

Responses of stream macroinvertebrates to Bt maize leaf detritus

CATHERINE P. CHAMBERS,¹ MATT R. WHILES,^{1,6} EMMA J. ROSI-MARSHALL,² JENNIFER L. TANK,³ TODD V. ROYER,⁴
NATALIE A. GRIFFITHS,³ MICHELLE A. EVANS-WHITE,⁵ AND AMBER R. STOJAK¹

¹Department of Zoology and Center for Ecology, Southern Illinois University, Carbondale, Illinois 62901 USA

²Department of Biology, Loyola University Chicago, Chicago, Illinois 60626 USA

³Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556 USA

⁴School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405 USA

⁵Division of Biology, University of Arkansas, Fayetteville, Arkansas 72901 USA

Abstract. In the midwestern United States, maize detritus enters streams draining agricultural land. Genetically modified Bt maize is commonly planted along streams and can possibly affect benthic macroinvertebrates, specifically members of the order Trichoptera, which are closely related to target species of some Bt toxins and are important detritivores in streams. The significance of inputs of Bt maize to aquatic systems has only recently been recognized, and assessments of potential nontarget impacts on aquatic organisms are lacking. We conducted laboratory feeding trials and found that the leaf-shredding trichopteran, *Lepidostoma liba*, grew significantly slower when fed Bt maize compared to non-Bt maize, while other invertebrate taxa that we examined showed no negative effects. We also used field studies to assess the influence of Bt maize detritus on benthic macroinvertebrate abundance, diversity, biomass, and functional structure in situ in 12 streams adjacent to Bt maize or non-Bt maize fields. We found no significant differences in total abundance or biomass between Bt and non-Bt streams, and trichopterans comprised only a small percentage of invertebrate biomass at all sites (0–15%). Shannon diversity did not differ among Bt and non-Bt streams and was always low (H' range = 0.9–1.9). Highly tolerant taxa, such as oligochaetes and chironomids, were dominant in both Bt and non-Bt streams, and macroinvertebrate community composition was relatively constant across seasons. We used litterbags to examine macroinvertebrate colonization of Bt and non-Bt maize detritus and found no significant differences among litter or stream types. Our in situ findings did not support our laboratory results; this is likely because the streams we studied in this region are highly degraded and subject to multiple, persistent anthropogenic stressors (e.g., channelization, altered flow, nutrient and pesticide inputs). Invertebrate communities in these streams are a product of these degraded conditions, and thus the impact of a single stressor, such as Bt toxins, may not be readily discernable. Our results add to growing evidence that Bt toxins can have sublethal effects on nontarget aquatic taxa, but this evidence should be considered in the context of other anthropogenic impacts and alternative methods of pest control influencing streams draining agricultural regions.

Key words: agricultural stream; aquatic insect; aquatic macroinvertebrate; *Bacillus thuringiensis*; corn; *Cry1Ab*; genetically engineered crops; maize; midwestern stream (USA); shredder; transgenic; Trichoptera.

INTRODUCTION

The Corn Belt region of the midwestern United States is characterized by monoculture fields with degraded, low order streams draining the landscape. Maize (*Zea mays* L.) and other crops are planted adjacent to stream margins. After autumn harvest, maize detritus, including leaves, stalks, and cobs, remain on fields and can enter streams during storms through movements by wind and water. Inputs of maize detritus to headwater streams can range from 0.1–7.9 g ash-free dry mass (AFDM)/m² of stream channel per year (Rosi-Marshall et al. 2007).

Further, maize leaves and cobs are commonly found in agricultural streams (Stone et al. 2005), with standing stocks up to 6.4 g AFDM/m² (Rosi-Marshall et al. 2007).

In 2008, ~57% of the maize planted in the United States was Bt maize (NASS 2008). Bt maize is genetically modified to express crystalline (Cry) proteins from the bacterium *Bacillus thuringiensis* (Bt), which are expressed in all tissues of the plant throughout the growing season and persist in maize residues left on fields after harvest (Zwahlen et al. 2003). Cry proteins are toxic to the larval forms of some common agricultural pests because they bind to receptors in the gut and cause lethal septicemia when activated by the strongly alkaline midgut.

Whereas numerous studies have assessed nontarget effects of Bt endotoxins on terrestrial species (e.g., Losey

Manuscript received 9 April 2009; revised 20 November 2009; accepted 9 December 2009; final version received 12 January 2010. Corresponding Editor: A. K. Ward.

⁶ Corresponding author.

E-mail: mwhiles@zoology.siu.edu

et al. 1999, Wraight et al. 2000, Saxena and Stotzky 2001a, Stanley-Horn et al. 2001, Zangerl et al. 2001, Candolfi et al. 2004, Clark et al. 2006, Marvier et al. 2007), the potential effects of Bt toxins in aquatic systems are not well studied, although *Bacillus thuringiensis* var. *israelensis* (B.t.i.) has been used to control black fly larvae in streams, and studies indicate few effects on nontarget aquatic insects (Jackson et al. 1994). The Lepidoptera-specific Cry1Ab Bt endotoxin is of particular interest for aquatic ecosystems because some lepidopterans live in freshwater habitats, as do all North American members of the order Trichoptera, which are closely related to lepidopterans and have an alkaline mid-gut that is required for Bt toxins to be effective (Martin et al. 1982).

Many trichopterans are classified as shredders (Merritt et al. 2008), meaning they feed on coarse particulate organic detritus and, thereby, transform coarse materials into fine particles. Agricultural practices can decrease shredder biomass in adjacent streams by reducing riparian litter inputs and degrading in-stream habitat (Townsend et al. 1997, Hagen et al. 2006). However, shredding invertebrates such as trichopterans that do persist in agricultural streams could be an important link in incorporating maize and any associated toxins into stream food webs by converting coarse materials that enter the stream into fine particles that are utilized by filter feeders and collector-gatherers.

Rosi-Marshall et al. (2007) recently provided evidence that crop detritus and pollen enter agricultural streams, and that trichopterans fed Bt maize leaves in a laboratory setting had slower growth. However, that study was limited to one laboratory feeding experiment using one taxon and one variety of Bt maize detritus, and did not include a field examination of macroinvertebrate responses. Here, we build on Rosi-Marshall et al. (2007) by examining the effects of Bt maize leaf detritus on aquatic invertebrates in headwater streams using combined field and laboratory approaches. In the laboratory, we conducted multiple feeding trials, using two varieties of Bt maize detritus, with a leaf-shredding trichopteran, a shredding amphipod, and a snail. We hypothesized that trichopterans fed Bt maize detritus in the laboratory would have slower growth and higher mortality than those fed non-Bt detritus. In the field, we compared benthic macroinvertebrate diversity, abundance, biomass, and functional structure in 12 stream reaches adjacent to fields planted in either Bt or traditional (non-Bt) maize. We also measured invertebrate colonization of maize detritus using leaf packs containing either Bt or non-Bt leaves. We hypothesized that any differences in benthic and litterbag invertebrate communities in streams draining Bt maize and non-Bt maize fields would be most evident as differences in trichopteran diversity and abundance. Our field studies were designed to provide real-world relevance, while the

laboratory components allowed for a controlled assessment of Bt effects without confounding environmental factors.

METHODS

Study sites

We sampled agricultural streams in north-central Indiana in 2005 and 2006. This region is heavily agricultural, with 92% of land area in row crops, and Bt maize use is steadily increasing. In 2008, Bt maize comprised 62% of the total maize planted in Indiana, compared with 42% in 2007, 25% in 2006, and 15% in 2005 (NASS 2006, 2008). We sampled six streams during each year of the study (streams 1A–F in 2005, and streams 2A–F in 2006). The same six sites from 2005 could not be used in 2006 because of the yearly maize–soy crop rotations in the region. Greater than 200 m of each stream flowed through fields planted with either Bt maize or non-Bt maize on both sides. Crop types were consistent for at least 200 m along our reaches, but we cannot rule out potential influences of upstream crops (e.g., the possibility of Bt crops located upstream of our non-Bt reaches). In 2005, 1A–1D flowed through Bt maize fields, and 1E and 1F flowed through non-Bt fields; in 2006, sites 2A–2C flowed through Bt maize fields and 2D–2F flowed through non-Bt fields (Table 1).

