

# Experimental Detritus Manipulations Unite Surface and Cave Stream Ecosystems Along a Common Energy Gradient

Michael P. Venarsky,<sup>1,2\*</sup> Jonathan P. Benstead,<sup>1</sup> Alexander D. Huryn,<sup>1</sup>  
Brock M. Huntsman,<sup>1,3</sup> Jennifer W. Edmonds,<sup>4</sup> Robert H. Findlay,<sup>1</sup>  
and J. Bruce Wallace<sup>5</sup>

<sup>1</sup>Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487, USA; <sup>2</sup>Present address: Australian Rivers Institute, Griffith University, Nathan Campus, Sir Samuel, Griffith Building N78, 4.11, 170 Kessels Road, Nathan, Queensland 4111, Australia; <sup>3</sup>Present address: Department of Fish, Wildlife and Conservation Ecology, New Mexico State University, Las Cruces, New Mexico 88003, USA; <sup>4</sup>Nevada State College, Physical and Life Sciences, Henderson, Nevada 89002, USA; <sup>5</sup>Department of Entomology, University of Georgia, Athens, Georgia 30602, USA

## ABSTRACT

Subsidies of detritus from donor habitats are important energy sources for many ecosystems, but understanding their role in structuring recipient food webs requires comparative experimental studies along the full spectrum of detrital fluxes. Here we report results from an experimental addition of maize (*Zea mays* L.) litter to a detritus-poor cave stream ecosystem, which we then compare with analogous, past experiments using detritus-rich surface stream ecosystems that similarly have detritus-based food webs and extremely low *in situ* primary production. Bulk-tissue and compound-specific stable isotope analyses showed that maize litter carbon (C) was rapidly assimilated by microbes and transferred via suc-

cessive trophic levels to the top of the cave stream food web (omnivorous crayfishes and predatory salamanders). All trophic levels increased in abundance and biomass, but only facultative cave taxa, that is those also found in surface streams, contributed to this numerical response. The lack of response by obligate cave species presumably occurred because evolutionary trade-offs associated with adaptations to low-C environments constrained their population-level responses during the one-year period of the litter addition. Comparison of the responses of the cave community with the analogous litter manipulation experiments in surface streams showed strong convergence in the functional relationship between invertebrate and detritus biomass ( $R^2 = 0.72$ ,  $P < 0.0001$ ). Our results suggest that these seemingly disparate stream food webs lie along a single, common gradient of detritus supply, occupied at its extreme minimum by communities of obligate cave taxa adapted to low-energy environments.

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\*Corresponding author; e-mail: mvenarsky@gmail.com

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## INTRODUCTION

The majority of global net primary production is not consumed by herbivores, but enters the detrital pool that dominates most ecosystem energy budgets (Polis and Strong 1996; Cebrian 1999; Cole and others 2006). Many studies have shown how detrital resources influence ecological processes, including the support of more diverse, abundant, and productive consumer communities than could be maintained by in situ primary production (Moore and others 2004; Marczak and others 2007; Hagen and others 2012). Accordingly, the transport of detritus across ecosystem boundaries to recipient food webs plays an important role by supplying an abundant resource that exerts strong bottom-up control on community biomass and secondary production (Wallace and others 1999; Chen and Wise 1999). Allochthonous sources of detritus also contribute to food web stability (Huxel and McCann 1998; Moore and others 2004), while also linking ecosystems across ecological landscapes (Marcarelli and others 2011).

Most classic studies examining detrital dynamics have used adjacent recipient and donor ecosystems, with the former receiving detritus subsidies typically exceeding consumer demand (Wallace and others 1997; Cole and others 2006). Forest streams are quintessential examples of such donor-controlled, detritus-based ecosystems. In extreme cases, shading by the forest canopy results in vanishingly low levels of in-channel net primary production (for example,  $<0.5 \text{ g C m}^{-2} \text{ y}^{-1}$ ; Hains 1981) and secondary production is based almost entirely on imported leaf litter and dissolved organic C (DOC; Wallace and others 1997, 1999), with high rates of leaf litter inputs to some forest streams resulting in large detritus surpluses (for example,  $>50\%$  of annual detrital inputs) due to limited consumer demand (Benstead and others 2009). Although the detrital dynamics of forest stream ecosystems have received much attention (Tank and others 2010), these strongly linked, highly subsidized ecosystems alone do not provide insight into the extremes of recipient-donor ecosystem relationships. Cave stream ecosystems, for example, lack photosynthetic primary producers (see Sarbu and others 1996; Engel and others 2004 for descriptions of chemolithoautotrophy-based cave ecosystems) and are thus similar to forest stream ecosystems with low rates of primary production. Unlike forest streams, however, there is a gradient in connectivity between cave and surface ecosystems that both generally limits or-

ganic C supplies and consumer productivity and causes significant variability in productivity among caves (Hüppop 2000; Huntsman and others 2011b; Venarsky and others 2014). Importantly though, high rates of consumer demand relative to detritus availability within cave stream ecosystems may result in negligible surpluses, even in relatively productive caves with strong surface connections (Venarsky and others 2014).

Studies of the importance of the links between donor and recipient ecosystems forged by organic matter transport are relatively numerous. In contrast, few ecosystem-level studies have examined how recipient communities are affected across extreme ranges in detritus subsidies (Hagen and others 2012; Scharnweber and others 2014; Venarsky and others 2014). To our knowledge, the most comprehensive studies are those of Wallace and others (1997, 1999, 2015), which experimentally excluded and then replaced leaf litter in a naturally detritus-rich [for example,  $>0.5 \text{ kg}$  mean annual ash-free dry mass (AFDM)  $\text{m}^{-2}$ ] forest stream in western North Carolina, USA. The motivation for these studies was to provide an experimental, ecosystem-level evaluation of the importance of detritus to ecosystem productivity; they did indeed show a dramatic bottom-up effect that was propagated through all trophic levels examined, with most animal species declining in abundance and biomass when detritus was excluded.

Here we report the results of a complementary study in which we added leaf litter to a cave stream with low quantities of particulate organic matter (that is,  $<0.05 \text{ kg}$  AFDM  $\text{m}^{-2}$ ; Bluff River Cave, Alabama, USA). We monitored community structure of all trophic levels (microbes to top predators) in two stream reaches (upstream "reference reach," downstream "treatment reach") for 2 years. During the second year, maize litter (*Zea mays*) was added to the treatment reach to experimentally increase detrital resources. Maize litter was chosen because it is a readily available and palatable litter resource (Griffiths and others 2009; Venarsky and others 2012; Scharnweber and others 2014). As a C4 plant, maize is also enriched in  $^{13}\text{C}$  relative to the ambient C3 plant detritus, which allowed us to track the flow of its C through the cave food web using stable isotope analysis (Scharnweber and others 2014).

