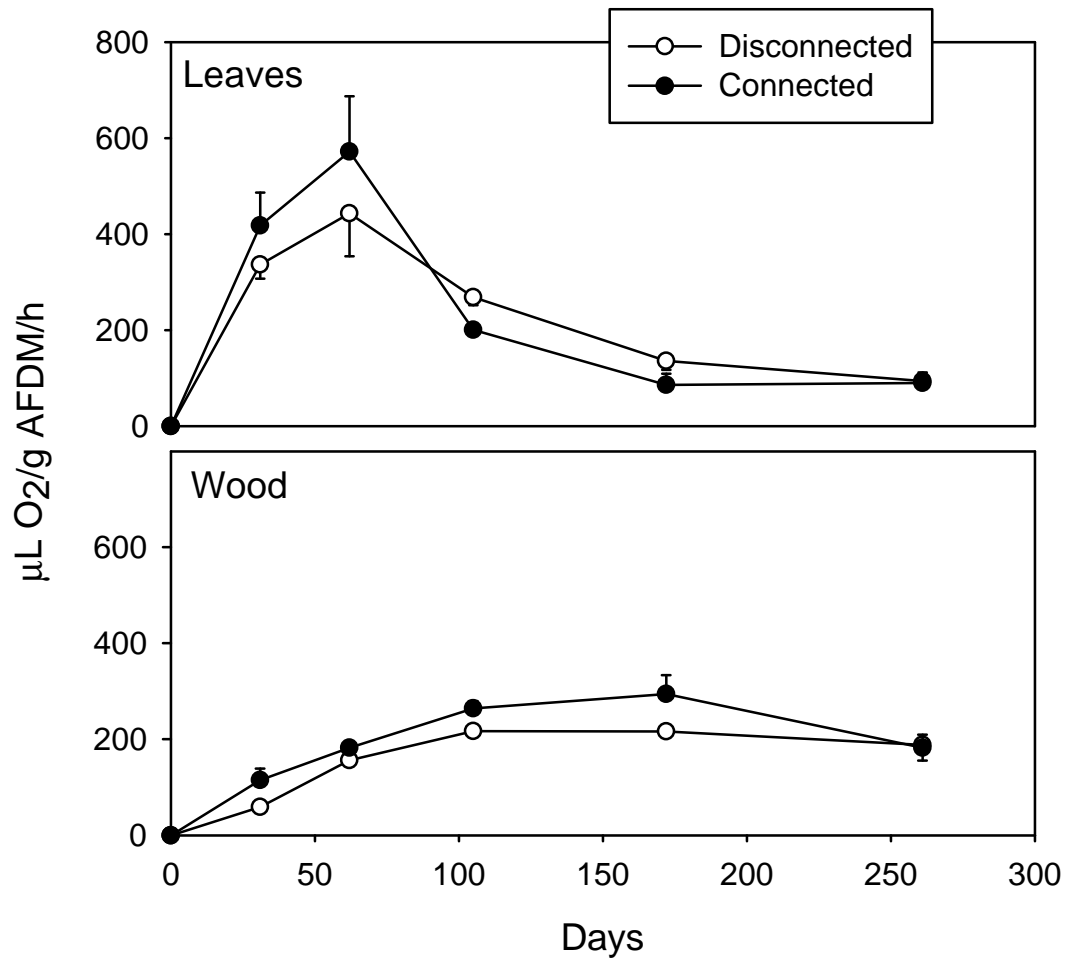


Figure 2.2. Respiration on leaves and wood over time.

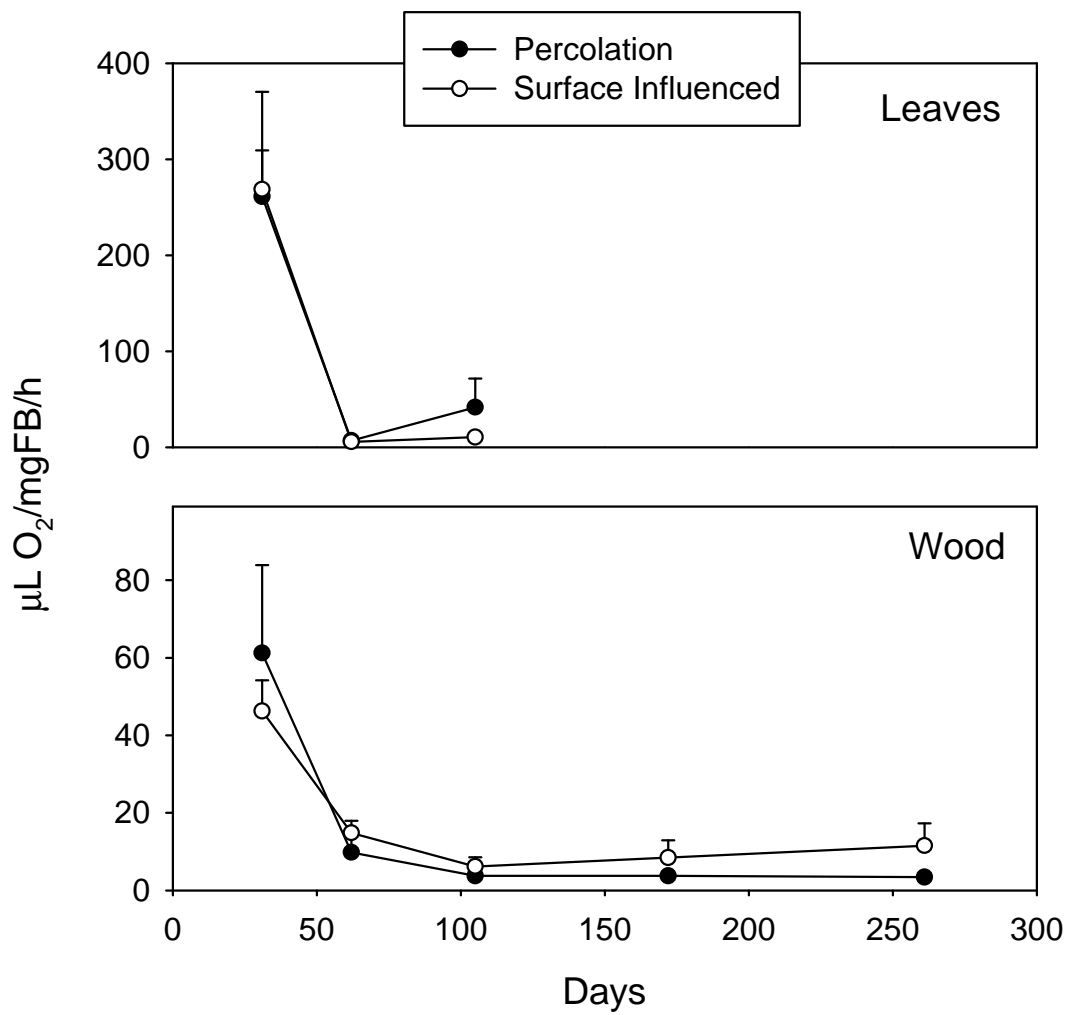


Figure 2.3. Change in respiration:fungal biomass (FB) ratio over time.

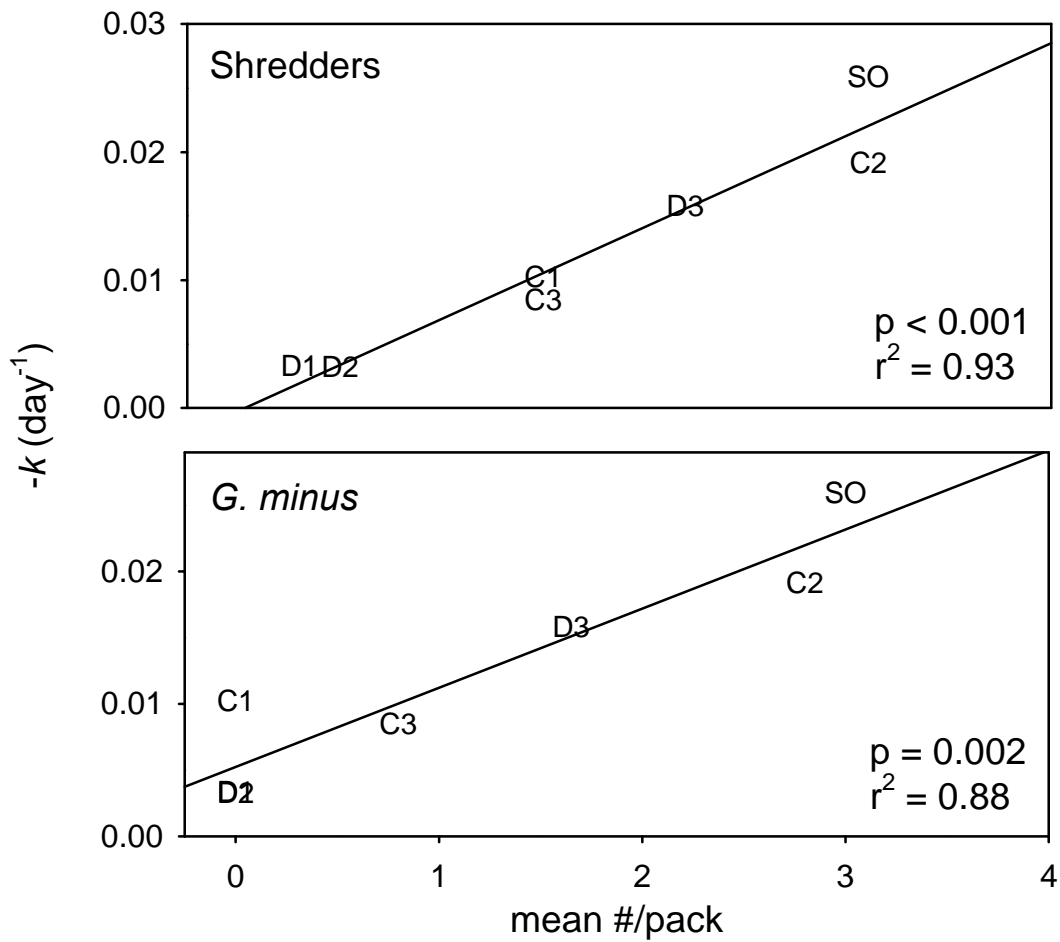


Figure 2.4. Relationship between leaf breakdown rate and shredder and *Gammarus minus* colonization of leaf packs.

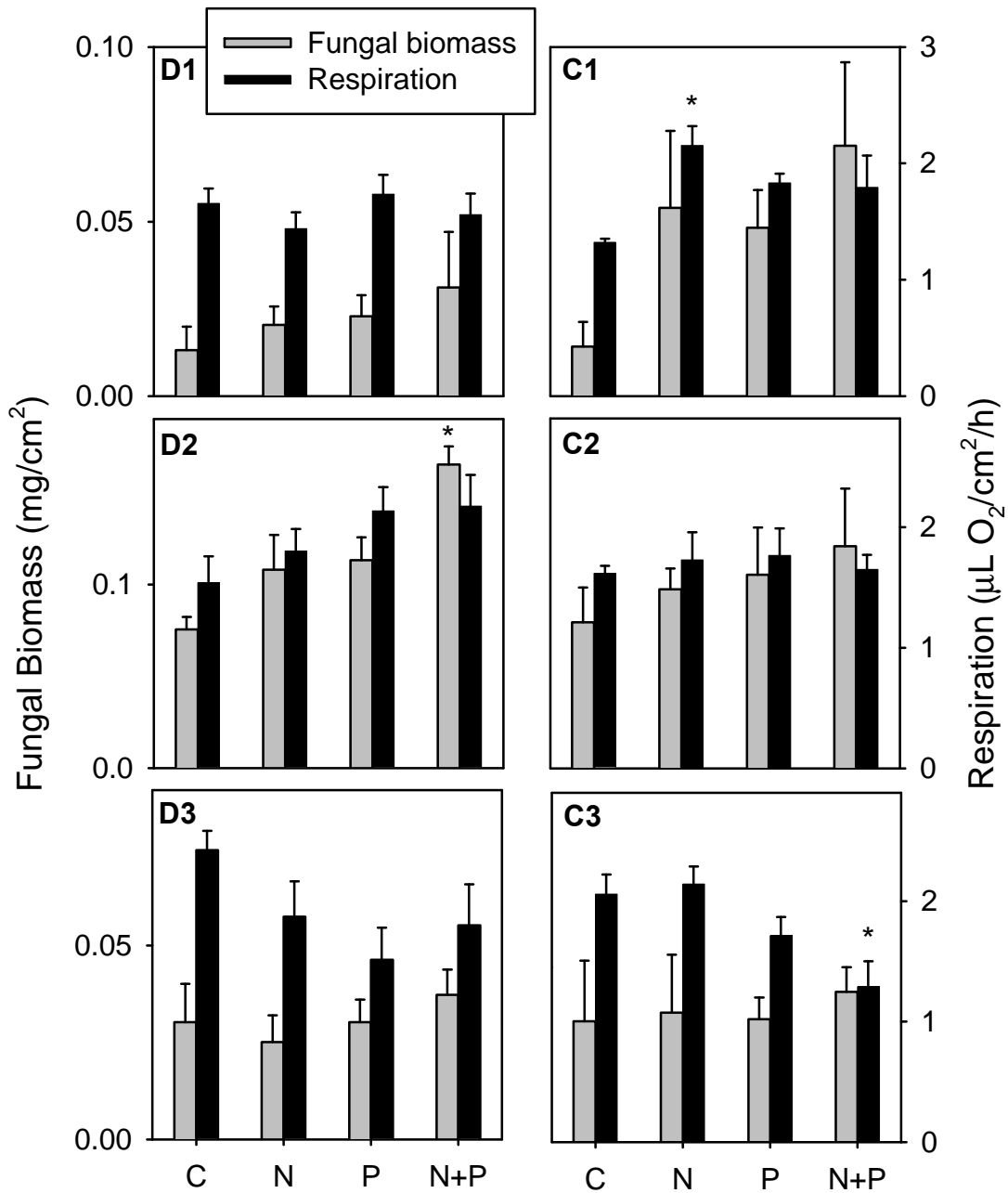


Figure 2.5. Effect of nutrient enrichment on fungal biomass and respiration on wood in each stream. C = control, N = NO<sub>3</sub> added, P = PO<sub>4</sub> added, and N+P = NO<sub>3</sub> and PO<sub>4</sub> added. Means + 1 SE are plotted. N = 5 for each bar. \* indicates treatment significantly different from the control (Dunn's procedure, p < 0.05).

