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Rapid decomposition of maize detritus in agricultural headwater streams

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Abstract. Headwater streams draining agricultural landscapes receive maize leaves (*Zea mays* L.) via wind and surface runoff, yet the contribution of maize detritus to organic-matter processing in agricultural streams is largely unknown. We quantified decomposition and microbial respiration rates on conventional (non-Bt) and genetically engineered (Bt) maize in three low-order agricultural streams in northwestern Indiana, USA. We also examined how substrate quality and in-stream nutrient concentrations influenced microbial respiration on maize by comparing respiration on maize and red maple leaves (*Acer rubrum*) in three nutrient-rich agricultural streams and three low-nutrient forested streams. We found significantly higher rates of microbial respiration on maize vs. red maple leaves and higher rates in agricultural vs. forested streams. Thus both the elevated nutrient status of agricultural streams and the lability of maize detritus (e.g., low carbon-to-nitrogen ratio and low lignin content) result in a rapid incorporation of maize leaves into the aquatic microbial food web. We found that Bt maize had a faster decomposition rate than non-Bt maize, while microbial respiration rates did not differ between Bt and non-Bt maize. Decomposition rates were not negatively affected by genetic engineering, perhaps because the Bt toxin does not adversely affect the aquatic microbial assemblage involved in maize decomposition. Additionally, shredding caddisflies, which are known to have suppressed growth rates when fed Bt maize, were depauperate in these agricultural streams, and likely did not play a major role in maize decomposition. Overall, the conversion of native vegetation to row-crop agriculture appears to have altered the quantity, quality, and predictability of allochthonous carbon inputs to headwater streams, with unexplored effects on stream ecosystem structure and function.

Key words: agriculture; allochthonous inputs; Bacillus thuringiensis; *carbon cycling; decomposition; genetically engineered crops; leaf breakdown; maize; microbial respiration; Midwestern United States; organic matter; streams.*

Introduction

Unlike headwater streams in forested areas where inputs of allochthonous detritus are an important basal resource (Vannote et al. 1980), headwater streams draining agricultural landscapes are believed to be supported primarily by autochthonous carbon from in-stream primary producers (Wiley et al. 1990). However, maize (*Zea mays* L.) is a dominant row crop planted in the Midwestern United States, covering 36.6×10^6 ha in 2007 (NASS 2007), and may represent an important allochthonous subsidy to agricultural streams. After crop harvest, maize byproducts (leaves, stalks, and cobs) are commonly left on fields and can enter streams via wind and surface runoff (see Plate 1). Our previous research has shown that inputs of maize byproducts to agricultural streams have a range of 0.1–7.9 g ash-free dry mass (AFDM)·m^{-2}·yr^{-1}, resulting in benthic standing stocks of maize leaves and cobs up to 6.4 g AFDM/m^2 (Rosi-Marshall et al. 2007). In comparison, detrital inputs and standing stocks in typical temperate-forest headwater streams may be two to three orders of magnitude greater (Webster and Meyer 1997) than maize detrital inputs and standing stocks in agricultural streams. The contribution of crop byproducts to organic-matter processing in agricultural streams has not been investigated and may challenge the notion that agricultural streams in the Midwestern United States are fueled by autochthonous production with few allochthonous contributions.

Organic-matter processing can also be influenced by the quality of allochthonous inputs that enter a stream. Traditionally, this occurred via species replacement and selective breeding, but genetic engineering now allows for additional molecular-scale changes to the chemistry of plant material within a species. The planting of genetically engineered crops in the Midwestern United

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TABLE 1. Physical and chemical characteristics of three agricultural streams in northwestern Indiana, USA.

Stream	Location	Discharge (L/s)†	Gradient (m/m)	Wetted width (m)‡	Temperature range (°C)§	Nutrient concentration¶		
						SRP (μg/L)	NH_4^+-N (μg/L)	NO_3^--N (mg/L)
1C	40°41′56″ N, 87°25′12″ W	312.6 (42.2)	0.0011	4.1 (0.4)	−1.1–14.6	9.2 (3.0)	14.1 (5.4)	3.3 (1.3)
1E	40°36′34″ N, 87°11′25″ W	155.4 (38.0)	0.0008	3.0 (0.5)	−1.0–13.1	17.4 (4.3)	13.9 (2.6)	4.8 (2.4)
1F	40°37′19″ N, 87°25′39″ W	83.4 (26.9)	0.0019	2.6 (0.3)	0.0–15.3	7.1 (3.0)	24.5 (12.5)	4.7 (2.3)

Note: SRP is soluble reactive phosphorus; data are means with SE in parentheses.
† Discharge was measured four (streams 1C, 1E) or six (stream 1F) times.
‡ Wetted width was measured once, and error reflects variation in width along the study reach.
§ Temperature was monitored continuously over the study period.
¶ Nutrient concentrations were measured three times.