Site selection of Bt vs. non-Bt streams was based on information provided by farmers and later confirmed using a commercial double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) on maize leaves sampled from adjacent fields. Study streams were typical of the region and were fairly similar in overall physical attributes (e.g., channelized agricultural “ditches”), but did encompass a range of discharges (Table 1). Further site description details are presented in Griffiths et al. (2009).

Basic water chemistry parameters and channel and substrate features were assessed at each site using standard stream sampling techniques (e.g., Hauer and Lamberti 2006). Dissolved oxygen concentrations, temperature, and conductivity were monitored periodically in each stream using dataloggers installed for 2–3 day intervals. Water samples were collected for nutrient analyses from each stream before and after pollen shed and before and after harvest each year. We filtered water samples through 0.7- μ m pore size Whatman GF/F glass-fiber filters (Whatman, Florham Park, New Jersey, USA) into acid-washed bottles, and froze them until analyses. We measured nitrate-N concentrations using a DIONEX 600 ion chromatograph (DIONEX Corporation, Sunnyvale, California, USA) with ED50 electrochemical detector and AS14A guard and analytical columns. We measured ammonium-N concentrations using the phenol hypochlorite method (Solorzano 1969) and soluble reactive phosphorus (SRP) concentrations using the molybdate/antimony method (Murphy and Riley 1962).

TABLE 1. Physical and chemical characteristics of the study streams in north-central Indiana, USA.

Site	Mean summer discharge (L/s)	Mean summer velocity (m/s)	Mean wetted width (m)	Mean depth (cm)	Substrate composition (%)				Summer DO minimum (mg/L)	Summer DO maximum (mg/L)	Mean specific conductivity (uS/cm)	Mean NO ₃ ⁻ -N (mg/L)	Mean SRP (ug/L)	Mean NH ₄ ⁺ -N (ug/L)
					Pebble	Gravel	Sand	Silt						
1A	0.3	0.01	1.10	18	12	22	45	32	2.72	9.50	504.7	10.14	6.0	22.0
1B	NA	NA	1.90	28	0	0	8	92	NA	9.19	592.7	3.71	45.0	79.0
1C	75.0	0.17	4.10	21	23	22	53	2	4.38	11.62	633.1	0.95	15.0	12.0
1D	15.8	0.03	3.40	26	11	46	40	2	4.17	14.53	463.9	0.04	10.0	12.0
1E	3.3	0.02	3.00	29	18	39	31	14	1.06	15.53	597.4	0.04	22.0	3.0
1F	6.6	0.07	2.60	15	20	28	45	16	7.21	11.46	468.3	0.05	13.0	11.0
2A	424.0	0.16	8.42	19	13	35	34	13	5.92	8.47	596.8	2.18	3.4	4.8
2B	57.0	0.19	3.84	14	10	61	27	2	4.98	8.64	649.1	2.51	5.4	30.0
2C	20.9	0.06	2.72	20	8	17	33	45	5.19	15.76	630.6	3.54	6.5	73.2
2D	195.0	0.09	6.98	24	0	25	61	15	5.39	9.32	617.2	1.83	4.9	18.0
2E	16.8	0.07	1.14	15	12	31	41	17	5.21	15.42	734.3	10.20	4.5	3.3
2F	137.0	0.21	3.51	11	14	31	49	6	6.38	16.96	627.4	0.95	2.0	1.2

Notes: Sites numbered "1" were sampled in 2005, and those numbered "2" were sampled in 2006. Stream sites 1A–1D flowed through Bt maize fields, and 1E and 1F flowed through non-Bt maize fields. Sites 2A–2C flowed through Bt maize fields, and 2D–2F flowed through non-Bt maize fields. Average depth and substrate composition were estimated during invertebrate sampling. Key to abbreviations: DO, dissolved oxygen; NO₃⁻-N, nitrate; SRP, soluble reactive phosphorus; NH₄⁺-N, ammonium. "NA" indicates that data are not available because of a lack of flow (discharge) or equipment malfunction (DO).

Laboratory feeding trials

Building on our prior results from feeding experiments (Rosi-Marshall et al. 2007), we performed additional feeding trials to test for potential effects of Bt maize on growth and mortality on multiple taxa of freshwater invertebrates. We used leaves from Bt (two varieties; Table 2) and non-Bt maize plants, as well as silver maple (*Acer saccharinum*) leaves, and allowed animals to feed freely. Feeding trials were carried out separately on two shredding taxa (*Lepidostoma liba* [Trichoptera: Lepidostomatidae] and *Hyaella azteca* [Amphipoda: Hyaellidae]) and one scraping snail (*Gyraulus* [Pulmonata: Planorbidae]).

Lepidostoma liba feeding trials were carried out in early spring of 2006 (see Rosi-Marshall et al. 2007) and in early spring of 2007. *Lepidostoma* spp. are common shredders in headwater streams and have been shown to be important processors of litter in a variety of stream systems (Grafius and Anderson 1979, 1980, Whiles et al. 1993). For *L. liba* feeding trials, we filled aerated plastic growth chambers (9 cm height × 12 cm width × 9 cm depth) with 1 L of stream water and 500 mL of sand. Lights were set on a 10:14 hour L:D cycle and temperature was maintained at 12°C to mimic field conditions at the time of year individuals were collected. In 2006, we used Bt maize collected from fields adjacent to stream 1A and non-Bt maize collected from fields adjacent to stream 1E. In 2007, we used non-Bt maize collected near stream 2E, silver maple (*Acer saccharinum*) leaves collected in Carbondale, Illinois, and used a different type of Bt maize from fields adjacent to stream 2B in addition to the first type from 1A collected in 2005 (used in the 2006 feeding trials) to ensure the validity of the 2006 results (see Table 2). Maple leaf trials were added in 2007 so that we could make comparisons with invertebrates reared on a common

food source found in less disturbed headwater streams in this region.

Our main objective for feeding trials and litterbag experiments was to use materials that had similar digestibility and nutritional value, so we selected Bt and non-Bt maize leaves that had similar C:N ratios (Table 2). Even within the same isolines, Bt maize can have higher percentage lignin than non-Bt maize (Saxena and Stotzky 2001b), so we also chose Bt and non-Bt leaves with similar lignin content (Table 2). Percentage carbon (C) and nitrogen (N) were measured with a Costech elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) and percentage lignin was assessed by the Dairy One Forage Testing Laboratory (Ithaca, New York, USA).

We air dried leaves for feeding trials at room temperature for at least one month. We then pre-conditioned maize leaves in separate aerated aquaria with filtered stream water for one week. Maple leaves used in *H. azteca* and 2006 *L. liba* trials were conditioned for two weeks longer than maize leaves to make nutritional quality more similar; dried maple leaves have a higher C:N than maize and increased conditioning time for maple reduced this difference. We collected *L. liba* from

TABLE 2. Properties of maize leaves used in the 2006 feeding trials of *Lepidostoma liba*, *Hyaella azteca*, and *Gyraulus* sp., and the 2007 feeding trials of *L. liba*.

Stream	Trial year	Bt/Non-Bt	Variety	C:N	Lignin (%)
1E	2006	non-Bt	Pioneer 35Y68	24.6	6.3
1A	2006, 2007	Bt	Crow 4635	20.1	5.7
2B	2007	Bt	Agventure	19.4	5.1
2E	2007	non-Bt	not available	22.3	3.8

Note: Senesced maize leaves were collected during harvest from fields adjacent to the indicated study streams.

undisturbed spring seeps in southern Illinois and measured total body length of individuals by gently removing a small portion of the posterior end of the case so that each end of the body was visible. We then placed four individuals averaging 3.5 mm in length (ranging from 2.5 to 4.5 mm) in each chamber in a random fashion. Eight chambers were used for each treatment type. All chambers started with four leaf disks (14 mm diameter) of the respective preconditioned treatment leaf litter, and we added four leaf disks equally to each chamber as necessary (every ~3 days) over the 29-day trial so that food was not limiting. Initial and final lengths of individuals were measured from digital pictures using Image J software (Rasband 2006) to estimate length to the nearest 0.01 mm. We estimated initial and final AFDM of each individual using length–mass relationships (Benke et al. 1999) and used the instantaneous growth equation (Benke and Huryn 2006) to estimate daily growth rates of individuals, which were then averaged for each chamber. Mortality and growth were assessed at the end of each trial in order to minimize disturbance and stress during the experiments.