### Chapter 3: Food web structure and the role of bacterial carbon in cave streams.

#### Abstract

We examined trophic structure and the roles of particulate organic matter and bacterial carbon in three cave stream food webs using stable isotope natural abundance ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) ratios and a  $^{13}\text{C}$  acetate tracer addition. Cave stream food webs consisted of three trophic levels: detritus, primary consumers, and predators.  $^{13}\text{C}$  acetate was rapidly taken up in the streams with uptake lengths and rates similar to that in surface streams. Epilithon was highly labeled as were several snails (*Fontigens tartarea*, *Gyraulus parvus*, and *Physa* sp.) that were feeding on epilithon. The stygophilic amphipod *Gammarus minus* and the stygobitic isopod *Caecidotea holsingeri* were primary consumers feeding on FBOM and epilithon. Two stygobitic amphipods, *Stygobromus emarginatus* and *S. spinatus*, and a stygobitic planarian, *Macrocotyla hoffmasteri*, were predators in the streams. The  $^{15}\text{N}$  isotopic signature of DOM, epilithon, and animals suggested that dissolved organic matter originating from surface soils supported stream food webs, including streams with coarse particulate organic matter input from the surface.

**Keywords:** cave, stream, stable isotope, food webs, microbial loop,  $^{13}\text{C}$ ,  $^{15}\text{N}$

## **Introduction**

Food webs and energy flow in ecosystems have long been used to study ecosystem structure and function. The complexity of energy sources and transfers in ecosystems can make food web analysis difficult, but an understanding of the roles of production and consumption is central to community ecology (Polis 1994). Energy transfer through detritus is an important alternative to direct consumption of plants in many terrestrial and aquatic ecosystems. Forested streams and groundwater systems, in particular, depend on detritus as an energy source because autochthonous production is low or absent (e.g., Fisher and Likens 1973, Vannote et al. 1980). Riparian vegetation shades streams, lowering in-stream primary production, but terrestrial plants fuel stream food webs by providing coarse particulate organic matter (CPOM), primarily leaves and wood, to the stream (Fisher and Likens 1973, Minshall et al. 1983). Consumers may use CPOM directly or indirectly by grazing microbial films on the detritus (Cummins 1974), and stream communities are tightly linked to the availability of CPOM (Wallace et al. 1999). Microbial loops, in which dissolved organic carbon (DOC) from detritus in the stream or from groundwater is passed onto consumers through microbial films, are also important components of stream food webs (Meyer 1994, Hall and Meyer 1998).

Like forested surface streams, cave streams rely on detritus for energy. Food limitation appears to be an important force in the evolution and ecology of cave animals (Culver 1982), yet relatively little is known about trophic dynamics in caves. Cave streams are exclusively heterotrophic because light is absent, and, like forested surface streams, cave streams rely on particulate or dissolved organic matter imported from the surface for energy (Culver 1985). Chemoautotrophic production in some cave systems results in high energy availability and also high invertebrate diversity (Sarbu et al. 1996). However, chemoautotrophic groundwater systems are relatively rare and they function differently from most karst ecosystems that use detritus for energy. CPOM enters cave streams through large openings and should be a high quality food source in the energy-poor cave environment. Cave streams fed by water percolating through soil and small fractures in the bedrock do not receive CPOM input and contain only fine particulate organic matter (FPOM) and dissolved organic matter as potential energy sources.

Studying detrital pathways in surface streams is complicated by the presence of both autotrophic and heterotrophic pathways, complex detrital input, and large food webs. Stable isotopes have emerged as a useful tool for untangling complex food webs in many ecosystems, including streams. Studies have used both isotope natural abundance and isotope tracers to examine stream food-webs. Natural abundance studies rely on the characteristic fractionation of isotopes, typically of C, N, or S, during assimilation. Such studies have successfully identified consumer-food relationships in surface streams (e.g., Rounick, et al. 1982, Peterson, et al. 1993), but rely on distinct differences in the isotopic signature of each food source, which often do not occur. Tracer studies have proven useful by allowing researchers to label particular compounds and trace their pathway through the food web (e.g., Peterson et al. 1997, Hall et al. 1998, Hall and Meyer 1998). Studies combining natural abundance ratios and tracer addition (e.g., Mulholland *et al.* in press) have proven particularly useful by taking advantage of the benefits of both approaches.

Cave streams may provide a means of understanding detritus processing in streams by acting as surrogates for the detritus-driven portion of stream food webs. Compared to surface streams, cave streams have: 1) simplified food webs with only detritus as an energy source; 2) similar physical attributes (substrate, flow, riffle-pool sequence); 3) similar community trophic structure (detritus, biofilms, collectors, shredders, scrapers, predators); 4) relatively stable environments; and 5) discrete inputs of organic matter. We used natural abundance ratios of C and N in conjunction with tracer  $^{13}\text{C}$  addition to study food web structure and potential energy sources to cave stream food webs. Our goals were to: 1) clarify trophic structure and feeding relationships among organic matter and animals in cave streams; 2) examine the role of bacterial carbon in stream food webs; 3) investigate the influence of CPOM on stream food webs by comparing cave streams with and without CPOM input.

## **Methods**

### *Study Sites*

We conducted the study in early spring 1999 in Organ Cave, Greenbrier County, West Virginia. Organ cave contains >60 km of passage and a stream network that drains an 8.1km<sup>2</sup> basin. Streams in the cave range from 1<sup>st</sup> to 3<sup>rd</sup> order and vary in connectivity to the surface. Some streams are directly connected to the surface by large openings and consequently receive

CPOM (leaves and wood) from terrestrial vegetation. Other streams are fed only by water that percolates through overlying soil and bedrock and receive DOM and FPOM but not CPOM. There is little evidence of chemoautotrophic production or guano input from bats in the cave streams, so coarse, fine, and dissolved organic matter are the only sources of energy for the stream food webs.

We chose 3 streams, Sively 2, Sively 3, and Jones Canyon, that span the range of CPOM input to Organ Cave streams and contain the bulk of the aquatic species found in the drainage network. These streams are small, 1<sup>st</sup> order streams that flow over bedrock, gravel, and silt. Sively 2 receives the largest amount of CPOM from the surface, including leaf and wood fragments (Table 3.1). Sively 3 is weakly connected to the surface and contains a small amount of CPOM, generally small pieces of wood. Jones Canyon is fed strictly by percolating water and contains no CPOM. FBOM standing stocks were similar in all streams and DOC is similar in Sively 2 and Jones Canyon, but relatively high in Sively 3 (Table 3.1).

The streams contain obligate (stygobite) and facultative (stygochile) cave species as well as some surface (epigean) species although not all animals are present in each stream. Stygobitic animals include amphipod (*Stygobromus emarginatus* and *S. spinatus*) and isopod (*Caecidotea holsingeri*) crustaceans, a planarian (*Macrocotyla hoffmasteri*), and a snail (*Fontigens tartarea*). *Gammarus minus*, a stygophilic amphipod found in surface streams and cave streams, occurs in Sively 2 and Sively 3 only, presumably as a result of its invasion history (Culver et al. 1994). Epigean invertebrates sometimes found in the streams include snails (*Gyraulus parvus* and *Physa* sp.), caddisflies (*Dolophilodes*), and mayflies (*Acentrella*).

### *<sup>13</sup>C tracer release*

We used a tracer addition of <sup>13</sup>C 1-sodium acetate to label bacteria in each stream from 22 April - 20 May 1999. Marriotte bottles containing <sup>13</sup>C 1-sodium acetate dissolved in deionized H<sub>2</sub>O were used to deliver <sup>13</sup>C to the stream at a concentration of ~1 μg<sup>13</sup>C/L (~7 μg acetate/L). Acetate drip rate and injectate concentration were adjusted every other day to maintain a constant <sup>13</sup>C concentration during the release according to changes in stream discharge. Discharge was measured by multiplying stream cross sectional area by velocity, measured with a flow-meter, at the downstream end of the study reach in Sively 2 and Sively 3. We measured discharge in Jones Canyon by directing the entire stream flow into a graduated

cylinder with a piece of metal flashing for 30 s. There should have been no carbon enrichment effect caused by the acetate release because the acetate DOC addition to each stream was small relative to background DOC (0.06, 0.03, and 0.11% of background in Sively 2, Sively 3, and Jones Canyon, respectively).

The method assumes only bacteria take up the  $^{13}\text{C}$  acetate. It is unlikely that fungi took up the label because fungal half-saturation constants for labile DOC are  $\sim 0.1\text{-}1\text{ mmol/L}$  (Newell 1984), which is above the  $\sim 0.1\text{ }\mu\text{mol/L}$  acetate concentration we created in our streams. Bacteria, on the other hand, have half saturation constants of  $\sim 0.001\text{ }\mu\text{mol/L}$  (Newell 1984), well below the acetate concentration we created in the stream. Some abiotic sorption of the label to biofilms probably occurred, however, (Hall and Meyer 1998), found abiotic sorption of  $^{13}\text{C}$  acetate to stream sediments was minimal during similar releases in surface streams.

#### *Sample collection, processing, and analysis*

One day prior to the release, we collected detrital and invertebrate samples along a 55-65m reach in each stream to measure background  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Triplicate samples, from the top, middle, and bottom of the reach, were taken of epilithon, FBOM, and larger invertebrates. Composite samples of leaves, wood scrapings, and animals (5-15 individuals) too small for individual analysis (*F. tartarea*, *G. parvus*, *Physa*, *M. hoffmasteri*, and *S. spinatus*) were also collected throughout the study reach. One dissolved organic matter sample was collected in Sively 3 and Jones Canyon. One L of water was filtered through a  $0.45\text{ }\mu\text{m}$  filter, acidified with concentrated HCl to pH  $\sim 2.5$  to remove inorganic C, and allowed to evaporate at  $40^\circ\text{C}$  in a beaker. After the water had evaporated, the film left in the beaker was collected and analyzed. Natural abundance  $^{13}\text{C}$  and  $^{15}\text{N}$  were used to establish probable trophic relationships using an expected  $\delta^{13}\text{C}$  enrichment of  $\sim 1\text{ ‰}$  between an organism and its food and a  $\delta^{15}\text{N}$  enrichment of  $\sim 2\text{-}4\text{ ‰}$  between trophic levels (Ehleringer et al. 1986, Peterson and Fry 1987, Keough et al. 1996)

In each stream we established stations 5, 10, 20, 40, and 55-65m downstream of the release point for collection during and after the  $^{13}\text{C}$  release. After 28 days, composite samples of 3-15 detrital or animal samples were collected within 2m upstream or downstream of each station. The release was then stopped and collections were made again at the same stations 2 (day 30) and 12 (day 40) days after the release was stopped.

At each station, epilithon was collected by brushing 3-5 rocks with a nylon brush in a small amount of water. FBOM was collected from 5 random locations at each station from the top 1cm of the stream bed using a turkey baster. The FBOM slurry was then passed through a 1mm sieve in the field. In the lab, the epilithon and FBOM slurries were filtered onto pre-ashed 0.45 um glass fiber filters and rinsed 5 times with deionized water to remove any loosely bound  $^{13}\text{C}$  acetate. Epilithon and FBOM were scraped from the filters, taking care not to include filter particles in the sample, and placed into glass vials. Because cave sediments contain large amounts of inorganic C as  $\text{CaCO}_3$ , a few drops of 30% HCl were added to each epilithon and FBOM sample to drive off any inorganic C. At each station, several leaf pieces were collected by hand and the soft outer layer of 3-5 wood pieces was scraped with a knife. Leaves and wood were rinsed 5 times with deionized water and placed in glass vials. All organic matter samples were then dried at 40 °C.

Invertebrates were collected by hand, returned live to the lab, and kept at ambient stream temperature for 24h to allow animals to clear most of their gut contents. Invertebrates were then rinsed 5 times with deionized water and dried at 40 °C. Snails were treated with a few drops of 30% HCl to remove inorganic C in their shells before drying. Not all taxa could be collected at every station on given dates, so not all taxa are well represented in our samples.

Dried organic matter and animal samples were ground with a glass rod. Samples were analyzed by mass spectrometry and, for each sample, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were calculated as:

$$\delta(^{13}\text{C} \text{ or } ^{15}\text{N}) = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000 \text{ (Eq. 1)}$$

where R is the  $^{13}\text{C}/^{12}\text{C}$  or  $^{14}\text{N}/^{15}\text{N}$  ratio.

