Effects of organic matter and season on leaf litter colonisation and breakdown in cave streams

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SUMMARY

1. Low organic matter availability is thought to be a primary factor influencing evolutionary and ecological processes in cave ecosystems. We examined links among organic matter abundance, macroinvertebrate community structure and breakdown rates of red maple (*Acer rubrum*) and corn litter (*Zea mays*) in coarse- (10×8 mm) and fine-mesh (500-μm) litter bags over two seasonal periods in four cave streams in the south-eastern U.S.A.

2. Organic matter abundance differed among cave streams, averaging from near zero to 850 g ash-free dry mass m^{-2}. Each cave system harboured a different macroinvertebrate community. However, trophic structure was similar among caves, with low shredder biomass (2–17% of total biomass).

3. Corn litter breakdown rates (mean $k = 0.005$ day^{-1}) were faster than red maple (mean $k = 0.003$ day^{-1}). Breakdown rates in coarse-mesh bags ($k = 0.001$–0.012 day^{-1}) were up to three times faster than in fine-mesh bags ($k = 0.001$–0.004 day^{-1}). Neither invertebrate biomass in litter bags nor breakdown rates were correlated with the ambient abundance of organic matter. Litter breakdown rates showed no significant temporal variation. Epigean (surface-adapted) invertebrates dominated biomass in litter bags, suggesting that their effects on cave ecosystem processes may be greater than hypogean (cave-adapted) taxa, the traditional focus of cave studies.

4. The functional diversity of our cave communities and litter breakdown rates are comparable to those found in previous litter breakdown studies in cave streams, suggesting that the factors that control organic matter processing (e.g. trophic structure of communities) may be broadly similar across geographically diverse areas.

Keywords: bottom-up, decay, decomposition, donor control, resource subsidy

Introduction

Cave streams are quintessential donor-controlled ecosystems representing one endpoint along a heterotrophic spectrum (Polis & Strong, 1996; Simon, Benfield & Macko, 2003). Lacking photoautotrophs, the productivity of most cave ecosystems (except those based on chemolithoautotrophy; Sarbu, Kane & Kinkle, 1996; Sarbu, 2001; Engel *et al.*, 2004) depends on organic matter from the surface (Poulson & Lavoie, 2001). The amount and type of organic matter imported into a cave are largely determined by its connectivity to the surface (Poulson & Lavoie, 2001). Systems weakly connected to the surface typically receive fine particulate and dissolved organic matter, while systems that are well connected via cave entrances and sink holes may receive relatively high inputs of dissolved, fine and coarse particulate organic matter (e.g. leaves and woody debris, Poulson & Lavoie, 2001). Regardless of connectivity, the quantity and quality of these inputs are often low compared to surface streams because of a lack of direct riparian inputs and prior biological processing in surface and soil habitats, resulting in the characterisation of cave ecosystems as being energy-limited (Culver, 1982; Culver, Kane & Fong, 1995; Poulson & Lavoie, 2001; Graening & Brown, 2003). As a consequence of differences in cave-surface connectivity, however, gradients in resource availability exist that may influence the biomass and diversity of species found within caves. These

differences in community structure may ultimately affect cave ecosystem processes.

A few studies have attempted to examine the links among organic matter inputs, community structure and ecosystem processes in cave systems. Schneider, Christman & Fagan (2011) showed that organic matter quality (leaf litter versus rat carcasses) influenced terrestrial community structure in cave 'pits' (i.e. vertical cave passages with direct connections to the surface). Huntsman *et al.* (2011b) found that growth rates and production of the obligate cave salamander *Gyrinophilus palleucus* (McCrady) were positively related to amount of organic matter in cave streams, presumably because of higher prey availability. In a study of groundwater systems, Datry, Malard & Gibert (2005) reported that aquifers recharged by storm water received higher inputs of dissolved organic matter and had higher densities and diversities of invertebrates, suggesting that organic matter supply may influence groundwater community structure. Similar patterns have been observed in both phreatic aquifers and cave streams affected by organic pollution (Sinton, 1984; Smith *et al.*, 1986; Madsen, Sinclair & Ghiorse, 1991; Notenboom, Plénet & Turquin, 1994; Simon & Buikema, 1997; Sket, 1999).

Fewer studies have examined how organic matter abundance affects ecosystem processes in cave streams. Cooney & Simon (2009) used a microcosm experiment to show that amendment of dissolved organic matter increased microbial productivity and enzyme activity on cave-stream rocks. The processing of particulate organic matter in caves has also received some attention. Simon & Benfield (2001) found that cave streams with direct upstream connections to the surface (e.g. cave entrances and sinkholes) had high organic matter inputs, communities dominated by epigean (surface) and stygophilic (species that reproduce in both cave and surface habitats) taxa and faster decomposition of white oak (*Quercus alba* L.) litter. In contrast, streams without large upstream openings to the surface had lower organic matter standing stocks, were dominated by hypogean (cave specialist) taxa and had slower rates of litter breakdown. Differences in litter breakdown rates among cave streams were attributed to the degree of surface connection, which controlled both organic matter inputs and the biomass of surface taxa. Relationships between organic matter abundance and litter breakdown are not simple, however. For example, a regression analysis of data reported by Simon & Benfield (2001) revealed no significant relationship between organic matter abundance and litter breakdown rates among the five cave streams they studied (M. P. Venarsky, unpubl. data). Similarly, Huntsman, Venarsky

& Benstead (2011a) found no correlation between organic matter abundance and carrion breakdown rates in four cave streams, suggesting that factors additional to the abundance of organic matter influence rates of carrion processing in caves.

Here, we examined the linkages among organic matter abundance (i.e. a surrogate for resource supply), the structure of litter macroinvertebrate communities and litter breakdown rates during two seasonal periods in four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A. These ecosystems contained different amounts of organic matter, allowing the test of three related hypotheses. First, macroinvertebrate abundance is often higher in groundwater systems receiving high inputs of organic matter compared with those receiving low inputs (Datry *et al.*, 2005). We hypothesised that macroinvertebrate biomass in litter packs would be positively correlated with ambient organic matter abundance because caves with higher quantities of organic matter are likely to support more macroinvertebrates. Second, because positive correlations between litter breakdown rates and consumer biomass have been shown for cave and surface streams (Simon & Benfield, 2001; Hieber & Gessner, 2002), we predicted that litter breakdown rate would be positively correlated with consumer biomass. Third, because many environmental characteristics in cave systems vary little temporally (e.g. light, humidity and temperature; Juberthie, 2001; Culver & Pipan, 2009), we predicted that consumer biomass and the rate of litter breakdown would also not vary temporally. The design of our study allowed us to quantify how organic matter abundance affects cave stream macroinvertebrate communities and how variation in community structure affects rates of organic matter processing, a key ecosystem process in cave and groundwater ecosystems.