Hyaella azteca was used as a representative noninsect shredder. *Hyaella* is a widely distributed and common benthic amphipod that is frequently used in USEPA toxicology tests because of its tolerance for a wide range of environmental variables (Smith 2001). They are omnivorous, eating all types of animal and plant material, and can shred decomposing leaves. *Hyaella azteca* have much shorter generation times than *L. liba* and *Gyraulus*, averaging ~10 days per brood (Smith 2001). *H. azteca* were obtained from the Southern Illinois University Toxicology Laboratory cultures, and trials were set up in early spring of 2007 following the general *L. liba* procedure, with some minor modifications. *H. azteca* cultures were passed through 375- and 500- μ m sieves to obtain ~6-day-old individuals (EPA 2000). We ran *H. azteca* trials for only 7–10 days in order to limit reproduction. Eight replicate chambers of each of the three food types were used. We used the same Bt maize, non-Bt maize, and silver maple leaves that were used in *L. liba* trials, and 10 individuals were placed in each chamber. Growth was estimated using the same procedures as for *L. liba*.

Gyraulus is a common, small, pulmonate snail that scrapes biofilms from substrata and submerged vegetation (Smith 2001). *Gyraulus* individuals were collected from a small forested pond in southern Illinois and used in feeding trials in spring of 2007. *Gyraulus* trials followed procedures for *L. liba*, using disks from the same Bt and non-Bt maize leaves, with the following minor changes: 10 individuals were placed in each of eight chambers of Bt leaves and eight chambers of non-Bt leaves and because *Gyraulus* is planispiral, changes in shell width (measured at the widest point) were used to estimate growth.

Benthic macroinvertebrates and benthic organic matter

To estimate invertebrate diversity, abundance, and biomass, we collected five benthic cores in random locations in each of the 12 stream reaches in summer (preharvest) and in fall (1–3 weeks after harvest, when maize detritus input is likely highest). We used a 20 cm diameter stovepipe core sampler, pushed far enough into the substrate to form a seal, and removed substrata down to ~10 cm. Substrata composition was visually estimated and recorded as percentage composition based on a modified Wentworth scale (Cummins 1962). Average water depth was measured 5–10 cm upstream from where the core was placed. Materials were placed in a graduated bucket and volume was recorded. The contents of the bucket were then elutriated through a 250- μ m sieve. Materials retained on the sieve were preserved in 10% formalin. Materials remaining in the bucket were visually inspected for mollusks and other negatively buoyant taxa; if present, they were removed by hand and added to materials on the sieve. We collected a 100-mL subsample of material that passed through the sieve for estimating amounts of very fine (<250 μ m) particulate organic matter (VFPOM).

Samples for VFPOM were kept on ice no longer than three days before laboratory processing where the subsample was passed through a pre-weighed 47-mm glass-fiber filter (pore size 1.6 μ m). Filters were then dried for at least 48 hours at 50°C, weighed, ashed in a muffle furnace at 500°C for 2–3 h, then reweighed to obtain ash-free dry mass (AFDM), which is the difference between the ash and dry masses. AFDM values were corrected for subsample size and then converted to grams AFDM per square meter using the known volume of material in the core and area of the core.

In addition to VFPOM, we measured coarse (>1 mm) and fine (250 μ m–1 mm) fractions of organic matter within the core samples to quantify potential food sources available to macroinvertebrate consumers. We separated material from the core samples into coarse (>1 mm) and fine (<1 mm and >250 μ m) fractions using nested sieves and sorted coarse organic materials by type (maize leaves, maize stalks, maize cobs, deciduous seeds, deciduous leaves, and other material). Materials were processed in the same manner as VFPOM to obtain milligrams AFDM. Invertebrates found in coarse fractions were counted, measured to the nearest millimeter (body length), and identified (usually to genus), using Merritt et al. (2008) and Smith (2001).

The fine fraction of each sample was usually subsampled using a Folsom wheel, generally to 1/8–1/32 of the total, depending on the amount of material in the sample. Invertebrates were removed, identified, and measured in the same fashion as coarse fractions, and remaining fine particulate organic matter (FPOM) was processed to estimate grams AFDM per square meter. Invertebrate abundances and organic matter masses were corrected for subsample fractions.

We estimated biomass of invertebrates using length-mass regressions from Benke et al. (1999) or relationships developed for taxa found in these systems following procedures of Benke et al. (1999). To compare invertebrate communities, we calculated richness, EPT richness (richness of members of the generally pollution intolerant orders Ephemeroptera, Plecoptera, and Trichoptera), and Shannon diversity for all streams on all dates following procedures of Barbour et al. (1999). We also categorized invertebrates by functional feeding group based on Merritt et al. (2008).

Maize litterbag invertebrates

We used standard litterbag techniques (Benfield 2006) to quantify the community composition of invertebrate taxa colonizing and, therefore, possibly feeding on Bt and non-Bt maize in litter bags. After harvest in 2005, senesced maize leaf litter was collected from fields bordering each site and air dried. We paired Bt and non-Bt maize leaves to be placed in each stream based on similarities in lignin percentages and C:N (see Griffiths et al. 2009). Plastic mesh citrus bags (30×20 cm, 1×0.25 cm mesh size) were filled with 13 g dry mass of Bt or non-Bt leaves, and were anchored to the stream bottom in areas of similar velocity. We placed a total of 21 bags with Bt maize leaves and 21 bags with non-Bt maize leaves in each stream. Three bags were retrieved on days 0, 3, 7, 13, 25, 37, and 70 to estimate decomposition rates (see Griffiths et al. 2009). We processed bags from days 3, 13, and 25 for invertebrates, whereby we rinsed the entire contents of each bag through nested 1-mm (coarse fraction) and 250- μ m (fine fraction) sieves. Coarse and fine fractions were preserved in 10% formalin and later processed, identified, and measured in the same manner as benthic cores to estimate macroinvertebrate diversity and biomass.

Because a Bt identification error was not discovered prior to the litterbag experiment, stream 1D maize leaves were erroneously labeled as non-Bt and placed in litterbags in streams 1A and 1B. Therefore, the final litterbag study design only involved stream 1C (Bt) and streams 1E and 1F (non-Bt), which did not have maize leaves from 1D in any litterbags.

Statistical analyses

A Student's *t* test was used to assess statistical differences in daily growth rates between treatment groups in laboratory feeding trials, using chambers as replicates. Streams were treated as replicates for all analyses of benthic and litterbag data. We analyzed all organic matter data as well as macroinvertebrate abundance, biomass, and diversity data using two-sample *t* tests to assess differences between stream types (Bt and non-Bt) and between seasons (summer and fall) within stream types, and each year was analyzed separately. Two-sample *t* tests were also used to compare macroinvertebrates found in Bt and non-Bt maize litterbags, as well as in Bt and non-Bt maize