We calculated the ratio of tracer  $\delta^{13}\text{C}$  in a consumer to tracer  $\delta^{13}\text{C}$  in potential food to determine potential feeding relationships (Mulholland et al. in press). The ratio should equal 1.0 if the organism fed exclusively on one food source and if the organism C was in isotopic equilibrium with its food (the trophic fractionation effect is removed by subtracting background values). Ratios <1.0 indicate the animal used a less enriched food source or had not reached isotopic equilibrium. Ratios >1.0 indicate an organism used a more enriched food source. Ratios at each station where both consumers and food were labeled above background on day 28 were averaged to calculate ratios for each consumer-food source pair. For animals that appeared

to be using two organic matter sources, a mixing model was used to estimate the proportion of each food source in its diet:

$$F_x = \frac{d^{13}C_a - d^{13}C_y}{d^{13}C_x - d^{13}C_y} \quad (\text{Eq. 2})$$

where  $F_x$  is the fraction of carbon from source  $x$ ,  $d^{13}C_a$  is the d28  $\delta^{13}C$  value of the animal minus background  $\delta^{13}C$ , and  $d^{13}C_x$  and  $d^{13}C_y$  are the d28  $\delta^{13}C$  values of food sources  $x$  and  $y$ , respectively, minus background  $\delta^{13}C$  (Gearing 1991).

### *<sup>13</sup>C acetate transport and uptake*

We calculated the acetate uptake length in each stream using the slope of the linear regression of  $\ln(\delta^{13}C)$  of epilithon versus distance downstream ( $x$ ):

$$\ln(\delta^{13}C_x) = \ln(\delta^{13}C_o) - kx \quad (\text{Eq. 3})$$

where  $\delta^{13}C_x$  is the epilithon  $\delta^{13}C$  value at distance  $x$  below the injection site (Hall and Meyer 1998). Thirty was added to each  $\delta^{13}C$  value to raise it above zero for  $\ln$  transformation. Only sites whose  $\delta^{13}C$  values were above background levels were used in the regressions.  $^{13}C$  uptake length ( $S$ ), the average distance  $^{13}C$  acetate traveled downstream before uptake, was calculated as the inverse of the slope ( $k$ ) (Newbold et al. 1981). We used epilithon for calculations because it was most highly labeled, should represent the primary point of  $^{13}C$  uptake in the stream, and should not be displaced downstream under normal flow because it is attached to rocks.

$^{13}C$  uptake rate ( $U$ ) in each stream was calculated as:

$$\sum_{x=1}^{20} U_x = (C_x \times Q \times 3600s / h) / S \quad (\text{Eq. 4})$$

where  $C_x$  is the concentration of acetate  $^{13}C$  (in mg/L) at each meter ( $x$ ) below the release site,  $Q$  is the average stream discharge, in L/s, during the release, and  $S$  is the  $^{13}C$  uptake length (Newbold et al. 1981, Hall and Meyer 1998).  $C_x$  was calculated using  $C_o$  (average  $^{13}C$  concentration at the release site) and  $S$  in Eq. 3.  $U_x$  was summed for a 20-m reach in each stream and divided by the area of each stream reach to determine  $^{13}C$  acetate uptake per square meter per hour. We used a 20m reach in the calculation because it was the maximum distance in which epilithon was reliably labeled above background in each stream.

We estimated epilithon C turnover time ( $T_t$ ) using the loss of tracer  $\delta^{13}C$  (sample  $\delta^{13}C$  minus background  $\delta^{13}C$ ) in epilithon between days 30 and 40. We assumed a logarithmic

decline in the  $^{13}\text{C}$  label and calculated  $T_t$  as  $1/\text{slope}$  for the regression of  $\delta^{13}\text{C}$  versus time for each station. For each stream,  $T_t$  values from each station were then averaged.

## Results

### *Natural abundance ratios*

Differences in the  $\delta^{15}\text{N}$  values between the base (detritus) and the top (*M. hoffmasteri* or *Stygobromus*) of the food web were 12.6, 11.4 and 7.6 ‰ in Sively 2, Sively 3, and Jones Canyon, respectively. Detritus and organisms and generally fell into groups representing 3 trophic levels in each stream (Figure 3.1). DOM, FBOM, and epilithon were the likely food sources for primary consumers as they were from 2 - 4 ‰ lower than *Caecidotea holsingeri*, *Dolophilodes*, *F. tartarea*, *G. minus*, *G. parvus*, and *Physa*. Leaves and wood were more than 4 ‰ lower than all invertebrates (Figure 3.1). *Macrocotyla hoffmasteri*, *S. emarginatus*, and *S. spinatus* were within 2 - 4 ‰ more enriched than one or more primary consumers and appear to be predators.

Probable animal-food pairs based on background  $\delta^{13}\text{C}$  values were generally consistent with the presumed trophic levels based on  $\delta^{15}\text{N}$  values. *Caecidotea holsingeri*, *Dolophilodes*, *F. tartarea*, *G. minus*, *G. parvus*, and *Physa* were  $\sim 1$  ‰  $\delta^{13}\text{C}$  enriched relative to epilithon, FBOM, or wood (Table 3.2). *Macrocotyla hoffmasteri*, *S. emarginatus*, and *S. spinatus* were most likely eating primary consumers (Table 3.2). Because most taxa were  $\sim 1$  ‰ more enriched than several potential foods, specific consumer-food pairs could not be distinguished using background  $\delta^{13}\text{C}$ .

### *Detrital labeling and $^{13}\text{C}$ uptake*

Leaves and wood were not labeled above background, except for wood at 5 m in Sively 2 (Figure 3.2). Epilithon, on the other hand, was highly labeled in all three streams by day 28 and epilithon  $\delta^{13}\text{C}$  decreased exponentially downstream (Figure 3.3). Epilithon most highly labeled in Sively 3 where tracer  $\delta^{13}\text{C}$  was 2 and 7 times higher than in Sively 2 or Jones Canyon, respectively.  $^{13}\text{C}$  uptake length calculated from epilithon  $\delta^{13}\text{C}$  was very short in Sively 2 and  $\sim 5$ -7 times longer in Sively 3 and Jones Canyon, respectively (Table 3.3).  $^{13}\text{C}$  uptake rate was highest in Sively 2 (Table 3.3), intermediate in Jones Canyon, and lowest in Sively 3. FBOM was labeled above background at a much lower level than epilithon, and the label decreased

exponentially downstream (Figure 3.4). FBOM labeling was highest in Sively 3 where tracer  $\delta^{13}\text{C}$  2-2.5 times higher than in Sively 2 or Jones Canyon.

In all three streams, epilithon and FBOM were generally more labeled two days after the release was stopped than on day 28 (Figures 3.5, 3.6, 3.7). By 12 days post-release, epilithon and FBOM  $\delta^{13}\text{C}$  fell to levels equal to or below day 28 values. Mean epilithon turnover time was highest in Sively 2 but was not significantly different among streams (ANOVA,  $p > 0.05$ ).

### *Invertebrate labeling*

All invertebrates were labeled above background, but the magnitude and longitudinal and temporal pattern of  $^{13}\text{C}$  enrichment varied among taxa. *Dolophilodes* was uncommon in the streams, but  $\delta^{13}\text{C}$  at 10 m in Sively 2 reached 302‰, 329‰ higher than its background  $\delta^{13}\text{C}$  and ~3 times the maximum epilithon  $\delta^{13}\text{C}$  at that site. The snails *F. tartarea*, *G. parvus*, and *Physa* were all highly labeled and their longitudinal pattern of labeling was similar to that of epilithon in each stream (Figure 3.3). The temporal pattern of *F. tartarea*  $\delta^{13}\text{C}$  followed a pattern similar to epilithon:  $\delta^{13}\text{C}$  increased to day 30 then fell to roughly day 28 levels by 12 days post-release (Figures 3.5 and 3.7). *Gyraulus parvus* and *Physa*  $\delta^{13}\text{C}$  increased or remained roughly the same as day 28 values after the release was stopped (Figure 3.6)

In both Sively 3 and Sively 2, *G. minus* was less enriched than the snails and epilithon. Individuals at the site closest to the release were less labeled than those at downstream stations (Figure 3.4). *G. minus* is very mobile and probably was moving above and below the release point, diluting the label in at the upper stations. Because of this, we did not include the upstream station in any further *G. minus* calculations. *Gammarus minus*  $\delta^{13}\text{C}$  values below the upper stations were slightly above FBOM but below epilithon, suggesting that *G. minus* was feeding on both foods. Using a mixing model incorporating epilithon and FBOM  $\delta^{13}\text{C}$ , we determined *G. minus*' diet consisted of 82% ( $\pm 10$ , SD) and 92% ( $\pm 23$ , SD) FBOM in Sively 2 and Sively 3, respectively.

*S. emarginatus* was less enriched than *G. minus* but followed a roughly similar longitudinal pattern in Sively 2 on day 28. *Caecidotea holsingeri*, *S. spinatus*, and *M. hoffmasteri* were enriched to slightly lower levels than *G. minus* but these animals were not as well represented in the sampling (Figure 3.4). *Gammarus minus*, *S. emarginatus*, and *S. spinatus*

$\delta^{13}\text{C}$  continued to increase or did not change at most stations up to 12 days post-release (Figures 3.5, 3.6, and 3.7)

*Invertebrate:food  $\delta^{13}\text{C}$  ratios:*

*Fontigens:epilithon* and *Gyraulus:epilithon* ratios were slightly below 1.0 suggesting that these snails had not yet reached isotopic equilibrium or were including a less enriched food, possibly FBOM, in their diets. The high *Fontigens:epilithon* ratio (2.91) in Sively 2 was due primarily to a high *Fontigens*  $\delta^{13}\text{C}$  value at 20m; the ratio calculated without that site included was 1.12 ( $\pm 0.19$ ). *Physa:epilithon* was close to 1.0 (Table 3.4) suggesting *Physa* was primarily consuming epilithon. *Gammarus:FBOM* exceeded 1.0 in both Sively 2 and Sively 3 (Table 3.4), indicating *Gammarus* was consuming a more highly enriched food source, probably epilithon, in addition to FBOM. *Macrocotyla hoffmasteri:prey* ratios were near of slightly above 1.0 for *G. minus*, *S. spinatus*, and *F. tartarea* (Table 3.4). *Stygobromus emarginatus:prey* ratios were well below 1.0 for both *F. tartarea* and *G. minus*.

## **Discussion**

The pattern of  $^{13}\text{C}$  acetate labeling by epilithon and FBOM in our streams was similar to that observed in a forested headwater stream (Hall and Meyer 1998). Epilithon in cave streams should be primarily bacterial films because there is no light to support algae and little POM to support fungi. High labeling of cave epilithic biofilms is not surprising because bacteria on rocks should rely heavily on DOC as a carbon supply. Lower labeling of FBOM, compared to epilithon, suggests bacteria in the sediments were using both DOC and particulate organic matter as carbon substrates. Bacteria in surface stream sediments have been shown to rely on both streamwater DOC and sediment organic matter (Bott *et al.* 1984). We assumed fungi would not take up  $^{13}\text{C}$  acetate because we added it at very low concentration (Hall and Meyer 1998). Leaves and wood, whose biofilms are dominated by fungi (Weyers and Suberkropp 1996), were not highly labeled in our streams supporting our assumption. Leaves and wood were not labeled in a similar  $^{13}\text{C}$  release in a surface stream (Hall and Meyer 1998).

$^{13}\text{C}$  uptake lengths and rates in the cave stream were similar to those measure by Hall and Meyer (1998) in a forested stream. Rapid DOC uptake in the cave streams is not surprising considering the scarcity of organic C in caves. Our measured uptake lengths and rates

overestimate natural DOC uptake because acetate should be more labile than bulk DOC in the streamwater. The stream with CPOM input, Sively 2, had a short uptake length and intermediate labeling of epilithon and FBOM, indicating bacteria in the stream were quickly removing  $^{13}\text{C}$  acetate from the water column. In Jones Canyon, relatively long  $^{13}\text{C}$  uptake length and low epilithon and FBOM labeling suggest there was little bacterial activity in the stream. In Sively 3, uptake length was long, but surprisingly, epilithon and FBOM labeling was very high compared to Sively 2. Longer uptake length may have been due to higher discharge and perhaps lower bacterial biomass or activity per unit area in Sively 3. Hall and Meyer (1998) found epilithon and sediment bacteria were more highly labeled in litter excluded surface streams than in reference streams. They concluded bacteria were relying more heavily on streamwater DOC than on POC in the sediments in the litter excluded streams. Bacteria on FBOM in Sively 3 may have been relying more on streamwater DOC than bacteria in the other streams did, causing the higher label. This does not explain differences in epilithon labeling between streams because epilithon should use only DOC. We do not have data about DOC quality in our streams, but if DOC were of higher quality in Sively 2, possibly from leaves and wood in the stream, epilithic bacteria in that stream may have been using more natural DOC, reducing the  $^{13}\text{C}$  label, than in Sively 3 where DOC was more abundant, but perhaps of lesser quality.