Methods

Study sites

Four streams in hydrologically separate cave systems were studied. Tony Sinks, Salt River and Jess Elliot caves are in Jackson County, AL, U.S.A. Big Mouth Cave is in Grundy County, TN, U.S.A. Distances between caves ranged from 9 to 76 km. These caves were chosen for the following four reasons. First, their communities appeared similar in structure upon initial inspection (i.e. they all contained cave salamanders and isopods and cave and surface crayfish). Second, organic matter abundance differed among caves because of differing connectivity to surface systems. Third, visual surveys of surface

habitats in the recharge area of each cave suggested human disturbance was minimal (e.g. no clear-cuts, little residential development and few roads within the study catchments). Lastly, physical characteristics of the study reaches within and among cave streams were similar. Stream width ranged between 5 and 10 m, while depth ranged from 20 to 80 cm and the substratum was composed of a mixture of sand and gravel interspersed with exposed areas of bedrock. All study reaches were between 200 and 600 m (horizontally) from the nearest known cave entrance and ranged from 70 to 90 m in length. Cave streams did not have known direct aquatic connections (e.g. upstream cave entrances) to surface habitats during base-flow conditions.

Litter colonisation and breakdown rates

Rates of litter breakdown were measured using bags containing red maple (*Acer rubrum* L.) and corn (*Zea mays* L.) litter. Choice of these two species allowed us to examine the effects of litter quality on breakdown rate; both species break down relatively rapidly (Webster & Benfield, 1986; Griffiths *et al.*, 2009) but their C : N ratios differ [red maple C : N = 53 (Ostrofsky, 1997), corn C : N = 24–28 (Griffiths *et al.*, 2009)]. Corn litter was also included to examine its potential as a litter source in a separate reach-scale detritus addition study (M. P. Venarsky, unpubl. data). Red maple litter was collected shortly after abscission from the Coweeta Hydrologic Laboratory (Macon County, NC, U.S.A. and Tuscaloosa County, AL, U.S.A.). Senesced corn litter from non-Bt plants (i.e. a variety that has not been genetically modified to contain *Bacillus thuringiensis* genes) was collected from an agricultural field near Salem, IL, U.S.A.

Litter of both species was air-dried, and *c.* 4 g (±0.1 g) was placed into each of 14×12 cm fine- (500-µm) and coarse-mesh (10×8 mm) bags. The different mesh sizes controlled invertebrate access to the leaf litter. The fine-mesh bags allowed access by only small-bodied invertebrates (e.g. microcrustaceans, Chironomidae and Oligochaeta), while coarse-mesh bags allowed access by both small- and large-bodied taxa (e.g. crayfish and late instar Ephemeroptera). Litter bags ($n = 100$) were deployed among five stations (five litter bags per litter species per mesh size per station) in each stream. Stations were separated by 5–10 m, except in Jess Elliot Cave where two stations were separated by 85 m. Litter bags were tethered to nylon string at 0.25-m intervals and anchored in the stream by a sandbag. To assess handling loss, three litter bags of each litter species and mesh size were retrieved on the day of deployment. On each of 10

sampling dates, one bag of each litter species and mesh size was retrieved from each station in each of the four caves ($n = 5$ of each combination). Litter bags were stored on ice from the time of collection to processing (*c.* 24 h). To assess temporal variation in litter breakdown rates, the litter-bag study was conducted twice, from January to July 2008 and from July to December 2008 (to January 2009 for Tony Sinks). Red maple litter from the different source locations was not mixed between experimental periods; litter from Macon County, was used during the first experimental period and litter from Tuscaloosa County, for the second period. No effects of different maple litter sources were detected (see Results).

Water temperature and depth were recorded in each stream every 30 min using a Solinst Barologger model 3001 data logger (Solinst, Georgetown, ON, Canada). The coefficient of variation (CV) around average daily water levels was calculated in each cave to quantify flow variability; a higher CV indicates more variable hydrology. CV around water depths could not be calculated for the first deployment date because of instrument failure.

Water samples were collected from each stream on each sampling date and analysed for NH_4^+-N, NO_3^--N and soluble reactive phosphorus (SRP). All water samples were filtered through glass-fibre filters (0.7-µm nominal pore size). Water samples were transported to the laboratory on ice and frozen until analysis. Temporal trends in organic matter abundance were examined by quantifying organic matter on eight dates (four per incubation period) using a 490-cm^2 corer placed at two random points within 4 m of each litter-bag station. The corer was inserted into the stream bottom, large organic matter was removed and the substratum disturbed to a maximum depth of 4 cm. Remaining suspended organic matter was removed from the water column of the corer via 10 sweeps of the water column with a 250-µm mesh net.

Sample processing

Contents of litter bags were placed into a 3.8-L bucket containing *c.* 2 L of water. The contents were agitated to dislodge macroinvertebrates from litter, which was removed by hand. The water was then sieved (250-µm), additional leaf material was removed and invertebrates retained on the sieve preserved in 5% formalin. Organic matter from both the litter bags and cores was processed identically. Organic matter was dried at 60 °C to constant mass and combusted at 500 °C for 6 h. Combusted material was wetted, re-dried and weighed. The difference between oven-dried mass and ash mass was used to estimate the ash-free dry mass (AFDM) of organic matter.

For water samples, concentrations of NH_4^+-N were measured using the orthophthaldialdehyde fluorometric method (Holmes *et al.*, 1999 as modified by Taylor *et al.*, 2007). Concentrations of NO_3^--N were measured using ion chromatography (Dionex ICS 2000 Ion Chromatograph; APHA, 1998). SRP concentrations were quantified using the ascorbic acid method (Murphy & Riley, 1962).

Macroinvertebrates were identified to the lowest possible taxonomic level (usually genus for insects and molluscs, class or order for microcrustaceans). Because species-level identifications were not made, we were unable to classify definitively all specimens into hypogean (cave-adapted species) or epigean (surface species) groups. Specimens were identified as hypogean when (i) they could be identified to genus; (ii) they lacked eye structures and (iii) the genus was known to contain hypogean species. The insect taxa (Table 1) identified in this study have previously been reported from caves (e.g. Brussock, Willis & Brown, 1988; Simon & Benfield, 2001), but are probably epigean species able to survive in cave streams (Romero, 2009). Body lengths of macroinvertebrates were measured to the nearest mm. Biomass was estimated using published length–mass relationships (Calow, 1975; Culver *et al.*, 1985; Leeper & Taylor, 1998; Benke *et al.*, 1999; Doroszuk *et al.*, 2007; Lemke & Benke, 2009; Huntsman *et al.*, 2011a). Taxa were categorised into functional feeding groups after Merritt, Cummins & Berg (2008).