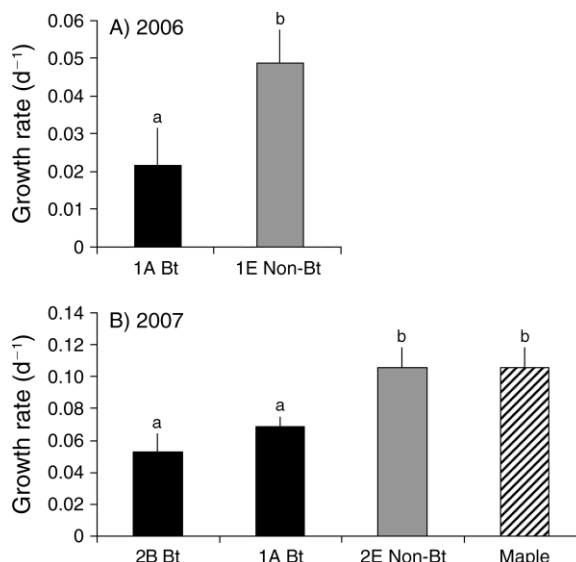


FIG. 1. Average daily growth rates (mean + SE) of the leaf-shredding trichopteran *Lepidostoma liba* in feeding trials performed during (A) 2006 (data from Rosi-Marshall et al. 2007) and (B) 2007. In 2006, senescent Bt (*Bacillus thuringiensis*) maize leaves were collected from fields along stream 1A, and non-Bt maize was collected from stream 1E. In 2007, Bt maize leaves were collected from stream 2B, non-Bt leaves were collected from stream 2E, and the 2006 Bt leaves from stream 1A as well as silver maple (*Acer saccharinum*) leaves were used. Lowercase letters above bars indicate significant differences between litter types ($P < 0.05$, with Bonferroni correction when multiple comparisons were made). Growth rates are instantaneous [$\text{growth} = \ln(\text{final mass}/\text{initial mass})$] based on changes in individual mass over time.

streams. Results of all analyses were considered significant at $P < 0.05$; in cases where multiple comparisons were made (e.g., 2007 *L. liba* feeding trials comparing Bt maize, non-Bt maize, and maple), a Bonferroni correction ($\alpha/\text{number of comparisons}$) was used to adjust α accordingly (Zar 1999). Log transformations were applied where necessary to meet assumptions of normality. Because field data were variable and sample sizes were low, results from field studies were considered marginally significant trends at $P < 0.10$. All statistical analyses were performed using SAS 9.1 (SAS Institute 2005).

RESULTS

Laboratory feeding experiments

In general, there was low mortality in the *L. liba* feeding trials. In 2006, two *L. liba* individuals from separate growth chambers with 1A Bt maize died, while there was no mortality in non-Bt treatments. In 2007, six individuals fed Bt maize from 1A died, while none died from the 2B Bt maize; three individuals died in maple treatments and two died in non-Bt maize treatments in 2007. Overall, there were no significant trends in mortality between food type treatments during either

TABLE 3. Average organic matter standing stocks in northwestern Indiana streams flowing through Bt and non-Bt fields.

Category	2005 AFDM								2006 AFDM			
	Summer				Fall				Summer			
	Bt (mg/m ²)		Non-Bt (mg/m ²)		Bt (mg/m ²)		Non-Bt (mg/m ²)		Bt (mg/m ²)		Non-Bt (mg/m ²)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
VFPOM	239.3	135.8	515.2	124.2	933.6	675.3	467.8	68.2	435.1	135.6	251.6	24.7
FPOM	32.4	16.6	24.0	7.9	56.8	51.2	12.9	7.4	42.1	26.9	35.2	9.8
Total CPOM	110.5	53.8	38.9	7.6	137.5	57.5	49.8	26.4	51.2	16.7	54.0	35.6
Corn (all parts)	1.6	0.6	0.4	0.3	3.0	2.3	1.6	1.3	0.8	0.8	0.0	0.0
Deciduous leaves	9.2	8.6	0.1	0.1	4.8	3.1	0.7	0.6	0.5	0.2	0.6	0.6
Grass	15.3	15.3	1.4	0.7	24.7	4.0	1.8	0.9	9.8	5.2	12.6	6.7
Macrophytes	1.8	1.3	7.4	6.1	2.8	1.0	0.1	0.1	4.4	4.4	0.4	0.4
Amorphous organic	59.5	31.9	19.2	6.9	98.3	50.5	25.9	6.5	21.7	11.1	12.2	6.9
Roots	10.9	6.1	4.3	3.5	2.7 ^a	1.7	12.2 ^b	2.4	4.3	2.2	1.6	1.6
Seeds	1.1 ^c	0.6	1.2 ^c	0.5	0.2 ^d	0.1	2.9 ^d	2.4	4.9 ^{ac}	1.5	0.4 ^{bc}	0.4
Wood	11.1 ^c	2.1	4.9 ^c	1.6	1.0 ^d	0.3	4.6 ^d	3.7	3.4	1.7	22.8	22.8
Filamentous algae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.4	3.4	3.3

Notes: There were four Bt streams and two non-Bt streams in 2005, and there were three Bt and three non-Bt streams in 2006. Superscripts a and b indicate differences between stream type; superscripts c and d indicate differences between season within stream type and year. Key to abbreviations: VFPOM, very fine particulate matter (<250 μm); FPOM, fine particulate organic matter (250 μm –1 mm); CPOM, coarse particulate organic matter (>1 mm).

year. However, for both years, *L. liba* individuals grew significantly more slowly (2005 1A Bt = 0.022 d⁻¹, 2005 1D non-Bt = 0.049 d⁻¹; 2006 2B Bt = 0.053 d⁻¹, 2006 1A Bt = 0.069 d⁻¹, 2006 2E non-Bt = 0.106 d⁻¹, 2006 maple = 0.106 d⁻¹) when fed Bt maize compared to non-Bt maize (2005 Bt vs. non-Bt, $t_{15} = 2.2$, $P = 0.049$; 2006 1A Bt vs. non-Bt, $t_{15} = 2.5$, $P = 0.0008$; 2006 2B Bt vs. non-Bt, $t_{15} = 0.3$, $P = 0.005$; Fig. 1). In 2007, individuals fed non-Bt maize litter had similar growth rates to those fed maple leaves ($t_{15} = 1.1$, $P = 0.99$; Fig. 1).

There was no mortality in *Hyaella* or *Gyraulus* growth experiments. *Hyaella* growth did not differ when fed Bt vs. non-Bt maize ($t_{15} = 0.4$, $P = 0.71$). Growth of *Hyaella* fed on maple did not differ from either type of maize (Bt vs. maple $t_{15} = 0.8$, $P = 0.391$, non-Bt vs. maple $t_{15} = 1.5$, $P = 0.132$). *Gyraulus* growth also did not differ between Bt and non-Bt maize treatments ($t_{15} = 0.1$, $P = 0.962$).

Benthic organic matter

Benthic organic matter in all sites was always dominated by VFPOM, which comprised 57–91% of total organic matter, ranging from 239.3 mg AFDM/m² to 1562.1 mg AFDM/m². Fine particulate organic matter (FPOM) ranged from 12.9 mg AFDM/m² to 61.3 mg AFDM/m², making up 1–9% of total organic matter. Neither VFPOM nor FPOM differed among stream types or seasons (Table 3).

Total coarse particulate organic matter (CPOM) estimates ranged from 38.9 mg AFDM/m² to 210.1 mg AFDM/m² and comprised 1–26% of total benthic organic matter. Of CPOM categories, only roots in the fall of 2005 ($t_5 = 11.3$, $P = 0.020$) and seeds in the summer of 2006 ($t_5 = 2.16$, $P = 0.049$) differed between stream types. Maize CPOM did not differ with season or

by stream type, and standing stocks were generally low, ranging from 0% to 3% of total CPOM (Table 3).

Benthic macroinvertebrates

A total of 57 macroinvertebrate taxa were identified in benthic core samples. In general, communities were dominated by tolerant taxa typical of degraded streams such as oligochaetes, chironomids, and physid snails. Oligochaetes contributed an average of 30% of total macroinvertebrate abundance across sites, while Ostracoda and non-Tanypodinae midges contributed 22% and 23% of total abundance, respectively. Abundance estimates of individual taxa did not vary greatly by stream type (Table 4). In 2005, abundances of all taxa were similar between Bt and non-Bt streams. In 2006, *Simulium* (Diptera: Simuliidae) ($t_5 = 1.3$, $P = 0.001$) and *Tropisternus* (Coleoptera: Hydrophilidae) ($t_5 = 1.3$, $P = 0.008$) larvae were the only taxa with significant differences; both were less abundant in streams adjacent to Bt and non-Bt maize fields. Trichopterans were only moderately abundant, with average densities ranging from 113 to 618 individuals/m² across stream types and years, and abundances did not differ significantly between Bt and non-Bt streams in either year (all $P > 0.05$). Further, the majority of the trichopterans in these streams were filter-feeding hydro- psychids, rather than leaf-shredding taxa.