Increase in the epilithon label 2 days after the release stopped may have been due to uptake of  $^{13}\text{C}$  left in storage in the stream and low epilithon C turnover. Loss of the  $^{13}\text{C}$  label from epilithon and FBOM was surprisingly slow given the rapid turnover rates of bacteria. Abiotic sorption of DOC to biofilms may have occurred (McDowell 1985, McKnight et al. 1992); however, most DOC uptake by sediments is biotic (Lock and Hynes 1976, Dahm 1981).  $^{13}\text{C}$  taken up by bacteria may have been incorporated into exopolymers, which may have a much longer turnover rate than bacteria (Hall and Meyer 1998), rather than respired. Regardless of the mechanism, the acetate DOC was immobilized for relatively long periods of time in the biofilms. Immobilization, either biotic or abiotic, of DOC by stream and hyporheic sediments can be high (Fiebig and Lock 1991, Fiebig and Marxsen 1992). If bacteria rapidly respire DOM C, microbial loops are inefficient and are not important energy sources for higher trophic levels (Pomeroy and Wiebe 1988). Because DOC was immobilized in cave microbial films, rather than rapidly lost as  $\text{CO}_2$ , the microbial loop may more efficiently transfer energy from DOM to invertebrate

consumers in cave streams. This is demonstrated by the high labeling of snails (*F. tartarea*, *G. parvus*, *Physa*) in our study.

*Fontigens tartarea*, *G. parvus*, and *Physa* are found on rocks in the streams and feed on epilithon as scrapers. Natural abundance  $^{15}\text{N}$  values place them one trophic level above epilithon and their high  $^{13}\text{C}$  enrichment and longitudinal pattern strongly tie the snails to epilithon.

*Dolophilodes* was also highly labeled, with  $\delta^{13}\text{C}$  values above epilithon. *Dolophilodes* constructs a very fine net capable of filtering bacteria-sized particles (Malas and Wallace 1977) and was probably feeding on suspended bacteria and DOC colloids in the water column that would have been highly labeled. These caddisflies are commonly found in the cave streams, but their populations are probably sustained by recruitment from the surface. Even if they are not persistent populations, they are participating in the stream food web as filterers, a niche apparently not occupied by any cave species.

The surface form of *G. minus* is generally considered a facultative shredder but has a broad diet including leaves, FBOM, suspended particulate organic matter, and biofilms (Haley 1997, Mulholland et al. in press). The trophic position of *G. minus* in cave streams has been somewhat ambiguous. The cave form of *G. minus* shreds leaves in the laboratory and apparently in cave streams as well (Chapter 2). Culver et al. (1991) found that *G. minus* is a predator of *C. holsingeri* in Sively 3 and in laboratory streams. Our  $^{15}\text{N}$  and tracer  $^{13}\text{C}$  results suggest *G. minus* is feeding primarily on FBOM and, to a lesser extent, epilithon. *Gammarus minus* may be a predator or shredder when prey and leaves are available, but *C. holsingeri* were rare in our streams and most leaves were small, refractory pieces. Wood was abundant in Sively 2, but neither  $^{15}\text{N}$  nor tracer  $^{13}\text{C}$  results suggested a direct link between *G. minus*, or any other animals, and wood.

Based on natural abundance  $^{15}\text{N}$ , there are 3 invertebrate predators in Organ Cave streams: *M. hoffmasteri*, *S. emarginatus*, and *S. spinatus*. Tracer  $^{13}\text{C}$  showed *M. hoffmasteri* could have been preying on *F. tartarea*, *G. minus*, or *S. spinatus*. *S. spinatus* is not a likely prey item because *M. hoffmasteri* and *S. spinatus* had similar  $\delta^{15}\text{N}$ . Had *M. hoffmasteri* been feeding on *S. spinatus* it would have been relatively  $^{15}\text{N}$  enriched. The stygophilic planarian *Phagocata gracilis* feeds on newly hatched *G. minus* (Jenio 1972), and *M. hoffmasteri* may do the same in Sively 2 and Sively 3. However, we cannot exclude *F. tartarea* or *C. holsingeri* as possible prey in some streams.

Ratios of *S. emarginatus*  $\delta^{13}\text{C}$  to potential prey  $\delta^{13}\text{C}$  were below 1.0, but *S. emarginatus* apparently was not at isotopic equilibrium with its prey. *Stygobromus emarginatus*'  $\delta^{13}\text{C}$  continued to increase up to 10 days post-release. Stygobitic amphipods can grow quite slowly (Dickson 1979) and *S. emarginatus* is relatively large, so it probably did not turn over all of its C during the release. Previous research in Sively 2 suggested that *S. emarginatus* could be a predator of *G. minus* (Culver et al. 1991).

We were surprised that *S. spinatus* would be a predator in these streams given its small size, ~5mm length. The only primary consumers in Jones Canyon, where *S. spinatus* occurs, are *C. holsingeri* and *F. tartarea*. *S. spinatus* and *C. holsingeri* showed no predator-prey interaction in field experiments in Jones Canyon and another Organ Cave stream (Culver et al. 1991). This leaves *F. tartarea* as the only likely food source for *S. spinatus* in Jones Canyon. *S. spinatus* also could be acting as a scavenger, feeding on dead or disabled *C. holsingeri*.

### *Food Webs*

The food webs in all three streams are quite similar despite differences in CPOM input (Figure 3.8). In each stream there are two pathways from organic matter to consumers: epilithon to snails and FBOM/epilithon to *Gammarus minus* and *C. holsingeri*. One or more predators feed on primary consumers in each stream, but the specific predator-prey pathways are not clear. Leaves and wood are not primary foods for any of the cave stream animals. Leaf and wood  $\delta^{15}\text{N}$  in our streams (+2 - +4 ‰) were similar to leaf and wood values in surface streams (-4 to +1 ‰; Fry 1991, Mulholland et al. in press), but the  $\delta^{15}\text{N}$  values for primary consumers (>+8.9 ‰) in all of the cave streams were beyond the values expected (+4 - +8 ‰) if they were feeding on leaves or wood. Natural  $\delta^{15}\text{N}$  of all animals in our streams (8.9 to 16.0 ‰) was high compared to  $\delta^{15}\text{N}$  of animals in surface streams (range = ~0 to 8 ‰; Fry 1981). Epilithon and FBOM also were  $^{15}\text{N}$  enriched compared to similar material in surface streams (<4 ‰; Fry 1991, Peterson et al. 1993, Mulholland et al. in press). The  $\delta^{15}\text{N}$  of epilithon, FBOM, and animals in the streams is more consistent with a food web based on soil organic matter, which is relatively  $^{15}\text{N}$  enriched (range -4 to +14 ‰; Peterson and Fry 1987) than with a food web based on terrestrial leaves and wood. DOM  $\delta^{15}\text{N}$  was equal to or slightly below epilithon in Sively 3 and was below epilithon and FBOM in Jones Canyon. DOM in Sively 2 and Sively 3 may be a mix of organic matter leached from soils above the cave and DOC from leaves and wood in the stream (Meyer *et al.*

1997). DOM in Jones Canyon must come exclusively from soil because there is no CPOM in the stream.

Soils should be the source of FBOM in Jones Canyon (Figure 3.8). FBOM in Sively 2 and Sively 3 may be a mixture of wood and DOM-derived material. Bacteria in Sively 2 and Sively 3 sediments that used DOM from the water column and POM from leaves or wood could explain the intermediate FBOM  $\delta^{15}\text{N}$  value in those streams. Bacteria in surface stream sediments derive C from both POM and DOM in the water column (Bott et al. 1984). FPOM tracer  $^{13}\text{C}$  labeling also was intermediate between epilithon, which uses DOM only, and wood. In comparison, FBOM in Jones Canyon, which has no CPOM, had a  $\delta^{15}\text{N}$  similar to DOM and epilithon. Wood input appears to influence Organ Cave streams indirectly as a source of FBOM rather than being consumed by animals. Wood placed in Sively 2, Sively 3, and Jones Canyon broke down slowly and showed no evidence of invertebrate feeding (Chapter 2). Leaves may be a patchy resource in space and time than can be used directly when available. Whole leaves placed in Sively 2 and Sively 3 quickly lost mass and were shredded by invertebrates, particularly *G. minus* (Chapter 2). However, during our study, leaves were only available as small, refractory fragments that were probably nutritionally similar to wood. Leaves placed in Jones Canyon lost mass slowly, at a rate similar to wood (Chapter 2). The Jones Canyon food web, which naturally does not receive CPOM input, apparently is not equipped to efficiently use leaves and wood.

### *Summary*

Previous research has shown that sediment organic matter and microorganisms are important food sources for cave invertebrates (Ginet 1960, Dickson 1979). Our results suggest soil organic matter is the carbon source fueling bacterial films on cave stream sediments. These bacteria, in turn, are supporting consumers and predators:  $^{13}\text{C}$  acetate was immobilized in epilithon and primary consumers and predators were  $^{13}\text{C}$  labeled. Bacterial carbon is important in surface streams, where it makes up a large portion of the energy requirements of many stream invertebrates (Hall and Meyer 1998). The pattern of  $^{13}\text{C}$  uptake and bacterial carbon use is similar in cave and surface streams suggesting heterotrophic pathways are similar in the two systems. Although cave streams have fewer species than most surface streams, cave streams contain a similar number of trophic levels and most detrital-based feeding groups (scrapers,

collectors, and predators). DOM alone supported a fairly complex food web in one stream, Jones Canyon, and was important even in streams with CPOM input. The similarity between surface and cave streams and the distinct DOM and POM sources suggest cave streams can be useful surrogates for studying DOM driven pathways in streams.

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## References

- Bott, T. L., L. A. Kaplan and F. T. Kuserk. 1984. Benthic bacterial biomass supported by streamwater dissolved organic matter. *Microbial Ecology* 10:335-344.
- Culver, D. C. 1985. Trophic relationships in aquatic cave environments. *Stygologia* 1:43-53.
- Culver, D.C. 1982. *Cave Life: Evolution and Ecology*. Harvard University Press, Cambridge, MA. 189p.
- Culver, D. C., D. W. Fong and R. W. Jernigan. 1991. Species interactions in cave stream communities: experimental results and microdistribution effects. *American Midland Naturalist* 126:364-379.
- Culver, D. C., W. K. Jones, D. W. Fong and T. C. Kane. 1994. Organ Cave karst basin. 451-473 in J. Gibert, D. L. Danielopol and J. A. Stanford (eds). *Groundwater Ecology*. Academic Press, San Diego.
- Cummins, K. W. 1974. Structure and function of stream ecosystems. *BioScience* 24:631-641.
- Dahm, C. N. 1981. Pathways and mechanisms for removal of dissolved organic carbon from leaf leachate in streams. *Canadian Journal of Fisheries and Aquatic Sciences* 38:68-76.
- Dickson, G. W. 1979. The importance of cave mud sediments in food preference, growth, and mortality of the troglobitic amphipod crustacean *Crangonyx antennatus* Packard (Crangonyctidae). *Crustaceana* 36:129-140.
- Ehleringer, J.R., P.W. Rundel and K.A. Nagy. 1986. Stable isotopes in physiological ecology and food web research. *Trends in Ecology and Evolution* 1:42-45.
- Fiebig, D. M. and M. A. Lock. 1991. Immobilization of dissolved organic matter from groundwater discharging through the stream bed. *Freshwater Biology* 26:45-55.
- Fiebig, D. M. and J. Marxsen. 1992. Immobilization and mineralization of dissolved free amino acids by stream-bed biofilms. *Freshwater Biology* 28:129-140.
- Fisher, S. G. and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecological Monographs* 43:421-439.
- Fry, B. 1991. Stable isotope diagrams of freshwater food webs. *Ecology* 72:2293-2297.
- Gearing, J.N. 1991. The study of diet and trophic relationships through natural abundance  $\delta^{13}C$ . 201-218 in D.C. Coleman and B. Fry (eds). *Carbon Isotope Techniques*. Academic Press, San Diego.

- Ginet, R. 1960. Ecologie, écologie et biologie de *Niphargus* (Amphipodes Gammaridés hypogés). *Annales de Biospeologie* 15:127-376.
- Haley, C.J. 1997. Comparisons of secondary production, life history, and mouthpart functional morphology between two populations of the amphipod *Gammarus minus*. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA. 93 p.
- Hall, R. O., B. J. Peterson and J. L. Meyer. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. *Ecosystems* 1:283-298.
- Hall, R. O. and J. L. Meyer. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79:1995-2012.
- Jenio, F. 1972. The *Gammarus* of Elm Spring, Union County, Illinois (Amphipoda:Gammaridae). Ph.D. dissertation, Southern Illinois University, Carbondale, IL. 182 p.
- Keough, J.R., M.E. Sierszen and C.A. Hagley. 1996. Analysis of a Lake Superior coastal food web with stable isotope techniques. *Limnology and Oceanography* 41:136-146.
- Lock, M. A. and H. B. N. Hynes. 1976. The fate of "dissolved" organic carbon derived from autumn-shed maple leaves (*Acer saccharus*) in a temperate hard-water stream. *Limnology and Oceanography* 21:436-443.
- Malas, D. and J. B. Wallace. 1977. Strategies for coexistence in three species of net-spinning caddisflies (Trichoptera) in second-order Appalachian streams. *Canadian Journal of Zoology*. 55:1829-1840.
- McDowell, W. H. 1985. Kinetics and mechanisms of dissolved organic carbon retention in a head water stream. *Biogeochemistry* 1:329-352.
- McKnight, D.M., K.E. Bencala, G.W. Zellweger, G.R. Aiken, G.L. Feder, and K.A. Thorn. 1992. Sorption of dissolved organic carbon by hydrous aluminum and iron oxides occurring at the confluence of Deer Creek and the Snake River, Summit County, Colorado. *Environmental Science and Technology* 26:1388-1396.
- Meyer, J. L. 1994. The microbial loop in flowing waters. *Microbial Ecology* 28:195-199.
- Meyer, J.L., J.B. Wallace, S.L. Eggert. 1997. Leaf litter as a source of dissolved organic carbon in streams. *Ecosystems* 0:1-11.
- Minshall, G. W., R. C. Petersen, K. W. Cummins, T. L. Bott, J. R. Sedell, C. E. Cushing and R. L. Vannote. 1983. Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs* 53:1-25.