States has become commonplace; in 2007, 49% of maize planted in the United States was genetically engineered to express *Bacillus thuringiensis* (Bt)-derived crystalline (cry) proteins (NASS 2007). Bt maize engineered to express Cry1Ab protein targets lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*), by modifying epithelial receptors on the midgut, causing the gut contents to leak into the hemolymph (Whalon and Wingerd 2003). The adverse effects of Bt maize on nontarget terrestrial organisms, such as monarch butterflies, have received much attention in recent years (Losey et al. 1999, Stanley-Horn et al. 2001, Marvier et al. 2007); however, the effects of transgenic crops on nontarget aquatic organisms are understudied and warrant further investigation. Our previous research has shown that caddisflies (*Lepidostoma liba*) fed genetically engineered maize leaves in the laboratory exhibit suppressed growth rates compared to their counterparts fed conventional maize (Rosi-Marshall et al. 2007). If Bt maize is detrimental to aquatic microbial or invertebrate assemblages in situ, organic-matter decomposition and overall carbon cycling may also be affected in streams that drain fields planted with genetically engineered maize.

Our goal was to examine the decomposition of allochthonous organic matter in agricultural streams. We also wanted to explore how technological advances in agriculture (e.g., genetic engineering) might affect the rate at which crop detritus is incorporated into stream food webs. We addressed these questions by comparing decomposition and microbial respiration rates on Bt and conventional (non-Bt) maize in agricultural streams. Because microbial activity is likely a significant driver of organic-matter decomposition in high-nutrient, low-gradient agricultural streams, we also wanted to examine the extent to which litter quality and dissolved-nutrient availability affected the rate at which crop detritus is respired. We predicted that maize leaves would be a high-quality substrate for microorganisms because of their simple leaf structure, low lignin content (4.5%–6.3%) and low C:N ratio (24.0–38.8) compared to a number of temperate deciduous-leaf species (Ostrofsky 1997). Additionally, high inorganic nitrogen and phosphorus concentrations typical of agricultural streams (Howarth et al. 1996, Kemp and Dodds 2001, Royer et al. 2004) should support high microbial activity. We investigated how substrate quality and in-stream nutrient concentrations influence microbial respiration on maize by comparing respiration on maize vs. red maple (*Acer rubrum*) leaves in nutrient-rich agricultural vs. low-nutrient forested streams.

METHODS

Study sites

We quantified decomposition rates and associated microbial respiration rates on Bt and non-Bt maize in three low-order streams (stream names: 1C, 1E, 1F, as in Rosi-Marshall et al. [2007]) in northwestern Indiana, USA, an intensively cultivated region of the Midwestern corn belt, with approximately 96% of land area planted in row crops (NASS 2002). Prior to European settlement, this landscape was dominated by wetlands, tallgrass prairie, and mixed oak and beech–maple forests (Welch 1930, Gordon 1936). Frequent dredging of agricultural streams for effective drainage has decreased channel complexity and removed structures that retain organic matter. These study streams are typical of low-gradient, Midwestern agricultural streams in that they have deeply incised channels, uniform widths, and high nitrate concentrations (Table 1).

To examine the interacting influences of high-nutrient agricultural streams and high-quality maize detritus on microbial respiration, we selected six low-order streams, three draining primarily forested land (stream names: Bear, Sand, Swan) and three draining agricultural land (stream names: Fenner, Greegs, Haney) in the Kalamazoo River basin in southwestern Michigan, USA, which is the closest basin to northwestern Indiana that contains both of these land-use types. Due to the patchiness of land use within the Kalamazoo River basin, the three forested streams are located in the western part of the basin, and the three agricultural streams are located in the eastern part of the basin. However, the replicate streams are still located close to each other (maximum distance between sites = 36 km). Further, the maximum distance between agricultural streams is 17 km, and the maximum distance between forested streams is 14 km. Generally, the three agricultural streams had higher nitrate concentrations

and lower coarse benthic organic-matter standing stocks than did the three forested streams (Table 2).

Water chemistry

To compare nutrient concentrations in streams within and across land-use categories, we collected water samples for nutrient analyses at three time periods throughout the decomposition study in Indiana and three periods throughout the microbial respiration study in Michigan. We filtered water samples through 0.7-μm Whatman GF/F glass-fiber filters (Whatman, Florham Park, New Jersey, USA) into acid washed bottles, and froze the samples at −30°C until analysis. 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).

Benthic organic-matter standing stocks

To compare the amount of benthic carbon in streams of differing land use, we collected benthic organic-matter samples in our three forested and three agricultural streams in Michigan at the end of the study. We sampled coarse (CBOM, >1 mm diameter) and fine (FBOM, 52 μm–1 mm diameter) benthic organic matter with a 314-cm^2 core at five locations throughout the study reach. Both CBOM and FBOM samples were dried at 60°C for 48 h, and then combusted in a muffle furnace at 500°C for 1 h. We calculated ash-free dry mass (AFDM) as the difference between ash mass and dry mass.

Decomposition and microbial respiration on Bt and non-Bt maize

Prior to harvest in late October 2005 we collected dried Bt and non-Bt maize leaves from maize plants that were growing adjacent to our study streams in Indiana. We determined the nutritional quality of collected Bt and non-Bt maize leaves by measuring percentage carbon (C) and nitrogen (N) using a Costech Elemental Analyzer (Costech Analytical Technologies, Valencia, California, USA) and percentage lignin via the Dairy One Forage Testing Laboratory (Ithaca, New York, USA). To control for extraneous factors that may affect decomposition and respiration rates, we then paired Bt and non-Bt maize hybrids to be placed in each stream based primarily on similar percentage lignin, and secondarily on similar percentage carbon (C), percentage nitrogen (N), and C:N ratios (Table 3). While previous studies have controlled for isolines by growing Bt maize in experimental plots or greenhouses, field studies do not always allow for such control. We used field-grown maize hybrids in the decomposition experiment to represent the variety of maize planted in the landscape, and thus the maize detritus that is likely to enter headwater streams.