Total biomass ranged from 4.0 to 5.8 g AFDM/m² in 2005 and 9.5 to 11.7 g/m² in 2006. Mollusks, dipterans, and oligochaetes were dominant contributors to total macroinvertebrate biomass in most sites. Similar to patterns of abundance, there were no differences in total macroinvertebrate biomass during both years between stream types and very few differences in biomass of individual taxa between Bt and non-Bt streams (Table 5). Although not significant for annual averages, non-

TABLE 3. Extended.

2006 AFDM			
Fall			
Bt (mg/m ²)		Non-Bt (mg/m ²)	
Mean	SE	Mean	SE
1562.1	1263.5	334.1	54.2
61.3	38.4	45.5	29.3
88.8	51.3	210.1	159.2
0.0	0.0	0.9	0.6
0.1	0.1	1.6	0.8
60.7	52.2	89.1	71.0
2.9	1.9	16.1	16.1
18.2	5.1	57.0	37.5
6.2	5.1	12.4	12.4
0.0 ^d	0.0	0.0 ^d	0.0
0.0	0.0	31.6	24.3
0.7	0.1	1.4	1.1

Tanypodinae midges had higher biomass in Bt streams compared to non-Bt streams in fall of 2005 ($t_5 = 1.8$, $P = 0.007$) and *Chrysops* (Diptera: Tabanidae) showed a trend of lower biomass in Bt streams compared to non-Bt streams in fall 2005 ($t_5 = 1.5$, $P = 0.060$; Table 5).

Community composition in all streams was dominated by collector-gatherers during both years, which

accounted for 42–77% of total biomass across sites (Table 6). There were no significant differences in functional feeding group composition between stream types at any time (all $P > 0.05$; Table 6), but some seasonal patterns were evident. In 2005, shredders ($t_{11} = 1.8$, $P = 0.024$) increased significantly from summer to fall (Table 6). In 2006, scrapers showed a trend of decreasing from summer to fall ($t_{11} = 1.2$, $P = 0.07$; Table 6).

Shannon diversity was low overall (H' ranges = 0.9–1.9) and did not vary significantly by stream type or with season (all $P > 0.05$; Table 7). Total richness did not vary by stream type (all $P > 0.05$). Richness decreased from summer to fall in 2005 ($t_5 = 2.0$, $P = 0.023$), but not in 2006 ($t_6 = 0.9$, $P = 0.199$; Table 7). Richness of EPT taxa ranged from 3.0 to 6.0 and did not vary by stream type (Table 7).

Litterbag macroinvertebrates

The stream adjacent to Bt maize fields had a more diverse community ($H' = 2.2$) associated with litterbags than the streams adjacent to non-Bt fields ($H' = 0.9$) on day 13 ($t_2 = 3.1$, $P = 0.039$), regardless of litter type (filled with Bt or non-Bt maize), but diversity did not differ with stream type (flowing through Bt or non-Bt fields) on day 3 (Bt $H' = 1.9$, non-Bt $H' = 0.7$; $t_2 = 4.1$, P

TABLE 4. Annual average abundance of macroinvertebrate taxa in Bt and non-Bt streams in northwestern Indiana during 2005 and 2006.

Taxon	2005				2006			
	Bt (ind./m ²)		Non-Bt (ind./m ²)		Bt (ind./m ²)		Non-Bt (ind./m ²)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Tricladida	595.5	17.4	1586.0	1352.5	1980.5	627.5	1575.5	6.5
Nematoda	128.0	99.0	92.5	9.0	36.5	18.0	71.0	32.5
Annelida								
Hirudinea	67.5	42.5	9.0	9.0	373.5	255.5	424.0	177.0
Oligochaeta	10 409.0	307.5	6510.5	4352.5	26 442.5	9060.5	24 374.0	8694.0
Crustacea								
Amphipoda	0.0	0.0	138.0	65.5	114.5	89.0	73.0	50.5
Copepoda	1365.0	100.6	4097.0	2611.5	2092.0	1564.0	883.5	527.0
Cambaridae	14.0	6.5	3.0	0.4	24.0	13.5	15.5	4.0
Ostracoda	46 392.0	22 278.0	8151.0	4369.0	3322.0	182.8	941.5	424.5
Insecta								
Coleoptera	1532.5	1396.0	983.0	1061.0	733.0	490.5	426.0	76.0
Diptera	3630.0	2373.0	2480.0	479.5	34 557.5	10 151.0	22 812.5	8475.5
Ephemeroptera	697.5	388.0	231.5	45.0	1580.0	662.0	683.5	18.0
Hemiptera	0.0	0.0	28.5	22.0	1.5	1.5	0.0	0.0
Odonata	363.5	67.0	14.7	12.5	445.5	314.0	55.5	49.5
Plecoptera	492.0	334.0	344.0	255.0	899.5	616.5	1.5	1.5
Trichoptera	618.0	575.0	297.0	53.0	325.0	32.8	113.0	93.5
Mollusca								
Corbiculidae	144.0	78.0	43.0	29.5	149.0	104.0	17.0	13.0
Physidae	371.5	238.0	1887.0	397.0	59.0	46.0	14.0	11.0
Sphaeriidae	17.0	13.5	5.0	4.0	31.0	24.0	0.0	0.0
Lymnaeidae	64.0	49.5	22.5	17.5	10.0	8.0	17.0	13.0
Planorbidae	55.5	43.0	28.0	11.5	0.0	0.0	0.0	0.0
Hydrobiidae	227.0	159.0	41.5	32.5	69.0	27.0	83.0	65.0
Planorbidae	3.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Unionidae	1.5	1.5	0.0	0.0	3.0	3.0	0.0	0.0

Note: There were four Bt streams and two non-Bt streams in 2005, and there were three Bt and three non-Bt streams in 2006.

TABLE 5. Annual average biomass of macroinvertebrate taxa in Bt and non-Bt streams in 2005 and 2006.

Taxon	2005 AFDM biomass				2006 AFDM biomass			
	Bt (mg/m ²)		Non-Bt (mg/m ²)		Bt (mg/m ²)		Non-Bt (mg/m ²)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Tricladida	129.0	85.4	199.8	22.5	178.2	18.7	442.3	52.7
Nematoda	0.1	0.0	0.0	0.0	0.3	0.0	5.9	2.6
Annelida								
Hirudinea	8.1	0.7	4.0	1.5	3.5	0.0	12.9	7.6
Oligochaeta	284.2	262.2	476.7	56.4	416.2	42.7	232.6	203.6
Crustacea								
Amphipoda	4.4	1.9	0.0	0.0	0.0	0.0	8.5	6.9
Copepoda	1.5	1.3	1.7	1.4	1.8	0.6	1.2	1.0
Cambaridae	128.7	118.0	42.9	5.3	265.7	20.1	86.6	19.6
Ostracoda	206.8	46.9	19.2	1.9	28.1	3.6	7.3	5.1
Insecta								
Coleoptera	152.3	98.6	99.2	15.5	62.6	6.7	243.1	32.0
Diptera	288.6	37.5	549.7	75.3	803.6	144.4	1018.4	124.6
Ephemeroptera	12.6	7.1	2.1	1.4	46.8	7.3	50.6	10.5
Hemiptera	0.0	0.0	1.6	0.0	12.8	9.1	0.0	0.0
Odonata	38.6	8.2	9.8	7.1	9.1	3.6	0.2	0.0
Plecoptera	165.5	23.6	97.7	87.3	916.9	0.5	0.7	0.0
Trichoptera	12.2	4.8	4.9	4.9	33.6	4.2	118.1	32.9
Mollusca								
Corbiculidae	414.6	49.7	282.0	82.9	202.5	24.0	3.2	1.9
Physidae	302.5	36.5	151.8	16.8	503.2	20.2	4.5	0.5
Sphaeriidae	1.9	1.1	0.6	0.0	2.0	1.2	2.1	1.1
Lymnaeidae	145.2	26.0	15.0	1.6	22.8	9.3	0.0	0.0
Planorbidae	707.2	90.8	0.2	0.0	1.6	0.5	0.0	0.0
Hydrobiidae	132.4	13.8	0.0	0.0	70.7	6.7	4.9	0.5
Planorbidae	36.6	9.6	0.0	0.0	1.2	0.9	0.0	0.0
Unionidae	0.0	0.0	0.0	0.0	1259.7	149.8	0.0	0.0

Note: There were four Bt streams and two non-Bt streams in 2005, and there were three Bt and three non-Bt streams in 2006.