Data analysis

Differences in benthic organic matter abundance among caves and sampling dates were assessed using two-way ANOVA. Litter breakdown rate (-*k*) was calculated by linear regression of natural-log transformed data (% AFDM remaining) against elapsed days. A *k*-coefficient was estimated for each litter species and mesh type at each station. A paired *t*-test was used to compare breakdown rates (*k*-coefficients) of corn and maple litter. Differences in breakdown rates among mesh sizes, incubation periods and caves within each litter type were assessed using three-way ANOVA. Three-way ANOVAs were run separately for the two litter species. Following all ANOVAs, a Bonferroni pair-wise *post hoc* comparison was conducted. Within each litter species, mesh size and incubation period, simple linear regression was used to assess the effect of (i) organic matter abundance on macroinvertebrate biomass and breakdown rate and (ii) macroinvertebrate biomass on breakdown rate. Data were transformed ($log_{10} + 1$) as needed to meet the assumptions of statistical analyses. All parametric analyses were conducted using Data Desk version 6.1 (Data Description Inc., 1996).

Three multivariate techniques in PRIMER (Version 6; Plymouth Marine Labs, Plymouth, U.K.; Clarke & Warwick, 2001) were used to compare macroinvertebrate community structure within and among caves: analysis of similarities (ANOSIM), non-metric multidimensional scaling (nMDS) ordinations and similarity percentages (SIMPER). Similarity matrices were first computed using the Bray–Curtis coefficient on log_{10}-transformed biomass data. A series of two-way nested ANOSIMs was then conducted to reduce the number of factors in the original data set, with each single factor (litter type, mesh size, incubation period, sampling date and station identity) nested within the cave factor. Ultimately, none of these five factors significantly affected community structure within each cave (see Results). Consequently, for each cave we averaged each taxon's biomass from each station (i.e. across litter types, mesh sizes and incubation periods) and compared this reduced data set to the original using the RELATE routine in PRIMER. We used the station-level mean taxon biomass data in all subsequent analyses of community structure (i.e. as five replicates within each cave). First, we used nMDS to generate graphical summaries of the relationships in community structure among caves. Samples with higher similarity are plotted closer to one another; a stress value indicates how accurately the plot represents the actual multivariate relationships (values <0.1 are optimal, values >0.2 are less useful; Clarke & Warwick, 2001). Bubble plots were incorporated into the nMDS plots to illustrate trends in community composition among caves. Second, we performed one-way ANOSIMs on the mean taxon biomass data to test for an effect of cave identity on community structure. Finally, we used the SIMPER routine to identify those taxa that contributed disproportionately to the overall dissimilarity in community structure among caves. Two values were calculated in this analysis: (i) the average contribution of an individual taxon to overall dissimilarity and (ii) its standard deviation across samples. The ratio of these two values indicates how consistently the taxon discriminates among caves.

Results

Mean daily water temperature and NH_4^+-N, NO_3^--N and SRP concentrations varied little among caves or incubation period (Table 2). The CV for the daily water-level profiles in Tony Sinks, Salt River, Jess Elliot and Big Mouth caves were 0.13, 0.15, 0.02 and 0.13, respectively. The CV for Big Mouth Cave was driven by three dates

Table 1 Mean (1 SD) macroinvertebrate biomass (mg dry mass) in leaf packs incubated in four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A.

	Big Mouth	Jess Elliot	Salt River	Tony Sinks
Nematoda	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
Mollusca				
Bivalvia				0.001 (0.011)
Gastropoda				<0.001 (0.002)
Oligochaeta	0.002 (0.013)	<0.001 (0.002)	0.007 (0.066)	0.953 (5.792)
Ostracoda	<0.001 (0.002)			0.042 (0.157)
Cladocera				
Chydoridae	0.001 (0.008)		<0.001 (0.001)	0.001 (0.005)
Non-chydorid Cladocera				<0.001 (0.001)
Copepoda				
Cyclopoida	0.008 (0.021)	0.006 (0.016)	0.013 (0.032)	0.024 (0.049)
Harpacticoida	<0.001 (0.001)	<0.001 (0.001)	<0.001 (<0.001)	<0.001 (0.002)
Malacostraca				
Isopoda				
Caecidotea*	0.209 (0.808)	0.229 (0.953)	0.372 (1.176)	0.298 (1.195)
Lirceus				0.273 (1.102)
Amphipoda				0.036 (0.473)
Crangonyx*[†]		0.011 (0.121)		0.006 (0.073)
Stygobromus*[†]		0.008 (0.112)		
Insecta				
Plecoptera				
Allocapnia[†]	0.002 (0.032)		0.003 (0.046)	
Ephemeroptera				
Eurylophella	0.002 (0.023)	0.002 (0.023)	0.016 (0.120)	0.028 (0.230)
Paraleptophlebia	0.002 (0.029)		0.005 (0.071)	0.083 (0.421)
Diptera				
Dixidae	<0.001 (0.002)			
Ceratopogonidae				0.001 (0.015)
Chironomidae				
Tanypodinae				
Genus A	0.003 (0.034)	0.044 (0.137)	0.107 (0.335)	0.120 (0.363)
Genus B	0.008 (0.101)	0.306 (0.880)	0.017 (0.089)	0.297 (0.671)
Tanytarsini				
Micropsectra	0.003 (0.013)		0.001 (0.007)	0.003 (0.018)
Chironomini				
Polypedilum[†]	0.013 (0.062)	0.001 (0.010)	0.131 (0.590)	1.683 (5.157)
Paratendipes	0.008 (0.038)		0.002 (0.023)	0.009 (0.078)
Chironomus[†]	0.001 (0.011)		0.010 (0.085)	0.006 (0.063)
Dicrotendipes	<0.001 (0.006)			
Orthocladiinae				
Corynoneura	<0.001 (0.005)			<0.001 (0.003)
Parametriocnemus	0.005 (0.038)	0.017 (0.073)	<0.001 (0.003)	0.023 (0.110)
Heleniella	0.001 (0.005)			0.005 (0.039)
Rheocricotopus			0.003 (0.025)	0.003 (0.026)
Diplocladius	0.005 (0.042)	<0.001 (0.002)	0.005 (0.039)	
Eukiefferiella	0.001 (0.008)	0.002 (0.013)		

*Hypogean (i.e. cave-adapted) taxa.

