Soil Biology & Biochemistry 57 (2013) 349–355

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

In-field rates of decomposition and microbial communities colonizing residues vary by depth of residue placement and plant part, but not by crop genotype for residues from two Cry1Ab Bt corn hybrids and their non-transgenic isolines

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ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form
7 September 2012
Accepted 10 September 2012
Available online 4 October 2012

Keywords:
Bt corn
Plant residue decomposition
Soil microbial communities
T-RFLP
Bacteria
Fungi
Micro-arthropods

ABSTRACT

The adoption of Bt corn has been largely overshadowed by concerns about their unintended effects on human health and the environment. Residues of transgenic Bt crops decomposed more slowly than their non-transgenic isolines in one laboratory study, although no mechanism to explain these observations was proposed. If Bt crop residues were to decompose more slowly in field soils, changes in carbon cycling and nutrient availability could result. We compared the in-field decomposition rates and diversity of decomposers colonizing residues of two Cry1Ab Bt corn hybrids, active against the European corn borer, with their non-transgenic isolines in litterbags placed in a Nebraska field. After five months, we found no significant differences in either the rates of residue mass loss or in the bacterial, fungal or micro-arthropod communities colonizing the transgenic versus the non-transgenic residues. Instead, both residue mass loss and detritivore colonizers were significantly affected by residue placement (surface versus buried) and plant part, demonstrating that environmental factors and residue quality, not the presence of the Cry1Ab protein, were the key drivers of residue decomposition and detritivore colonization in this study.

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1. Introduction

After 16 years of commercialization, the environmental safety of transgenic crops continues to be a subject of debate. Public concern over the cultivation of transgenic crops centers on their potential impact on: (i) human and other animal health, (ii) associated plant genetic resources, (iii) other, unintended environmental impact(s), and (iv) for insecticidal crops, their potential effects on non-target species (Thies and Devare, 2007). In 2009, 86% of the cultivated corn in the US consisted of transgenic corn about 70% of which was Bt corn (NASS, National Agricultural Statistics Service-Report, 2010); since then, Bt corn cultivation has expanded to South America, Asia and Europe, where its use increased by 26% in 2011 (James, 2011).

Bt corn has been genetically modified by incorporating Cry insecticidal genes derived from the soil bacterium *Bacillus thuringiensis* into the plant genome. The Cry1Ab Bt protein, toxic to the European corn borer (*Ostrinia nubilalis* Hubner), can be released into the soil through root exudates in some plants (Saxena et al., 2004), and its insecticidal activity can persist for as long as 6 months in some soils (Stotzky, 2005). The Bt protein may also be released by decomposing plant residues incorporated into soil after crop harvesting. Thus far, no adverse effects have been reported on the abundance or diversity of bacterial or fungal communities in the rhizosphere of Bt corn (Blackwood and Buyer, 2004; Devare et al., 2004, 2007) or in soils into which ground residues have been added (Saxena and Stotzky, 2001a). Decreased rates of respiration from soils in which residues from six Bt crops had been incorporated were observed in one laboratory incubation (Flores et al., 2005), although no mechanism to explain these observations was proposed.

The decomposition of plant litter is critical for many ecosystem processes such as nutrient cycling and soil formation (Swift et al., 1979). The rate at which these processes take place is influenced by the quality of litter, i.e., its nutrient content and relative proportions of different structural carbon (C) compounds, the physical-chemical environment, and the biota (Horwath, 2007). On average, lignin content of corn residues varies between 4 and 13% with roots having the highest concentration followed by stalks, cobs and leaves, respectively (Xue et al., 2011; Tarkalson et al., 2008). Due to its resistance to enzymatic attack, higher lignin- and polyphenol-

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http://dx.doi.org/10.1016/j.soilbio.2012.09.011

containing plant materials reduce organic C loss and increase the residence time of organic matter in soils (Adl, 2003). Transgenic plants may differ potentially from their parent lines in their biochemical composition. Cry1Ab Bt corn, for example, was shown to contain a higher lignin content than its respective non-Bt isoline (Saxena and Stotzky, 2001b; Fang et al., 2007), although other studies have not substantiated these results (Jung and Sheaffer, 2004; Tarkalson et al., 2008; Daudu et al., 2009; Xue et al., 2011).