= 0.116) or day 25 (Bt $H' = 1.6$, non-Bt $H' = 1.2$; $t_2 = 0.9$, $P = 0.066$). Shannon diversity did not differ with litter type in bags on day 3 (Bt $H' = 1.0$, non-Bt $H' = 1.1$; $t_5 = 0.1$, $P = 0.997$), day 13 (Bt $H' = 1.3$, non-Bt $H' = 1.4$; $t_5 = 0.1$, $P = 0.867$), or day 25 (Bt $H' = 1.4$, non-Bt $H' = 1.4$; $t_5 = 0.6$, $P = 0.471$).

Total invertebrate biomass in litterbags did not differ between litter type or stream type on any of the three

dates we examined ($P > 0.1$ for all; Chambers 2008). However, total biomass on day 25 (average \pm SE for all litter and stream types = 148.26 ± 6.97 mg AFDM/litterbag) was over 2 \times higher than on day 3 (57.26 ± 3.93 mg AFDM/litterbag) and \sim 60% higher than on day 13 (92.56 ± 5.06 mg AFDM/litterbag). Trichopterans represented $<10\%$ of the total invertebrate biomass in litterbags in any stream on any date.

TABLE 6. Average biomass and percentage composition of invertebrate functional feeding groups, and averages of selected community metrics (based on abundances) in Bt and non-Bt study streams in northwestern Indiana.

Invertebrate feeding group	2005 AFDM biomass								2006 AFDM biomass							
	Summer				Fall				Summer				Fall			
	Bt (mg/m ²)	%	Non-Bt (mg/m ²)	%	Bt (mg/m ²)	%	Non-Bt (mg/m ²)	%	Bt (mg/m ²)	%	Non-Bt (mg/m ²)	%	Bt (mg/m ²)	%	Non-Bt (mg/m ²)	%
Collector-gatherer	2583.4	64	2707.9	59	2066.9	44	2960.6	51	8325.1	77	4289.6	45	3076.6	42	8167.5	70
Filterer	381.3	10	406.5	9	878.1	19	429.4	7	189.6	2	144.1	2	3146.1	43	1752.1	15
Predator	381.5	10	722.8	16	721.5	15	425.0	7	600.9	6	3575.3	38	107.2	1	125.4	1
Scraper	600.4	15	765.8	17	500.9	11	1007.4	17	1398.0 ^c	13	1375.2 ^c	15	225.7 ^{d†}	3	472.0 ^{d†}	4
Shredder	59.6 ^c	1	22.6 ^c	0	578.2 ^d	12	970.4 ^d	17	294.2	3	66.3	1	822.6	11	1138.9	10
Total	4006.2		4625.6		4745.6		5792.9		10807.8		9450.5		11045.6		11658.9	

Notes: There were four Bt streams and two non-Bt streams in 2005, and there were three Bt and three non-Bt streams in 2006. There were no differences between stream types (all $P > 0.05$); biomass values followed by different superscript letters (c or d) are significantly different ($P < 0.05$) between seasons within stream type.

† $P < 0.10$.

TABLE 7. Averages of selected community metrics (based on abundances) in Bt and non-Bt study streams in northwestern Indiana.

Richness and composition	2005				2006			
	Summer		Fall		Summer		Fall	
	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt
Richness	27.0 ^c	25.0 ^c	22.0 ^d	19.0 ^d	27.0	21.0	21.0	16.0
EPT richness	4.5	4.0	3.0	3.0	6.0	4.3	6.0	6.0
Shannon index, H'	1.9	1.8	1.4	1.3	1.4	1.0	1.5	0.9
Composition (%)								
Oligochaeta	13.0	10.0	9.0	11.0	7.0	4.0	3.0	4.0
Chironomidae	9.0	17.0	6.0	19.0	64.0	41.0	18.0	55.0
Trichoptera	1.0	4.0	0.0	2.0	2.0	1.0	3.0	15.0

Notes: There were four Bt streams and two non-Bt streams in 2005, and there were three Bt and three non-Bt streams in 2006. There were no differences between stream types (all $P > 0.05$); values followed by different superscript letters (c or d) are significantly different ($P < 0.05$) between seasons within stream type. EPT richness is the number of taxa in the insect orders Ephemeroptera, Plecoptera, and Trichoptera.

The shredding stonefly *Allocapnia* (Plecoptera: Capniidae) was a minor contributor to total invertebrate biomass on all dates for streams 1C (Bt) (0.9–6%) and 1E (non-Bt) (0.4–2%). However, *Allocapnia* contributed 22–46% (11.34–46.55 mg AFDM/litterbag) of total invertebrate biomass in both litterbag types in stream 1F (non-Bt). Non-Tanypodinae chironomids (37–66%), hydrophilid beetles (1–7%), and physid snails (6–9%) also contributed significantly to total biomass on all dates.

Functional feeding group composition of invertebrates in litterbags did not differ between litter type and stream type ($P > 0.10$ for all comparisons on all dates; Chambers 2008), but there were temporal patterns in functional groups that were consistent across litter and stream types. Invertebrate biomass for all litter and stream types on day 3 was dominated by collector-gatherers (68%) and shredders (20%). Collector-gatherers (47%) were also dominant on day 13, but shredders increased and were also well-represented (37%), mostly due to increases in *Allocapnia*, which comprised 34% of total biomass (31.7 ± 19.7 mg AFDM/litterbag). Collector-gatherers (43%) and shredders (35%) were also dominant on day 25, with *Allocapnia* still dominating shredder biomass and trichopterans poorly represented.

DISCUSSION

Feeding trials

Our laboratory results build on evidence provided by Rosi-Marshall et al. (2007) that Bt maize detritus can negatively influence leaf-shredding trichopterans. Using the same methods as Rosi-Marshall et al. (2007), we performed an additional series of feeding trials, which included another variety of Bt maize, and observed the same pattern of significantly lower growth rates for *Lepidostoma liba* individuals fed Bt maize compared to non-Bt maize. Although we found only sublethal effects, our results suggest the potential for significant population-level consequences because slower growth of freshwater invertebrates can result in reduced adult size

and fecundity (e.g., Vannote and Sweeney 1980, Rausell et al. 2000). Decreases in growth and fecundity could eventually lead to a decrease in leaf-shredding trichopteran numbers over time and could, therefore, influence essential ecosystem services such as leaf litter decomposition in headwater streams (Wallace and Webster 1996).

Our experimental chambers were not flow through systems, and thus leaching from leaf disks may have elevated Bt toxin concentrations in the water in chambers compared to what would occur in streams. However, we also conditioned leaf materials in stream water for one week before placing them in experimental chambers. Based on results from Griffiths et al. (2009), using the same litter materials collected at the same time and from the same crop fields that we used in our feeding trials, ~60% of the Bt toxin leaches from Bt maize litter within one hour of immersion in water, but leaching slows dramatically after that and ~20% of the initial concentration is still present after 70 days. This confirms that the toxin was still present in the detritus we used in our feeding trials, but at a fraction of the original concentration of fresh, dried material. Thus, invertebrates in streams adjacent to Bt crop fields may be exposed to materials with much higher concentrations of Bt toxins than we used, but for relatively short periods of time based on leaching rates.

Benthic macroinvertebrate communities and variability among streams

While all the study streams we selected were similar in morphology, it was inherently difficult to simultaneously control for consistent discharge, velocity, width, depth, substrate composition, and riparian features across sites. In addition to potential Bt effects, the few differences we observed between Bt and non-Bt streams appeared most likely linked to differences in stream physical characteristics and food resource availability. For example, higher abundance of predatory *Tropisternus* larvae in non-Bt streams during 2006 was likely linked to prey availability rather than the absence of Bt maize, as there was ~40% higher biomass of small collector-gatherer taxa (e.g.,

Copepoda, Oligochaeta, Chironomidae) in non-Bt streams during this same time.