We tested three predictions. First, we anticipated that the experimental maize litter subsidy would have a bottom-up effect on the entire cave stream community. We expected increases in prokaryotic (archaea and bacteria) and micro-eukaryotic biomass (fungi, ciliates) to be propagated to higher

trophic levels (insects, crustaceans, and vertebrates). Second, we predicted that community structure at all trophic levels would change following the maize litter subsidy. More specifically, we expected the population-level responses of the metazoans to vary according to life-history strategy (obligate vs. facultative cave species). Obligate cave species have characteristics presumed to be adaptations to energy limitation, including low metabolic activity, growth rates and fecundity (Poulson and Lavoie 2000; Hüppop 2000; Gibert and DeHarveng 2002). Facultative cave species maintain populations in both surface and cave streams (Poulson and Lavoie 2000; Hüppop 2000, 2005, Fernandes and others 2016) and have relatively high growth rates and fecundity, which are apparent adaptations to energy-rich surface environments (Poulson and Lavoie 2000; Hüppop 2000, 2005). Consequently, we expected that our experiment would increase biomass of both obligate and facultative cave species, but that facultative species would show greater responses via immigration and reproduction (Hüppop 2000; Culver and Pipan 2009). Third, we predicted that the litter addition would cause macroinvertebrate biomass in the cave stream to converge with that observed in forested headwater surface streams (Wallace and others 1997, 1999, 2015). The cave streams (this study) and forested headwater surface streams (Wallace and others 1997, 1999, 2015) included in our comparative analysis are all located in the southern Appalachian Mountains (~235 km apart) and thus share key ecological characteristics (Table S1), with each food web dependent on detrital subsidies due to either very low or non-existent *in situ* primary production. Thus, this comparison removed the confounding influence of landscape position (that is, surface vs. subterranean) and allowed an examination of the fundamental influence of organic matter fluxes on recipient ecosystem community structure.

## METHODS

### Study Site

Our litter addition experiment was conducted in the stream that flows through Bluff River Cave, Jackson Co., Alabama, USA (Table S1). Bluff River Cave has approximately 1200 m of accessible passage ways (height ~20 m, width ~10 m), of which about 1000 m is stream channel ranging from 0.5 to 4 m in width and from 0.2 to 0.8 m in depth at baseflow. Water depth can exceed 9 m during spates. Two 100-m stream reaches, separated by 25 m, were chosen:

an upper reference reach and a lower treatment reach. These stream reaches are approximately 0.4 stream km from the cave entrance and substrate composition throughout both study reaches consisted of sand and gravel with small areas (<20 linear meters of stream) of exposed bedrock. See Appendix S1 and Table S1 for further information. Land cover of the cave stream recharge area (analogous to surface stream watershed or catchment area) consists of dense deciduous forest, with essentially no urbanization or intensive agriculture. Cave stream flows in this region vary with season, with relatively high and variable discharge from fall to spring (that is, November to April) and low stable flows through the late spring and summer months (that is, May to October; M. P. Venarsky, *personal observation*). Coarse particulate organic matter sampled from this cave stream is usually highly processed but is presumably derived from the dominant hardwoods of the recharge area [for example, oak (*Quercus* spp.), shagbark hickory (*Carya ovata*), sugar maple (*Acer saccharum*), eastern red cedar (*Juniperus virginiana*)].

Bluff River Cave stream provides habitat for obligate and facultative cave animals. Obligate cave animals include species of the isopod genus *Caecidotea*, and the amphipod genera *Crangonyx* and *Stygobromus*, the crayfish *Cambarus hamulatus*, and the salamander *Gyrinophilus palleucus*. Facultative cave animals include the crayfish *Cambarus tenebrosus*, larval insects (primarily Diptera: Chironomidae, Ephemeroptera and Plecoptera), and the salamander, *Eurycea cirrigera*. Obligate cave stream species of Ephemeroptera, Plecoptera, and Diptera have not been identified in our study region, but a few taxa have been identified in Europe (Gibert and DeHarveng 2002; Figueroa and López-Rodríguez 2010; Andersen and others 2016). Colonization by facultative stream-dwelling taxa is likely via sinking streams (Ray 2005).

### Experimental Design

Our study used a before–after control–impact (BACI) sampling design (Stewart-Oaten and others 1986). Both reaches were sampled monthly from February 2009 to February 2011. Maize litter was added to the treatment reach beginning in February 2010 and augmented monthly thereafter to maintain a target of approximately 500 g dry mass (DM) maize litter m<sup>-2</sup>. We installed 20 mesh barriers (5-m intervals) perpendicular to the stream channel to minimize maize litter loss from the treatment reach. We constructed mesh barriers from 1 × 0.5-m pieces of netting (mesh size 2.5 × 1.5 cm) and used sandbags as anchors. Loss

of maize litter from the treatment reach was minimal under baseflow conditions, but additions of maize litter were required the week following two large flood events. The maize variety used (variety W5280: Wyffels Hybrids Inc., Geneseo, Illinois, USA) did not contain the *Bt* toxin, which has been shown to affect some aquatic invertebrates negatively (Rosi-Marshall and others 2007).

### Microbial Biomass and Community Structure

Microbial biomass was estimated using phospholipid phosphate (PLP) analysis. Community structure was determined using phospholipid fatty acid (PLFA) and terminal restriction fragment length polymorphism (T-RFLP) analyses. On each sampling date, three sediment cores (3.9 cm diameter  $\times$  2 cm depth) were taken from each study reach, transported on ice to the laboratory, frozen, lyophilized, and then stored in a  $-20^{\circ}\text{C}$  freezer until PLP and PLFA analysis. In addition, three sediment cores (5-mL syringe to a depth of 2 cm) were collected from each study reach with gloved hands, placed into sterile cryovials, immediately flash-frozen, and stored at  $-80^{\circ}\text{C}$  until processed for T-RFLP analysis (see Appendix S2 for detailed methods).

### Metazoan Community Analysis

On each sampling date, 10 sediment cores (22.5 cm diameter, 15 cm or to bedrock in depth) were taken within each study reach and used to estimate organic matter mass and microcrustacean and macroinvertebrate (amphipods, isopods, and insects) densities. Crayfish and salamander densities were estimated by placing block nets at the ends of each study reach and capturing individuals with dip nets during three passes. The snout-vent length for salamanders and ocular carapace length for crayfish was recorded ( $\pm 1$  mm) prior to release.