- Mulholland, P.J., J.L. Tank, D.M. Sanzone, W.M. Wollheim, B.J. Peterson, J.R. Webster and J.L. Meyer. In press. Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a  $^{15}\text{N}$  tracer addition. *Journal of the North American Benthological Society*.
- Newbold, J. D., J. W. Elwood, R. V. O'Neill and W. VanWinkle. 1981. Measuring nutrient spiraling in streams. *Canadian Journal of Fisheries and Aquatic Sciences* 38:860-863.
- Newell, S.Y. 1984. Bacterial and fungal productivity in the marine environment: a contrastive overview. *Colloques Interationaux Centre National Recherches Scientifique (Marseilles)* 331:133-139.
- Peterson, B.J., M. Bahr and G.W. Kling. 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. *Canadian Journal of Fisheries and Aquatic Sciences* 54:2361-2367.
- Peterson, B., B. Fry, L. Deegan and A. Hershey. 1993. The trophic significance of epilithic algal production in a fertilized tundra river ecosystem. *Limnology and Oceanography* 38:872-878.
- Peterson, B. J. and B. Fry. 1987. Stable carbon isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293-320.
- Polis, G. A. 1994. Food webs, trophic cascades and community structure. *Australian Journal of Ecology* 19:121-136.
- Pomeroy, L.R. and W.J. Wiebe. 1988. Energetics of microbial food webs. *Hydrobiologia* 159:7-18.
- Rounick, J. S., M. J. Winterbourn and G. L. Lyon. 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. *Oikos* 39:191-198.
- Sarbu, S. M., T. C. Kane and B. K. Kinkle. 1996. A chemoautotrophically based cave ecosystem. *Science* 272:1953-1955.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130-137.
- Wallace, J.B., S.L. Eggert, J.L. Meyer and J.R. Webster. 1999. Effects of resource limitation on a detrital-based ecosystem. *Ecological Monographs* 69:409-442.
- Weyers, H. S. and K. Suberkropp. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. *Journal of the North American Benthological Society* 15:408-420.

Table 3.1. Coarse (CBOM) and fine (FBOM) benthic organic matter standing stocks, suspended particulate organic matter (SPOM), and dissolved organic carbon (DOC) in the study streams.

<b>Variable</b>	<b>Sively 2</b>	<b>Sively 3</b>	<b>Jones Canyon</b>
CBOM (g AFDM/m <sup>2</sup> )	17.8	0.09	0
FBOM (g AFDM/m <sup>2</sup> )	48.6	28.2	37.4
SPOM (mg AFDM/L)	1.21	0.71	0.48
DOC (mg/L)	1.8	3.4	1.2

CBOM and FBOM are means of 10 samples, date. SPOM and DOC are means of 5-11 dates, 1998-99.

Table 3.2. Difference between background  $\delta^{13}\text{C}$  of consumers and potential food sources. Only consumer-food combinations close to expected  $\delta^{13}\text{C}$  difference of  $\sim 1\text{‰}$  are shown.

Site	Consumer	Food	Difference (‰)
Sively 3	<i>G. rugosa</i>	FBOM	1.3
Sively 3	<i>G. rugosa</i>	wood	1.4
Jones Canyon	<i>C. holsingeri</i>	epilithon	1.8
Jones Canyon	<i>C. holsingeri</i>	FBOM	0.7
Sively 2	<i>Dolophilodes</i>	leaves	1.0
Jones Canyon	<i>F. tartarea</i>	epilithon	1.4
Sively 3	<i>G. minus</i>	FBOM	1.8
Sively 2	<i>G. minus</i>	FBOM	1.8
Sively 2	<i>G. minus</i>	wood	0.7
Jones Canyon	<i>M. hoffmasteri</i>	<i>C. holsingeri</i>	1.3
Sively 2	<i>M. hoffmasteri</i>	<i>F. tartarea</i>	0.6
Jones Canyon	<i>M. hoffmasteri</i>	<i>S. spinatus</i>	0.5
Jones Canyon	<i>Physa</i>	epilithon	0.8
Sively 3	<i>S. emarginatus</i>	<i>G. rugosa</i>	1.1
Sively 2	<i>S. emarginatus</i>	<i>F. tartarea</i>	1.0
Sively 3	<i>S. emarginatus</i>	<i>F. tartarea</i>	0.7
Sively 3	<i>S. emarginatus</i>	<i>G. minus</i>	0.6
Jones Canyon	<i>S. emarginatus</i>	<i>M. hoffmasteri</i>	0.8
Jones Canyon	<i>S. emarginatus</i>	<i>S. spinatus</i>	1.4
Jones Canyon	<i>S. spinatus</i>	<i>C. holsingeri</i>	0.8
Jones Canyon	<i>S. spinatus</i>	<i>F. tartarea</i>	1.2

Table 3.3. Stream size, average  $^{13}\text{C}$  concentration,  $^{13}\text{C}$  uptake length and rate, and epilithon turnover time ( $T_t$ ) in each stream.

<b>Variable</b>	<b>Sively 2</b>	<b>Sively 3</b>	<b>Jones Canyon</b>
average discharge (L/s)	0.43	1.19	0.03
average width (m)	0.3	1.0	0.2
average $^{13}\text{C}$ concentration (mg/L)	0.0011	0.0011	0.0013
$^{13}\text{C}$ uptake length (m)	5.4	26.0	35.2
$^{13}\text{C}$ uptake rate (mg/m <sup>2</sup> /h)	0.23	0.04	0.19
epilithon $T_t$ (d)	17.0	13.3	12.7

Table 3.4. Consumer:potential food  $\delta^{13}\text{C}$  ratios on day 28 corrected for background  $\delta^{13}\text{C}$ . Ratios are means of values for each station (n ranges 2-5). Standard deviations are in parentheses.

Site	Consumer	Food	Ratio
Sively 3	<i>G. rugosa</i>	epilithon	0.68 (0.07)
Jones Canyon	<i>F. tartarea</i>	epilithon	0.49 (0.22)
Sively 2	<i>F. tartarea</i>	epilithon	2.91 (3.09)
Sively 3	<i>Physa</i>	epilithon	1.29 (0.44)
Sively 2	<i>G. minus</i>	FBOM	1.10 (1.02)
Sively 3	<i>G. minus</i>	FBOM	1.71 (0.93)
Sively 3	<i>M. hoffmasteri</i>	<i>Physa</i>	0.14 (0.13)
Sively 3	<i>M. hoffmasteri</i>	<i>G. rugosa</i>	0.30 (0.34)
Sively 3	<i>M. hoffmasteri</i>	<i>G. minus</i>	0.60 (0.59)
Jones Canyon	<i>M. hoffmasteri</i>	<i>S. spinatus</i>	1.19 (1.39)
Jones Canyon	<i>M. hoffmasteri</i>	<i>F. tartarea</i>	0.88 (1.11)
Sively 2	<i>S. emarginatus</i>	FBOM	0.52 (0.29)
Sively 2	<i>S. emarginatus</i>	<i>F. tartarea</i>	0.19 (0.02)
Sively 2	<i>S. emarginatus</i>	<i>G. minus</i>	0.58 (0.16)
Jones Canyon	<i>S. spinatus</i>	epilithon	0.22 (0.14)
Jones Canyon	<i>S. spinatus</i>	FBOM	1.82 (2.39)
Jones Canyon	<i>S. spinatus</i>	<i>F. tartarea</i>	0.44 (0.24)

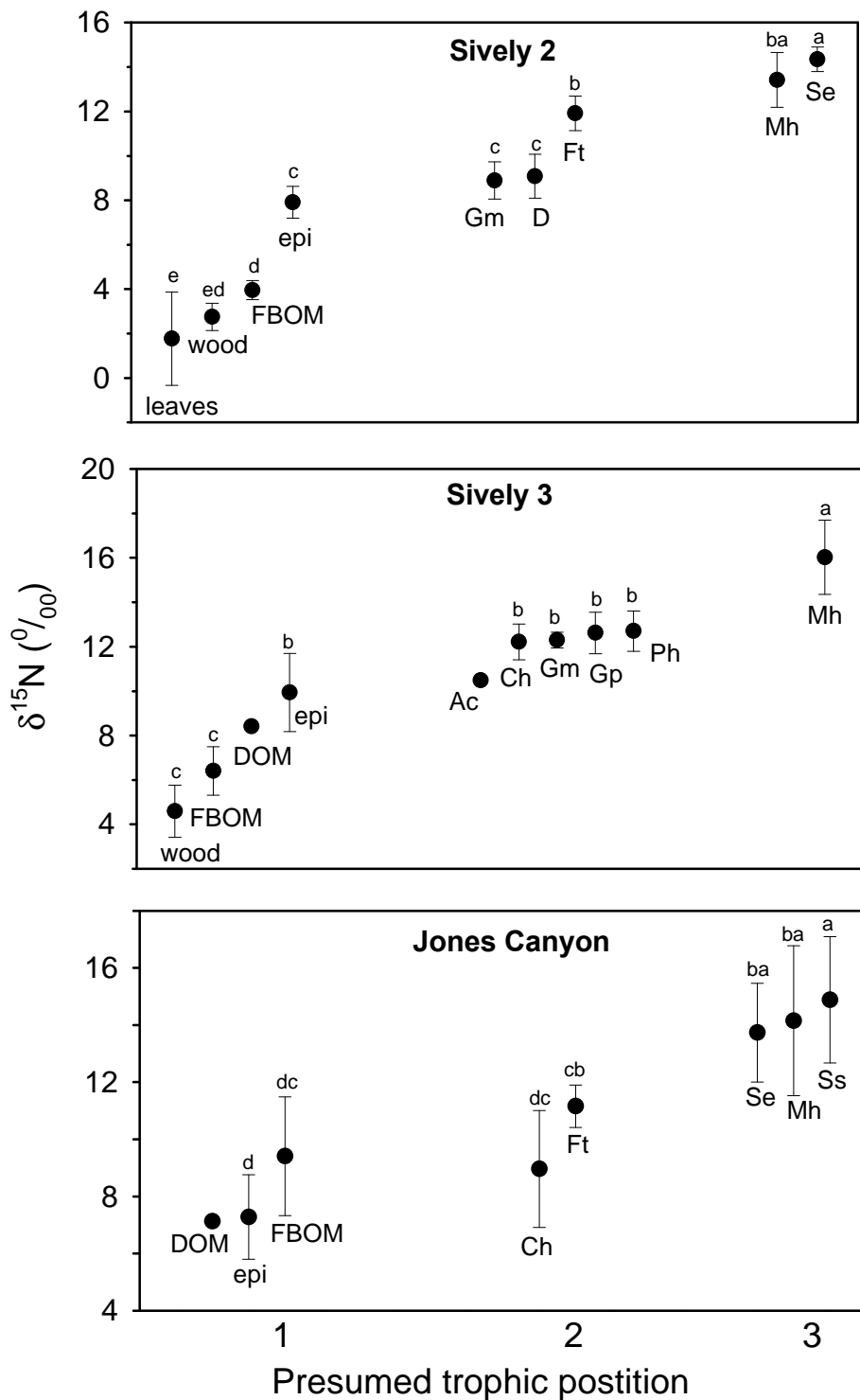


Figure 3.1. Presumed trophic position of detritus and animals. Symbols are mean  $\delta^{15}\text{N}$  values with 95% confidence intervals. Codes below symbols indicate taxa: Ac= *Acentrella*, B= snail B, Ch= *Caecidotea holsingeri*, D= *Dolophilodes*, Ft= *Fontigens tartarea*, Gm = *Gammarus minus*, Gp= *Gyraulus parvus*, Mh= *Macrocotyla hoffmasteri*, P= *Physa*, Se = *Styobromus emarginatus*, Ss= *S. spinatus*. Means with different letters above the symbols are significantly different (Duncan's,  $p < 0.05$ ).

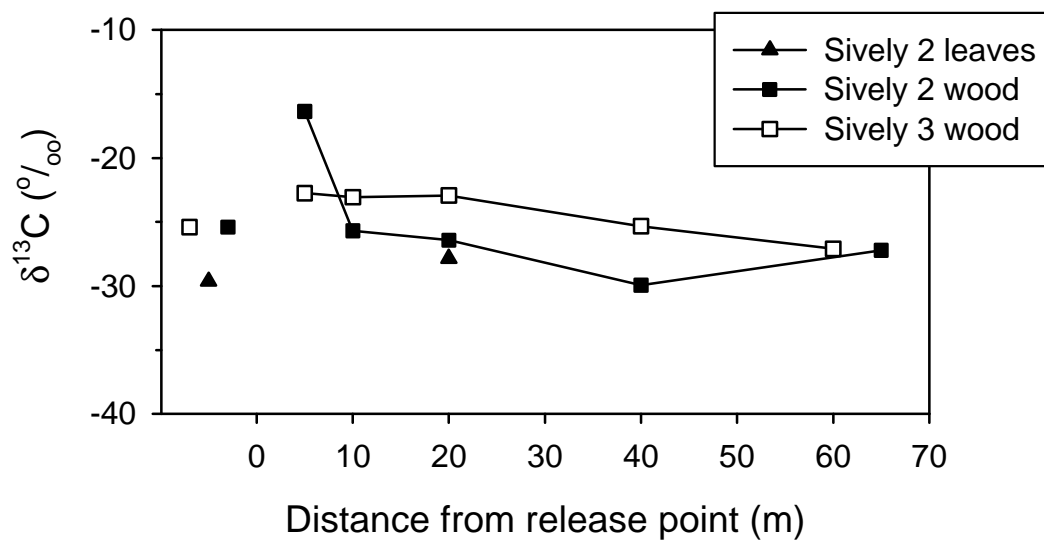


Figure 3.2. Leaf and wood  $\delta^{13}\text{C}$  along stream reaches on day 28. Symbols above 0m are unenriched composite background samples.