We air-dried maize leaves in the laboratory for a week prior to constructing litterbags. We cut maize leaves to an approximate size of 10 cm, and placed 13 g of maize leaves into nylon bags with a 1×0.25 cm mesh size. In each of three headwater streams draining agricultural fields in Indiana, we anchored 21 Bt and 21 non-Bt litterbags to the streambed in areas of similar stream velocity and retrieved three replicate litterbags on days 0, 3, 7, 13, 25, 37, and 70, from 4 November 2005 to 13 January 2006, which coincided with the time that maize leaves are most likely to enter streams. On each collection date, we removed ~1 cm^2 of maize from each Bt and non-Bt litterbag for a microbial respiration assay. We then rinsed litterbags over nested 250-μm and 1-mm sieves to remove sediments and invertebrates. We observed shredding invertebrates on the maize leaves throughout the decomposition experiment (C. Chambers, *unpublished data*). We dried the maize leaves in a drying oven at 60°C for 48 h, and then combusted them in a muffle furnace at 500°C for 1 h to determine AFDM. The decomposition coefficient ($-k$; d^{-1}) was calculated as the slope of the regression of the natural log of proportion AFDM remaining vs. time (Benfield 2006) for each litter type in each stream. To account for temperature differences across streams, we also expressed decomposition rates in degree days ($-k$: degree d^{-1}). We recorded water temperature every half hour in each stream using a HOBO temperature logger (Onset, Bourne, Massachusetts, USA) and calculated the cumulative degree days for each time period as the sum of the mean daily temperatures over that time period (Benfield 2006).

Interacting influence of land use and organic-matter type on microbial respiration

To determine whether high nutrient concentrations or substrate quality influenced microbial respiration on maize litter, we compared microbial respiration on maize and red maple leaves in agricultural and forested headwater streams. We placed 13 g of air-dried non-Bt maize leaves or 13 g of air-dried red maple leaves into litterbags, and anchored three litterbags of each litter type in each of three high-nutrient agricultural and three low-nutrient forested streams ($n = 36$ litterbags in total) in Michigan (Table 2), in areas of similar stream velocity. On days 5, 12, 19, 26, 33, 40, 54, and 68, from 1 February 2006 to 5 April 2006, we removed ~1 cm^2 of material from the same replicate litterbag in each stream for a microbial-respiration assay.

Microbial-respiration assay

Leaves collected from litterbags were returned to the laboratory, stored in stream water overnight at 4°C, and analyzed for microbial respiration the following day. We placed leaf samples of an approximate size of 1 cm^2 into 60 mL Falcon tubes filled (no air bubbles) with filtered

TABLE 2. Characteristics of the three forested and three agricultural streams in the Kalamazoo River basin, Michigan, USA, together with results of ANOVA.

Stream	Location	Discharge (L/s)	Temperature range (°C)	Nutrient concentration		
				SRP (μg/L)	NH_4^+-N (μg/L)	NO_3^--N (mg/L)
Forested						
Bear Creek	42°37′22″ N 85°55′04″ W	85.7 (7.2)	3.7–10.5	6.8 (0.5)	31.2 (2.7)	0.69 (0.07)
Sand Creek	42°35′36″ N 85°56′33″ W	39.0 (2.1)	2.8–10.2	5.0 (0.1)	17.9 (0.6)	0.46 (0.02)
Swan Creek	42°30′23″ N 85°59′33″ W	953.9 (139.0)	1.6–10.2	8.1 (0.9)	63.4 (19.0)	0.69 (0.07)
Agricultural						
Fenner Creek	42°33′34″ N 85°33′46″ W	142.5 (9.7)	2.6–5.9	6.3 (1.4)	82.2 (9.8)	8.6 (0.7)
Greegs Brook	42°34′01″ N 85°34′14″ W	123.7 (4.6)	2.9–6.4	19.1 (2.6)	111.4 (30.4)	8.0 (1.1)
Haney Drain	42°42′06″ N 85°38′01″ W	28.6 (4.9)	3.5–9.8	26.6 (16.4)	33.7 (9.9)	5.0 (1.0)
ANOVA results†		NS	NS	NS	NS	$P < 0.001$

Note: Discharge and nutrient concentrations were measured three times throughout the study period, temperature was measured on each collection date, and organic-matter standing stocks were measured at the end of the study. SRP is soluble reactive phosphorus, FBOM is fine benthic organic matter, CBOM is coarse benthic organic matter, and AFDM is ash-free dry mass. Data are means with SE in parentheses (the error associated with the three measurement periods).