[†]Potential shredder taxa.

near the end of the study. Removing these dates decreased the CV to 0.02. Tony Sinks Cave (807 g m^{-2}) had the highest amount of organic matter and Jess Elliot Cave (1 g m^{-2}) the lowest. Organic matter abundance was significantly different among caves ($F_{3,288} = 30.6$, $P < 0.001$; Fig. 1a). Organic matter did not vary significantly among sampling dates within each cave ($F_{9,288} = 1.7$, $P = 0.10$; Fig. 1b), and the cave × date interaction was

Table 2 Mean (±1 SD) daily temperature and NH_4^+-N, NO_3^--N, and soluble reactive phosphorus (SRP) concentrations in four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A. during two incubation periods

Cave	Inc	Temp (°C)	NH_4^+-N (µg L^{-1})	NO_3^--N (µg L^{-1})	SRP (µg L^{-1})
Big Mouth	1	13 ± 1	6 ± 5	367 ± 139	4 ± 5
	2	13 ± 1	11 ± 18	207 ± 117	2 ± 2
Jess Elliot	1	13 ± 0	BD	242 ± 50	5 ± 6
	2	14 ± 0	BD	231 ± 63	2 ± 3
Salt River	1	12 ± 1	3 ± 2	286 ± 83	5 ± 2
	2	13 ± 1	4 ± 2	265 ± 27	4 ± 3
Tony Sinks	1	14 ± 1	4 ± 3	259 ± 34	2 ± 3
	2	14 ± 1	7 ± 4	284 ± 39	1 ± 2

Inc, incubation period; Temp, temperature; BD, below detection.
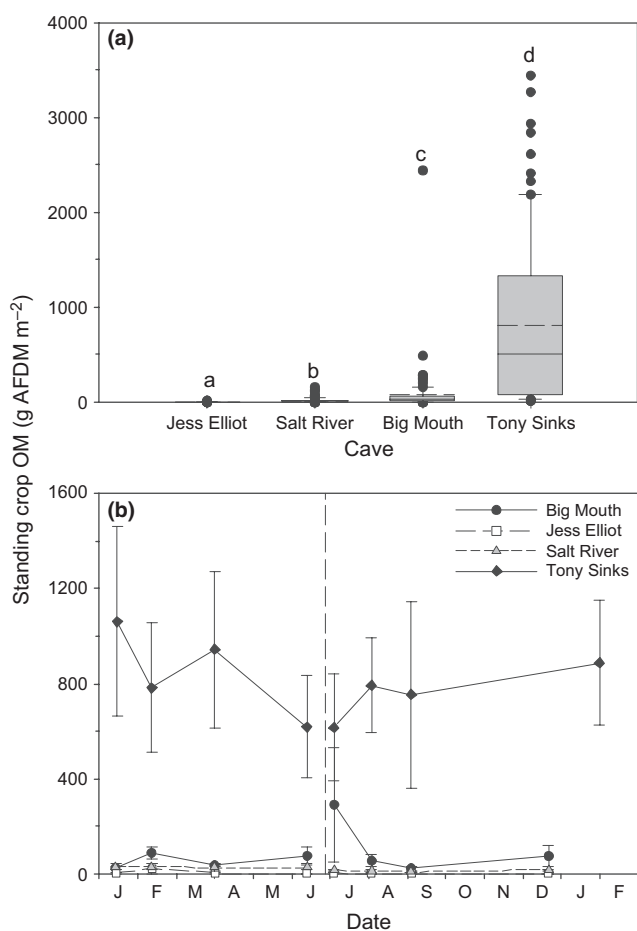
Fig. 1 (a) Box and whisker plot of organic matter (OM) abundance in four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A. Grey boxes are the 25th and 75th percentile, and whiskers are 5th and 95th percentiles. The dashed and solid lines within each box are the mean and median, respectively. Significant differences ($P < 0.05$) among caves are indicated by different letters. (b) Time series of OM abundance in each cave. Vertical dashed line separates incubation periods: left = incubation period 1 (January to July 2008), right = incubation period 2 (July to December 2008, or to January 2009 for Tony Sinks Cave).

not significant ($F_{19,288} = 0.8$, $P = 0.75$). The July peak in organic matter in Big Mouth Cave was driven by a single sample, rather than an increase in organic matter in all samples.

Macroinvertebrate community structure

Mean total macroinvertebrate biomass per bag for corn litter ranged from 0 mg DM in Big Mouth Cave to 31.4 mg DM in Tony Sinks Cave, and from 0 mg DM in Salt River Cave to 17.1 mg DM in Tony Sinks Cave for maple litter. With the exception of corn litter in coarse-mesh bags, litter bags in Tony Sinks Cave contained 2–20 times more macroinvertebrate biomass than the other caves by the end of each incubation period (Fig. 2a–d).

Thirty taxa were identified among the four caves (Table 1). Only three taxa were characterised as hypogean (*Caecidotea*, *Stygobromus* and *Crangonyx*). The mean biomass of hypogean taxa, which were dominated by *Caecidotea*, ranged from 0 to 3.1 mg DM per litter bag. The average contribution of hypogean taxa to total biomass per litter bag was 16% in Big Mouth Cave, 9% in Jess Elliot Cave, 14% in Salt River Cave and 10% in Tony Sinks Cave.

Several taxonomic groups (Chydoridae, Ostracoda, Cyclopoida, Harpacticoida and Oligochaeta) are known to contain both epigean and hypogean species (Romero, 2009). Thus, specimens belonging to these groups could not be confidently categorised as hypogean or epigean. Collectively, mean biomass per litter bag of these taxonomic groups ranged from 0 to 14.3 mg DM. On average, these taxa accounted for 22% of total biomass per litter bag in Big Mouth Cave, 12% in Jess Elliot Cave, 17% in Salt River Cave and 22% in Tony Sinks Cave.

Five taxa were identified as potential leaf shredders (*Allocapnia*, *Polypedilum*, *Chironomus*, *Stygobromus* and *Crangonyx*), of which the latter two are hypogean. Potential shredder biomass was dominated by *Polypedilum* and averaged from 0 to 18.7 mg DM per litter bag. On average, potential shredder biomass accounted for 5% of total biomass per litter bag in Big Mouth Cave, 2% in Jess Elliot Cave, 9% in Salt River Cave and 17% in Tony Sinks Cave (Table 1).