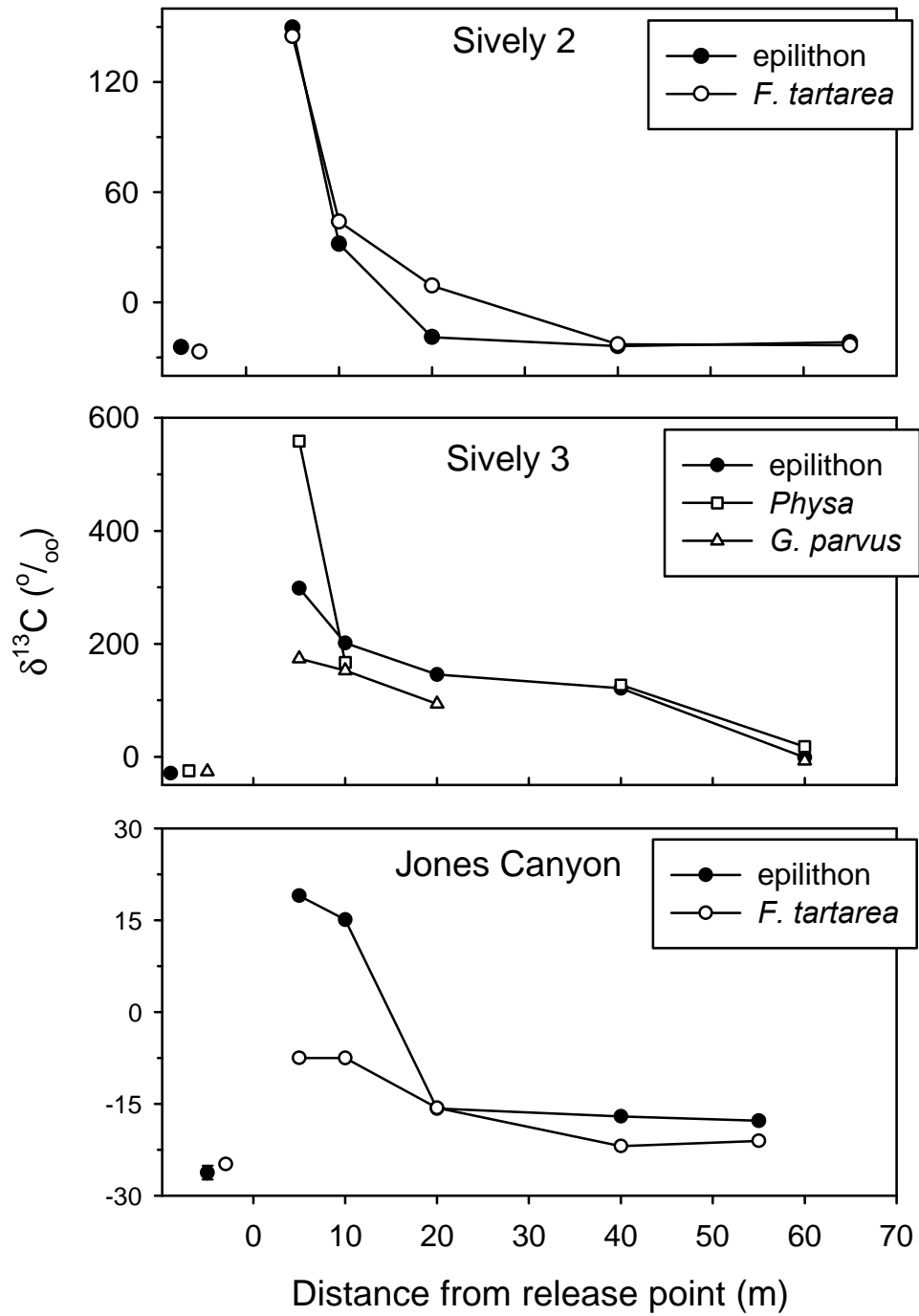


Figure 3.3. Epilithon and scraper  $\delta^{13}\text{C}$  along stream reaches on day 28. Symbols above 0m are unenriched background samples with 95% CI.

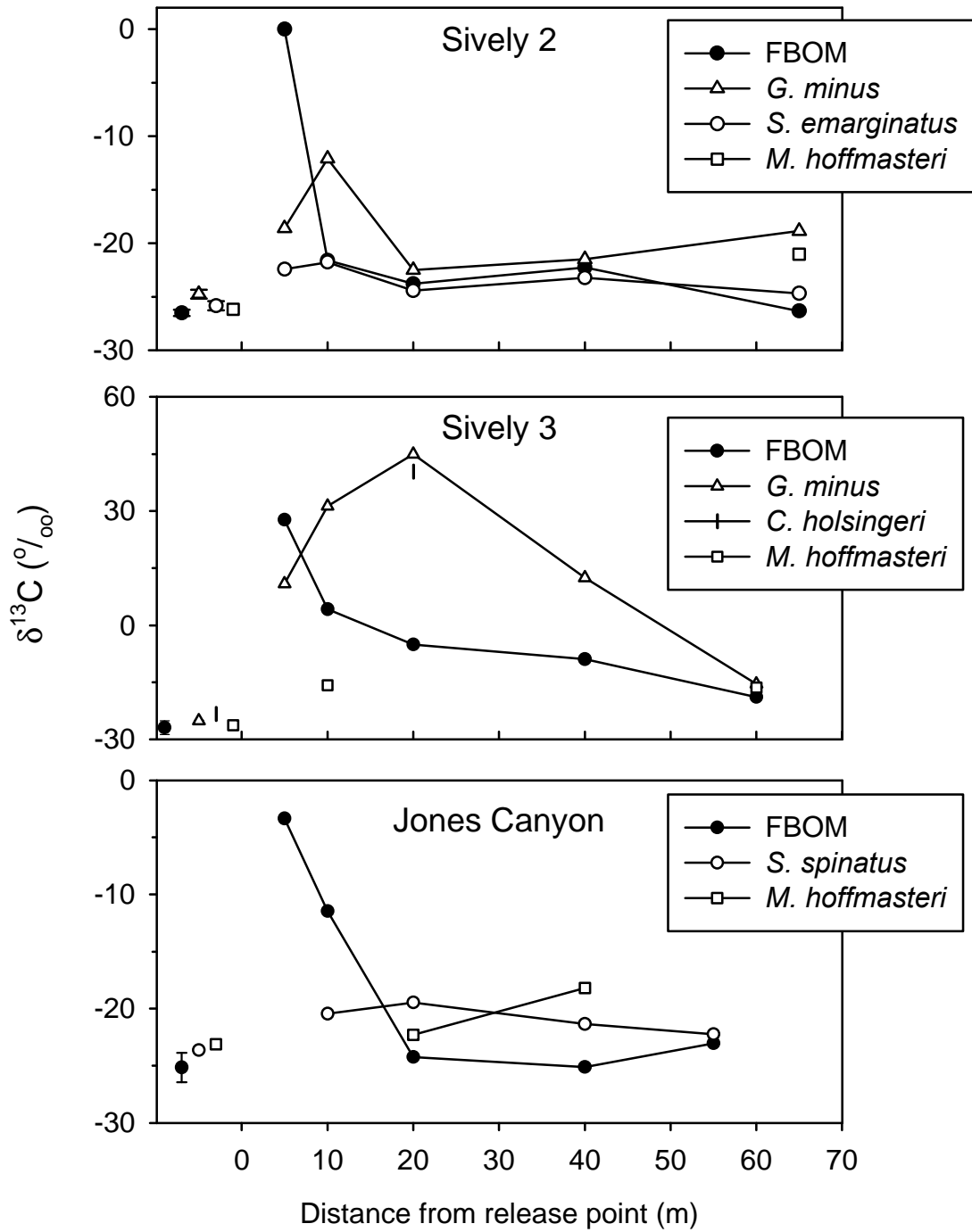


Figure 3.4. FBOM, collector, and predator  $\delta^{13}\text{C}$  along stream reaches on day 28. Symbols above 0m are unlabeled background samples with 95%CI (n=3).

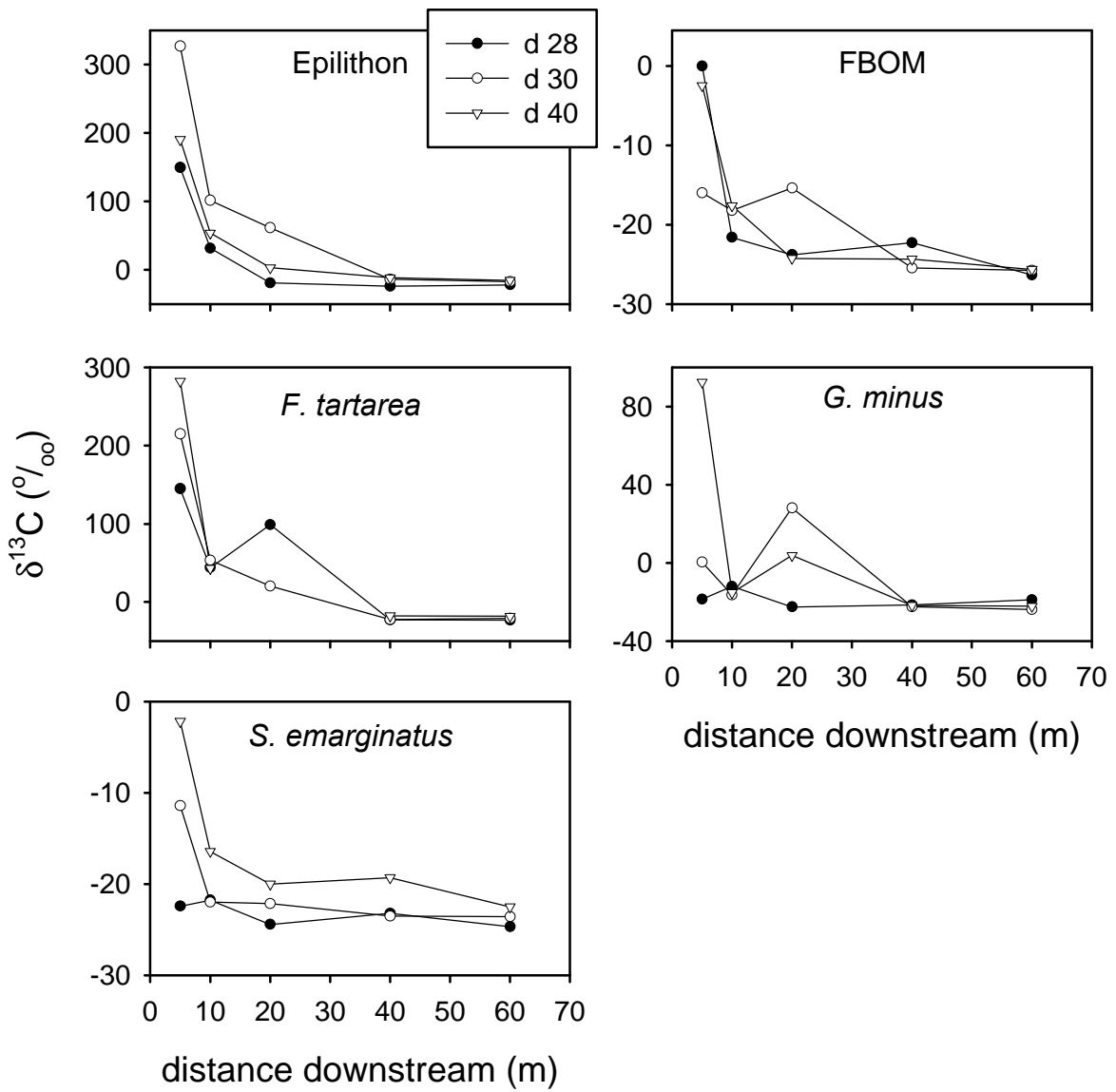


Figure 3.5. Longitudinal profiles of organic matter and consumer  $\delta^{13}\text{C}$  in Sively 2 on days 28, 30 and 40.