† Discharge, mean daily temperature, and nutrient concentrations were compared between land-use types using one-way repeated-measures ANOVAs, and benthic organic-matter standing stocks were compared between land-use types using one-way ANOVAs. Nonsignificant ANOVAs ($P > 0.05$) are indicated by NS.

stream water (GF/F, Whatman, Florham Park, New Jersey, USA). To compare microbial respiration on both leaf types among all streams throughout the study, samples were always incubated at room temperature (17°C–20°C) for 2 h in the dark. We quantified microbial respiration associated with litter material (expressed in mg O_2·[g AFDM]$^{-1}$·h^{-1}) by measuring the change in dissolved oxygen concentration over the 2-h incubation period using a hand-held dissolved-oxygen probe (DO200; Yellow Springs Instruments, Yellow Springs, Ohio, USA) (Hill et al. 2000). Tubes containing only stream water were included as blanks ($n = 3$ tubes per stream) to account for background changes in dissolved oxygen, which were minimal.

Cry1Ab protein analysis

To determine whether the Cry1Ab protein was present in Bt maize leaves throughout the decomposition experiment and to examine how Cry1Ab protein concentration varied over time, we determined the short-term (8 hours) and long-term (70 days) leaching rates of Cry1Ab from maize leaves immersed in flowing water. For the short-term experiment, we placed 13 g of air-dried Bt maize leaves into 5 litterbags, anchored the litterbags to the benthos in one agricultural stream, and collected a subsample of ~0.5 g from each litterbag every half hour over an 8-h period. For the long-term leaching experiment, we removed ~0.5 g from three replicate Bt litterbags (initial mass = 13 g) in each stream as part of the decomposition experiment. The litterbags were retrieved on days 0, 3, 7, 13, 25, 37, and 70, and subsamples were frozen at −30°C until protein analysis.

We determined the concentration of Cry1Ab protein in maize leaves using a commercial double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Maize leaves were thawed and oven dried at 60°C for 48 h. To extract Cry1Ab protein, we placed maize leaf samples in 1X PBST (phosphate buffered saline with the detergent Tween-20) in a 1 g-to-50 mL ratio. We homogenized maize leaf samples using a hand-held tissue homogenizer (BioSpec Products, Bartlesville, Oklahoma, USA), centrifuged samples at 10 000 rpm for 10 min, and used the supernatant for the ELISA analysis. An eight-point calibration curve ranging from 0.5 ng/mL to 100 ng/mL was created from the serial dilution of purified Cry1Ab protein (Abraxis, Warminster, Pennsylvania, USA). We included three buffer blanks to identify any potential contamination among samples. Samples were

TABLE 3. Chemical characteristics of Bt and non-Bt maize used in the decomposition experiment in northwestern Indiana, USA.

Stream	Litterbag treatment	Maize hybrid	Lignin (%)	Carbon (%)	Nitrogen (%)	C:N
1C	Bt	111-75rrBt Frontiersmen	4.8	47.5 (0.6)	1.7 (0.1)	27.9 (1.5)
	non-Bt	Pioneer35Y68	4.5	45.3 (1.6)	1.6 (0.1)	28.3 (1.0)
1E	Bt	Crows4635Bt	5.7	44.2 (0.5)	1.1 (0.1)	38.8 (2.9)
	non-Bt	Pioneer35Y68	6.3	47.5 (0.5)	1.9 (0.1)	24.6 (0.5)
1F	Bt	Agventure†	5.1	44.9 (1.2)	1.4 (0.1)	33.5 (1.6)
	non-Bt	Pioneer35Y68	5.1	46.0 (0.8)	2.0 (0.2)	24.0 (1.7)

Notes: Four replicates collected across the 200-m stream reach were analyzed for percentage carbon and nitrogen. Each stream received the same non-Bt maize hybrid (Pioneer35Y68); however, the chemical composition varied in each stream as the non-Bt maize hybrid came from three different source fields. Data are means with SE in parentheses.

† No specific hybrid information was available.

TABLE 2. Extended.

Organic-matter standing stock	
FBOM (g AFDM/m^2)	CBOM (g AFDM/m^2)
68.9 (58.5)	336.7 (147.4)
55.9 (30.2)	175.9 (104.3)
32.5 (26.7)	260.4 (109.3)
51.6 (23.5)	23.5 (13.3)
175.8 (141.3)	83.2 (77.0)
168.6 (39.8)	71.9 (32.4)
NS	$P = 0.03$

aliquoted in triplicate into a 96-well ELISA plate (Strategic Diagnostics, Newark, Delaware, USA), and the absorbance was read at 450 nm and 650 nm using a SpectraMax M2 microplate reader (Molecular Devices Corporation, Sunnyvale, California, USA); we subtracted the absorbance at 450 nm from the absorbance at 650 nm to correct for turbidity. The concentration of Cry1Ab protein was expressed as micrograms Cry1Ab per gram of dry maize leaf.

Statistics

We compared discharge and nutrient concentrations between agricultural and forested land-use types using one-way repeated-measures analysis of variance (RM ANOVA), and compared organic-matter standing stocks between land-use types using one-way ANOVA (Zar 1999). We used a nested analysis of covariance (ANCOVA), where maize types were nested within stream and time was the covariate, to determine whether decomposition coefficients ($-k$) differed significantly between Bt and non-Bt maize (Benfield 2006). Comparison of microbial respiration rates between Bt and non-Bt maize was carried out using a one-way nested RM ANOVA, where maize types were nested within stream. Microbial respiration rates on maize and red maple leaves and in agricultural and forested streams were analyzed using a two-way nested RM ANOVA, where streams were nested within land-use type and litter types were nested within stream. We used simple linear regressions to examine how microbial respiration rates influenced decomposition rates of maize leaves, and to examine how stream water nutrient concentrations influenced microbial-respiration rates (Zar 1999). Data were normalized to meet parametric assumptions using natural-log or square-root transformations. All statistical analyses were performed with SYSTAT 11.0 (SYSTAT 2004).