Plant residues remaining on the soil surface or incorporated into soil are the primary C and energy sources for heterotrophic soil microorganisms. Soil arthropods are responsible for the initial decomposition of residues because they fragment the litter, increasing the surface area of exposed tissue, which is then colonized by saprophytic bacteria and fungi (Seastedt, 1984). These soil heterotrophs produce and release a number of extracellular enzymes that hydrolyze various components of plant detritus and soil organic matter for use as C, nutrient and energy sources (Thies and Grossman, 2006). Typically, the abundance and composition of biological communities recovered from various sites are strongly related to the selective pressures present in the environment sampled, including the quality and quantity of organic substrates (Conn and Dighton, 2000). Copiotrophic bacteria tend to respond most rapidly to additions of simple compounds such as starch and sugars, while fungi and actinomycetes generally predominate when more resistant compounds are added. In addition, fungi are usually better colonizers of organic materials on the soil surface, while bacteria colonize more readily on the surfaces of substrates that are mixed into the soil (Horwath, 2007; Thies and Grossman, 2006). Thus, microbial communities responsible for decomposition processes, and changes in their community composition, could serve as sensitive indicators of variation in the quality (i.e., composition) of organic matter inputs.

Culturing organisms from environmental samples recovers only a fraction of the extant community members and thus is not a particularly robust tool for soil microbial community analysis. Culture-independent approaches, such as DNA fingerprinting or other molecular techniques, are widely employed, useful alternatives. Terminal restriction fragment length polymorphism (T-RFLP) analysis is based on amplifying target genes, such as the rRNA genes, from DNA extracted from environmental samples. In T-RFLP, either the forward or reverse PCR primer is labeled with a fluorescent molecule enabling the accurate sizing of the fragment proximal to the primer to be determined using DNA sequencing technologies. T-RFLP has been used successfully to analyze complex microbial communities from soil, marine sediments and rhizosphere samples (Thies, 2007). In addition, T-RFLP yields a greater number of operational taxonomic units, and is thus more discriminatory, than other common DNA fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE) (Jones and Thies, 2007). We used this approach to characterize and compare bacterial and fungal communities colonizing residues of Cry1Ab-producing Bt corn and non-transgenic near isolines of the Bt hybrids.

We sought to evaluate whether short-term decomposition dynamics, the decomposer micro-arthropod communities or the bacterial and fungal communities colonizing residues surfaces of two different hybrids of Cry1Ab Bt corn differ from those that colonize the surfaces of residues from their respective non-transgenic isolines under field conditions.

2. Material and methods

2.1. Site description and experimental design

This field experiment was carried out at the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE, USA. The soil at this site is a Holdrege silt loam (fine-silty, mixed, mesic Typic Argiustolls), with an organic matter content of 1.8% and a pH of 6.5 in the surface 20 cm. Litterbags were used to measure the decomposition rate of corn residues from 4 corn hybrids: Pioneer 34N44 Bt (Bt), Pioneer 34N43 (Non-Bt), NC+ 4990 Bt (Bt), and NC+ 4880 (Non-Bt) and 3 plant parts: cobs, stalks, and leaves; with 2 depths of litterbag placement: the soil surface and at 10 cm depth.

The four corn hybrid treatments were planted in the spring of 2003 in a ridge-till system in field plots (4.6 × 45.7 m) in a randomized complete block design with four replications. The plots were furrow-irrigated to meet 100% of the crop evaporation requirement. Nitrogen fertilizer was applied to match the University of Nebraska recommended rates of 218 kg N ha^{-1} as ammonium nitrate. Plant materials used to prepare litterbags were obtained from these four corn hybrids. Whole plant samples were collected from the field plots for each hybrid at physiological maturity in October, 2003. The plant samples were separated into cobs, stalks, and leaves for each hybrid, and then dried at 65 °C. Approximately 10 g of plant tissue from each plant part was placed separately in nylon net litterbags of dimensions 12.5 × 12.5 cm and 0.79 mm mesh and the weights recorded. After sealing, the litterbags were labeled with an aluminum tag containing the sample information that was tied to the bag with copper wire. On December 5th, 48 litterbags were placed at each of the two soil depths in duplicate ($n = 192$) and left for 5 months in the field trial plots from which the respective plant material originated, and where the same four hybrids continued to be planted in their respective plots. All 192 litterbags were collected and processed. One of the duplicate sets ($n = 96$) of litterbags remained in Nebraska and was used for mass loss analysis. The other duplicate set ($n = 96$) was boxed and shipped overnight to Cornell University, Ithaca, NY, for micro-arthropod and microbial community analyses.

2.2. Plant residue mass loss

One complete set of litterbags (kept in Nebraska, $n = 96$), was rinsed gently with tap water to remove any adhering soil then dried at 65 °C to constant dry weight. The samples were then placed in a muffle furnace and ashed at 500 °C for 2.5 h. The percent of plant material remaining was determined as follows:

$$\mathrm{PMR} = \frac{(A_{\mathrm{PRE}} - A_{\mathrm{POST}}) \div A_{\mathrm{PRE}} \times H_{\mathrm{PS}}}{I_{\mathrm{P}}} \times 100$$

Where, PMR = plant material remaining (%); A_{PRE} = weight of pre-ash sample (g); A_{POST} = weight of post-ash sample (g); H_{PS} = weight of harvested plant/soil from litterbag (g); and I_P = weight of initial litterbag plant sample (g).