Invertebrates were removed from samples in the laboratory, identified to the lowest practical taxonomic level, and their lengths measured ( $\pm 1$  mm). Biomass was estimated using length–mass relationships (Calow 1975; Culver and others 1985; Leeper and Taylor 1998; Benke and others 1999; Doroszuk and others 2007; Lemke and Benke 2009; Huntsman and others 2011a, b). Invertebrates were categorized as obligate or facultative cave taxa. Obligate cave taxa were identified by the lack of eyes and pigment (for example, *Caecidotea* sp., *Crangonyx* sp. and *Stygobromus* sp., and *Cambarus hamulatus*). All Ephemeroptera, Plecoptera, and Diptera were assumed to be facultative cave taxa

because obligate cave species for these groups are currently only known in Europe (Gibert and DeHarveng 2002; Figueroa and López-Rodríguez 2010; Andersen and others 2016). Some taxa could not be reliably placed into a category (oligochaetes, cyclopoids, harpacticoids, ostracods, nematodes, and cladocerans). These taxa, however, contributed negligibly to total biomass. Organic matter was sieved to retain the greater than 250  $\mu\text{m}$  fraction, which was dried to constant mass ( $60^{\circ}\text{C}$ ), weighed, and then combusted at  $500^{\circ}\text{C}$  for 6 h. The remaining ash was wetted, re-dried, and weighed. The difference between dry mass and ash mass estimated ash-free dry mass (AFDM).

### Food Web Structure

Incorporation of maize-derived C into the food web was tracked via stable isotope analysis. Samples of detritus and consumer tissues were collected from both study reaches on three dates prior to the litter addition and then every 1–2 months following litter addition. Samples were transported on ice to the laboratory, where invertebrates were lyophilized and stored at  $-20^{\circ}\text{C}$  until analysis for C and nitrogen (N) isotope composition (Analytical Chemistry Laboratory, University of Georgia, Athens, USA, and Marine Biological Laboratory, Stable Isotope Laboratory, Woods Hole, MA, USA, for low-mass samples). We analyzed whole-body samples (either individuals or composites of several individuals) for small taxa (for example, Diptera and Ephemeroptera) and muscle tissue for crayfish (cheliped) and salamanders (tail). N isotopes were used to assess relative trophic position of metazoans. Fatty acid methyl esters (FAMES, see Appendix S2) were used to determine maize-derived C contributions to microbial components of the food web. Compound-specific isotope analysis of FAMES was conducted using a Thermo Electron Isotope Ratio Mass Spectrometer (model Advantage V) fitted with an Agilent model 6890 gas chromatograph and a Thermo Electron GCC III.

### Data Analyses

Changes in variables before and after the litter addition were assessed using BACI analysis (Stewart-Oaten and others 1986; Schroeter and others 1993). For each variable of interest, the mean monthly value from the reference reach was subtracted from the corresponding treatment reach value and either a Student's paired *t* test (normally distributed data) or a Wilcoxon signed-rank test (non-normally distributed data) was used to compare effect sizes pre- and post-treatment. PLFA profiles were analyzed using principal component

analyses (PCA) after log transformation [ $\ln(x + 1)$ ] of weight percent fatty acid data. T-RFLP peak data were analyzed using non-metric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM; R Programming Team version 3.1.1).

We estimated the relative contributions of natural C3 litter and maize C4 litter to microbial, invertebrate and salamander production using  $\delta^{13}\text{C}$  data in a mixing model (R package Stable Isotope Analyses in R [SIAR]; Parnell and others 2010; Parnell and Jackson 2011; R Core Team 2013). SIAR uses a Bayesian framework incorporating uncertainty in all input data to produce probability estimates of source contributions to biomass. Our mixing models incorporated uncertainty in the  $\delta^{13}\text{C}$  of consumers (or microbial PLFAs) and the two C sources, as well as the trophic fractionation of C isotopes ( $\Delta\delta^{13}\text{C}$ ). Estimates of  $\Delta\delta^{13}\text{C}$  are not available for the microbial PLFAs and consumers that we sampled. Consequently, we calculated empirical fractionation factors by assembling all the  $\delta^{13}\text{C}$  data from the reference reach and the pre-addition treatment reach for C3 litter ( $n = 17$ ) and each PLFA group ( $n = 11\text{--}18$ ) or taxon ( $n = 1\text{--}23$ ) and bootstrapping ( $n = 999$ ) the difference between the two values to give a mean ( $\pm 1$  SD)  $\Delta\delta^{13}\text{C}$  for each PLFA group or taxon. We then applied this  $\Delta\delta^{13}\text{C}$  in subsequent SIAR calculations using source (C3 litter [ $n = 12$ ] and maize litter [ $n = 12$ ]) and consumer ( $n = 2\text{--}36$ )  $\delta^{13}\text{C}$  values in the post-addition treatment reach. This approach assumes that fractionation did not change with the addition of maize litter. Because we had no prior information to inform relative C3 and C4 contributions, we used the vague (Dirichlet-distributed) default prior. We combined all data across all dates when calculating  $\Delta\delta^{13}\text{C}$  and SIAR estimates of source contribution. Some PLFAs were grouped for analysis because they represented a single trophic group (fungi: 18:2 $\omega$ 6/19:0 and 18:3 $\omega$ 3; non-fungal protists: 20:4 $\omega$ 6 and 20:5 $\omega$ 3) or because their gas chromatograph peaks could not be separated with sufficient resolution (i15:0, a15:0 and i16:0; 16:0, 16:1 $\omega$ 7t, 16:1 $\omega$ 9/10:me16:0, 16:1 $\omega$ 7, 16:1 $\omega$ 5, i17:0, a17:0, and 17:0; 18:1 $\omega$ 5, 18:1 $\omega$ 7, 18:1 $\omega$ 9, and 18:1?). Each SIAR run consisted of 500,000 iterations (burn-in of 50,000 iterations) with the output thinned by a factor of 15.

### Comparison of Forested Headwater Surface Versus Cave Streams

We compared the results of our experiment, in which detritus was added to a low-C ecosystem, with an analogous past experiment in which

detritus was first removed from, and then added to, a light-limited, naturally high-C ecosystem that shared a similar regional pool of potential colonizers (that is, southern Appalachian Mountains). Data from our study of the Bluff River Cave stream were thus combined with data from past experimental litter exclusions and additions in forested headwater streams (C53, C54, C55) at Coweeta Hydrologic Laboratory Southern Research Station (Wallace and others 1997, 1999, 2015) to examine relationships between mean annual organic matter storage and macroinvertebrate biomass. Equivalent datasets from three other reference cave streams in northeastern Alabama (Huntsman and others 2011a, b; Venarsky and others 2012) were used in the analyses to provide context. We did not expand our analysis to other datasets because equivalent organic matter and invertebrate biomass data (that is, annual means derived from monthly benthic samples from the same biogeographic region) are not widely available. All data were analyzed using simple linear regression (R Programming Team version 3.1.1).