RESULTS

Decomposition and microbial respiration on Bt and non-Bt maize

Bt maize broke down at a faster rate than non-Bt maize ($-k_{Bt} = 0.021$ d^{-1} and $-k_{nBt} = 0.015$ d^{-1}; nested ANCOVA: $F_{1,108} = 7.95$, $P = 0.006$; Fig. 1), and varied across the three Indiana streams (nested ANCOVA: $F_{2,108} = 3.36$, $P = 0.04$), with the fastest decomposition rate measured in stream 1E ($-k_{Bt} = 0.024$ d^{-1}) and the slowest decomposition rate found in stream 1F ($-k_{Bt} = 0.012$ d^{-1}). When decomposition rates were expressed in degree days, maize-leaf decomposition was no longer different among streams (nested ANCOVA: $F_{2,108} = 1.93$, $P = 0.15$).

Microbial respiration rates did not differ between Bt and non-Bt maize (nested RM ANOVA: $F_{3,7} = 0.65$, $P = 0.61$; Fig. 2a), but varied across the three Indiana streams (nested RM ANOVA: $F_{2,7} = 8.45$, $P = 0.01$), with the highest respiration rates measured in stream 1E and the lowest respiration rates recorded in stream 1F. Respiration on Bt and non-Bt maize followed a similar temporal trend; microbial respiration increased from day 3 to day 13, reached a plateau between days 13 and 37, and then decreased by day 70 when there was little maize material left in the litterbags. During this period, microbial respiration rates varied more than two-fold, with the lowest respiration rate measured on day 3 (0.84 $\pm$ 0.06 mg O_2·[g AFDM]$^{-1}$·h^{-1}) and the highest respiration rate measured on day 13 (1.91 $\pm$ 0.11 mg O_2 ·[g AFDM]$^{-1}$·h^{-1}) (mean $\pm$ SE). Using data from all streams, there was a significant, positive relationship between maximum microbial respiration rates and decomposition rates when expressed by day ($r^2 = 0.80$, $P = 0.02$) or degree day ($r^2 = 0.77$, $P = 0.02$).

Short- and long-term leaching of Cry1Ab protein from submerged maize leaves

The short-term leaching experiment indicated that 61% of the Cry1Ab protein leached from maize leaves within the first hour of submergence and then maintained a constant concentration of ~2.0 μg Cry1Ab/g

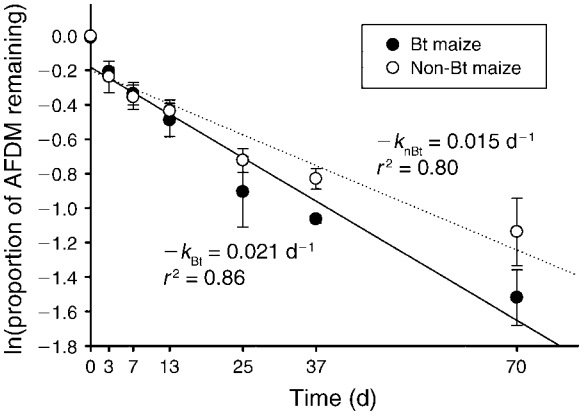

FIG. 1. Decomposition of Bt maize and non-Bt maize in three agricultural streams over a period of 70 days. The decomposition coefficients ($-k_{Bt} = 0.021$ d^{-1} and $-k_{nBt} = 0.015$ d^{-1}) differed significantly between Bt and non-Bt maize (nested ANCOVA: $F_{1,108} = 7.95$, $P = 0.006$). Data are means $\pm$ SE from replicate streams ($n = 3$ streams per data point); AFDM is ash-free dry mass.