2.3. Decomposer community structure

2.3.1. Micro-arthropods

To measure the abundance of active litter decomposer micro-arthropods, the contents of the complete set of litterbags ($n = 96$) sent to Cornell University were placed into modified Tullgren funnels set under 40 W light bulbs for 24 h. Mobile arthropods were collected in vials containing 70% ethanol placed at the base of each funnel. The specimens were stored at 4 °C in 70% ethanol until examined. The micro-arthropods were counted and identified to Class and Order using a Nikon SMZ-10 stereoscope at 40× magnification and following the Borror et al. (1989) micro-arthropod identification key.

2.3.2. Bacterial and fungal community analyses

2.3.2.1. DNA extraction. For each plant tissue type, i.e., leaves, cobs and stalks, approximately 5 g were taken from each litterbag and placed in vials containing 20 mL of 0.5 M sterile potassium phosphate buffer (pH 6.8), and shaken at moderate speed for 1 h in order to detach microbial colonizers from the plant tissue surfaces. The microbial suspension from each sample was sequentially dispensed in a 2 mL tube, centrifuged and decanted until a dense precipitate of biomass was obtained. DNA was extracted from approximately 300 μL of the biomass precipitate, obtained in the previous step, using the BIO 101 FastDNA® Spin Kit for Soil (Qbiogene Inc., Irvine, CA). An additional washing step with 5.5 M guanidine thiocyanate at step 6 was included to remove humic acids, as recommended by the manufacturer. The concentration of DNA extracted from each sample was quantified by measuring the fluorescence of ethidium bromide bound to DNA in individual extracts and comparing it to a standard curve developed using calf thymus DNA. Fluorescence was detected using a Fluor-S Multi-imager and concentrations estimated subsequently using the accompanying QuantityOne software (BioRad, Hercules, CA).

2.3.2.2. Bacterial PCR. DNA extracted from residue surface-colonizing organisms was amplified by PCR using the fluorescently-labeled forward primer (27f) 5′-/6-FAM/AGA GTT TGA TCM TGG CTC AG-3′ and reverse primer (1492r) 5′-TAC GGY TAC CTT GTT ACG ACT T-3′ (Lane, 1990), synthesized by Integrated DNA Technologies (Coralville, IA). These primers amplify the Bacterial 16S rRNA genes, resulting in products of approximately 1500 bp. Three, replicate 50 μL PCR reactions per sample were performed. Each reaction contained 7.5 ng of DNA template, 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM deoxy-nucleotide triphosphates (dNTPs), 0.1 mg mL^{-1} bovine serum albumin (BSA), 0.1 μM of each primer, and 0.05 U μL^{-1} of Taq DNA polymerase (all from Promega Corp., Madison, WI). Reactions were carried out in an MJ Research (Waltham, MA) thermal cycler using an initial denaturing step of 94 °C for 3 min followed by 30 cycles of the following program: denaturation at 94 °C for 30 s, primer annealing at 59 °C for 45 s, and extension at 72 °C for 60 s. A final extension at 72 °C for 15 min was performed after the thermal cycling was complete.

2.3.2.3. Fungal PCR. To analyze the communities of residue surface-colonizing fungi, the internal transcribed spacer (ITS) region between the 18S and 28S genes was amplified using 0.2 μM of fluorescently-labeled ITS1 forward primer 5′-/6-FAM/TCC GTA GGT GAA CCT GCG G-3′ and the ITS4 reverse primer 5′-TCC GTA GGT GAA CCT GCG G-3′ (White et al., 1990) in PCR reactions containing 15 ng of DNA template, 0.05 U μL^{-1} of Taq DNA polymerase, 3.0 mM $MgCl_2$, 1× PCR buffer, 0.6 mM dNTPs, 0.1 g L^{-1} BSA, and nuclease free water. Three, replicate 50 μL reactions were performed for each sample. The amplification program consisted of an initial denaturing step at 94 °C for 5 min, followed by 30 cycles of the following program: denaturation at 94 °C for 30 s, annealing at 51 °C for 45 s, and extension at 72 °C for 45 s. A final extension at 72 °C for 10 min was performed. Products of approximately 600 bp were obtained from the fungal ITS amplification. All PCR products were visualized by running them on a 1.5% agarose electrophoretic gel to verify amplification, then replicate PCR reactions were pooled and amplified DNA was quantified as described above.