Although maize litter was added to the treatment reach in Bluff River Cave and tree litter [tulip poplar (*Liriodendron tulipifera*), dogwood (*Cornus florida*), sweetgum (*Liquidambar styraciflua*)] was added to the forest stream studied by Wallace and others (2015), the effective nutritional qualities of maize and deciduous hardwood litter are generally similar (Rosi-Marshall and others 2007; Chambers and others 2010). Maize litter, however, has a relatively low C:N ratio (24–39) and lignin content (4.5–6.3%; Griffiths and others 2009) compared with the C:N ratios (range = 49–121) and lignin contents (range = 13–33%) of *L. tulipifera*, *C. florida* and *L. styraciflua* (Ostrofsky 1997).

## RESULTS

### Integration of Maize Litter C into the Food Web

Compound-specific stable isotope analysis of PLFAs indicated that on average 21% to 49% of C assimilated by PLFA-defined microbial functional groups was from maize litter (Figure S1, Table S2). PLFAs typically associated with bacteria showed the lowest levels of  $^{13}\text{C}$  enrichment, while fungal and phagocytic protist PLFAs showed higher levels of enrichment. Metazoans showed a larger range in the mean proportion of maize-derived C than microbes and protists (range 28% to 81%) and generally higher levels of enrichment. Most metazoan taxa sampled from the treatment reach following

the litter addition showed greater than 50% dependence on the maize-derived C, although some taxa showed a high degree of variability in the level of enrichment among individuals (for example, *C. hamulatus*, Plecoptera; Figure S1). The temporal pattern of enrichment showed rapid incorporation of maize-derived C into consumer tissues in the treatment reach, with a plateau of approximately  $-15\text{‰}$   $\delta^{13}\text{C}$  reached 125 d (18 June 2010; Figure 1A) following the litter addition. Once this plateau was reached, a clear separation in  $\delta^{13}\text{C}$  values between reaches was maintained for the duration of the study, with the exception of samples taken on December 15, 2010 (Figure 1A), which were likely affected by drift of individuals from the reference reach during high discharge caused by a major storm on November 30, 2010. The clear separation of  $\delta^{13}\text{C}$  values for consumers between reaches was also apparent for annual means calculated for individual taxa (Figure 1B).

### Population and Community Responses by Microbes

Following the litter addition, PLFA analyses indicated that fungal biomass increased by about 450%, on average, in the treatment reach while decreasing by 56% in the reference reach ( $P < 0.001$ ; Figure 2, Table S3). Phagocytic protist and bacterial biomass also increased (241% and 47%, respectively) in the treatment reach compared with the reference reach ( $P < 0.05$ ; Figure 2, data for protists not shown). A pairwise test of the bacterial T-RFLP profiles showed that communities in the treatment reach diverged from reference conditions following litter addition (ANOSIM;  $R$ -statistic = 0.46,  $P = 0.001$ , Figure S2A) and when compared with communities in both the reference and treatment reaches prior to litter addition (ANOSIM;  $R$ -statistic  $> 0.3$ ,  $P < 0.014$ ). The bacterial community of maize litter prior to its addition to the treatment reach did not drive this result (data not shown). Similarly, analyses of PLFA profiles indicated a significant shift in microbial and protist community structure following the litter addition, due to increases in the relative abundance of fungi, phagocytic protists, and aerobic bacteria (Figure S2B).

### Population and Community Responses by Metazoans

Mean richness of obligate cave taxa did not vary before (0.2 to 1.0 per sample) or after (0.2 to 0.8 per sample) the maize litter addition (Student's

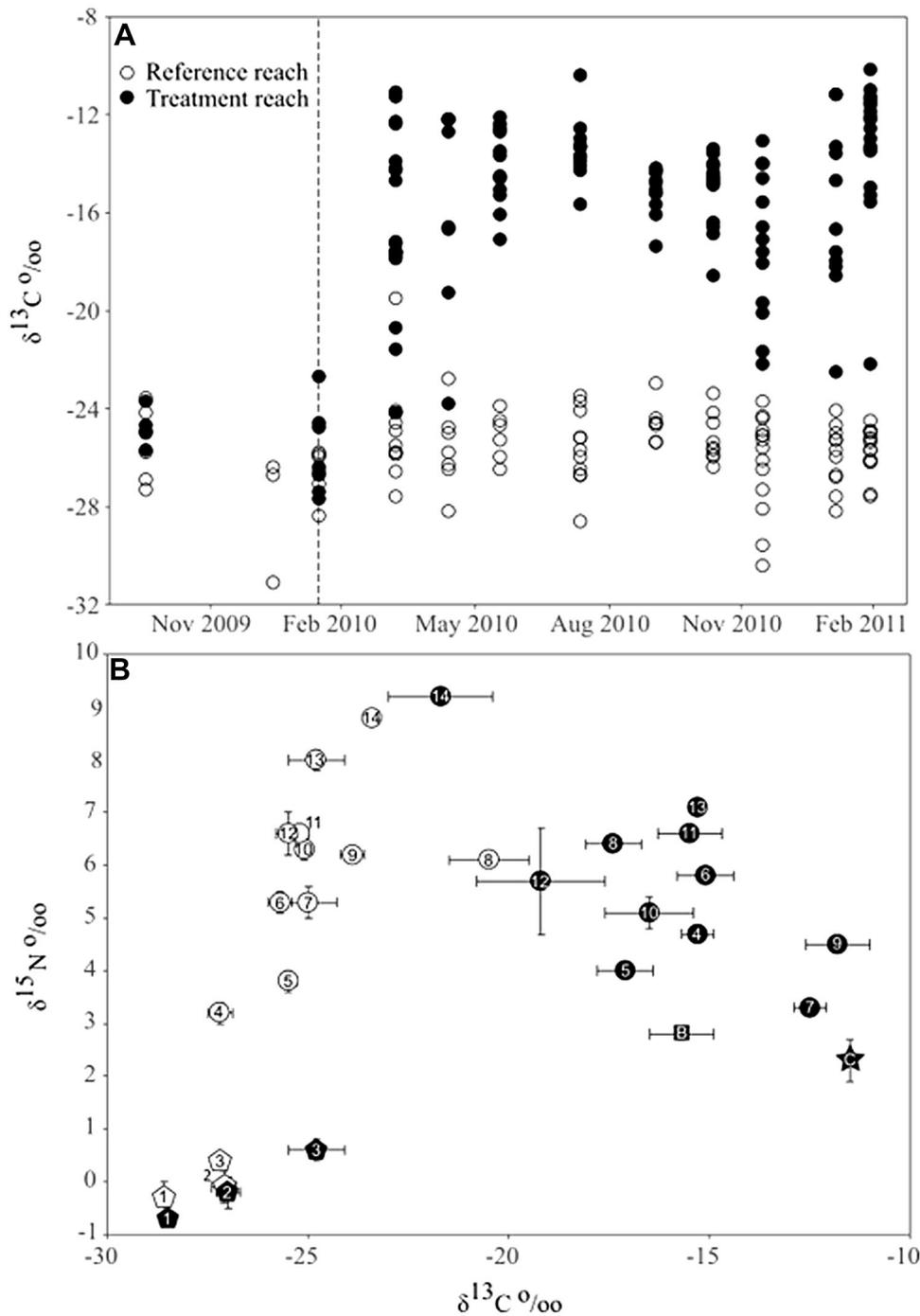
paired  $t$  test;  $P = 0.14$ ). The mean richness of facultative cave taxa, however, increased (1.1 to 3.6 vs 3.2 to 6.6 taxa per sample; Student's paired  $t$  test;  $P < 0.05$ , Figure S3).

