Applied Soil Ecology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

Impact of coleopteran targeting toxin (Cry3Bb1) of *Bt* corn on microbially mediated decomposition

C. Nicole Lawhorn [a], Deborah A. Neher [a,*], Galen P. Dively [b]

[a] Department of Earth, Ecological and Environmental Science, University of Toledo, 2801W. Bancroft St., Toledo, OH 43606, United States
[b] Department of Entomology, University of Maryland, 3112 Plant Sciences Bldg., College Park, MD 20742, United States

ARTICLE INFO

Article history:
Received 16 October 2008
Received in revised form 5 December 2008
Accepted 9 December 2008

Keywords:
Corn rootworm
Diabrotica
MON863
YieldGard

ABSTRACT

Genetically engineered corn expressing crystalline proteins for insect control and encoded by genes derived from soil bacterium *Bacillus thuringiensis* (*Bt*) are widely adopted in the United States. Among the seven different events of *Bt* corn available commercially, YieldGard® Rootworm (MON863) expresses a variant of the *cry*3Bb1 protein in the root tissue to control corn rootworm larvae. Although numerous laboratory and field studies show no unexpected ecological risks at the insect community-level above-ground, few studies have addressed the possible impact of *cry* proteins released from living or decaying roots of *Bt* corn on soil microbial communities. Here, we test the hypothesis that coleopteran-active *Bt* corn does not affect nontarget ecological processes, such as decomposition or the function of the associated saprophytic microbial community. Experimental treatments were: (1) a *Bt* hybrid; (2) a non-*Bt*, isogenic hybrid treated with a conventional soil insecticide; and (3) a non-*Bt*, isogenic hybrid without insecticide. Soil and root samples were collected at various times throughout 2 years from experimental plots to estimate microbial community function by quantifying activity of extracellular enzymes on 10 substrates. Decomposition was measured as mass loss by root decay in litter bags. *Bt* corn (MON863) exuding the *cry*3Bb1 toxin does not appear to have adverse effects on saprophytic microbial communities of soil and decaying roots or on decomposition. The addition of the soil insecticide had greater effects on microbial function in soil and decaying roots than *Bt* corn. Our results are similar to those found previously for the *cry*3Bb1 protein that showed no adverse effects on microbial community composition in controlled and natural environments. This field study is one of the first to report the use of extracellular enzyme assays to examine the effect of transgenic crops on the functional activity of microbes in soil and decaying roots.

1. Introduction

In 2007, 49% of the U.S. corn hectareage was planted to genetically engineered hybrids expressing single or stacked lepidopteran- and coleopteran-active proteins encoded by genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) (USDA-ERS, 2007). *Bt* corn is expected to pose little environmental impact due to the highly selective nature of the expressed proteins. Numerous laboratory tests have indicated no acute adverse effects on many nontarget organisms and results of 47 field studies have shown no unexpected ecological risks to above-ground insect communities (in reviews by O'Callaghan et al., 2005; Romeis et al., 2006; Marvier et al., 2007; Wolfenbarger et al., 2008). Despite the wide adoption of *Bt* corn and current knowledge about its nontarget effects, only a few studies have assessed the possible impact of the insecticidal proteins on soil microbial communities. These studies showed no or less impact of the lepidopteran-active *cry*1Ab protein on microbial community structure than other environmental factors, such as plant age or heterogeneity of field properties (Blackwood and Buyer, 2004; Devare et al., 2004; Baumgarte and Tebbe, 2005; Fang et al., 2005). However, the nontarget effects of *Bt* corn hybrids expressing the coleopteran-active proteins on soil microbes have not been evaluated.

The first coleopteran-active *Bt* corn was registered as YieldGard® Rootworm (event MON863) by the Monsanto Company, and expresses a variant of *cry*3Bb1 protein in the root tissue to control corn rootworm larvae. There are several possible mechanisms by which rootworm-active *Bt* corn could affect soil microorganisms. First, *Bt* corn could alter soil microbial communities and the resultant decomposition rate of plant residue through increased lignin concentration in corn tissue (Saxena and Stotzky, 2001). The nontarget effects of lignin content of *Bt* corn hybrids expressing

* *Corresponding author.* Current address: Department of Plant and Soil Science, University of Vermont, 105 Carrigan Dr., Burlington, VT 05405, United States. Tel.: +1 802 656 0474; fax: +1 802 656 4656.
E-mail address: deborah.neher@uvm.edu (D.A. Neher).