Stream 1C, a Bt stream, was perhaps the most distinct from the other study streams; it had higher discharge and velocities, greater width, and a higher percentage of coarse substrata (e.g., pebbles) compared to the other 2005 study streams, and these factors likely resulted in higher invertebrate diversity. Stream 1C had higher Shannon diversity as well as higher trichopteran biomass compared to other study streams, the majority of which were *Hydropsyche* and *Cheumatopsyche*, which are net-spinning filter-feeders (Merritt et al. 2008). Filter-feeding trichopterans require stable substrata, e.g., cobbles or pebbles, in order to attach their nets, and they also need steady flows to deliver food into nets (Edington 1968, Wallace and Merritt 1980), conditions that were most consistently available in stream 1C. Higher abundances of trichopterans in stream 1C (a Bt stream) appeared to contradict our prediction and laboratory results. However, patterns in stream 1C were driven by members of the family Hydropsychidae. Hydropsychids, unlike the leaf-shredding trichopterans we used in feeding trials, are filter feeders that feed on organic seston, which is derived from a variety of sources (Wallace and Merritt 1980, Wotton 1994) and, therefore, may not be negatively influenced by the presence of Bt detritus. However, this group of trichopterans could be influenced by other potential Bt inputs, such as pollen during brief periods of pollen shed (Rosi-Marshall et al. 2007).

Maize detritus in agricultural streams

Maize detritus is apparently available as food for detritivores for only a relatively short amount of time, as decomposition rates leave <50% of mass remaining after 25 days (Griffiths et al. 2009). Maize leaves placed in the stream soften quickly and tear apart easily within a few days, particularly in riffle areas (C. Chambers, *personal observation*). Maize inputs into streams tend to be temporally variable, yet they potentially occur throughout the year, particularly when no-till agricultural practices are used. Rapid maize decomposition rates may explain why benthic core samples showed no significant increase in maize content from the summer to the fall and why overall standing stocks in stream channels were consistently low.

The distribution of maize detritus in agricultural streams is linked to the altered hydrology of these systems. Our study streams are heavily channelized with little ability to dissipate stream energy, and they contain few retentive structures; therefore, organic materials are commonly scoured from the stream beds during storm events. As a result, maize detritus may not be available for an extended amount of time in one area, but it does have potential to move downstream once it has entered the channel (Rosi-Marshall et al. 2007). For example, immediately following spring flooding, we observed large accumulations of maize stalks, leaves, and cobs

around bridge abutments, tree trunks, and other stream obstructions. Stalks and cobs persist longer than leaves, but become clumped in stream channels and along banks, so they were not common in benthic samples. Nonetheless, large accumulations of these materials persist in highly localized patches along streams.

Invertebrate assemblages in litterbags changed over time, illustrating subtle dynamics in the response of invertebrates to the addition of food resources. When maize litterbags were first available (day 3), a limited number of small-bodied taxa were present in bags. Biomass then increased greatly with time to day 25. Collector-gatherers were the dominant early colonists, but they were also present later in the colonization cycle; this group includes some dominant taxa that are abundant year-round in these streams, such as chironomids. The increase in predator biomass in litterbags over time coincided with the increase in total invertebrate biomass, suggesting that predators were tracking prey in the litterbags. The contribution of shredders to total litterbag biomass also increased over time, and this pattern was driven almost exclusively by the shredding stonefly *Allocapnia*. Increasing biomass of plecopterans (mostly *Allocapnia*) from summer into winter months was also evident in benthic core samples. The prevalence of *Allocapnia* in litterbags suggests that this shredder responds relatively rapidly to patchily distributed detrital resources and thus may be food limited in these streams. Given that *Allocapnia* dominate the shredder group, and other shredders are poorly represented, this taxon is probably the only significant contributor to invertebrate-mediated litter decomposition in these agricultural streams, which is an important management consideration.

Bt effects and other stressors to agricultural streams

We suggest that the discrepancy between our field and laboratory results reflects the general scarcity of leaf-shredding trichopterans in our study streams. *Pycnopsyche* (Limnephilidae), *Nectopsyche* (Leptoceridae), and *Phryganea* (Phryganeidae) were the only case-building trichopterans we encountered in these streams, and all were scarce. Further, of these three taxa, only *Pycnopsyche* is consistently classified as a shredder, whereas the other two are considered more omnivorous (Wiggins 1996). Based on our laboratory results, which suggest that only trichopterans are negatively influenced by Bt detritus, the general scarcity of leaf-shredding trichopterans in these highly altered and degraded streams precluded our ability to detect any potential effects of Bt detritus in the field. We also note that by selecting sites based on adjacent crop plantings, we could not account for all plantings located upstream of our non-Bt streams and, therefore, cannot rule out potential influences of upstream inputs of Bt, particularly following storm events, which could transport Bt and other materials from upstream.

Our study constituted an intensive investigation of a limited number of study streams, and there were inherent statistical limitations to this approach. In particular, relatively low replication, which was exacerbated by the misidentification of one of the crop fields, and inherent high variability in invertebrates within and among streams, limited our ability to identify statistically significant patterns (e.g., high probabilities of Type II errors). Numerous taxa showed consistent trends between sites over multiple years (e.g., Ostracoda, Corbiculidae, and Physidae), but high variability in abundances precluded significant results. Future studies may consider sampling a greater number of sites less frequently, but this approach may still be limited by the high spatiotemporal variability that we observed in these systems.

The overall health, or lack thereof, of our study streams undoubtedly influenced our findings and contributed to the disparity between field and laboratory results. The streams we examined are highly degraded systems that are impacted by multiple stressors. High nutrient inputs from fertilizer applications, coupled with increased sedimentation, altered flow regimes, ongoing conventional pesticide use, and degraded riparian habitats, are major stressors to these systems (Cooper 1993). Individually and combined, these factors likely reduced invertebrate abundance, biomass, and diversity, and precluded the presence of a healthy, diverse assemblage of shredders. With multiple stressors, which at this point have persisted for decades, communities in these streams are unlikely to reflect solely the influences of a single stressor such as Bt toxin. Remaining taxa in these systems may also have developed some resistance. Insects can develop resistance to Bt toxins over time, and complete resistance has been documented in 15 generations (Bauer 1995, Ferre and Van Rie 2002).

While our results provide further evidence for some adverse effects of Bt maize detritus on an important component of stream-dwelling communities, they should be considered in the context of modern agricultural practices and alternatives. Traditional pest management methods (e.g., direct applications of various pesticides) can have significant negative impacts on a wide variety of nontarget species, including many aquatic insects (Cooper 1993), whereas the negative consequences of Bt detritus were sublethal effects on a species that is relatively closely related to the target pest. Based on the specific conditions we studied, our results suggest that further research on potential effects of Bt toxins on stream ecosystem structure and function is warranted before informed decisions regarding the costs and benefits of the use of genetically engineered Bt crops, relative to available alternatives, can be made. In particular, field studies are needed that isolate Bt toxin effects from other potential stressors, a task that was nearly impossible in our study streams. Such studies would allow for more comprehensive assessments of

risks and related decision making with regard to the planting of Bt crops, which is rapidly increasing.

ACKNOWLEDGMENTS

We thank J. Stanton for field and laboratory assistance, and S. Peterson and J. Uzzuardo for assistance processing samples in the laboratory. We also thank J. Bricker, Extension Educator at Purdue University; R. J. Steele, County Surveyor; and numerous private land owners for assistance in selecting and accessing study sites. Two anonymous reviewers provided comments that greatly improved this manuscript. This project was funded by the National Science Foundation (DEB-0415984).