Total macroinvertebrate biomass increased significantly following the maize litter addition (Wilcoxon signed-rank test;  $P < 0.001$ , Figure 3E, Table S4). The biomass of obligate cave species showed no response (Student's paired  $t$  test;  $P = 0.09$ , Figure 3A). The biomass of facultative cave species, however, increased significantly (Wilcoxon signed-rank test;  $P < 0.001$ , Figure 3B). With regard to large-bodied macroinvertebrates, the biomass of the facultative cave crayfish *C. tenebrosus* increased after the litter addition (Wilcoxon signed-rank test;  $P = 0.02$ , Figure 3D), whereas the biomass of the obligate cave crayfish *C. hamulatus* showed no significant change (Student's paired  $t$  test;  $P = 0.85$ , Figure 3C, Table S5). Finally, the biomass of the facultative cave salamander *Eurycea cirrigera* increased following the litter addition (Wilcoxon signed-rank test;  $P = 0.001$ , Figure 3F, Table S5). The response of the obligate cave salamander *G. pallescens* could not be tested due to low capture rates.

Although the biplot of mean  $\delta^{13}\text{C}$  versus  $\delta^{15}\text{N}$  values for consumers and basal resources shows clear separation of  $\delta^{13}\text{C}$  values between the reference and treatment reaches (exceptions include the two highly mobile crayfish species and naturally occurring organic matter; Figure 1B), a regression of mean  $\delta^{15}\text{N}$  values for consumers occurring in both reaches yielded a slope of 0.92 ( $\delta^{15}\text{N}$  treatment reach =  $0.92 \times \delta^{15}\text{N}$  reference reach + 0.05;  $R^2 = 0.90$ ,  $P < 0.0001$ ,  $n = 15$ ) showing a nearly 1:1 correspondence, indicating that the trophic structure of metazoan consumers remained similar following the maize litter addition (Figure 1B). The range of  $\delta^{15}\text{N}$  values measured between basal categories of organic matter ( $-0.7\text{‰}$ ) and the top consumer (*Cambarus hamulatus*:  $9.2\text{‰}$ ) suggests that three trophic levels of metazoan consumers were present (assuming a  $3.4\text{‰}$  shift in  $\delta^{15}\text{N}$  values between trophic levels; Hershey and others 2006).

### Comparing Organic Matter Manipulations in Forest and Cave Streams

The maize litter addition to Bluff River Cave stream and approximately 8 years of organic matter exclusion and coarse woody debris removal from a forest stream ("organic matter exclusion," hereafter; Wallace and others 2015) resulted in similar biomass of macroinvertebrates in these ecosystems

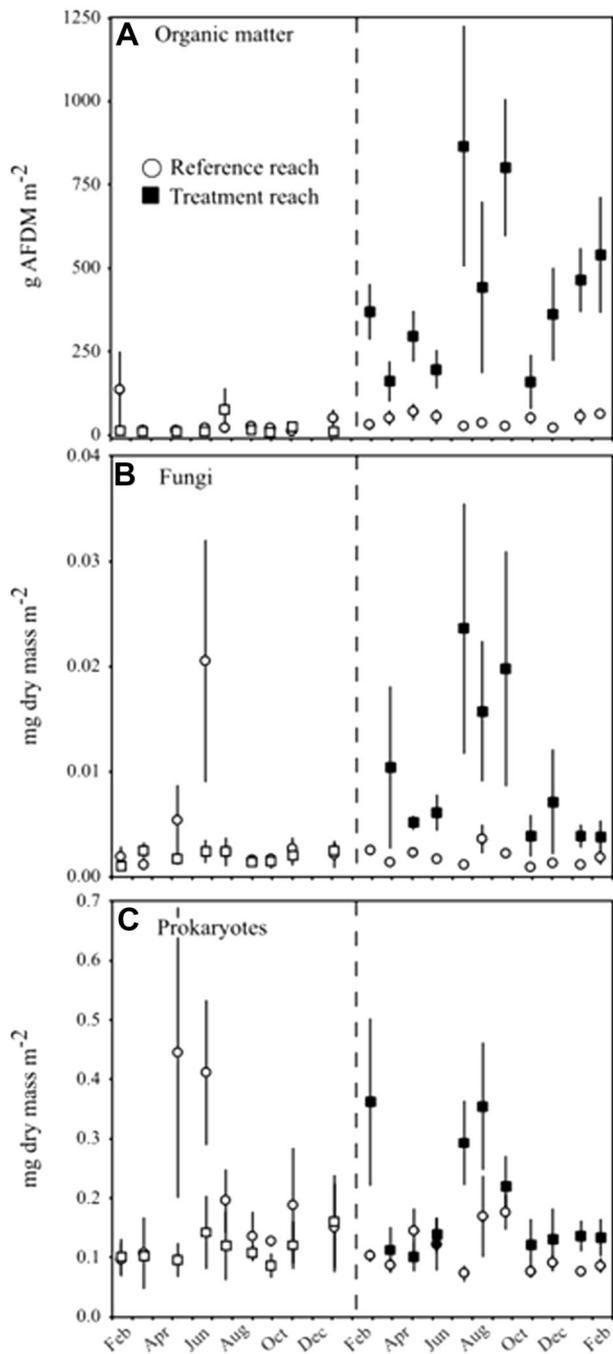


**Figure 1. A**  $\delta^{13}\text{C}$  values of individual consumers in the reference and treatment reaches of Bluff River Cave, Alabama, USA, before and after the addition of maize litter. The dashed line indicates the start of the maize litter addition. Highly mobile species (that is, those capable of moving both downstream and upstream) were excluded from the time series (= *Eurycea* sp., *Cambarus tenebrosus* and *C. hamulatus*). **B** Biplot showing mean  $\delta^{15}\text{N}$  versus  $\delta^{13}\text{C}$  values ( $\pm 1$  SE) for basal resources (star, square, and pentagon) and consumers (circle) in the reference and treatment reaches of Bluff River Cave, Alabama, USA. C maize litter (star), B microbial biofilm (square) in the treatment reach following litter addition, 1 C3 plant litter (pentagon), 2 wood (pentagon), 3 fine benthic organic matter (pentagon), 4 non-Tanypodinae (Diptera: Chironomidae), 5 Oligochaeta, 6 Tanypodinae (Diptera: Chironomidae), 7 Ephemeroptera, 8 *Cambarus tenebrosus*, 9 Isopoda, 10 *Caecidotea* sp., 11 *Eurycea* sp., 12 Plecoptera, 13 *Crangonyx* sp., 14 *Cambarus hamulatus*. Data points obscure some error bars.