The nested two-way ANOSIMs (factors nested within each cave) of the entire biomass data set ($n = 800$) showed that the following five variables did not significantly affect community structure within each cave ($P > 0.05$): incubation period (R-statistic = 0.02), mesh size (R-statistic = 0.04), station identity (five per cave, R-statistic = 0.01), litter species (R-statistic = 0.01) and date within incubation period (R-statistic = 0.11). The reduced
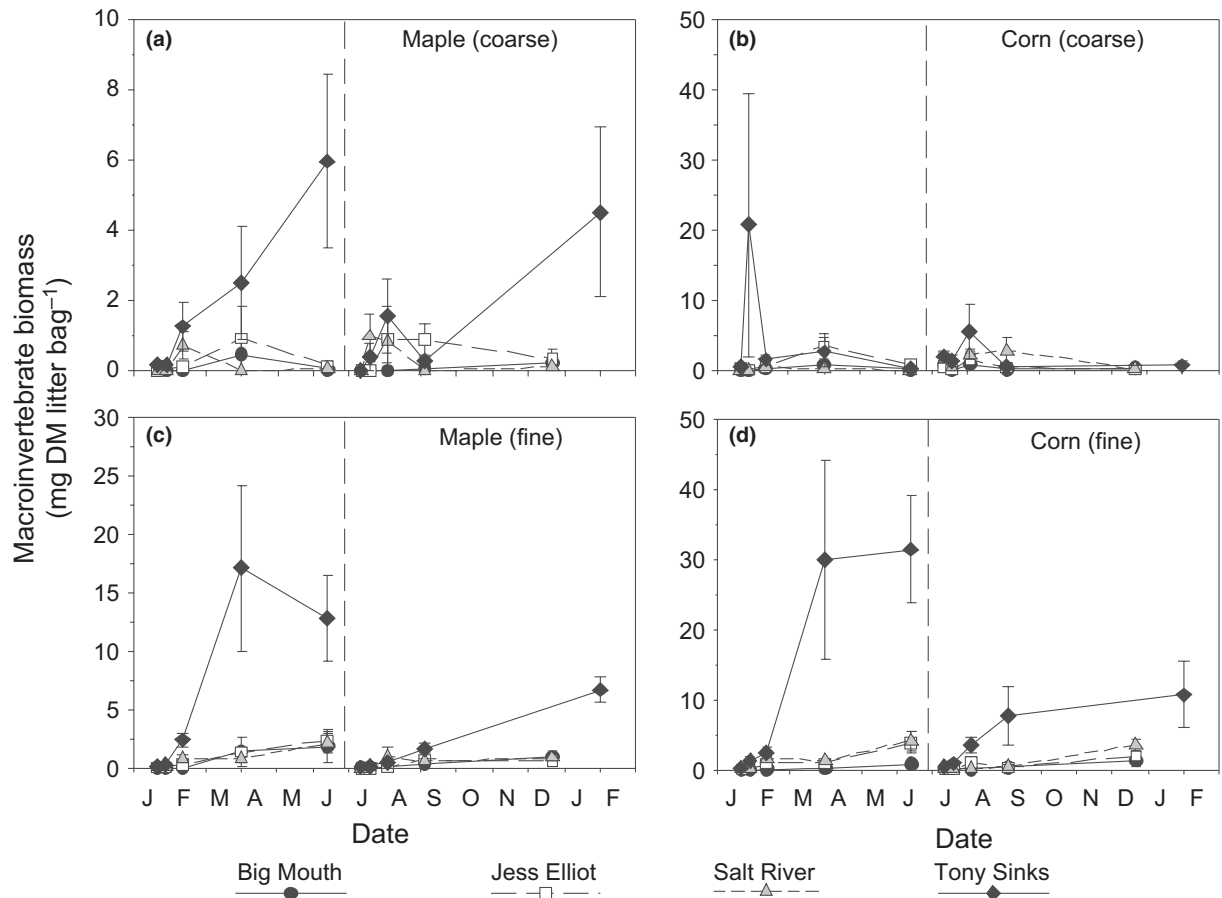
Fig. 2 Mean (±1 SE) macroinvertebrate biomass per g ash-free dry mass (AFDM) of leaf litter remaining in litter bags placed in four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A. (a, b) Coarse-mesh litter bags containing maple and corn litter, respectively. (c, d) Fine-mesh litter bags containing maple and corn litter, respectively. Vertical dashed line separates incubation periods: left = incubation period 1 (January to July 2008), right = incubation period 2 (July to December 2008, or to January 2009 for Tony Sinks Cave).

data set for each cave obtained by averaging the biomass of each taxon from both litter species, mesh sizes and incubation periods across each station was very similar to the original data set (RELATE, Spearman's ρ = 0.70, $P < 0.05$).

Animal community structure differed significantly among caves (*R*-statistic = 0.65, P = 0.001), with pair-wise ANOSIM (*R*-statistics = 0.53–0.90, P = 0.008–0.016) and nMDS both indicating significant differences in community structure (Fig. 3). Six taxa accounted for 79–87% of the overall dissimilarity among all pair-wise cave comparisons (Fig. 4). *Lirceus* (Isopoda) occurred only in Tony Sinks Cave, which also contained an unusually high biomass of macroinvertebrates, particularly Oligochaeta and *Polypedilum* (Fig. 4). Among the remaining caves, Salt River Cave had a high biomass of Tanypodinae genus A and *Polypedilum*, while Tanypodinae genus B was the dominant taxon in Jess Elliot Cave. Macroinvertebrate
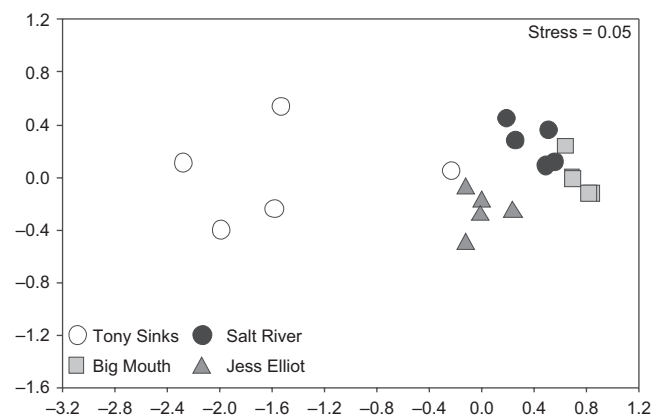

