

Nematode diversity in soil from a field trial with decomposing Bt cotton expressing Cry1Ac and Cry2Ab2 protein

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Abstract

The quality of decomposing plant materials may affect the soil community structure. The aim of the study was to determine the impact of decomposing Bt cotton and its isoline on soil nematode diversity. Bt cotton (06Z604D), isoline (99M03) and HART 89M (local non-Bt cotton cultivar) were planted for two seasons in a completely randomized block design in a confined field trial at Mwea, Kenya. After harvest the plant material was incorporated into soil and the nematode diversity was determined. The presence of Bt protein was evaluated using ELISA and insect bioassays. Abundance of bacteria feeding nematodes was significantly ($p < 0.05$) high but to a smaller extent in the Bt cotton plots (53.7% and 52% in the first and second season respectively) than in isoline (42.8% and 45% in the first and second season respectively). Insect bioassays detected Bt protein in the Bt cotton plots during the entire decomposition period in both seasons. There were no significant differences in nematode trophic groups composition between isoline and HART 89M. The effect of Cry2Ab2 and Cry1Ac protein in decomposing Bt cotton litter on soil nematodes was minimal. The study provides a basis for future studies on the impact of genetically engineered plants on soil nematodes in Kenyan agroecosystems.

Additional key words: *Bacillus thuringiensis*; biosafety; *Helicoverpa armigera*; nematodes.

Introduction

Transgenic crops are commercially cultivated in different countries and one of the main traits expressed in the plants is insect resistance. Cultivation and commercial release of transgenic crops has been a contentious issue due to the novelty of the technology. There are public concerns on the potential effects of transgenic crops on human health and the environment. The ecological concerns include gene flow to local cultivars, resistance development, invasiveness and effects on non target organisms such as nematodes (Mina *et al.*, 2008). Nematodes are among the most important organisms that act as intermediaries in soil decomposition. The bacteriovorous and fungivorous nematodes

are involved in bacterial and fungal channels of decomposition in response to changes in the microbial composition which is affected by addition of detritus (Ruess & Ferris, 2004). Organic matter is an important source of soil nutrients and its quality and quantity may negatively or positively affect the soil fauna. Decomposing transgenic plants may have a direct effect on soil microorganisms through exposure to the Bt protein and indirectly through unintended changes in the plant make up during transformation (Mina *et al.*, 2008). According to Saxena & Stotzky (2001), Bt cotton decomposes less than its near isogenic line. They further argue that a decrease in decomposition would result in improvement of soil structure and reduction in soil erosion due to accumulation of organic matter. They

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Abbreviations used: α (scale parameter); Bt (*Bacillus thuringiensis*); CI (channel index); cp (colonizer-persister); EI (enrichment index); ELISA (enzyme linked immunosorbent assay); KARI (Kenya Agricultural Research Institute); MAB (months after burying); MI (maturity index); NARL (National Agricultural Research Laboratories); PRC (principal response curve); SI (structure index).

however acknowledge the risk posed to non target organisms (NTOs) due to the extended exposure time. Escher *et al.* (2000) reported that Bt maize had higher N levels, and lower levels of lignin than non-Bt isolate. Decomposition of organic materials depends on their quality and C:N ratios. Nitrogen is mineralized when the C:N ratio of organic matter is < 20:1 but it is immobilized in microbial biomass when material has a C:N ratio > 20:1 (Ferris & Matute, 2003). According to Motavalli *et al.* (2004) transgenic crops may alter the rate of organic matter decomposition and N mineralization.

Bt protein from decomposing material can persist in soil due to binding to clay and organic matter. Crystal (Cry) proteins from Bt cotton and Bt corn may remain in soil up to 140 and 350 days respectively (Palm *et al.*, 1996; Sims & Ream, 1997). Decomposing Bt cotton residues can result in accumulation of 1.6 mg of Cry2A protein per kg of soil (Sims & Holden, 1996). Some studies have reported changes in microbial communities in decomposing transgenic material. According to Castaldini *et al.* (2005), Bt corn residues that were buried for 4 months affected eubacterial communities, endophytes and soil respiration. In other studies, fungi were present in higher numbers in decomposing Bt cotton than in non-Bt cotton. Gupta & Watson (2004) attributed this increase to exposure of the fungi to Cry1Ac protein. In addition, there were significant differences in the utilization of C between bacteria in Bt cotton and non-Bt cotton rhizosphere. There were more fungivorous nematodes present in buried litter of transgenic birch than in non-transgenic litter (Donegan *et al.*, 1997). Vauramo *et al.* (2006) also reported higher nematode populations in transgenic silver birch but Kotilainen *et al.* (2005) reported lower numbers. Other studies reported no significant effect of decomposing transgenic crops on soil organisms (Griffiths *et al.*, 2007; Zwahlen *et al.*, 2007; Honemann *et al.*, 2008).