0929-1393/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.apsoil.2008.12.003

*cry*3Bb1 protein have not been evaluated (Flores et al., 2005). Furthermore, although several *in situ* experiments have been performed on the persistence of *cry* proteins in soil, relatively little is known on their effects on microbe-mediated processes, such as decomposition (O'Callaghan et al., 2005; Lehman et al., 2008). The possibility also exists that *Bt* crops may cause changes in microbial populations which could lead to alterations in nutrient cycling in soil (Motavalli et al., 2004). Secondly, soil microorganisms can come in direct contact with *Bt* proteins released into soil, either by active secretion or passive leakage from living roots (Schmalenberger and Tebbe, 2002; Chevallier et al., 2003; Lynch et al., 2004).

The objective of this study was to determine if coleopteran-active *Bt* corn (MON863) affects nontarget saprophytic microbial communities by examining the responses of extracellular enzyme activity in soil and root samples throughout the growing season. This approach tests whether or not the expressed *cry*3Bb1 protein changes microbial processes associated with substrate utilization. Chemistry of decaying roots (lignin and cellulose levels) was performed to investigate whether nutrients utilized by microbes were affected, which would reveal possible disturbances between above- and below-ground processes.

2. Materials and methods

2.1. Site description

The experimental site was a 9.7-ha field consisting of six contour strips, each approximately 1.6 ha, located at the University of Maryland Research and Education Center, Beltsville, MD. Soil types consisted of Sunnyside fine sandy loam and Galestown-Evesvoro loamy sand, with mean (±1 SD) pH and organic matter of 6.0 (±0.2) and 1.2% (±0.2), respectively. Previous cropping practices consisted of alternating strips of non-transgenic corn and soybean under no-tillage cultivation.

2.2. Treatments

In 2003, three treatments arranged in a Latin square design were established in 0.4-ha plots within the first, third, and fifth contour strips of the field. The treatments were: (1) *Bt* corn (event MON863 YieldGard® Rootworm) expressing the *cry*3Bb1 protein; (2) non-*Bt* near-isogenic hybrid treated with a soil insecticide tefluthrin (Force®), as a positive control; and (3) the untreated, non-*Bt* near-isogenic hybrid as a negative control. The remaining adjacent strips (second, fourth, and sixth) were planted with soybean. The treatment plots within each strip were planted side-by-side without buffers. In 2004 and 2005, three replicates of the same treatments were planted in alternate strips of surface residue remaining from the previous year's soybean crop. During each year, plots were planted no-till during early May and managed according to recommended fertility and herbicide regimes.

2.3. Sample collection

Corn roots were collected at anthesis in each treatment plot and washed free of soil during August 2003 and July 2004. During each year, 42 Saran mesh litter bags (26 cm × 14 cm; 1 mm by 1.5 mm mesh size) containing 100 g of intact root material were buried in the soil (15-cm depth) within the central area of each plot. An additional 20 samples of *Bt* and non-*Bt* root material were weighed and then dried to estimate a wet to dry weight conversion factor. Subsets of eight litter bags were removed from each plot after successive times of incubation (year 1: Sept 03, Oct 03, Nov 03, Apr 04, May 04; year 2: Sep 04, Oct 04, Nov 04, Apr 05, May 05). Initial and remaining dry weight masses of root material at each sampling date were calculated using the wet to dry weight conversion factor.

Smaller samples (5–8 g) of wet root tissue from four of the eight litter bags in each plot were collected at each sampling date and frozen at −80 °C for enzyme and chemical assays. Composite samples of soil cores (2-cm in diameter, 10-cm deep) were also collected within the central area of each plot before planting (April), during late Aug, and at harvest (Oct). Samples were shipped in insulated bags to preserve soil community characteristics at the time of sampling. Upon return to the laboratory, subsamples (~2 g) from each bag were immediately frozen at −80 °C for enzyme assays.