Fig. 3 Non-metric multidimensional scaling ordination plot of macroinvertebrate litter-bag communities from four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A. Data points are based on $\log_{10}$-transformed macroinvertebrate biomass data that were averaged across stations within each cave (see text).
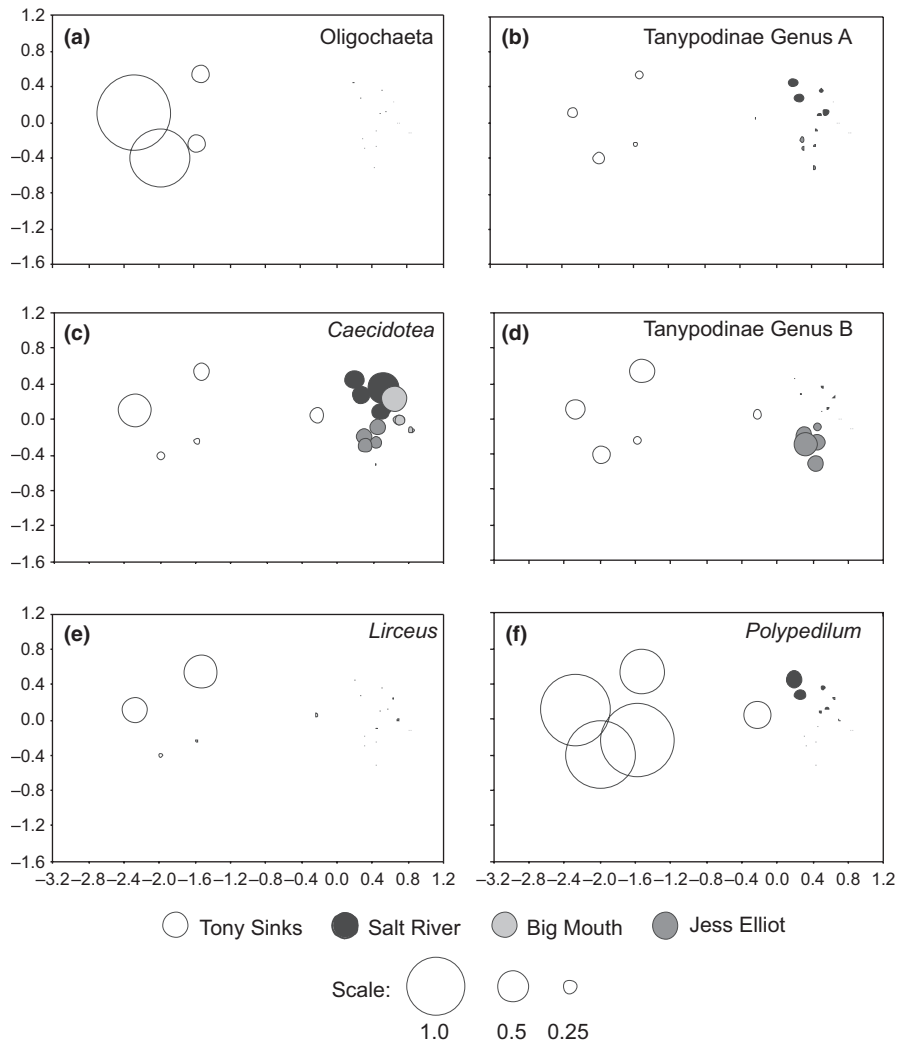
Fig. 4 Non-metric multidimensional scaling ordination plots based on $\log_{10}$-transformed biomass of six taxa that contributed 79–87% to the overall dissimilarity among all pair-wise cave comparisons between cave sites. The diameter of each circle is proportional to the taxon's biomass in each cave. (a) Oligochaeta, (b) Tanypod genus A, (c) *Caecidotea*, (d) Tanypod genus B, (e) *Lirceus* and (f) *Polypedilum*. Stress = 0.05 for all figures.

biomass in Big Mouth Cave was dominated by *Caecidotea* (Fig. 4). The biomass of *Caecidotea* (Isopoda), the only member of this group of taxa classified as hypogean, was similar among caves (Fig. 4).

Litter breakdown rates

The breakdown rate of corn litter in coarse-mesh bags (mean 0.007 day^{-1}, range 0.004–0.012) was, on average, double that in fine-mesh bags (mean 0.003 day^{-1}, range 0.002–0.004; Fig. 5a,b). The breakdown rate of red maple litter in coarse-mesh bags (mean 0.004 day^{-1}, range 0.001–0.012) was, on average, four times faster than that in fine-mesh bags (mean 0.001 day^{-1}, range 0.001–0.003; Fig. 5a,b).

Breakdown rates within each mesh size were similar between incubation periods in each cave (three-way ANOVA *post hoc* pair-wise comparisons, $P > 0.05$), allowing incubation periods within each mesh size and cave to be pooled. Two-way ANOVAs conducted on cave and mesh size gave similar results for both corn and red maple litter: cave ($F_{3,72} = 13.7$, $P < 0.001$ for both litter types), mesh size ($F_{1,72} = 349$ and 100, corn and maple, respectively, $P < 0.001$), and cave × mesh size interaction ($F_{3,72} = 4$, $P < 0.02$ for both litter types). Breakdown rates of leaf litter in fine-mesh bags were slower than those in coarse-mesh bags (Fig. 5). The average corn litter breakdown rate ($k = 0.005$ day^{-1}) was faster than that of red maple [$k = 0.003$ day^{-1}; Fig. 5 (paired *t*-test (79) = 14.1, $P < 0.001$)].
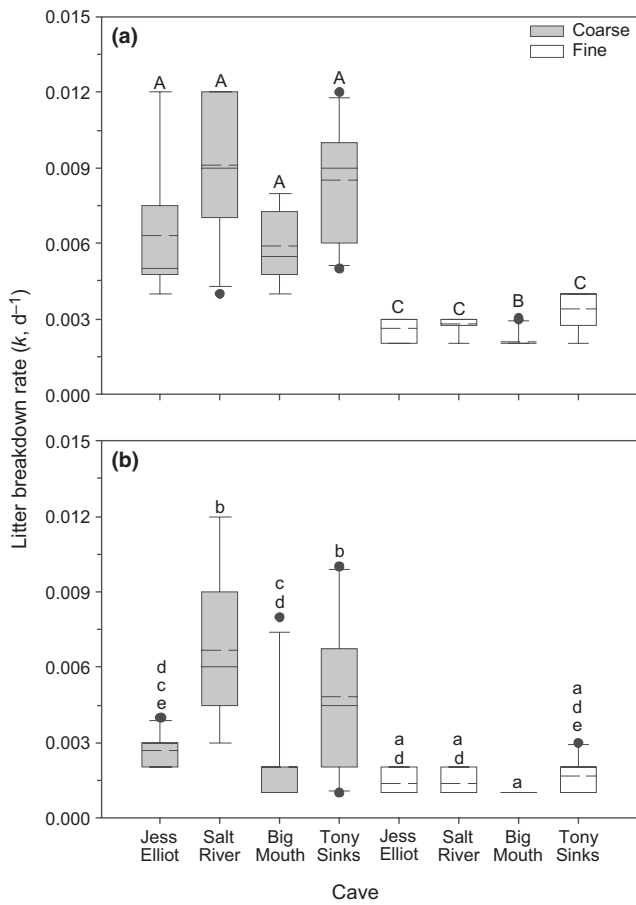
Fig. 5 Box and whisker plot of litter breakdown rate (k, day^{-1}) of corn (a) and red maple (b) in coarse- and fine-mesh bags in four cave streams in north-eastern Alabama and south-eastern Tennessee, U.S.A. Boxes are the 25th and 75th percentile, and whiskers are 5th and 95th percentiles. The dashed and solid lines within each box are the mean and median, respectively. Significant differences ($P < 0.05$) among caves and mesh sizes are indicated by different letters. Note that different case letters do not indicate significant differences between litter types.