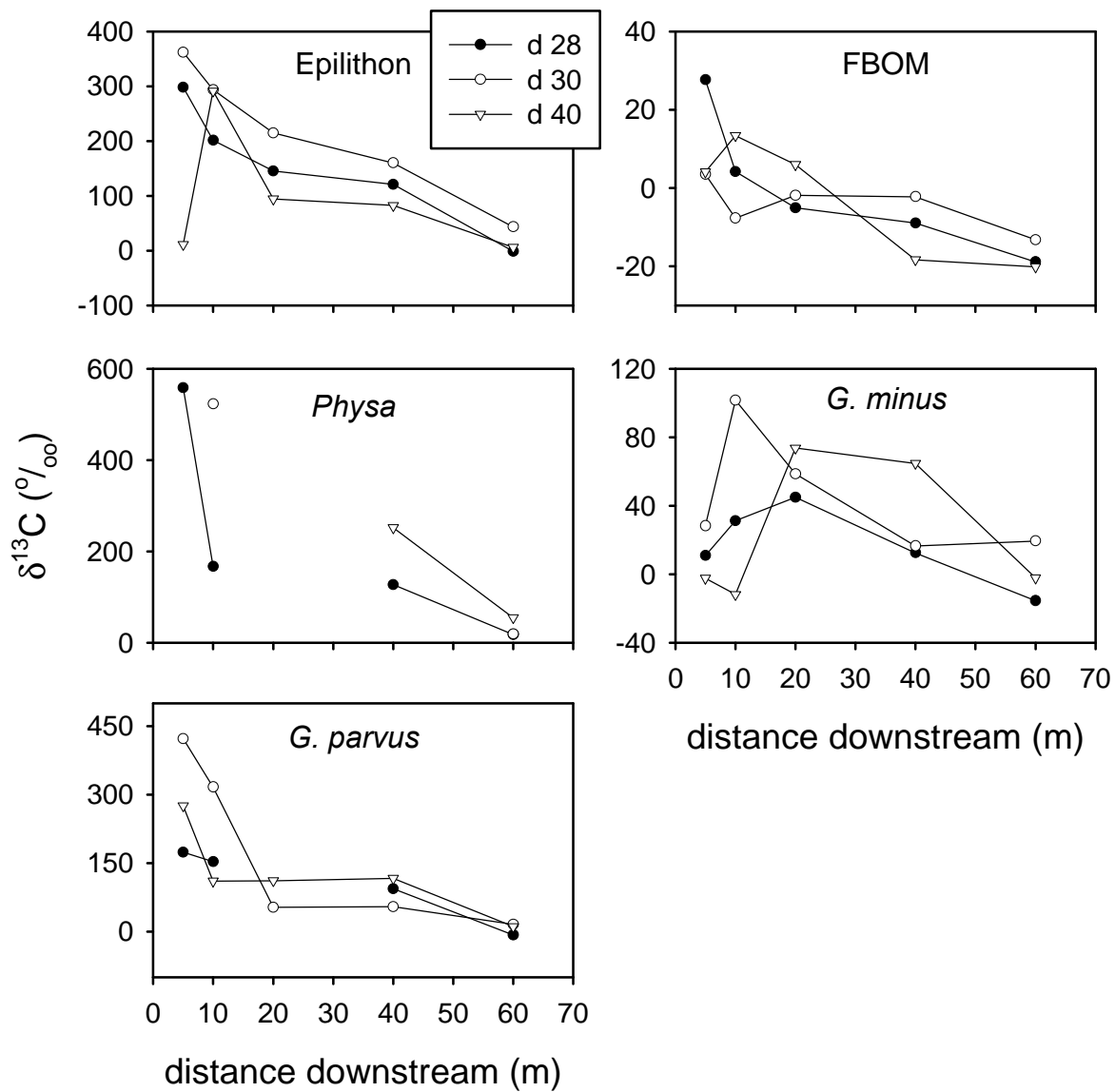


Figure 3.6. Longitudinal profiles of organic matter and consumer  $\delta^{13}\text{C}$  in Sively 3 on days 28, 30 and 40.

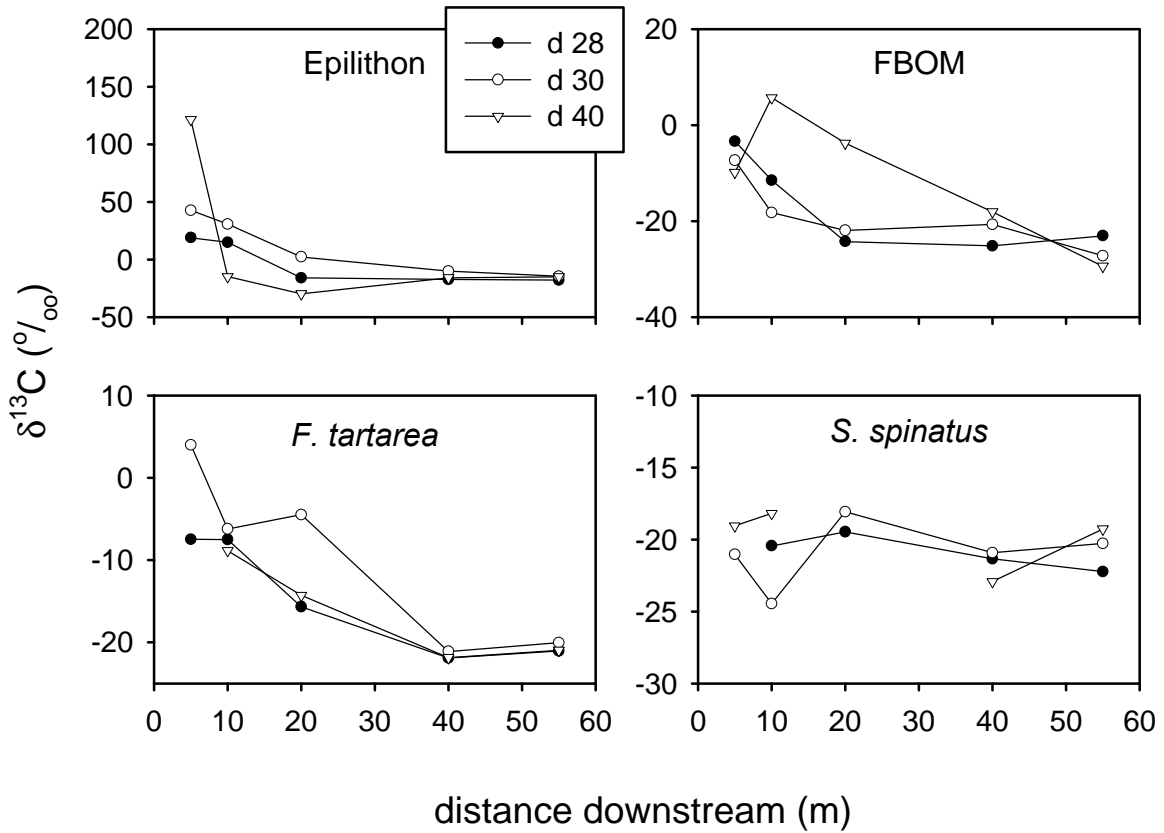


Figure 3.7. Longitudinal profiles of organic matter and consumer  $\delta^{13}\text{C}$  in Jones Canyon on days 28, 30 and 40.

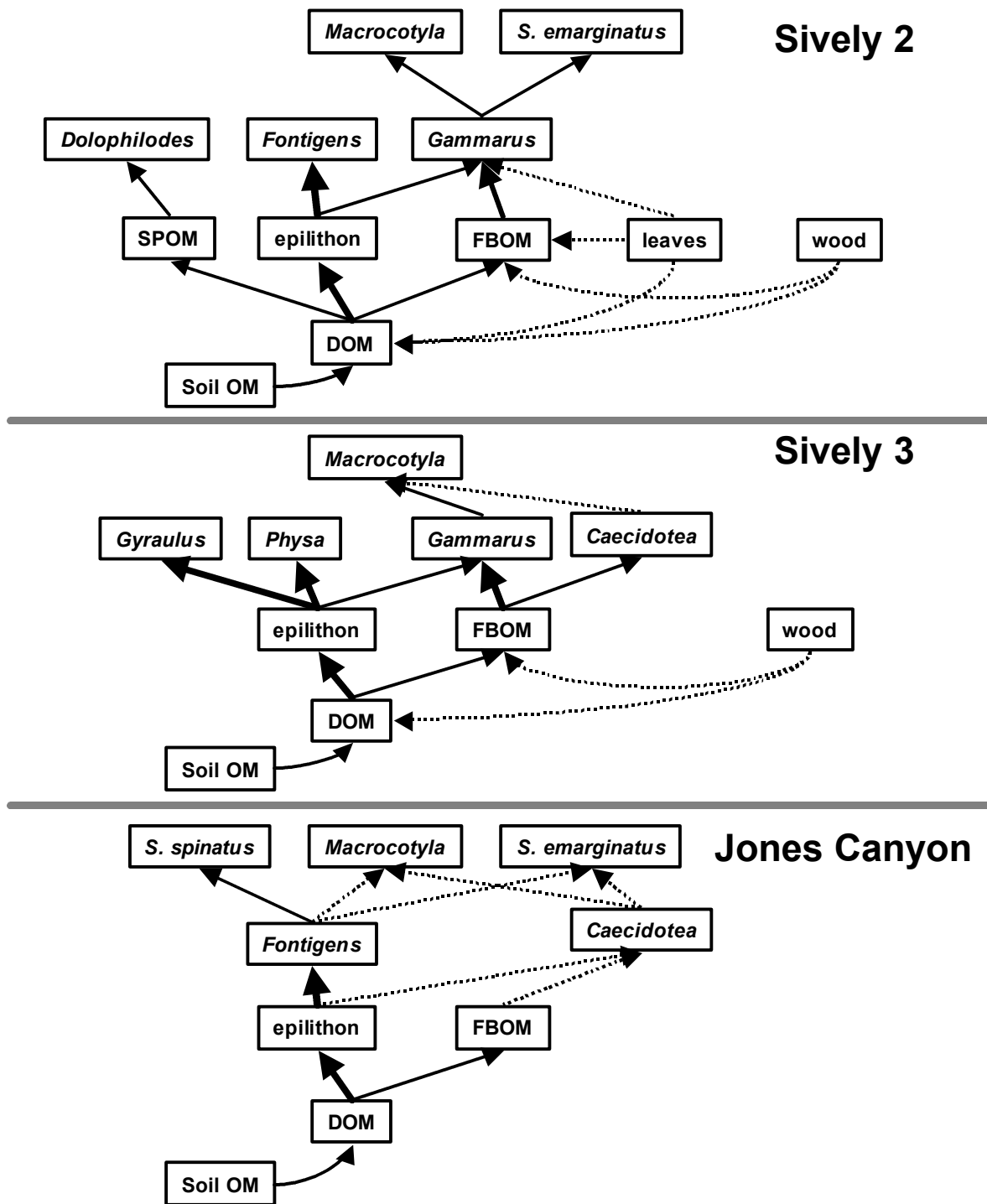


Figure 3.8. Food webs in each cave stream. Arrow thickness indicates importance of food source to a consumer. Dashed arrows indicate uncertain pathways.

## Summary

Bacteria appear to be an important energy source for higher trophic levels in karst groundwater. Factors that regulate bacterial activity and production in karst have the potential to control foodwebs by determining energy availability. Flooding, in particular, could be an important controlling factor by scouring senescent biofilms and renewing C, oxygen, and nutrient supplies underground. Bacterial density in the Dorvan aquifer responded to changing discharge much like bacteria in surface streams respond to floods (Chapter 1). Floods scoured bacteria from the interior of the aquifer and possibly overlying soil, increasing bacterial density in the water column during peak discharge. Density of respiring bacteria in the water column was relatively stable, possibly because active biofilms are less susceptible to scouring. Patterns of DOC and microbial hydrolytic activity were not clearly related to discharge. Microbial activity may be dictated by DOC quality rather than DOC quantity. Future research should focus on: 1) changes in DOM quality over time and across spatial zones; 2) multiple-scale examination of biofilms from the level of individual stones to riffles/pools to structural zones; and 3) the role of soil systems in providing DOM input to karst aquifers.

Coarse particulate organic matter is an alternate energy source to DOM in cave streams that are linked to the surface by large openings. Leaves and wood added to cave streams were consumed or transformed to FPOM at similar rates and by the same mechanisms as in surface streams (Chapter 2). Microbial films, especially fungi, colonized CPOM in a predictable manner and removed leaf and wood C from the stream through respiration. Shredding invertebrates accelerated leaf breakdown by consuming leaves and transforming CPOM to smaller particles. Distribution of shredding invertebrates can be an important determinant of organic matter breakdown in drainage networks: CPOM was quickly lost in Organ Cave streams with shredders and more persistent in streams without shredders. Wood lost mass slower than leaves in most cave streams and may represent a more stable C source over time. Wood breakdown in Organ Cave streams was not speeded by nutrient enrichment probably because nutrients levels in streamwater were high. Cave streams efficiently retain CPOM which is probably used close to its entry point to the subsurface. As a result of efficient CPOM retention, DOM and FBOM should be important food in streams distant from entry points where CPOM is unlikely to be transported.

Trophic structure in cave streams is similar to that of the detritus-based portion of surface stream food webs: there are ~3 trophic levels and animals in cave streams use many of the same feeding strategies (biofilm scrapers, collectors, shredders, predators) used by surface stream invertebrates. Cave streams appear to be good models for studying energy transfer from microbial loops to consumers in streams (Figure 4.1). In cave streams, DOM was used by bacteria in biofilms where DOM C was immobilized for a relatively long time. Primary consumers fed on epilithic and sediment biofilms, incorporating bacterial C, which was in turn consumed by predators. Natural abundance  $^{15}\text{N}$  data suggested soil organic matter was the primary fuel for the microbial loop and consumers in cave streams (Figure 4.1 B,C). Wood and leaf fragments were not used directly by animals in Organ Cave streams, but they may indirectly contribute to consumers by producing FBOM and DOM in the streams (Figure 4.1B). Whole leaves probably are a high-quality, but unreliable, energy source in cave streams: animals shredded leaves placed in the streams, but large leaf fragments were rare in Organ Cave streams. The CPOM → shredder pathway exists in cave streams, but may only be used during short time periods when leaves are available or in small spatial areas where leaves enter caves. In Organ Cave, the stygophile *Gammarus minus* or epigeal insects shredded leaves when they were present, but in streams with stygobites only, leaves were not shredded. Unlike in surface streams where specific taxa act as shredders and have life cycles adjusted to CPOM timing (Cummins et al. 1989), animals in caves probably switch feeding roles from collectors to shredders when leaves are available or simply do not use leaves directly. Inefficient use of CPOM in streams containing only stygobites supports the idea of a loss of dietary flexibility with increasing time in caves (Dickson 1979, Culver 1985).