2.4. Extracellular enzyme assays

Activity of 10 hydrolytic enzymes (Table 1) produced by bacteria involved in the hydrolysis of carbon, nitrogen, and phosphorous from detrital organic matter was quantified. Aminopeptidase assays were chosen to target amino acids that are abundant in the *cry*3Bb1 gene inserted in *Bt* corn, and/or those changed from non-*Bt* corn (Ditto, 2002). The protocol of Saiya-Cork et al. (2002) was followed. In brief, 100 ml of 50 mM (pH 5.0) acetate buffer was added to 1.0 g of soil or 0.5 g of roots to and homogenized. Eight replication wells of 200 μl aliquots per sample were dispensed into 96-well microplates. A 50 μl portion of substrate solution containing fluorogenically labeled substrates was added to each well. Microplates were incubated in a dark incubator at 20 °C for 18 h. Fluorescence was quantified using a microplate fluorometer (FLx800, Bio-Tek Instruments, Inc., Winooski, VT, USA) with 360 nm excitation and 460 nm emission filters. Corrections were made for negative controls and quenching. Activity is expressed in units of nmol h^{-1} g^{-1}.

2.5. Root chemistry

Contents of cellulose and lignin were determined in subsamples of root tissue from May and September 2004. No samples from 2003 to 2004 growing season were analyzed due to sample loss. Samples of root tissue (>2 g) were dried for 24 h at 90 °C, then homogenized and chopped in a blender (Waring Commercial

Table 1
Hydrolytic extracellular enzymes assayed in soil and decaying root samples.

Enzyme class	Enzyme	Major substrate	Product released from polymer	Elements released
Glycosidases	(-1,4-cellobiohydrolase	Cellulose	Cellobiose	C
	(-1,4-glucosidase	Starch	Glucose	C
	(-1,4-glucosidase	Cellulose, cellobiose	Glucose	C
	(-1,4-N-acetylglucosaminidase (NAGase)	Chitin, chitobiose	N-acetylglucosamine (NAG)	C, N
	(-1,4-xylosidase	Xylan, hemicellulose	Xylose	C
Esterases	Phosphatase	Nucleic acid, phospholipid	Phosphate	P
Aminopeptidases	L-alanine aminopeptidase	Protein	Alanine	C, N
	L-glycine aminopeptidase	Protein	Glycine	C, N
	L-leucine aminopeptidase	Protein	Leucine	C, N
	L-proline aminopeptidase	Protein	Proline	C, N

Please cite this article in press as: Lawhorn, C.N., et al., Impact of coleopteran targeting toxin (Cry3Bb1) of *Bt* corn on microbially mediated decomposition. Appl. Soil Ecol. (2009), doi:10.1016/j.apsoil.2008.12.003

Table 2
Effect of treatment on 10 enzyme (effect of month and treatment) substrate activities (nmol h^{-1} g^{-1}) in soil collected in April, August and October of 2003. Means ± 1 SE (n = 18, 3 times × 3 plots × 2 samples per plot). p-values are in the right three columns for single degree of freedom contrasts for *Bt* corn and non-*Bt* corn with insecticide (PC = positive control comparison), *Bt* corn and non-*Bt* corn without insecticide (NC = negative control comparison), and non-*Bt* corn with insecticide and non-*Bt* corn without insecticide (I = insecticide effect).

Enzyme	Mean nmol h^{-1} g^{-1} (±1 SE)			p-values		
	Bt	non-*Bt* − I	non-*Bt* + I	PC	NC	I
(-1,4-cellobiohydrolase	82.5 (46.33)	47.8 (16.05)	62.7 (21.13)	0.423	0.721	0.656
(-1,4-glucosidase	5.0 (3.03)	2.7 (0.70)	3.1 (0.39)	0.495	0.455	0.156
(-1,4-glucosidase	91.3 (16.47)	87.4 (16.59)	101.3 (16.72)	0.426	0.850	0.326
(-1,4-N-acetylglucosaminidase	87.8 (17.25)	83.4 (16.29)	86.0 (13.15)	0.564	0.811	0.415
(-1,4-xylosidase	31.7 (12.34)	23.1 (4.79)	27.6 (4.88)	0.265	0.831	0.365
Phosphatase	50.9 (14.64)	40.4 (8.58)	52.2 (9.37)	0.449	0.935	0.402
L-alanine aminopeptidase	24.4 (7.68)	21.0 (6.39)	26.5 (8.43)	0.175	0.845	0.122
L-glycine aminopeptidase	13.0 (4.80)	10.4 (3.87)	1.3 (3.89)	0.858	0.549	0.437
L-leucine aminopeptidase	11.4 (3.35)	10.9 (3.45)	58.0 (46.25)	0.460	0.656	0.239
L-proline aminopeptidase	4.5 (1.65)	4.9 (1.99)	7.4 (2.70)	0.877	0.464	0.376