Corn litter breakdown rate was similar among caves for each mesh size ($P > 0.05$, Fig. 5a), except for fine-mesh bags in Big Mouth Cave ($P = 0.03$). While the fine-mesh bags containing corn litter in Big Mouth Cave had a statistically slower breakdown rate, the size of the difference (c. 0.001 day^{-1}) was small (Fig. 5a). Maple litter breakdown rate was less consistent than that of corn litter (Fig. 5b). Maple in fine-mesh bags had a similar rate among caves ($P > 0.05$), and the rate was also similar to that in coarse-mesh bags in Big Mouth and Jess Elliot caves ($P > 0.05$). Breakdown of maple litter in coarse-mesh bags was fastest in Salt River and Tony Sinks caves and slowest in Big Mouth and Jess Elliot caves ($P > 0.05$). Mean organic matter abundance per cave was not a significant predictor of breakdown rates of either litter

type or mesh size ($F_{1,2}$ = 0.05–4.93, r^2 = 2–71%, P = 0.2–0.9). Furthermore, mean macroinvertebrate biomass was not a significant predictor of breakdown rates of either litter type in coarse- or fine-mesh bags ($F_{1,2}$ = 0.06–8.10, r^2 = 3–80%, P = 0.1–0.8).

Discussion

We found a large range (c. 0–850 g AFDM m^{-2}) in the abundance of organic matter among the cave streams in our study. A few previous studies have reported the abundance of organic matter in cave streams from c. 30 to 130 g AFDM m^{-2} (Simon & Benfield, 2001, 2002; Simon, Pipan & Culver, 2007). The large range in organic matter abundance shown in our study illustrates how resource supply can both vary greatly among caves within a limited geographic area and in some cases can be similar to that in many surface stream types (e.g. arid/semi-arid, deciduous and boreal streams; Jones, 1997). Factors that contribute to this variability in organic matter abundance among cave ecosystems are unknown, but are likely to be a combination of landscape features (e.g. topology and plant community structure), cave structure (e.g. depth of cave and size of voids and fractures in the surrounding bedrock) and climate (e.g. precipitation and hydrology). For example, the large quantities of organic matter in Tony Sinks Cave were probably the result of strong surface connections. This cave contained several entrances, both vertical and horizontal, that intersected intermittent stream channels. Additionally, large ceiling fissures were present in portions of the cave, below which large deposits of organic matter were observed.

In surface streams, the quantity of organic matter is known to influence macroinvertebrate biomass (Dobson & Hildrew, 1992; Wallace *et al.*, 1999; Negishi & Richardson, 2003). In our study, however, no significant correlation was found between ambient organic matter biomass and macroinvertebrate biomass in litter bags. Nevertheless, Tony Sinks Cave had both the largest amount of organic matter and the highest macroinvertebrate biomass per litter bag. Similarly, Huntsman *et al.* (2011b) reported that benthic macroinvertebrate biomass was higher in cave streams with higher quantities of organic matter. Together, these studies show that organic matter abundance can influence macroinvertebrate biomass in cave streams.

Epigean taxa dominated the communities in all caves in this study, while shredding taxa contributed little to total biomass. Other litter breakdown studies in cave streams have also reported a relatively high biomass of epigean taxa, with shredding taxa composing a small percentage of the overall community (Brussock *et al.*, 1988; Galas

et al., 1996). The absence of shredding taxa in cave systems is probably because of the combined limitation in both habitat access and resource availability (e.g. coarse particulate organic matter). Active colonisation by shredding insect taxa via oviposition is reduced in cave systems because of the limited number of openings to surface habitats. Additionally, perennial aquatic surface habitats are not always present in recharge areas, thereby limiting the possibility of cave streams being actively (e.g. via drift) or passively (e.g. displacement because of floods) colonised by both insect and crustacean (e.g. amphipods) shredding taxa. Simon & Benfield's (2001) study illustrates how connectivity and resource availability interact to structure cave stream communities. Cave streams with direct upstream surface connections received higher inputs of coarse particulate organic matter and had higher shredder abundances (epigean and stygophilic taxa) than disconnected cave streams that only received fine particulate organic matter. Similar to Simon & Benfield (2001), we found the highest shredder biomass in Tony Sinks Cave, which had both the highest organic matter abundance and the strongest apparent surface connection.

The breakdown of organic matter in cave and surface streams is mediated by the same processes, including leaching, microbial processing, consumption and physical fragmentation because of high water velocities (Webster & Benfield, 1986; Simon & Benfield, 2001). Despite differences among caves in organic matter abundance and total macroinvertebrate biomass per litter bag, breakdown rates within each litter type and mesh size were similar except for red maple in coarse-mesh bags in Salt River and Tony Sinks caves. An absence of shredders can affect litter breakdown rates in surface systems (Wallace, Vogel & Cuffney, 1986; Hieber & Gessner, 2002; Eggert & Wallace, 2003), and previous studies in cave systems have attrib-

uted slow leaf litter breakdown rates to the absence of large shredding taxa (Brussock *et al.*, 1988; Galas *et al.*, 1996; Simon & Benfield, 2001). The generally low shredder biomass found in the present study was likely to be a significant factor driving similarities in breakdown rates among cave streams.