The most commonly used conceptual model for streams, the River Continuum Concept (RCC; Vannote *et al.* 1980), does not fit cave streams well. The RCC relies on changes in the linkage between riparian vegetation and streams as stream size increases. Linkage between riparian vegetation and cave streams relies on geology and hydrology, not changing stream order. Unlike larger order streams in surface drainages, cave streams cannot shift towards primary production. Predictions about changing P/R ratios in the RCC are not applicable to cave streams because P/R is always <1 in caves, with the possible exception of chemoautotrophically based caves. Under the RCC, animal feeding modes shift from shredding to collecting with distance downstream as CPOM input decreases. This may apply in some situations where

CPOM enters in headwater cave stream. Finally, diversity in cave streams is highest in headwater streams (Fong and Culver 1994) rather than in mid-order streams as predicted by the RCC.

The ecosystem expansion and contraction model (Stanely et al. 1997) may be better alternative to the RCC for describing karst ecosystems. The model organizes an ecosystem into a hierarchy of different sized patches ranging from small stream segments to whole drainage networks. As dry and wet periods alternate, patches are disconnected and reconnected, influencing ecosystem structure and function. A patch-based model is easily applied to karst aquifers which are organized from small fractures to conduit systems to large aquifer structural zones (Mangin 1994). During ecosystem expansion, rising water fills voids in the aquifer and enhances connection within structural zones and between the drainage and surface systems (soils, terrestrial vegetation). During dry periods, the ecosystem "contracts" and surface systems become disconnected from the aquifer, followed by disconnection between and within structural zones. Physical (temperature, nutrients, etc.) and biotic (community composition, energy flow, etc.) should be affected by variation in connectivity among patches. For example, differences in connectivity between groundwater and surface water explain patterns of biodiversity in karst aquifers (Gibert 1986). Species interactions also differ among patches ranging from individual stones to drainages are examined (Culver et al. 1991). An ecosystem expansion-contraction model of karst aquifers requires much more information, particularly about nutrient and energy fluxes and community composition at a range of spatial and temporal scales. However, such a model should help capture the dynamic nature of linkages within karst aquifers and between aquifers and surface systems.

## References

- Culver, D. C. 1985. Trophic Relationships in aquatic cave environments. *Stygologia* 1:43-53.
- Culver, D. C., D. W. Fong and R. W. Jernigan. 1991. Species interactions in cave stream communities: experimental results and microdistribution effects. *American Midland Naturalist* 126:364-379.
- Cummins, K.W., M.A. Wilzbach, D.M. Gates, J.B. Perry, and W.B. Taliaferro. 1989. Shredders and riparian vegetation: leaf litter that falls into streams influences communities of stream invertebrates. *Bioscience* 39:24-30.
- Dickson, G. W. 1979. The importance of cave mud sediments in food preference, growth, and mortality of the troglobitic amphipod crustacean *Crangonyx antennatus* Packard (Crangonyctidae). *Crustaceana* 36:129-140.
- Fong, D.W. and D.C. Culver. 1994. Fine-scale biogeographic differences in the crustacean fauna of a cave system in West Virginia, USA. *Hydrobiologia* 287:29-37.
- Gibert J. 1986. Ecologie d'un systeme karstique jurassien. Hydrogéologie, dérive animale, transits de matières, dynamique de la population de *Niphargus* (Crustacé Amphipode). *Memoires de Biospeologie* 13:1-379.
- Mangin A. 1994. Karst Hydrogeology. In: Gibert J, Danielopol DL, Stanford JA, editors. *Groundwater Ecology*. San Diego:Academic Press, Inc. p 43-67.
- Stanley E.H., S.G. Fisher, and N.B. Grimm. 1997. Ecosystem expansion and contraction in streams. *BioScience* 47:427-435.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130-137.

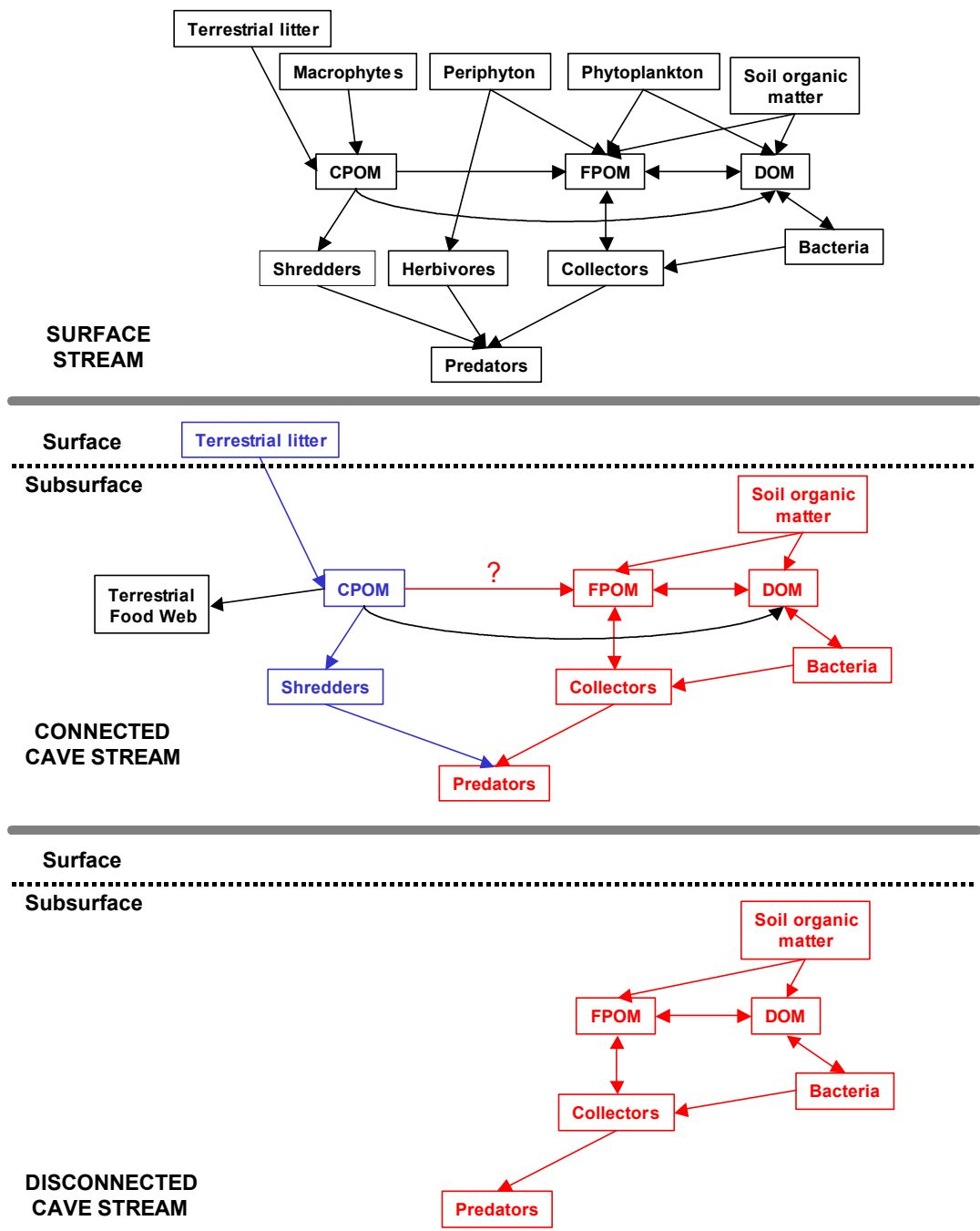


Figure 4.1. Energy flow in surface streams and cave streams (Modified from Allan 1995). Primary pathways in cave streams are shown in red. Spatially or temporally patchy pathways are shown in blue.

**Curriculum Vitae**  
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- 1994-2000 Ph.D., Virginia Polytechnic Institute and State University.  
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- 1998-present Research Coordinator, Distributional survey of the federally endangered isopod, *Lirceus usdagalun* in Virginia. U.S. Fish and Wildlife Service
- 1994-1996 Research Coordinator, Distributional survey of the cave milliped *Pseudotremia cavernarum* in Virginia. Virginia Department of Inland Fisheries and Wildlife
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2. Hervant, F., J. Mathieu, H. Barré, K.S. Simon and C. Pinon. 1997. Comparative study on the behavioral, ventilatory and respiratory responses of hypogean and epigean crustaceans to long-term starvation and subsequent feeding. *Comparative Biochemistry and Physiology* 118A (4):1277-1283.
3. Simon, K.S. 1995. Notes on the Distribution of *Pseudotremia cavernarum* Cope. *Virginia Journal of Science* 46(3): 205-206.

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2. Simon, K.S. 1995. Biology of Caves in Virginia and the Central Appalachians. Pages 157-175, in C. Zokaites, K.S. Hansen, and S.E. Vermeulen (eds.) *Underground in the Appalachians. A Guidebook for the 1995 Convention of the National Speleological Society.*

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3. Simon, K.S., J. Gibert, P. Petitot and R. Laurent. 1998. Spatial and Temporal Patterns of Carbon and Microbial Transport in a Karst Aquifer. 1998 National Speleological Society Convention. Seawannee, TN.
4. Simon, K.S. 1995. Distribution of the Ellett Valley Millipede (*Pseudotremia cavernarum* Cope) in Virginia. 1995 National Speleological Society Convention. Blacksburg, VA.
5. Simon, K.S. and A.L. Buikema Jr. 1994. Effects of Septic System Effluent on Macroinvertebrate Populations and Their Food Resources. 1994 National Speleological Society Convention. Brackettville, TX.

6. Simon, K.S. and A.L. Buikema Jr. 1994. Effects of Septic Systems on a Cave Aquatic Community. The 2<sup>nd</sup> International Conference on Groundwater Ecology. Atlanta, GA.
7. Simon, K.S. and A.L. Buikema Jr. 1993. Preliminary Investigation of the Effects of Organic Enrichment on a Cave Aquatic Community. The Virginia Academy of Science. Norfolk, VA.
8. Simon, K.S. and E.C. Martin. 1991. Colonization Sequence of Defaunated Substrates by Benthic Macroinvertebrates. The Ohio Academy of Science. Columbus, OH.
9. Ward, B., L. Rutherford, K.S. Simon, S. Braunschweig, and A.L. Buikema, Jr.. 1996. Tutorial Development in Teaching Biology. Biology in Action: New Approaches to Teaching and Learning in Science. NSF. Radford, VA.

**Posters:**

1. Simon, K.S., J. Gibert and R. Laurent. 1997. Transfer of bacteria, invertebrates, and nutrients in a karst aquifer. Karst Waters Institute conference: Conservation and Protection of the Biota of Karst. Nashville, TN.
2. Simon, K.S. and A.L. Buikema Jr. 1994. Biotic and Abiotic Responses of a Cave Aquatic System to Septic System Outfall. Presented by A.L. Buikema Jr. at the 15th Annual Meeting of the Society of Environmental Toxicology and Chemistry.

**Laboratory Guides and Teaching Aides:**

1. Simmons, G.M., J.R. Webster, A. Schultheis, and K. Simon. 1997. Methods for Use in Freshwater Ecology.
2. Simon, K.S., A.L. Buikema Jr., S. Braunschweig and B. Ward. 1995. Fetal pig dissection computer tutorial and practice test. General and Principle of Biology laboratories, Virginia Tech.  
<http://www.biol.vt.edu/coursepages/genbiol/tutorials/pigdissection/pig.html>
3. Virginia Tech Freshwater Ecology Course Homepage:  
<http://www.biol.vt.edu/departments/faculty/freshwater/freshwater.html>

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2. Simon, K.S. Carbon dynamics in detritus-based systems: can cave streams be used to understand energy flow in surface streams? Continued Funding: Sigma Xi Grant-in-Aid of Research, May 1998; \$700.00
3. Simon, K.S. VPI&SU, GSA Travel Fund for travel to NABS 1998, Prince Edward Island, Canada; \$265.00
4. Simon, K.S. Carbon dynamics in detritus-based systems: can cave streams be used to understand energy flow in surface streams? Sigma Xi Grant-in-Aid of Research, May 1997; \$600.00
5. Simon, K.S. Leaf and wood breakdown in cave streams. Graduate Research Development Project Grant, VPI&SU, August 1997; \$500.00
6. Simon, K.S. Flux of bacteria, carbon and invertebrates in karst aquifers: A comparative study of North American and European karsts. National Speleological Society Research Grant, June 1997; \$250.00

7. Simon, K.S. Structural and functional response of a cave aquatic community to organic enrichment. Virginia Academy of Science Research Grant, June 1993; \$786.00
8. Simon, K.S. Trophic interaction of the isopod *Caecidotea recurvata* in the aquatic cave community. Graduate Research Development Project Grant, VPI&SU, February 1993; \$300.00
9. Simon, K.S. Effects of organic enrichment on trophic dynamics of a cave stream community. National Speleological Society Research Grant, December 1992; \$300.00

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