Blender Model: 31BL92, New Hartford, CT), and 0.5 g of sample was placed into a pre-weighed 15 ml glass test tube. Distilled water (6 ml) was added to each test tube; tubes were placed in a sonicating water bath at 60 °C for 30 min, and then centrifuged at 820 × g for 15 min in a swinging bucket centrifuge (International Model HN, Needham HTS, MA, USA). The supernatant was removed by suctioning, and the process was repeated four more times with water and, then, five times with 95% ethanol. After the final ethanol rinse, samples were dried overnight (24 h) at 50 °C and placed in a desiccator to avoid absorption of atmospheric moisture. Cellulose and lignin content were quantified according to Moorhead and Reynolds (1993), with slight modifications. Briefly, 2 ml of concentrated (72%) sulfuric acid was added to tubes containing the ethanol-extracted samples and incubated for 1 h at 30 °C. Material was transferred in 56 ml of distilled water to a 125 ml flask and autoclaved for 1 h at 120 °C. Autoclaved samples were suctioned onto a pre-weighed Millipore filter (Whatman 542 fine-grade 5.5 cm hardened ashless filter papers), placed into a pre-weighed aluminum boat, and oven-dried at 90 °C for 24 h. Cellulose content was estimated as the difference in dry weights between the pre- and post-acid digested material. Lignin was estimated as the difference between the pre- and post-oven-dried (500 °C for 24 h) weights.

2.6. Statistical analysis

A mixed model analysis of variance (SAS/STAT Release 8.00, SAS Institute Inc., Cary, NC, USA) was performed separately on decaying roots and soil data to test for treatment effects on different enzyme activities and mass loss of roots by decomposition through time. Enzyme activity was transformed as $\log_e(x + 0.1)$, and the proportion of mass loss was transformed as arcsine of the square root. The row and column plot positions of the Latin square design were modeled as random factors. The repeated measures option was used to adjust for autocorrelation effects among sampling dates. Orthogonal contrasts were used to compare the three combinations of the treatments. One-way analysis of variance was performed on lignin and cellulose content to test for treatment effects.

3. Results

Soil collected from plots of *Bt* corn or non-*Bt* isoline corn, whether treated with or without insecticide, showed no significant differences in substrate utilization for any of the 10 substrates (Table 2). In contrast, activity levels of three of 10 substrates varied in buried intact roots (Table 3). Differences in enzyme activity were apparent between corn hybrids and use of insecticide. Potential activity of alanine was greater in *Bt* than non-*Bt* treatments, whereas activity of leucine and glycine were greater in non-*Bt* than *Bt* treatments. Potential activity of leucine and glycine were both greater when non-*Bt* corn was treated with insecticide.

Percent mass remaining of buried roots was affected by management ($p < 0.001$), genetics ($p < 0.001$), and insecticide ($p = 0.002$) in 2003–2004 season (Fig. 1). Decomposition was faster and progressively greater in non-*Bt* corn without insecticide, non-*Bt* corn with insecticide, and *Bt* corn (Fig. 1). However, no treatment effects ($p = 0.774$) were observed in 2004–2005 growing season. Lignin ($p = 0.614$) and cellulose content ($p = 0.362$) were similar among *Bt* corn, non-*Bt* isoline treated with insecticide, and

Table 3
Effect of treatment on 10 enzyme (effect of month and treatment) substrate activities (nmol h^{-1} g^{-1}) in decaying roots collected in two seasons (1: Sept 03, Nov 03, Apr 04, May 04; 2: Jul 04, May 05). Means ± 1 SE (N = 36, 6 times × 3 plots × 2 subsamples per plot) with statistical significance are bolded. p-values are in the right three columns for single degree of freedom contrasts for *Bt* corn and non-*Bt* corn with insecticide (PC = positive control comparison), *Bt* corn and non-*Bt* corn without insecticide (NC = negative control comparison), and non-*Bt* corn with insecticide and non-*Bt* corn without insecticide (I = insecticide effect).