2.3.2.4. Restriction digests. Pooled PCR products derived from bacterial and fungal DNA amplifications, respectively, were hydrolyzed in separate reactions using the restriction enzymes HhaI and MspI for fungal ITS amplicons, and HhaI and Sau96I for bacterial 16S rRNA gene amplicons in 15 μL reactions containing: 5 ng of amplified DNA, 10 U of restriction enzyme, 3 μL of the appropriate 10× buffer, and 0.002 mg μL^{-1} BSA. The reactions were incubated at 37 °C for 4.5 h, followed by an inactivation step at 68 °C for 15 min. The restricted products were purified using PERFORMA® DTR edge plates (Edge BioSystems, Gaithersburg, MD), then evaporated and re-suspended in a mixture containing 990 μL of 99% formamide and 10 μL of the LIZ500 size standard (Applied Biosystems, Foster City, CA). The lengths of the fluorescently-labeled terminal fragments were determined using an ABI3730 capillary DNA sequencer (Applied Biosystems) at the Cornell University Biotechnology Resource Center in Ithaca, NY.

2.4. Data analysis

Data were analyzed by use of a general linear model (GLM) ANOVA to evaluate differences in residue mass loss between hybrids, plant parts and placements. Total abundance of micro-arthropods, as well as abundance of individual taxa, was analyzed using a permutational multivariate analysis of variance (perMANOVA) to test significance of the experimental factors (i.e., hybrid, plant part, and placement), and assess the relative proportion of variation that each factor contributed (Anderson, 2001). T-RFLP electropherograms, obtained from the ABI3730 DNA sequencer, were analyzed initially using GeneMapper 3.0 software (Applied Biosystems). Only peaks sized between 50 and 500 bp were used in the statistical analyses. T-RFLP profiles were used to generate presence/absence matrices used in the perMANOVA analyses to test significance of the experimental factors and the relative proportion of variation that each factor contributed. The level of significance for all analyses was set at $\alpha = 0.05$. Principal component analysis (PCA) was used to visualize the fungal and bacterial community composition patterns, while detrended correspondance analysis (DCA) (Gauch, 1982) was used to graphically represent the micro-arthropod abundance data. All statistical analyses were performed using R software version 2.15.1, and PerMANOVA analyses were performed using the *adonis* function in the *vegan* package with the Bray–Curtis distance measure and 1000 permutations (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Plant residue mass loss

There were no significant differences in residue mass loss between the Bt and non-Bt corn hybrids, nor between the NC and Pioneer hybrids (Table 1). However, residues placed at 10 cm depth decomposed significantly faster than those placed on the soil surface and leaves decomposed significantly faster than stalks, which decomposed significantly faster than cobs (Table 1). Litter material placed belowground decomposed about 55% faster on average than the same litter material placed on the soil surface. Likewise, on average, leaves decomposed 20% faster than stalks and 35% faster than cobs. Of the three plant parts and two field placements evaluated, surface-placed cobs decomposed the least, while the highest mass loss (65%) was observed for leaves placed 10 cm belowground after five months in the field.

3.2. Decomposer community composition

3.2.1. Micro-arthropods

There were no significant differences between either hybrids or genotypes (i.e., NC vs Pioneer or Bt vs non-Bt) ($p = 0.465$) in the total abundance of micro-arthropods colonizing the plant residues. However, the placement of the residues (surface vs buried), significantly ($p = 0.001$) influenced the total abundance of decomposer micro-arthropods extracted from the litter material

Table 1
Residue mass loss for different plant parts either buried at 10 cm depth or placed on the soil surface from 4 corn hybrids after 5 months in the field in Nebraska.

Plant component	Field placement	Hybrid	Mean mass loss (%) ($n = 4$)	Group mean	Group SD[a]
Leaves	Buried	NC+ 4880	67.0	64.7a	3.4
		NC+ 4990 Bt	66.2		
		Pioneer 34N43	59.7		
		Pioneer 34N44 Bt	65.9		
	Surface	NC+ 4880	35.3	37.0b	4.0
		NC+ 4990 Bt	35.7		
		Pioneer 34N43	42.9		
		Pioneer 34N44 Bt	34.1		
Stalks	Buried	NC+ 4880	34.2	36.5b	2.7
		NC+ 4990 Bt	36.0		
		Pioneer 34N43	35.5		
		Pioneer 34N44 Bt	40.5		
	Surface	NC+ 4880	24.0	24.6c	1.2
		NC+ 4990 Bt	25.3		
		Pioneer 34N43	23.2		
		Pioneer 34N44 Bt	25.8		
Cobs	Buried	NC+ 4880	26.2	24.5c	1.9
		NC+ 4990 Bt	21.8		
		Pioneer 34N43	24.7		
		Pioneer 34N44 Bt	25.2		
	Surface	NC+ 4880	8.0	5.3d	3.3
		NC+ 4990 Bt	0.5		
		Pioneer 34N43	5.7		
		Pioneer 34N44 Bt	6.9		

Variance components[b]	F-value	Pr > F
Block	0.91	0.4415
Hybrids	0.46	0.7134
Plant parts	243.45	<0.000000
Placement	216.25	<0.000000
Plant parts × placement	11.63	<0.000044

Different letters within a column represent significant differences at $p = 0.05$.
[a] Standard deviation of the mean.
[b] Probabilities are given for all main effects and the only interaction component that was significant.