Breakdown rates of corn and red maple litter spanned a wide range of values ($k = 0.001$–0.012 day^{-1}). Mesh size significantly affected breakdown rate, which was generally faster and more variable in coarse-than in fine-mesh litter bags. The difference in breakdown rate among mesh sizes was unlikely to have been attributed to shredder exclusion because (i) shredder biomass was universally low (2–17% of total biomass per litter bag) among all cave stream communities; (ii) large-bodied shredders (e.g. crayfish, plecopterans, trichopterans, amphipods) were not well represented in the coarse-mesh litter bags and (iii) the dominant potential shredder was a small-bodied dipteran (*Polypedilum*) capable of colonising both coarse and fine-mesh litter bags (Table 1). Slower litter breakdown in fine-mesh bags have been attributed to anaerobic conditions because of reduced gas exchange (Cummins *et al.*, 1980; Webster & Benfield, 1986). Compaction of leaf litter occurred within our litter bags, which potentially generated anaerobic conditions in the centre of fine-mesh bags. However, macroinvertebrate biomass in fine-mesh bags was similar to or higher than in coarse-mesh bags, and there was no evidence of anaerobic conditions (e.g. blackened litter). A more probable cause of the slower breakdown in fine-mesh bags was the greater retention of litter fragments from physical and invertebrate processing. The relatively high breakdown rates of litter in coarse-mesh bags in Tony Sinks and Salt River were probably attributed to the effects of flooding. The large CV for discharge in these

Table 3 Selected published breakdown rates (k, day^{-1}) for corn (*Zea mays*) and red maple (*Acer rubrum*) from surface and cave streams. Note that in Simon & Benfield's (2001) study, breakdown rates were examined in caves streams that had either 'direct' (i.e. upstream cave entrances or sinkholes) or 'indirect' (i.e. small cracks and fissures) connections to surface environments

Litter type	k (day^{-1})	Habitat	Mesh size (mm)	Study
Non-BT corn (*Z. mays*)	0.015	Surface	10 × 10	Rosi-Marshall *et al.* (2007)
	0.015	Surface	10 × 2.5	Griffiths *et al.* (2009)
	0.015–0.07	Surface	7 × 11	Swan *et al.* (2009)
	0.004–0.012	Cave	10 × 8	This study
	0.002–0.004	Cave	0.5 × 0.5	This study
Red maple (*A. rubrum*)	0.007–0.014	Surface	5 × 5	Benfield *et al.* (2001) and citations therein
	0.008–0.009	Surface	5 × 5	Eggert & Wallace (2003)
	0.005	Surface	1 × 1	Gulis & Suberkropp (2003)
	0.005–0.025	Surface	5 × 5	Greenwood *et al.* (2007)
	0.007–.015	Surface	5 × 5	Webster *et al.* (2001) and citations therein
	0.001–0.012	Cave	10 × 8	This study
	0.001–0.003	Cave	0.5 × 0.5	This study

systems indicates that floods occurred frequently, potentially accelerating coarse-mesh breakdown rates via fragmentation and abrasion because of high water velocities (Canton & Martinson, 1990).

Mean corn litter breakdown rates estimated in this study were 2–20× lower than past estimates for surface streams (Table 3). Not surprisingly, the available surface studies of corn litter breakdown have been in agricultural streams (Rosi-Marshall *et al.*, 2007; Griffiths *et al.*, 2009; Swan *et al.*, 2009), with higher nutrient concentrations (e.g. N and P) and shredder abundances than the cave streams used in this study. The oligotrophic state of our cave streams, coupled with low shredder biomass, probably contributed to the relatively low breakdown rates of corn litter measured in our study.

Mean breakdown rates for maple litter in our study streams were either similar to or lower than estimates made using similar methods in surface systems (Table 3) with comparable nutrient concentrations. Shredder abundances were higher in the surface stream studies, which is likely to have contributed to faster litter breakdown. Litter breakdown estimates from our fine-mesh bags were two to five times slower than estimates reported in a study, by Gulis & Suberkropp (2003), that used 1-mm mesh bags to minimise macroinvertebrate colonisation. While the smaller mesh size used in our study may have contributed to slower breakdown via increased fragment retention, limited microbial colonisation may have also played a role. Microbial abundance and diversity have been reported to be lower in both hyporheic habitats and aquifers than in surface streams (Ellis, Stanford & Ward, 1998; Crenshaw, Valett & Tank, 2002; Krauss *et al.*, 2003). Simon & Benfield (2001) also found that fungal biomass was lower on leaves in cave streams without direct upstream surface connections. Thus, the relatively low rates of litter breakdown in fine-mesh bags in our study may have been caused by low microbial activity.

Environmental variables (e.g. light and temperature) within cave ecosystems have traditionally been viewed as stable compared to surface systems (Juberthie, 2001), which should reduce seasonal patterns of ecosystem processes. In cave systems, temporal stability in the structure of macroinvertebrate communities and the ecological processes they mediate is probably affected substantially by reduced seasonality in environmental cues (e.g. light and temperature), continuous resource availability (which may be small or large) and reduced immigration of epigean taxa because of limited surface connectivity. Accordingly, organic matter abundance, macroinvertebrate community structure and litter breakdown rates did not differ between incubation periods in

our study. Unlike most cave streams, many temperate surface systems receive seasonal pulses of detritus following plant senescence (e.g. forest headwater streams). Seasonal changes in temperature, light, hydrology and resource availability have been linked to temporal patterns in community structure, which have in turn been shown to affect organic matter processing in surface systems (e.g. Benstead & Huryn, 2011). The macroinvertebrate communities of cave streams, which contain few shredders (Brussock *et al.*, 1988; Galas *et al.*, 1996; Simon & Benfield, 2001), appear to process organic matter at a roughly constant rate year-round. To date, we are aware of only one other study that has examined temporal patterns in groundwater communities. Similar to our study, Farnleitner *et al.* (2005) showed that microbial communities in two karst springs showed little to no temporal variability in structure, suggesting that their ecological function may also have been stable through time.

Historically, limited resource inputs have been considered to be the primary factor influencing the ecology and evolution of hypogean communities. The results from our study offered mixed support for this hypothesis. Organic matter abundance was correlated neither with consumer biomass in litter bags nor with litter breakdown rates. However, Tony Sinks Cave had the highest organic matter abundance and consumer biomass, suggesting that resource inputs can influence cave community biomass without corresponding effects on function (e.g. inclusion of shredding taxa). Organic matter abundance does not appear to be the primary factor influencing the presence or absence of taxa among caves in this study because Jess Elliot, Salt River and Big Mouth caves all contained different communities despite marginal differences in organic matter abundance. However, the distinct community found in Tony Sinks Cave (e.g. indicated by its distinct grouping in the nMDS plots, Fig. 3) does suggest that organic matter abundance may play some role. Thus, in addition to organic matter abundance, other cave (e.g. surface connectivity, cave morphology and recharge area) and surface (e.g. topography and vegetation type) characteristics should be quantified to understand better the factors controlling cave community composition.

Hypogean taxa have traditionally been the focus of cave studies. Nevertheless, our study suggests that their role in energy flow, nutrient cycling and food web dynamics might be relatively small in many cave systems. Hypogean taxa represented only a small proportion of the consumer biomass found in our litter bags. In another recent study, Huntsman *et al.* (2011b) showed that epigean prey contributed *c.* 50–100% of the production of the obligate cave salamander *G. palleucus* (McCrady) in two

south-eastern U.S.A. caves. Thus, the inclusion of the entire community, epigean and hypogean, in studies of cave ecosystems will be necessary to understand the ecological processes that occur within them.

Acknowledgments

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