Product	Year	Mean nmol h^{-1} g^{-1} (±1 SE)			p-values		
		Bt	non-*Bt* − I	non-*Bt* + I	PC	NC	I
Phosphate	***[a]	102.0 (17.65)	83.4 (17.08)	107.2 (20.90)	0.705	0.128	0.247
β-glucoside	n.s.	327.9 (52.24)	293.3 (40.94)	272.6 (32.70)	0.702	0.378	0.616
NAG	*	218.6 (37.86)	196.2 (34.63)	198.6 (36.50)	0.772	0.683	0.901
β-cellobiose	*	189.1 (28.74)	178.9 (27.38)	167.8 (25.70)	0.630	0.487	0.823
β-xylose	n.s.	121.2 (35.21)	107.0 (24.99)	76.7 (16.12)	0.628	0.842	0.501
α-glucoside	***	9.0 (1.72)	9.7 (2.15)	11.9 (2.76)	0.487	0.294	0.710
Alanine	***	71.2 (27.22)	74.2 (23.21)	85.7 (43.59)	0.566	0.081	0.242
Leucine	n.s.	89.0 (25.55)	68.7 (16.86)	98.7 (35.18)	0.764	0.017	0.007
Proline	***	120.2 (57.25)	84.9 (23.25)	94.2 (34.31)	0.477	0.762	0.689
Glycine	***	77.4 (34.97)	44.5 (16.43)	60.4 (16.52)	0.588	0.078	0.023

[a] n.s.: $p < 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Please cite this article in press as: Lawhorn, C.N., et al., Impact of coleopteran targeting toxin (Cry3Bb1) of *Bt* corn on microbially mediated decomposition. Appl. Soil Ecol. (2009), doi:10.1016/j.apsoil.2008.12.003

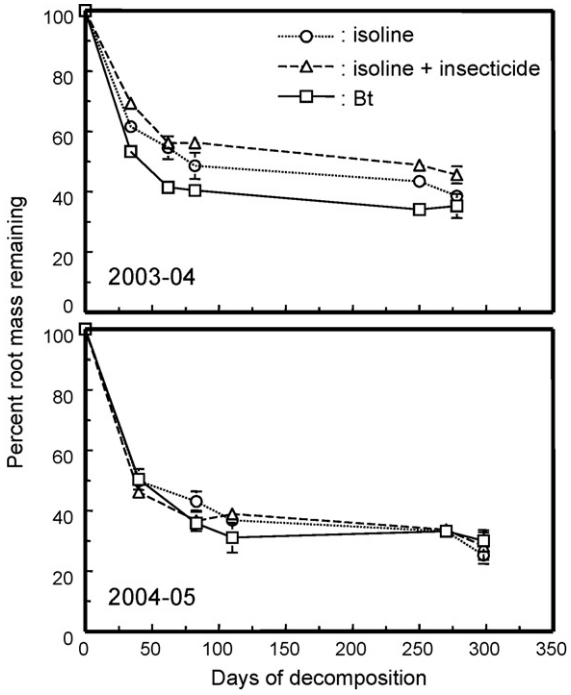

Fig. 1. Percent root mass remaining in litter bags collected from plots of the *Bt* hybrid, non-*Bt* hybrid treated with insecticide, and non-*Bt* hybrid without insecticide over 2003–2004 and 2004–2005 growing seasons. Standard error bars are illustrated; they are smaller than the symbols if they are not visible.

untreated, non-*Bt* isoline treatments whether measured in early or late season.

4. Discussion

The *Bt* hybrid had no significant effect on extracellular enzyme activity, suggesting that saprophytic microbial activities were affected minimally, if at all, by possible exposure to the *cry*3Bb1 protein in the soil. These results agree with the study by Devare et al. (2004) that reported no negative effects of the *cry*3Bb1 protein on microbial biomass, activity, or bacterial community structure using terminal restriction fragment length polymorphism (T-RFLP) analysis. However, this study used extracellular enzyme assays to focus on the possible changes in functional activity of soil microbes.