(Table 2). Similarly, significant differences ($p = 0.006$) were observed in the abundance of micro-arthropods colonizing the three plant parts, i.e., leaves, cobs, and stalks. These results were consistent with the analyses for individual taxa (data not shown). No effect of block was observed in any of the analyses; therefore, it was not included as a factor in the perMANOVA analysis. Detrended correspondence analysis of the total abundance of micro-arthropods (Fig. 1A) showed distribution of the data by field placement along the DCA1 axis, with buried residues grouping on the right side and surface residues on the left; however, no grouping was observed due to plant part or hybrid (Fig. 1B). According to the statistical analysis, field placement and plant part accounted for 58.5% of the total variation observed (Table 2). The more commonly observed arthropods, in order of abundance, were

Table 2
PerMANOVA R^2 and p-values for micro-arthropod, bacterial and fungal communities[a] colonizing the surface of residues (leaves, stalks and cobs) of two corn hybrids and their Bt counterparts, placed on the soil surface or at 10 cm depth in a Nebraska field.

	p-Values			R^2 values		
	Micro-arthropod ($n = 96$)	Bacteria ($n = 56$)	Fungi ($n = 57$)	Micro-arthropod	Bacteria	Fungi
Hybrid	0.465	0.403	0.337	0.018	0.015	0.013
Field placement	0.001	0.001	0.001	0.437	0.077	0.229
Plant component	0.006	0.001	0.001	0.148	0.135	0.122
Residuals				0.395	0.771	0.634

[a] R^2 values represent the relative proportion of variation each factor contributes to the total variation in the dataset. $p = 0.05$.

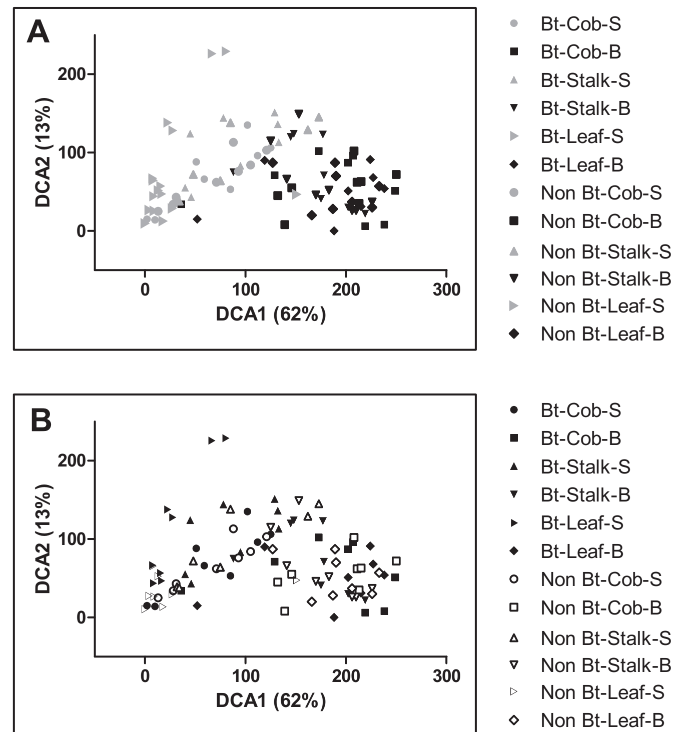

Fig. 1. Detrended correspondence analysis of total abundance of micro-arthropods colonizing three types of plant residues (leaves, stalks and cobs) of two Cry1Ab Bt corn varieties and their non-transgenic isolines, either buried at 10 cm depth or left on the soil surface. Panel A shows data points coded by plant part and placement. Panel B shows the same data coded by hybrid, plant part and placement.

mites, springtails and coleoptera. Mites accounted for 89% of the total number of specimens extracted, of which 67% were Prostigmata. Springtails accounted for 8% of the total, which were primarily Poduromorpha, while Coleoptera represented only 2.3% of the total number of arthropods extracted. Among the plant parts, leaf residues hosted the highest proportion of the total number of micro-arthropods (65%), followed by cobs (23%) and stalks (13%). With respect to placement, residues left on the soil surface had the highest number of arthropods (68%) compared to buried residues (32%) (data not shown).