[2-tailed *t* test, log transformation,  $P > 0.13$ ; Figure 4A, compare open diamonds (forest stream following organic matter exclusion and removal) versus black squares (cave stream treatment reach post-addition)], although organic matter mass was greater in the forest stream ( $P < 0.0001$ ). The addition of leaf litter to the forest stream, following organic matter exclusion, resulted in a convergence of both organic matter (that is, no significant difference, 2-tailed *t* test, log transformation,

$P > 0.05$ ) and macroinvertebrate biomass [ $P > 0.30$ ; Figure 4A, compare gray diamonds (forest stream following litter addition) and closed squares (cave stream reach following litter addition)] between the cave and surface streams.

Regression analysis revealed that a linear function best described the macroinvertebrate–organic matter relationship (mass  $\text{m}^{-2}$ ) among surface and cave streams ( $R^2 = 0.72$ ,  $P < 0.0001$ ; Figure 4A). Nevertheless, there was a significant decline in the



**Figure 2.** Mean (bars are standard errors) of **A** organic matter, **B** fungal biomass, and **C** prokaryotic biomass measured in the Bluff River Cave stream. Data from the reference reach are indicated by *open circles*. Data from the treatment reach are indicated by *open* (pre-litter addition) and *closed squares* (post-litter addition). Vertical dashed line indicates beginning of litter addition. One data point immediately following the litter addition in plot b (fungal biomass) was an extreme outlier [mean: 0.223, SE 0.131; mg dry mass  $m^{-2}$ ] and was excluded from the graph to allow patterns in other months to be more easily observed. Removing this data point from the BACI analyses did not affect conclusions.

ratio of macroinvertebrate biomass to organic matter ( $R^2 = 0.43$ ,  $P < 0.0001$ ; Figure 4B), with higher ratios (that is,  $>2$  mg  $g^{-1}$  AFDM) at organic matter levels below about 100 g AFDM  $m^{-2}$  and lower ratios (that is,  $<2$  mg  $g^{-1}$  AFDM) at organic matter levels above about 100 g AFDM  $m^{-2}$ . This shift in ratios was associated with a parallel change in community structure, from one dominated by obligate cave taxa or co-dominated by obligate and facultative cave taxa at organic matter levels below about 100 g AFDM  $m^{-2}$  to one dominated by facultative cave taxa at levels above about 100 g AFDM  $m^{-2}$  (Figure 4C).

## DISCUSSION

Our results provide experimental support for C limitation of a cave stream community by ambient rates of resource supply. Added C from maize litter was rapidly assimilated by microbes and promptly transferred to successive trophic levels in the Bluff River Cave food web, although direct assimilation of C from maize litter by some metazoan taxa may also have occurred (for example, McGrath and Matthews 2000). Biomass at all trophic positions subsequently increased, but taxon responses apparently depended on life-history strategies associated with cave specialization. Densities of obligate cave taxa did not change, presumably due to trade-offs associated with adaptation to low-C environments (for example, low metabolic activity, growth rates, and fecundity; Gibert and DeHarveng 2002), whereas facultative cave taxa showed robust numerical responses. Notably, the increase in total invertebrate biomass mirrored the results of complementary resource manipulations in surface streams. This convergence reveals the primacy of energy limitation in shaping cave community structure, as well as commonalities between surface and subterranean ecosystems that lie in different positions along a gradient of energy supply.

Our experimental addition of maize litter to the Bluff River Cave stream ecosystem resulted in significant alterations to the structure of the microbial community, particularly changes in the relative abundance of aerobic bacteria, fungi, and protists compared to reference conditions. Although bacterial biomass increased, the response was small ( $\sim 0.5$ -fold increase) compared with fungi ( $> four$ -fold increase) and protists ( $> two$ -fold increase). The relatively aggressive response by fungi was presumably due to the maize litter being both a high-quality resource (that is, low C:N ratio and lignin content) and an ideal substrate to support fungal