The results also were similar to those reported for *cry*1Ab protein, which showed no adverse effects on microbial community composition in controlled environments (Blackwood and Buyer, 2004; Griffiths et al., 2006; Icoz and Stotzky, 2008). Although there were no main *Bt* effects of the toxin detected with phospholipids fatty acid methods, a significant interaction of the toxin with soil texture was noted when employing community-level physiological profiles (Blackwood and Buyer, 2004) or 16S rRNAs (Fang et al., 2005). Furthermore, toxins from *B. thuringiensis* did not affect the growth of bacteria or algae in pure and mixed cultures (Koskella and Stotzky, 2002).

The addition of the conventional insecticide had greater effects on microbial function in soil and decaying roots than *Bt* corn. Tefluthrin decreased decomposition (Fig. 1) and increased presence of aminopeptidases, leucine, and glycine. These results probably reflect a change in the demand in nutrients by microbes, resulting from stress imposed by the insecticide, which was not present in the other treatments. Tefluthrin is a synthetic pyrethroid related to naturally occurring pyrethrum, a botanical insecticide. It does not contain leucine or glycine (Syngenta Corporation (www.syngentacropprotection-us.com), so the insecticide alone does not explain the elevated levels of leucine and glycine products. In Devare et al. study (2004), soil respiration was reduced in soils treated with tefluthrin, but this treatment had no effect on nitrogen mineralization potential or short-term nitrification rates.

In decaying root samples, no significant differences were found in the activity of the majority of the enzymes, with the exception that the production of alanine was greater, and leucine and glycine less in *Bt* hybrids. Similar to other studies, no effect of the *Bt* hybrid on phosphatase activity was observed (Stotzky, 2004; Wu et al., 2004). Increased enzyme activity suggests increased microbial activity (Chang and Yoo, 1986), which suggests that the microbial population responsible for decomposition might have changed. For example, Mulder et al. (2006) observed altered utilization of D-glucosaminic acid and L-arginine in *cry*1Ab hybrids grown in mesocosm experiments. Alternatively, physiological demand for nitrogen may have increased in the microbial community.Rate of decay of *Bt* corn was faster than the decay rate of roots from the non-*Bt* treatments during 2003–2004 growing season, but this trend was not repeated in the second growing season. Rain events were more common in the first growing season, which might explain the faster rates of decay. These results support those of Griffiths et al. (2005), who also showed faster decomposition of *Bt* than near-isoline corn, and of Cortet et al. (2006), who suggested that climatic conditions and soil properties may affect decomposition more than corn hybrid. No statistically significant differences in rates of decomposition were observed in *Bt*-cotton (Lachnicht et al., 2004) or *cry*1Ab *Bt* corn (Hopkins and Gregorich, 2003; Lehman et al., 2008). Decomposition was also slowest in the non-*Bt* hybrid with insecticide during the first growing season, which agrees with a reduced soil respiration rate observed with a field experiment with tefluthrin insecticide (Devare et al., 2004).

Quantity of lignin or cellulose in decaying roots was similar among treatments. If the content of lignin was greater in *cry*3Bb1 hybrids, these hybrids would tend to have a greater percent root mass remaining through time than the other samples. These results are contrary to studies reporting greater lignin content in above-ground parts of *cry*1Ab *Bt* corn hybrids compared to non-*Bt* hybrids (Saxena and Stotzky, 2001; Stotzky, 2004). Others have demonstrated that lignin content depends on analytical methodology (Jung and Sheaffer, 2004) and transformation event (Mungai et al., 2005). Greater lignin content in the *cry* protein may partly explain the reduced decomposition observed in the *Bt* hybrid than its near-isoline. Another difference is that this study performed assays on decaying intact roots, whereas previous decomposition studies used whole or fragmented leaves (Cortet et al., 2006; Mulder et al., 2006) or measured soil respiration as a surrogate of decomposition (Saxena and Stotzky, 2001; Devare et al., 2004; Stotzky, 2004; Castaldini et al., 2005; Flores et al., 2005). Roots would more likely contain higher concentrations of *cry*3Bb1 protein than fragmented leaf parts.In conclusion, *Bt* corn (event MON863) producing the *cry*3Bb1 toxin did not have significant effects on the activities of microbial communities in soil or root tissue and their role in decomposition. These findings are consistent with previous laboratory and field studies of the effects of *cry*3Bb1 protein of microbial communities. However, the lack of significant effects on soil microorganisms may be explained, at least partially, by the tremendous genetic diversity of soil microorganisms (Torsvik et al., 1990). Nonetheless, further examinations of potential nontarget effects are merited on other events of coleopteran-active *Bt* corn before it can be concluded that there are no negative ecological impacts on soil communities or processes.