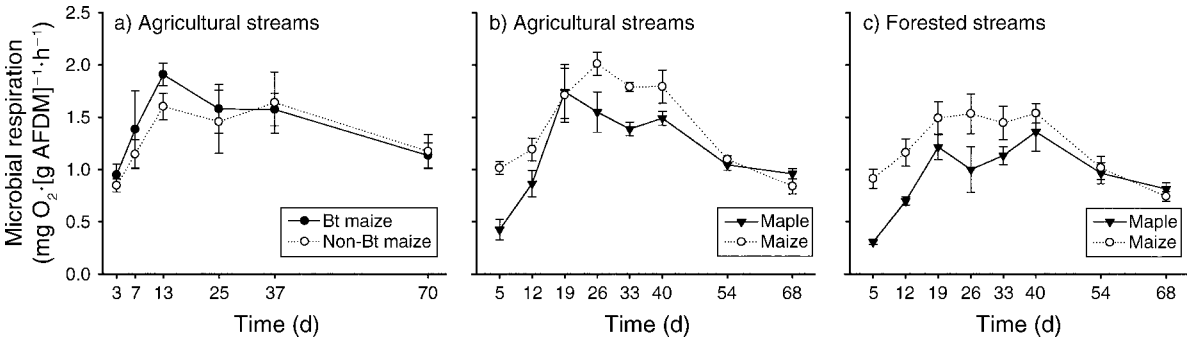

FIG. 2. Microbial respiration: (a) on Bt maize and non-Bt maize in three agricultural streams over a period of 70 days; and (b, c) on conventional maize and red maple leaves in (b) three agricultural streams and (c) three forested streams, over a period of 68 days. Microbial respiration on Bt and non-Bt maize did not differ over time (nested RM ANOVA: $F_{3,7} = 0.65$, $P = 0.61$). Respiration was greater on maize leaves vs. red maple leaves (nested RM ANOVA: $F_{1,23} = 42.9$, $P < 0.001$) and was greater in agricultural vs. forested streams (nested RM ANOVA: $F_{1,23} = 22.3$, $P < 0.001$). Data are means ± SE from replicate streams ($n = 3$ streams per data point).

dry maize leaf for the remaining 7 hours (Fig. 3 inset). When measured over 70 days, Cry1Ab protein leaching followed an exponential decay model (Fig. 3), but even after 70 days, 20% of the initial Cry1Ab protein was still present in maize leaves, indicating that the Cry1Ab protein was present throughout the decomposition and respiration experiment. Cry1Ab concentrations were variable among maize leaves collected on the same day, and while it appears that more Cry1Ab protein is present at day 3 than at hour 8, the mean Cry1Ab concentrations were not significantly different (one-way ANOVA: $P = 0.38$).

Interacting influence of land use and organic-matter type on microbial respiration

There were significant differences in substrate quality between litter types as microbial respiration was higher on maize leaves than on red maple leaves (nested RM ANOVA: $F_{1,23} = 42.91$, $P < 0.001$; Fig. 2b, c). Stream-water nutrients also appeared to influence microbial respiration rates as respiration was significantly higher in agricultural streams (Fig. 2b) compared to forested streams (nested RM ANOVA: $F_{1,23} = 22.34$, $P < 0.001$; Fig. 2c). There was no interaction between litter type and land use (nested RM ANOVA: $F_{1,23} = 0.15$, $P = 0.70$). Microbial respiration differed across streams within each land-use type (nested RM ANOVA: $F_{4,23} = 3.12$, $P = 0.03$); however, all streams followed a similar temporal pattern: respiration increased from day 5 to day 19, reached a plateau between days 19 and 40, and decreased by day 68 when there was little material left in the litterbags. Maximum microbial-respiration rates across streams were most strongly correlated with stream-water ammonium concentrations (slope = 0.19, $F_{1,10} = 4.18$, $r^2 = 0.30$, $P = 0.07$), and not correlated with stream-water soluble reactive phosphorus concentrations (slope = 0.19, $F_{1,10} = 2.04$, $r^2 = 0.17$, $P = 0.18$) or nitrate concentrations (slope = 0.10, $F_{1,10} = 2.82$, $r^2 = 0.22$, $P = 0.12$).

Average microbial-respiration rates on conventional maize leaves in agricultural streams in Michigan were similar to rates measured on conventional (non-Bt) maize in agricultural streams in Indiana (nested ANOVA: $F_{1,12} = 3.47$, $P = 0.09$), indicating that our respiration measurements are replicable across study areas. However, maximum microbial respiration rates were greater in Michigan agricultural streams than in Indiana (nested ANOVA: $F_{1,12} = 36.59$, $P = 0.03$), and the study streams in Michigan tended to have higher nitrate (nested ANOVA: $F_{1,12} = 38.21$, $P = 0.05$) and ammonium (nested ANOVA $F_{1,9} = 21.61$, $P = 0.001$) concentrations than our study streams in Indiana during the incubation period.

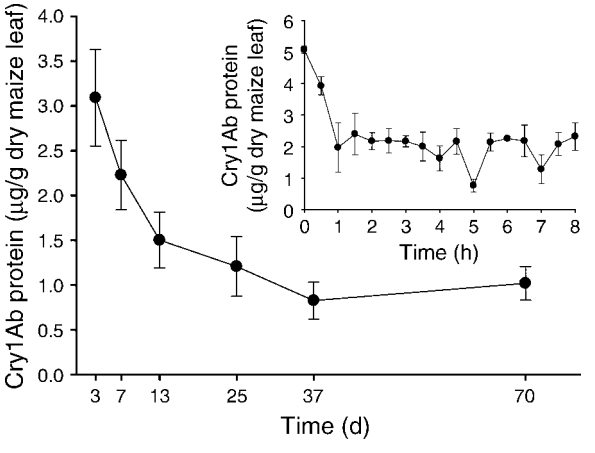

FIG. 3. Experiments quantifying short-term (inset) and long-term leaching of Bt-derived Cry1Ab protein from dried maize leaves in agricultural streams. Cry1Ab protein concentrations were measured in Bt maize leaves placed in one agricultural stream over an 8-h period in the short-term leaching experiment ($n = 5$ replicate litterbags) and in Bt maize leaves placed in three agricultural streams over 70 days in the long-term leaching experiment ($n = 3$ replicate litterbags per stream). Data are means ± SE from replicate litterbags (short-term) or replicate streams (long-term).