3.2.2. Bacterial and fungal communities

DNA of sufficient quality for use in PCR could not be obtained from any of the leaf residues. DNA from several stalk and cob samples also failed to amplify. The high state of decomposition of the plant tissues was likely the cause of the PCR inhibition observed. Community analysis data for bacteria ($n = 56$) and fungi ($n = 57$) colonizing cobs and stalks are presented here. PerMANOVA of the T-RFLP fingerprints of 16S rRNA gene fragments amplified from bacterial communities colonizing cobs and stalks of the two hybrids of transgenic Cry1Ab Bt corn and their respective non-transgenic isolines showed no significant effect related to hybrid for either the HhaI ($p = 0.403$) or Sau96I (data not shown) digests (Table 2). However, significant effects were observed for type of residue ($p = 0.001$) and field placement ($p = 0.001$) (Table 2 and Fig. 2); these two factors accounted for 21% of the total variation in the data, while hybrid only accounted for 1.5% (Table 2). PCA analysis, consistent with the PerMANOVA, showed separation of bacteria colonizing cobs and stalks along the PC1 axis, with stalks grouping on the left and cobs on the right side; while the PC2 axis showed separation of the data based on field placement with

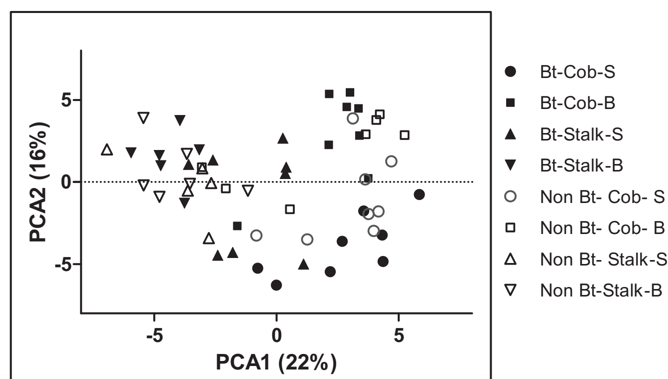

Fig. 2. Principal component analyses of T-RFLP fingerprints of bacterial communities colonizing the surface of cobs and stalks, either buried at 10 cm depth or left on the soil surface, of two Cry1Ab Bt corn varieties and their non-transgenic isolines.

buried samples on the top and surface-placed residues on the bottom of the graph (Fig. 2). No clustering according to hybrid was observed in the bacterial community analyses.

Likewise, PerMANOVA analysis of T-RFLP data of the ITS region of fungal colonizer communities revealed no significant effect of hybrid for either enzyme tested, i.e., HhaI ($p = 0.337$) or MspI (data not shown) (Table 2, Fig. 3). However, fungal communities were significantly influenced by the type of residue ($p = 0.001$) and field placement ($p = 0.001$) (Table 2). These two factors together represented 35.1% of the total variation captured in the data. The statistical analysis strongly correlated with the PCA representation; as shown in Fig. 3, the data are clearly separated by field placement along the PC1 axis, with communities from buried residues clustered on the left side and those of surface residues on the right, making field placement the strongest factor shaping the community. An interesting pattern is observed along the PC2 axis. Fungal communities colonizing buried residues are clustered together in the middle of the axis, but show little separation by type of residue, while on the surface, fungal communities colonizing stalks separated clearly from those colonizing the cobs (Fig. 3). As found with the micro-arthropod and bacteria analyses, fungal community composition was not significantly affected by genotype.

4. Discussion

Plant residue surfaces are areas of intense biological activity in soil. Microbial and faunal decomposition of plant residues determine the amount and timing of nutrient release and organic matter

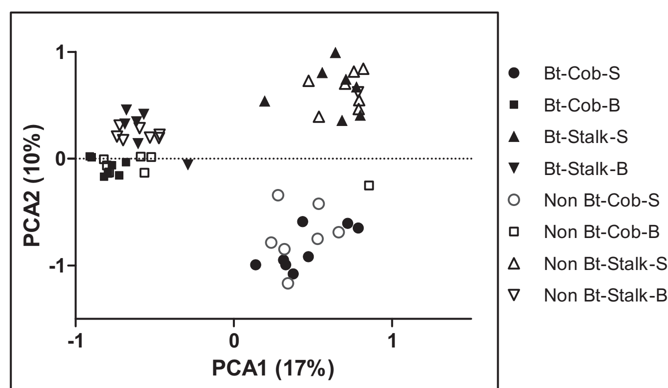

Fig. 3. Principal component analysis of T-RFLP fingerprints of fungal communities colonizing the surface of cobs and stalks, either buried at 10 cm depth or left on the soil surface, of two Cry1Ab Bt corn varieties and their non-transgenic isolines.

turnover (Horwath, 2007). The activity of decomposer communities depends strongly on resource bioavailability, microclimatic and edaphic conditions relative to the tolerance ranges of the organisms involved (Thies and Grossman, 2006; de Graaff et al., 2010). Our results show that neither the decomposition rate nor the composition of corn residue surface-colonizing decomposer communities was affected by the presence of Cry1Ab Bt protein in these residues. Instead, our results re-confirm the strong influence that residue quality has on its decomposition rate, and how strongly microbial community composition is affected by environmental conditions (Smalla et al., 2001), particularly variations in moisture and temperature related to litterbag field placement (Briar et al., 2012).