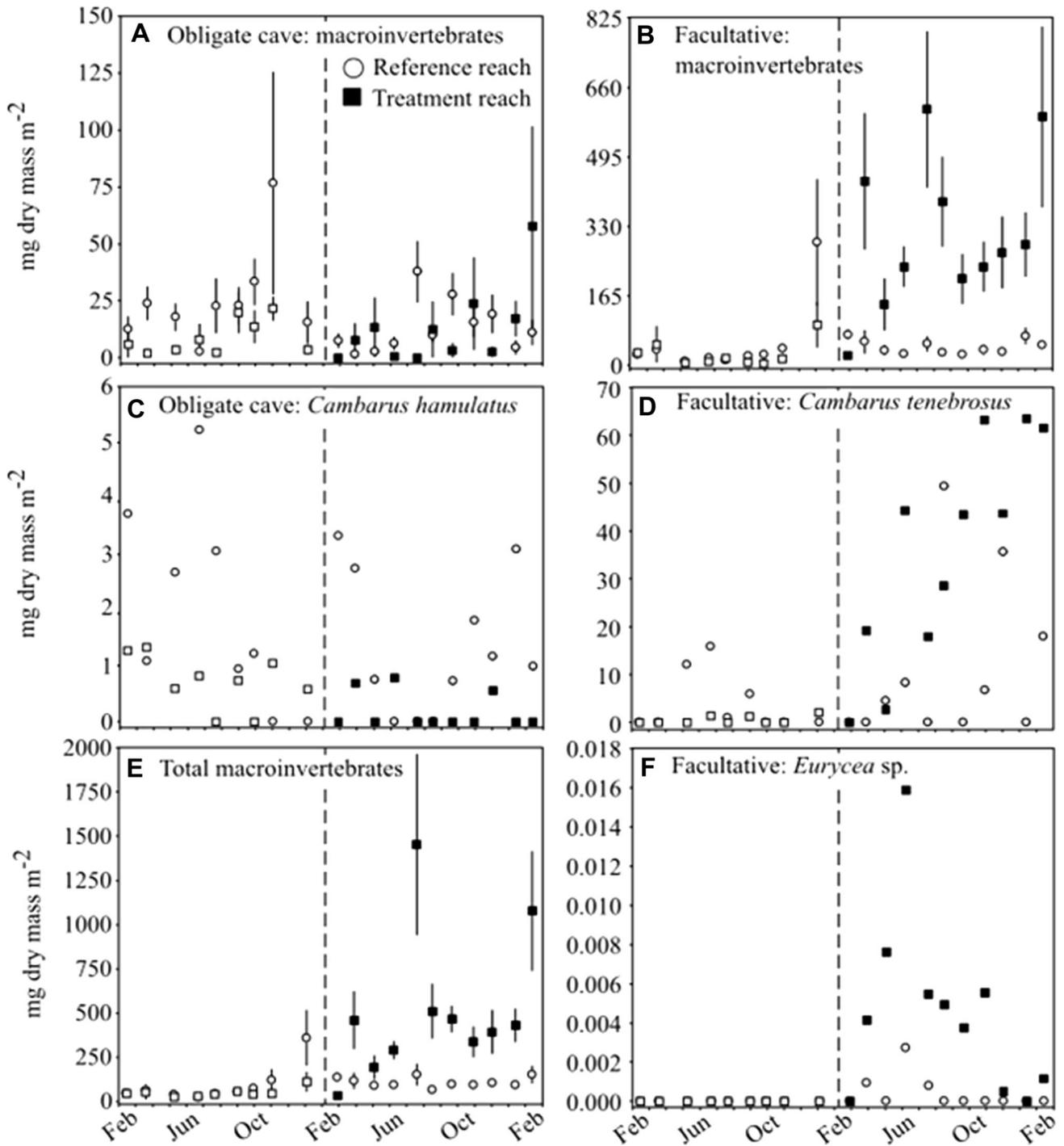
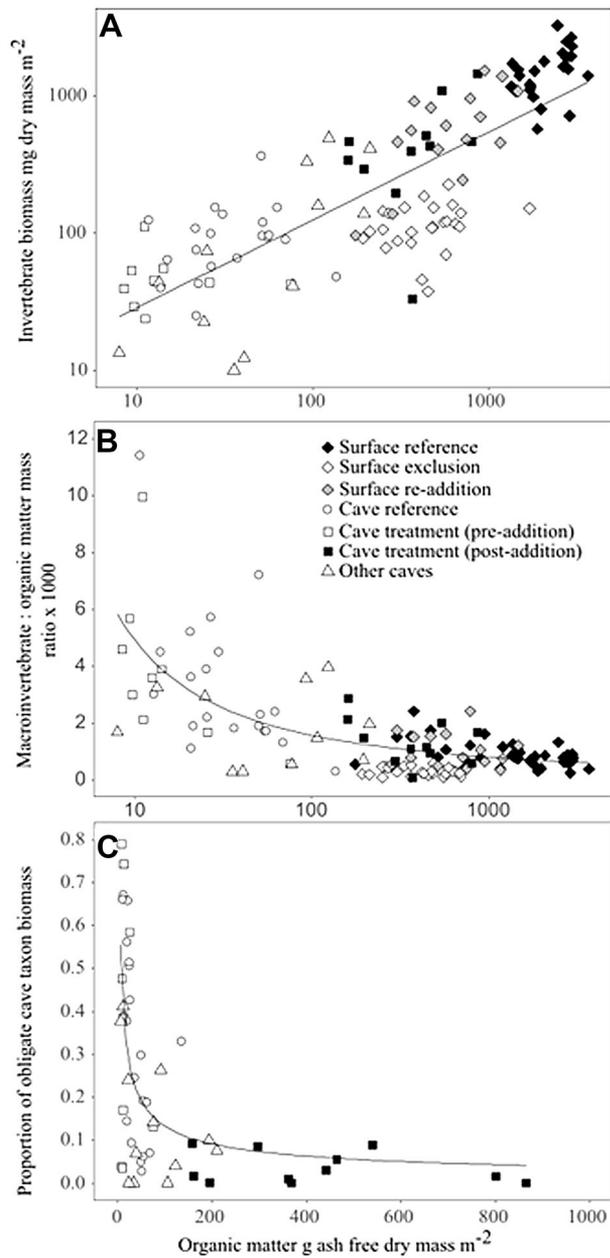


Figure 3. Mean biomass (bars are standard errors) of **A** obligate cave macroinvertebrates, **B** facultative cave macroinvertebrates, **C** *Cambarus hamulatus*, **D** *Cambarus tenebrosus*, **E** total macroinvertebrates, and **F** *Eurycea* sp. measured in Bluff River Cave stream. Data from the reference reach are indicated by open circles. Data from the treatment reach are indicated by open (pre-litter addition) and closed squares (post-litter addition). Vertical dashed line indicates beginning of litter addition.

growth via hyphal penetration of plant vascular tissue (Webster and Benfield 1986). Stable isotope analyses indicated that maize litter C was propa-

gated upward through at least three trophic levels (microbes, primary metazoan consumers, secondary metazoan consumers).



◀ **Figure 4.** **A** Relationship between macroinvertebrate biomass versus organic matter mass in cave and forested headwater surface streams ( $y = 64.59 + 0.65x$ ,  $R^2 = 0.72$ ,  $P < 0.0001$ ). **B** The ratio of macroinvertebrate biomass to organic matter mass for forest and cave streams ( $\times 1000$  to provide a convenient scale) versus organic matter (solid line is  $y = 6.47x^{-0.30}$ ,  $R^2 = 0.43$ ,  $P < 0.0001$ ). **C** The proportion of total macroinvertebrate biomass that was attributable to obligate cave species as a function of organic matter mass (solid line is  $y = 2.64x^{-0.75}$ ,  $R^2 = 0.53$ ,  $P < 0.0001$ ). AFDM = ash-free dry mass, DM = dry mass. **B** contains the legend for all graphs: reference reach (open circles) and treatment reach pre- (open squares) and post-litter addition (closed squares) in Bluff River Cave stream, three other cave streams in northeastern Alabama (open triangles; Huntsman and others 2011a, b; Venarsky and others 2012), and two forested headwater streams at Coweeta Hydrologic Laboratory Southern Research Station without (closed diamonds) and with (open diamonds) organic matter exclusion, and with organic matter exclusion followed by an experimental litter addition (gray diamonds; Wallace and others 2015).

ties, long life spans and low rates of growth and reproduction which precluded a strong numerical response (Aden 2005; Hüppop 2000, 2005). The different responses of the two sources of potential colonizers resulted in a rapid shift from the co-dominance of community biomass prior to our litter addition to a clear dominance by facultative cave taxa following the litter addition.