LITERATURE CITED

- Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment for use in wadeable streams and rivers: periphyton, benthic macroinvertebrates and fish. Second edition. U.S. Environmental Protection Agency, Washington, D.C., USA.
- Bauer, L. S. 1995. Resistance: a threat to the insecticidal crystal proteins of *Bacillus thuringiensis*. *Florida Entomologist* 78: 414–443.
- Benfield, E. F. 2006. Decomposition of leaf material. Pages 711–720 in F. R. Hauer and G. A. Lamberti, editors. *Methods in stream ecology*. Second edition. Academic Press, London, UK.
- Benke, A. C., and A. D. Huryn. 2006. Secondary production of macroinvertebrates. Pages 691–710 in F. R. Hauer and G. A. Lamberti, editors. *Methods in stream ecology*. Second edition. Academic Press, London, UK.
- Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* 18:308–343.
- Candolfi, M. P., K. Brown, C. Grimm, B. Reber, and H. Schmidli. 2004. A faunistic approach to assess potential side-effects of genetically modified *Bt*-Corn on non-target arthropods under field conditions. *Biocontrol Science and Technology* 14:2.
- Chambers, C. P. 2008. Potential impacts of transgenic crop residues on macroinvertebrate communities in agricultural streams. Thesis. Southern Illinois University, Carbondale, Illinois, USA.
- Clark, B. W., K. R. Prihoda, and J. R. Coats. 2006. Subacute effects of transgenic Cry1Ab *Bacillus thuringiensis* corn litter on the isopods *Trachelipus rathkii* and *Armadillidium nasatum*. *Environmental Toxicology and Chemistry* 25: 2653–2661.
- Cooper, C. M. 1993. Biological effects of agriculturally derived surface water pollutants on aquatic systems—a review. *Journal of Environmental Quality* 22:402–408.
- Cummins, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. *American Midland Naturalist* 67: 477–504.
- Edington, J. M. 1968. Habitat preferences in net-spinning caddis larvae with special reference to the influence of water velocity. *Journal of Animal Ecology* 37:675–692.
- EPA (Environmental Protection Agency). 2000. *Methods for measuring the toxicity and bioaccumulation of sediment associated contaminants with freshwater invertebrates*. U.S. Environmental Protection Agency, Washington, D.C., USA.
- Ferre, J., and J. Van Rie. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 47:501–533.
- Grafius, E., and N. H. Anderson. 1979. Population dynamics, bioenergetics, and the role of *Lepidostoma quercina* Ross

- (Trichoptera: Lepidostomatidae) in an Oregon woodland stream. *Ecology* 60:433–441.
- Grafius, E., and N. H. Anderson. 1980. Population dynamics and the role of two species of *Lepidostoma* (Trichoptera: Lepidostomatidae) in an Oregon coniferous forest stream. *Ecology* 61:808–816.
- Griffiths, N. A., J. L. Tank, T. V. Royer, E. J. Rosi-Marshall, M. R. Whiles, C. P. Chambers, T. C. Frauendorf, and M. A. Evans-White. 2009. Rapid decomposition of maize detritus in agricultural headwater streams. *Ecological Applications* 19: 133–142.
- Hagen, E. M., J. R. Webster, and E. F. Benfield. 2006. Are leaf breakdown rates a useful measure of stream integrity along an agricultural land use gradient? *Journal of the North American Benthological Society* 25:330–343.
- Hauer, F. R., and G. A. Lamberti. 2006. *Methods in stream ecology*. Academic Press, San Diego, California, USA.
- Jackson, J. K., B. W. Sweeney, T. L. Bott, J. D. Newbold, and L. A. Kaplan. 1994. Transport of *Bacillus thuringiensis* var. *israelensis* and its effect on drift and benthic densities of nontarget macroinvertebrates in the Susquehanna River, northern Pennsylvania. *Canadian Journal of Fisheries and Aquatic Sciences* 51:295–314.
- Losey, J. E., L. S. Raynor, and M. E. Carter. 1999. Transgenic pollen harms monarch larvae. *Nature* 399:214.
- Martin, M. M., J. J. Kukokmar, J. S. Martin, D. L. Lawson, and R. W. Merritt. 1982. Digestive enzymes of larvae of three species of caddisflies (Trichoptera). *Insect Biochemistry* 11: 501–505.
- Marvier, M., C. McCreedy, J. Regetz, and P. Kareiva. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science* 316:1475–1477.
- Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa, USA.
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 26:31–36.
- NASS (National Agricultural Statistics Service). 2006. Acreage. Agricultural Statistics Board, United States Department of Agriculture, Washington, D.C., USA.
- NASS (National Agricultural Statistics Service). 2008. Acreage. Agricultural Statistics Board, United States Department of Agriculture, Washington, D.C., USA.
- Rasband, W. S. 2006. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA. (<http://rsb.info.nih.gov/ij/>)
- Rausell, C., A. C. Martínez-Ramírez, I. García-Robles, and M. D. Real. 2000. A binding site for *Bacillus thuringiensis* Cry1Ab toxin is lost during larval development in two forest pests. *Applied and Environmental Microbiology* 66:1553–1558.
- Rosi-Marshall, E. J., J. L. Tank, T. V. Royer, M. R. Whiles, M. Evans-White, C. Chambers, N. A. Griffiths, J. Pokelsek, and M. L. Stephen. 2007. Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences USA* 104:16204–16208.
- SAS Institute. 2005. SAS version 9.1. SAS Institute, Cary, North Carolina, USA.
- Saxena, D., and G. Stotzky. 2001a. *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. *Soil Biology and Biochemistry* 33: 1225–1230.
- Saxena, D., and G. Stotzky. 2001b. Bt corn has a higher lignin content than non-Bt corn. *American Journal of Botany* 88: 1704–1706.
- Smith, D. G. 2001. *Pennak's freshwater invertebrates of the United States*. Fourth edition. John Wiley and Sons, New York, New York, USA.
- Solorzano, L. 1969. Determination of ammonia in natural waters by phenylhypochlorite method. *Limnology and Oceanography* 14:799–801.
- Stanley-Horn, D. E., G. P. Dively, R. L. Hellmich, H. R. Mattila, M. K. Sears, R. Rose, L. C. H. Jesse, J. E. Losey, J. J. Obrycki, and L. Lewis. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proceedings of the National Academy of Sciences USA* 98:11931–11936.
- Stone, M. L., M. R. Whiles, J. A. Webber, K. W. J. Williard, and J. D. Reeve. 2005. Macroinvertebrate communities in agriculturally impacted southern Illinois streams: patterns with riparian vegetation, water quality, and in-stream habitat quality. *Journal of Environmental Quality* 34:907–917.
- Townsend, C. R., C. J. Arbuckle, T. A. Crowl, and M. R. Scarsbrook. 1997. The relationship between land use and physiochemistry, food resources and macroinvertebrate communities in tributaries of the Taieri River, New Zealand: a hierarchically scaled approach. *Freshwater Biology* 37:177–191.
- Vannote, R. L., and B. W. Sweeney. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *American Naturalist* 115:667–695.
- Wallace, J. B., and R. W. Merritt. 1980. Filter-feeding ecology of aquatic insects. *Annual Review of Entomology* 25:103–132.
- Wallace, J. B., and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* 41:115–139.
- Whiles, M. R., J. B. Wallace, and K. Chung. 1993. The influence of *Lepidostoma* (Trichoptera: Lepidostomatidae) on recovery of leaf-litter processing in disturbed headwater streams. *American Midland Naturalist* 130:356–363.
- Wiggins, G. B. 1996. *Larvae of the North American caddisfly genera (Trichoptera)*. University of Toronto Press, Toronto, Canada.
- Wotton, R. S. 1994. *The biology of particles in aquatic systems*. Lewis, Boca Raton, Florida, USA.
- Wraight, C. L., A. R. Zangerl, M. J. Carroll, and M. R. Berenbaum. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proceedings of the National Academy of Sciences USA* 97: 7700–7703.
- Zangerl, A. R., D. McKenna, C. L. Wraight, M. Carroll, P. Ficarello, R. Warner, and M. R. Berenbaum. 2001. Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proceedings of the National Academy of Sciences USA* 98:11908–11912.
- Zar, J. H. 1999. *Biostatistical analysis*. Fourth edition. Prentice Hall, Upper Saddle River, New Jersey, USA.
- Zwahlen, C., A. Hilbeck, P. Gugerli, and W. Nentwig. 2003. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology* 12:765–775.