4.1. Residue decomposition

Litter quality is among the major factors controlling its rate of decomposition (Swift et al., 1979; Adl, 2003; Horwath, 2007). The lack of significant differences in mass loss between the Bt and non-Bt corn residues over 5 months in the field suggests that the presence of the Bt protein in residues of the two Bt corn hybrids tested had no significant effect on the decomposer communities at this field site. Saxena and Stotzky (2001b) reported that Cry1Ab Bt corn biomass had 33–97% higher lignin content than its non-transgenic isoline, and suggested that this might lead to a reduced rate of residue decomposition in soil; similarly, Flores et al. (2005) reported that Cry1Ab Bt corn biomass and the biomass from rice, cotton, tobacco, canola and potato decomposed more slowly in soil than non-Bt biomass, as inferred from a reduced rate of CO_2 evolved from soil microcosms in which finely ground Bt plant biomass was added in laboratory trials. No hypothesis was put forward to explain these observations, but the authors stated that differences in soil microbial communities were likely not responsible for the differential decomposition of Bt and non-Bt plant biomass. Fang et al. (2007), on the other hand, reported that Bt residues did not affect cumulative soil CO_2 efflux, rates of soil CO_2 evolution, or microbial DGGE patterns, while substrate utilization patterns were altered. Given the ecological implications of transgenic plant materials decomposing more slowly in soil, and the controversial nature of research in this field, a number of decomposition studies conducted under field conditions have been undertaken in order to elucidate such discrepancies. None of these studies has found significant differences in either lignin content (Jung and Sheaffer, 2004; Griffiths et al., 2007; Tarkalson et al., 2008) or decomposition rates of Cry1Ab Bt residues as compared to non-Bt residues (Lachnicht et al., 2004; Hopkins and Gregorich, 2005; Cortet et al., 2006; Griffiths et al., 2007). Differential rates of decomposition of different plant parts, on the other hand is commonly observed and is expected considering that corn leaves contain more nitrogen (N) than corn stalks (Jung and Sheaffer, 2004; Tarkalson et al., 2008). The C:N ratio in crop residues often controls the rate at which residues are decomposed because decomposers, particularly heterotrophic bacteria, have a high cellular N requirement relative to their demand for structural C (Thies and Grossman, 2006). Hence, residues with a lower C:N are expected to decompose more rapidly than those with a higher C:N (Horwath, 2007).

Environmental conditions at the soil surface are harsh compared to conditions even a few centimeters below the surface, with high fluctuations in both moisture and temperature. In contrast, belowground is a more buffered environment, where temperature and moisture change more slowly in relation to the weather. The higher mass loss of buried plant residues compared to those placed on the soil surface is likely due to the higher microbial activity possible under the more buffered conditions prevailing below the soil surface.

4.2. Decomposer community composition

4.2.1. Micro-arthropods

Among the soil fauna, micro-arthropods influence litter decomposition rates directly by both fragmenting and consuming plant material (Seastedt, 1984). Fragmentation or comminution contributes to residue decomposition and mineralization processes by creating additional surface area for microbial colonization. Within a given environment, high densities of micro-arthropods have been correlated with increased quality and quantity of food resources. As anticipated, we observed that the abundance of micro-arthropods in the litterbags was proportional to the extent of decay and that the highest number of micro-arthropods was found colonizing the highest quality litter, i.e., corn leaves. In agreement with other studies (Elkins and Whitford, 1982; Honemann et al., 2008), we observed that mites and collembola accounted for about 95% of total number of micro-arthropods recovered. Changes in numbers of micro-arthropods were strongly related to physical-chemical variables such as soil moisture and temperature. However, no effect on micro-arthropod communities colonizing the residues was observed between the Bt and non-Bt genotypes, for either of the two hybrid pairs. Our findings are supported by those of Al-Deeb et al. (2003) who found that numbers of soil mites (Prostigmata, Mesostigmata, and Oribatei), collembola, and nematodes were similar in soil planted with Bt corn and soil planted with its non-Bt isoline. Similarly, Honemann et al. (2008) report no differences in micro-arthropod decomposer communities between nine corn varieties including Bt and non-Bt isolines. Our results suggest that the Cry1Ab protein present in the Bt corn residues was not toxic or inhibitory to the dominant decomposer micro-arthropods – mites and collembola – and indicate no significant difference in community composition between the two genotypes studied.