Acknowledgments

The work was supported by USDA-CSREES - Biotechnology Risk Assessment Research Grants Program 2002-39454-12773 and

Please cite this article in press as: Lawhorn, C.N., et al., Impact of coleopteran targeting toxin (Cry3Bb1) of *Bt* corn on microbially mediated decomposition. Appl. Soil Ecol. (2009), doi:10.1016/j.apsoil.2008.12.003

2003-33120-13957. We thank T.R. Weicht, A. Miller, and B.J. Darby for technical assistance.

References

Baumgarte, S., Tebbe, C.C., 2005. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810), and its effect on bacterial communities in the maize rhizosphere. Mol. Ecol. 14, 2539–2551.

Blackwood, C.B., Buyer, J.S., 2004. Soil microbial communities associated with Bt and non-Bt corn in three soils. J. Environ. Qual. 33, 832–836.

Castaldini, M., Turrini, A., Sbrana, C., Benedetti, A., Marchionni, M., Mocali, S., Fabiani, A., Landi, S., Santomassimo, F., Pietrangeli, B., Nuti, M.P., Miclaus, N., Giovannetti, M., 2005. Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Appl. Environ. Microbiol. 71, 6719–6729.

Chang, N.K., Yoo, J.H., 1986. Annual fluctuations and vertical distributions of cellulase, xylanase activity and soil microorganisms in a humus horizon of a *Pinus rigida* stand. Korean J. Ecol. 9, 141–231.

Chevallier, T., Muchaonyerwa, P., Chenu, C., 2003. Microbial utilization of two proteins adsorbed to a vertical clay fraction: toxin from *Bacillus thuringiensis* subsp. *tenebrionis* and bovine serum albumin. Soil Biol. Biochem. 35, 1211–1218.

Cortet, J., Andersen, M.N., Caul, S., Griffiths, B., Joffre, R., Lacroix, B., Sausse, C., Thompson, J., Krogh, P.H., 2006. Decomposition processes under Bt (*Bacillus thuringiensis*) maize: results of a multi-site experiment. Soil Biol. Biochem. 38, 195–199.

Devare, M.H., Jones, C.M., Thies, J.E., 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity, and diversity. J. Environ. Qual. 33, 837–843.

Ditto, M.D., 2002. Biotechnology Consultation Note to the File BNF No. 000075: Monsanto's Corn Rootworm Protected Corn, MON863 transformation event. U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition. [Online.] http://www.agbios.com/docroot/decdocs/bnfM075.pdf.

Fang, M., Kremer, R.J., Motavalli, P.P., Davis, G., 2005. Bacterial diversity in rhizospheres of nontransgenic and transgenic corn. Appl. Environ. Microbiol. 71, 4132–4136.

Flores, S., Saxena, D., Stotzky, G., 2005. Transgenic *Bt* plants decompose less in soil than non-*Bt* plants. Soil Biol. Biochem. 37, 1073–1082.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messean, A., Sausse, C., Lacroix, B., Krogh, P.H., 2005. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. Plant Soil 275, 135–146.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Cortet, J., Foggo, A., Hackett, C.A., Krogh, P.H., 2006. Soil microbial and faunal community responses to *Bt* maize and insecticide in two soils. J. Environ. Qual. 35, 734–741.

Hopkins, D.W., Gregorich, E.G., 2003. Detection and decay of the *Bt* endotoxin in soil from a field trial with genetically modified maize. Eur. J. Soil Sci. 54, 793–800.