Cave stream ecosystems that are both connected to surface habitats via sinking streams (Ray 2005) and that receive relatively large supplies of organic matter can host abundant assemblages of facultative cave taxa that may, in some cases, exceed the biomass of cave-obligate taxa (Simon and Benfield 2001; Venarsky and others 2012, 2014). The sources of facultative taxa in cave streams, however, remain uncertain but are logically restricted to two pathways—oviposition by adults within the cave (either arriving as immigrants or from reproducing populations) or drifting aquatic stages entering the cave from sinking streams. The emergence of adult mayflies and chironomid midges from populations colonizing the treatment reach was documented, but evidence of actual reproduction within Bluff River Cave is lacking and we are aware of no prior studies documenting reproduction by insects, in particular, from surface streams in cave habitats. The alternative route consists of colonizers drifting downstream from sinking streams. The temporal pattern of incorporation of maize-derived C into consumer tissues (Figure 1A) is consistent with colonization by drift, showing a relatively high range of variation in  $\delta^{13}\text{C}$  values among individuals from the treatment reach during periods of rela-

Our experiment also showed that population- and community-level responses of metazoan consumers varied according to colonizer source. The rapidity and magnitude of the biomass increase in facultative cave taxa (both macroinvertebrates and vertebrates) was surprising given the rather slow energetic tempo commonly assumed to characterize cave ecosystems. The facultative cave community showed a dramatic greater than tenfold increase in biomass less than two months following the litter addition. In contrast, there was no detectable change in biomass of obligate cave taxa, a result that we attribute to low population densi-

tively high and variable discharge (that is, December through April) and low variation in  $\delta^{13}\text{C}$  values during periods of low stable flows (that is, May–November). This pattern may logically be interpreted as indicating the arrival of new colonizers during conditions of high flow and the near absence of colonizers when discharge, and thus the downstream drift of individuals among reaches, is minimized.

The colonization of patches of leaf detritus by downstream drift is a common demographic phenomenon for populations of stream macroinvertebrates and is important for maintaining ecosystem function in patchy landscapes (Townsend 1989; García and others 2012). A likely mechanism explaining the dynamics of colonization observed for the treatment reach of Bluff River Cave following the litter addition was demonstrated by Richardson (1991), in which detritus supplies were manipulated among replicated stream-side channels, showing an unambiguous positive relationship between macroinvertebrate population size and detritus supply. Although rates of colonization by drifting macroinvertebrates (primarily Plecoptera, Diptera and Trichoptera) were similar among channels, the emigration rates of drifting individuals were lowest from channels with the largest supplies of detritus, which resulted in a net population increase (Richardson 1991). Regardless of uncertainty related to potential colonization pathways, the abundant invertebrates colonizing the litter in this cave were clearly processing and assimilating organic matter and likely had a significant and sustainable role in ecosystem processes (Huntsman and others 2011a, b; Venarsky and others 2012). The potential for facultative stream-dwelling taxa to colonize cave streams, as a function of surface connectivity and resource level, adds a largely unacknowledged component to the temporal and spatial complexity of cave stream food webs. Detailed information on the scope of this phenomenon among cave ecosystems is lacking, but given the large areas of karst terrane worldwide (Juberthie 2000) and the regular occurrence of sinking streams within such terranes (Ray 2005), significant activity of surface taxa within cave food webs may be relatively common and widespread.

Although caves are rightly viewed as specialized ecosystems due to the lack of photosynthesis, rates of photosynthesis may also be exceedingly low in heavily shaded forest streams that similarly have food webs based almost entirely on imported detritus (Benstead and others 2009). Ecological differences between cave stream food webs and surface stream food webs in forested landscapes

may thus be more closely related to the rate of detritus supply than to special attributes associated with cave ecosystems in particular (Sket 1973, 1986). Given the scope for colonization of cave streams by surface taxa (Simon and Benfield 2001; Venarsky and others 2012), perhaps it should not be surprising that the relaxation of resource limitation in such ecosystems may result in community structure that converges with that of surface streams (Sket 1973, 1986).

Our study experimentally shifted a cave stream community along a resource supply gradient toward higher donor–recipient fluxes. Importantly, we were able to compare our results with those of complementary experimental studies that manipulated the organic matter supply of a heavily shaded forest stream ecosystem representing an opposing end member along such a resource supply gradient (Wallace and others 1997, 1999, 2015). This comparison allowed us to address whether a fundamental difference between these ecosystems is the magnitude of fluxes of organic matter supplied from the donor ecosystem, rather than simply the landscape position of the recipient ecosystem (that is, surface vs. subterranean). Although the cave stream and forest stream ecosystems we studied obviously differ significantly in many physical attributes, they also possessed key similarities that we argue allow important insights from their comparison.

At the extremely low levels of organic matter characterizing southern Appalachian cave stream ecosystems (Simon and Benfield 2001; Venarsky and others 2014, this study), cave stream communities show relatively large contributions of obligate cave taxa to community biomass and high ratios of macroinvertebrate biomass to organic matter. Under conditions of moderate quantities of organic matter supplies (that is,  $>200\text{ g AFDM m}^{-2}$ ), combined with effective colonization pathways for surface taxa to the cave stream, however, the general taxonomic structure and biomass of the cave and forest stream communities converged, with both being dominated by surface taxa with equivalent ratios of macroinvertebrate biomass to organic matter. This result is significant in that it indicates that organic matter supply, rather than the lack of light, is a major organizing factor determining attributes of cave communities and ecosystems, a conclusion that echoes those of Sket (1986) and Culver and Pipan (2014).

Unlike the organic matter addition to the experimental cave stream reach, however, the exclusion and removal of organic matter from the forest stream (Wallace and others 2015) resulted in

ratios of macroinvertebrate biomass to organic matter that differed significantly from those of the cave stream. Rather than the high ratios characterizing cave streams, the ratios measured in the forest stream following litter exclusion were an order of magnitude lower. This result highlights a potentially key difference in the functional attributes of surface and cave stream ecosystems and suggests significant differences in the relative efficiencies of organic matter consumption by taxa inhabiting these different ecosystems, with obligate cave species being able to use organic matter of varying quality much more effectively than facultative cave and surface species. The apparent differences between ecosystems were relaxed in the cave stream following the organic matter addition that facilitated the colonization of facultative cave species. The lack of a parallel shift in the surface stream during organic matter exclusion (that is, an increase in the ratio of consumer biomass to detritus biomass) was presumably related to the absence of equivalent pathways allowing obligate cave species to colonize the experimental forest stream.

Wide variation exists in the amount of detritus supplied by donor to recipient ecosystems (Polis and others 1997, 2000). Consequently, a gradient of detritus fluxes among ecosystems can be identified and selected ecosystems have been experimentally moved along it by manipulating detritus inputs. Our comparison of the community responses of a cave stream and a forest stream subjected to detritus manipulations showed remarkable convergence in the functional relationships between animal biomass and energy availability. This result suggests that the food webs of cave and forest streams likely lie along a single, common gradient of detritus supply, occupied at its extreme minimum by communities of obligate cave taxa adapted to low-energy environments and at its maximum by surface taxa. These complementary experiments thus revealed ecological commonalities between cave and surface streams, conceptually uniting apparently disparate ecosystems along a trajectory of energy supply.

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