4.2.2. Bacterial and fungal communities colonizing the surface of corn residues

The results of the T-RFLP fingerprinting analyses indicate that the Cry1Ab protein contained in the tissues of the two Bt corn hybrids tested had no apparent effect on the overall composition of soil bacterial and fungal communities colonizing these residues' surfaces relative to either each other or to their respective non-transgenic isolines. This is consistent with the results of several other studies that have reported no changes in the abundance or composition of bacterial and fungal communities in field soils where Bt corn was planted or in soils amended with Bt corn biomass and incubated under glasshouse conditions (Saxena and Stotzky, 2001a; Blackwood and Buyer, 2004; Devare et al., 2004, 2007; Icoz et al., 2008). Similarly, the numbers of culturable bacteria and fungi, and the activity of enzymes involved in degrading Bt plant biomass did not differ significantly from those associated with non-Bt plant biomass, confirming laboratory observations that the Cry1Ab protein is not toxic to a spectrum of pure and mixed cultures of microbes (Koskella and Stotzky, 1997).

Although molecular community analysis techniques targeting the Domain Bacteria and the Phylum Fungi by using universal primers might mask changes in sub-populations important in decomposing crop residues, T-RFLP is considered a very effective method for revealing similarities and differences between biotic communities and has a higher sensitivity than other PCR-fingerprinting methods that are commonly used (Thies, 2007; Jones and Thies, 2007; Siqueira et al., 2010).

Bacterial and fungal communities colonizing the surface of residues placed aboveground were significantly different from those colonizing buried residues and clustered separately in PCA analysis of their T-RFLP fingerprints. Many environmental factors, such as soil temperature, oxygen availability, moisture, solar radiation, and soil nutrient content significantly influence soil microbial community composition, dynamics and activities (Thies and Grossman, 2006). These environmental factors differ dramatically at the soil surface as compared to the subsurface. For example, surface-placed litter is exposed to frequent and drastic fluctuations in moisture and temperature as compared to litter placed below the soil surface. The differences between soil surface and subsurface residue-colonizing communities were more pronounced for fungi than for bacteria, suggesting that a broader range of bacteria are tolerant to changes in microclimate than fungi or that soil bacteria are able to colonize residues in contact with the soil surface more successfully. These community-level differences and the mass loss data demonstrated that environmental factors were determining not only the composition of these microbial communities, but their activity as well.

In addition to the effect of field placement on microbial communities colonizing the surface of corn residues, the fungal and bacterial populations colonizing the surfaces of the different plant parts were also clearly different, except for fungal communities colonizing buried residues. This was likely due to differences in the nutrient content and relative proportions of structural C compounds in cobs as compared to stalks, which may select for different microbial colonizers (Adl, 2003) by acting as 'selective growth media'. For instance, it has been shown that plant tissue quality, including total phosphorus and total N concentrations, correlate strongly with the fungal communities observed at a site (Thormann et al., 2004). Also, in a litterbag study, Debusk and Reddy (2005) showed that nutrient content of the decomposing plant tissue was more strongly correlated to decomposition rate than the nutrient content of the surrounding soil.

The reduced rates of CO_2 evolution observed from soils in which Bt crop residues were incorporated in the laboratory study reported by Flores et al. (2005) set off a wave of public controversy as to what the fate of Bt crop residues might be in field soils. Their results fueled public and scientific interest in determining whether Bt residues might negatively affect soil microbial communities and/or interfere with nutrient cycling processes. Here we have tested the in-field residue decomposition rates, abundance and diversity of micro-arthropod colonizers and the composition of bacterial and fungal communities colonizing the surface of plant residues of two Bt corn hybrids, representing two different transformation events involving the Cry1Ab protein coding gene. In no case did we observe any changes in measured variables that were attributable to differences between either the hybrid (NC versus Pioneer) or the Bt/non-Bt genotype. Environmental factors related to residue field placement and structural/nutrient content differences between different plant parts were responsible for the significant differences observed in both residue decomposition rates and the composition of residue-colonizing decomposer communities. In summary, this study contributes to the current scientific data suggesting that cultivating Cry1Ab Bt corn is unlikely to result in any significant change in rates of residue turnover or cause significant changes in soil decomposer activity or community composition.

Acknowledgments

This work was supported by grants from the United States Agency for International Development Soil Management – Collaborative Research Support Program at Cornell University and the Anna Elliot Memorial Fund at the University of Nebraska. We thank Claudia Ospina and Daniel Peck, Cornell University – Geneva Agricultural Experiment Station, for their help in characterizing micro-arthropods in litterbag residues. We would also like to thank Steve W. Culman, Hugh Gauch Jr. and Felipe Golib for their guidance on the statistical analysis.

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