PLATE 1. A headwater stream draining maize fields in northwestern Indiana, USA. Large accumulations of maize detritus (lighter-colored material within the riparian grass) from the previous season are visible within the active channel of the stream. This material may have entered laterally from the landscape or have been deposited as flood waters receded. In either case, the maize detritus is subject to movement to the stream via wind and gravity or entrainment by the stream during moderate to high flows. Photo credit: N. Griffiths.

DISCUSSION

Row-crop agriculture has profoundly influenced the landscape of the Midwestern United States. The major effects of agriculture, such as soil erosion and nutrient enrichment of streams, are well known, whereas much less is understood about the effects of agricultural practices on organic-matter dynamics in streams. The basal resources of Midwestern agricultural streams tend to be dominated by autochthonous production throughout most of the year, with limited allochthonous inputs (Wiley et al. 1990). However, large masses of maize detritus can enter streams during floods and with high winds (N. Griffiths, *personal observation*) and can accumulate in substantial debris dams (see Plate 1) that may play a comparable role to leaf litter accumulations in forested streams. Crop detritus can therefore constitute an important but temporally variable substrate for microbial decomposers and invertebrate consumers, and its potential role is not well understood (Rosi-Marshall et al. 2007).

Microbial respiration and decomposition of maize detritus

Microbial-respiration rates on decomposing maize leaves in agricultural streams were 3–4 times higher than rates measured on a variety of deciduous leaves in temperate forested streams (e.g., Tank et al. 1993, Gulis and Suberkropp 2003, Stelzer et al. 2003), and were comparable to respiration rates on substrates in warmer, tropical streams (e.g., Ramirez et al. 2003, Abelho et al. 2005). Whereas high respiration rates in tropical streams are attributed to consistently warm stream water (Abelho et al. 2005), we suggest that the high respiration rates measured in this study were driven by a combination of favorable substrate quality/lability and the enriched nutrient status of the streams (Fig. 2b, c, Table 2).

The lability of maize leaves is reflected in their low C:N ratio (24.0–38.8) and low lignin content (4.5%–6.3%). An analysis of 48 deciduous species commonly found in temperate riparian zones showed C:N ratios varying from 16.8 (*Robina pseudoacacia*) to 120.8 (*Liquidambar styraciflua*), with deciduous leaves having on average a C:N ratio of 46.6 (Ostrofsky 1997). Heterotrophic microorganisms involved in leaf decomposition typically have lower C:N ratios than their food sources, and thus have a greater demand for N relative to C (Sterner and Elser 2002). Because the C:N ratio of maize leaves is low, especially when compared to other allochthonous sources, maize leaves appeared to be an excellent carbon substrate for microbial decomposers.

In addition to C and N content, lignin is a major predictor of microbial respiration and decomposition rates (Melillo et al. 1984, Gessner and Chauvet 1994, Royer and Minshall 2001). Lignin is a refractory compound that is resistant to microbial decay and typically ranges from 13% to 39% in deciduous leaves (Ostrofsky 1997). Specifically, in the context of our study, maize leaves had a much lower lignin content (4.5%–6.3%) than did red maple leaves (20%) (Ostrofsky 1997), which, along with lower C:N ratios, could partially explain the higher respiration rates that we observed on maize.

Microbial-respiration rates on maize leaves were also likely enhanced by the high dissolved inorganic-nutrient

concentrations in agricultural streams. Other studies have shown that the addition of nitrogen or both nitrogen and phosphorus increased microbial-respiration rates on allochthonous organic matter (Tank and Webster 1998, Gulis and Suberkropp 2003, Stelzer et al. 2003, Gulis et al. 2004). Similarly, Ramirez et al. (2003) found that microbial respiration on conditioned *Ficus* leaves increased along a natural phosphorus gradient in eight low-order streams in Costa Rica. In our study, microbial respiration was higher on both maize and red maple leaves in agricultural streams, supporting the hypothesis that higher nutrient concentrations increase microbial respiration; however, large temporal variation in inorganic-nitrogen and inorganic-phosphorus concentrations in our study streams may have resulted in the nonsignificant relationships between nutrient concentrations and respiration rates.

We predicted that the high microbial-respiration rates found on maize leaves would be reflected in fast decomposition rates. Despite high microbial respiration and a more labile carbon substrate, decomposition rates of maize ($-k_{Bt} = 0.021$ d^{-1} and $-k_{nBt} = 0.015$ d^{-1}) fell within the range of decomposition rates for deciduous leaves in temperate forested streams (*Rhododendron maximum*: $-k = 0.0016$ d^{-1} and *Cornus florida* $-k = 0.0316$ d^{-1}; Webster et al. 1999). We attribute the moderate decomposition rates of maize to a lack of shredding invertebrates in agricultural streams. Densities of shredding macroinvertebrates were low in the agricultural streams we examined (C. Chambers, *unpublished data*); a trend that has been documented in other agricultural streams (Huryn et al. 2002, Stone et al. 2005, Hagen et al. 2006). We cannot quantify the degree to which consumers in headwater agricultural streams are dependent on the input of crop detritus, but our results demonstrate that this material is rapidly decomposed by microorganisms when it enters streams. The input of maize detritus is highly stochastic (Rosi-Marshall et al. 2007), unlike the seasonally predictable input of deciduous leaf litter in forested streams. Because of the stochastic nature of the inputs and the rapid use of maize by microorganisms, aquatic invertebrates may not be able to rely on maize detritus as a primary food resource.