Icoz, I., Stotzky, G., 2008. Fate and effects of insect-resistant *Bt* crops in soil ecosystems. Soil Biol. Biochem. 40, 559–586.

Jung, H.G., Sheaffer, C.C., 2004. Influence of *Bt* transgenes on cell wall lignification and digestibility of maize stover for silage. Crop Sci. 44, 1781–1789.

Koskella, J., Stotzky, G., 2002. Larvicidal toxins from *Bacillus thuringiensis* subspp. *kurstaki*, *morrisoni* (strain *tenebrionis*), and *israelensis* have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae *in vitro*. Can. J. Microbiol. 48, 262–267.

Lachnicht, S.L., Hendrix, P.F., Potter, R.L., Coleman, D.C., Crossley, D.A., 2004. Winter decomposition of transgenic cotton residue in conventional-till and no-till systems. Appl. Soil Ecol. 27, 135–142.

Lehman, R.M., Osborne, S.L., Rosentrater, K.A., 2008. No differences in decomposition rates observed between *Bacillus thuringiensis* and non-*Bacillus thuringiensis* corn residue incubated in the field. Agron. J. 100, 163–168.

Lynch, J.M., Benedetti, A., Insam, H., Nuti, M.P., Smalla, K., Torsvik, V., Nannipieri, P., 2004. Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol. Fertil. Soils 40, 363–385.

Marvier, M., McCreedy, C., Regetz, J., Kareiva, P., 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316, 1475–1477.

Moorhead, D.L., Reynolds, J.F., 1993. Changing carbon-chemistry of buried creosote bush litter during decomposition in the northern Chihuahuan Desert. Am. Midl. Nat. 130, 83–89.

Motavalli, P.P., Kremer, R.J., Fang, M., Means, N.E., 2004. Impact of genetically modified crops and their management on soil microbially-mediated plant nutrient transformations. J. Environ. Qual. 33, 816–824.

Mulder, C., Wouterse, M., Raubach, M., Roelofs, W., Rutgers, M., 2006. Can transgenic maize affect soil microbial communities? PLOS Comput. Biol. 2, 1165–1172.

Mungai, N.W., Motavalli, P.P., Nelson, K.A., Kremer, R.J., 2005. Differences in yields, residue composition and mineralization dynamics of *Bt* and non-*Bt* maize. Nutr. Cycl. Agroecosys. 73, 101–109.

O'Callaghan, M.O., Glare, T.R., Burgess, E.P.J., Malone, L.A., 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. Annu. Rev. Entomol. 50, 271–292.

Romeis, J., Meissle, M., Bigler, F., 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24, 63–71.

Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol. Biochem. 34, 1309–1315.

Saxena, D., Stotzky, G., 2001. Bt corn has a higher lignin content than non-Bt corn. Am. J. Bot. 88, 1704–1706.

Schmalenberger, A., Tebbe, C.C., 2002. Bacterial community composition in the rhizosphere of a transgenic, herbicide-resistant maize (*Zea mays*) and comparison to its non-transgenic cultivar Bosphore. FEMS Microbiol. Ecol. 40, 29–37.

Stotzky, G., 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. Plant Soil 266, 77–89.

Torsvik, V., Gokøyr, J., Daae, F.L., 1990. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56, 782–787.

USDA-Economic Research Service, 2007. Adoption of genetically-engineered crops in the U.S., Washington, DC. [Online.] http://www.ers.usda.gov/Data/BiotechCrops/alltables.xls.

Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Watrud, L.S., 2008. Bt Crop effects on functional guilds of non-target arthropods: A meta-analysis. PLoS One 3, e2118. doi:10.1371/journal.pone.0002118.

Wu, W.X., Ye, Q.F., Min, H., Duan, X.J., Jin, W.M., 2004. Bt-transgenic rice straw affects the culturable microbiota and dehydrogenase and phosphatase activities in a flooded paddy soil. Soil Biol. Biochem. 36, 289–295.

Please cite this article in press as: Lawhorn, C.N., et al., Impact of coleopteran targeting toxin (Cry3Bb1) of *Bt* corn on microbially mediated decomposition. Appl. Soil Ecol. (2009), doi:10.1016/j.apsoil.2008.12.003