The effects of Bt on microbial decomposition

Genetic variation within tree species has been shown to affect ecosystem processes in terrestrial (e.g., Madritch and Hunter 2002, Schweitzer et al. 2004, Madritch et al. 2006) and aquatic (LeRoy et al. 2007) systems. We found that Bt maize decomposed at a faster rate than non-Bt maize ($k_{Bt} = 0.021$ d^{-1} and $-k_{nBt} = 0.015$ d^{-1}); however, we suggest that the difference in these rates is small and likely not ecologically significant, especially given that decomposition rates measured for single leaf species in different streams can be quite variable (e.g., Webster et al. 1999, Simon and Benfield 2001). Further, our ability to detect significant, albeit small, differences in decomposition rates between Bt and non-Bt maize is in part due to sufficient replication and resulting low variation in AFDM replicates at both the litterbag level within streams as well as among streams. We found that microbial respiration did not differ between Bt and non-Bt maize, suggesting that the Bt-derived Cry1Ab toxin does not adversely affect the aquatic microbial assemblage (e.g., fungi and bacteria) involved in maize-leaf decomposition. This result is similar to terrestrial studies that found no effects of Cry1Ab from Bt maize on soil microbial growth (Saxena and Stotzky 2001) or community structure (Griffiths et al. 2005). The environmental conditions that aquatic microorganisms experience may also influence whether they are affected by the Cry1Ab toxin. When microorganisms have a high demand for carbon, as may be the case under high-nutrient conditions, they may be less sensitive to molecular differences between maize hybrids, but we know of no previous studies that have tested this. A mode of action of Cry1Ab on microorganisms has not been proposed, and in fact the Cry1Ab protein may serve as a source of organic nitrogen or carbon for microbial assimilation. Saxena and Stotzky (2001) found that unbound Cry1Ab was readily utilized as an organic carbon and/or nitrogen source by microbial cultures in the laboratory. However, in our study we controlled primarily for percentage lignin, and secondarily for carbon and nitrogen content of Bt and non-Bt maize leaves, suggesting that the presence of Bt protein per se did not affect respiration or decomposition rates.

Bt maize targets the European corn borer, a member of the order Lepidoptera, and so most studies investigating the nontarget effects of Bt maize have focused on terrestrial lepidopterans, such as monarch butterflies (Losey et al. 1999, Stanley-Horn et al. 2001) and swallowtails (Wraight et al. 2000). Although aquatic lepidopterans are rare, the closely related trichopterans (caddisflies) are common in streams, and laboratory studies suggest they may be susceptible to the Cry1Ab toxin (Rosi-Marshall et al. 2007). Our data demonstrate that after 70 days the Cry1Ab toxin is still present in maize detritus decomposing in streams; therefore the effects of this toxin on invertebrates may be long lived. Results from our decomposition studies suggest that maize detritus enters the aquatic food web mainly via microbial assimilation and that microorganisms do not discriminate between conventional and genetically engineered maize. It may be that the microorganisms serve as a filter between the Cry1Ab toxin and higher trophic levels, but this possibility remains to be investigated. Additionally, agricultural streams in the Midwestern United States are generally degraded due to nutrient enrichment, sedimentation, channelization, and riparian alteration, and thus the addition of genetically engineered maize detritus to these systems may have fewer impacts to ecosystem function than would be expected in less disturbed systems.

Broader ecological implications

The conversion of native vegetation to row-crop agriculture appears to alter the quantity, quality, and predictability of allochthonous carbon inputs to headwater streams. In temperate forested streams, inputs of allochthonous organic matter are diverse and predictable, and thus constitute an important and dependable basal food resource in these systems. In contrast, agricultural streams receive maize detritus in unpredictable pulses primarily following storms, and the interacting effects of maize being a highly labile carbon source combined with the elevated nutrient status of agricultural streams result in the rapid incorporation of maize into the microbial food web. Genetic engineering may also alter the quality of maize detritus that enters streams, but in this study, we found that genetic-scale differences between maize hybrids did not negatively affect microbial respiration and decomposition rates in agricultural streams. In less-disturbed streams, organisms may distinguish between genetic-level differences that could potentially result in alterations to ecosystem-level processes (e.g., decomposition) (LeRoy et al. 2007).

Future introductions of genetically engineered organisms may not be limited to traditional agricultural systems. For example, geneticists have developed transgenic aspen trees with reduced lignin content (Hu et al. 1999) and efforts are underway to engineer pest- and fungal-resistant trees (Herrera 2005). Genetically engineered trees have also been approved under the Kyoto Protocol as a viable method for sequestering carbon dioxide from the atmosphere (Herrera 2005). With the inevitable movement and transfer of genetically engineered organisms across the ever-increasing human-dominated landscape, it is critical to understand detrital processing of these introductions, thereby quantifying the consequences not only to nontarget organisms, but also to ecosystem processes such as carbon cycling in both terrestrial and aquatic systems.

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