Different indices may be used in assessing the condition of soil incorporated with decomposing Bt cotton. The maturity index (MI) is used as an indicator of soil disturbances as a result of chemical or physical stressors and has a value of 1 for disturbed systems and 4 for undisturbed conditions (Ettema *et al.*, 1998). On the other hand, trophic groups and functional guilds provide a basis for describing the status of soil food webs (Ruess & Ferris, 2004). Nematode faunal analysis provides an insight into the enriched, basal and structured conditions of the soil food web. Bt protein or unintended effects of gene insertion resulting in alteration in the

plant makeup may negatively or positively affect the decomposer communities including nematodes which may affect belowground decomposition dynamics. In the present study we evaluated the impact of decomposing Bt cotton on soil nematode diversity and abundance.

Material and methods

The field trial was set up in Ndomba, Central province, Kenya (0° 35' S, 37° 20' 30" E). The soil contained 9% sand, 14% silt and 77% clay. The experiment was laid out in a completely randomized block design with 5 × 5 m plots of Bt (06Z604D), isolate (99M03) and HART 89M (local non-Bt cotton cultivar) separated by 3 × 3 m strips. Each treatment was planted in five rows per plot and replicated four times. After six months of growth, during the first (Dec 2009-June 2010) and second (July-Dec 2010) seasons, the plant materials were harvested and incorporated into soil up to a depth of 60 cm, in the first (July-Dec 2010) and second (January-July 2011) seasons. Maximum and minimum temperatures and rainfall were recorded during the decomposition period.

Soil was collected from the different treatments for two seasons at 1, 2, 3, 4, 5 and 6 months after burying (MAB). Soil subsamples were collected randomly from the inner rows of each plot and then pooled into one composite sample. The soil samples were then kept at -20°C until further use in ELISA and insect bioassays.

ELISA tests

One gram of each soil sample was analysed for the presence of Bt of protein using a QualiPlate™ Combo Kit (AP 051) for Cry1A and Cry2A (EnviroLogix, Portland, ME, USA) following the manufacturer's instructions. Quantification of Cry2Ab2 and Cry1Ac was determined using a spectrophotometer (Benchmark®, Bio-Rad, Hercules, CA, USA).

Insect bioassays

To serve as a reference standard, standard mortality bioassays were done, by rearing neonate larvae in various concentrations of the Cry1Ac protein, which caused 0-100% mortality (Head *et al.*, 2002), in the diet. Ten-fold dilutions of pure Cry1Ac (University of

Aarhus, Denmark) were prepared using distiller water to make concentrations of 100, 10, 1, 0.1, 0.01 and 0.001 $\mu\text{g g}^{-1}$. One gram of non-Bt soil was mixed with 4 mL of each dilution and then brought up to 20 mL using a chickpea based artificial diet (Gujar *et al.*, 2004). The soil-diet mixture was assayed with *Helicoverpa armigera* by introducing a single larva into a petri plate covered with a soft paper towel. For each concentration, there were four replicates and for each replicate 100 insects were used. Mortality and larval growth inhibition (failure of neonates to reach 3rd instar or failure of 3rd instar to reach 4th instar was assessed after 7 days. Standard mortality bioassays using Cry2Ab2 was not done due to unavailability of the protein.

To assay for Cry1Ac and Cry2Ab2 protein using *H. armigera*, soil samples were incorporated into the artificial diet and then presented to *H. armigera* neonates. One gram of soil from each sample was mixed with 4 mL of water and brought up to a volume of 20 mL using the artificial diet (Gujar *et al.*, 2004). Artificial diet with no soil sample was included as a control. The experimental conditions were kept at $68 \pm 5\%$ relative humidity, $25 \pm 1^\circ\text{C}$ with a photoperiod of 16 h of light: 8 h of dark. Mortality and larval growth inhibition was assessed after 7 days.

Nematode assays

Soil subsamples were collected (each from a 30 cm deep and 8 cm diameter area) randomly from the inner rows of each plot and then pooled into one composite sample. From this mixture, 200 mL of each soil sample was used for nematode extraction using centrifugal-floatation method (Jenkins, 1964). Nematodes were counted and identified to genus level under a compound microscope at a magnification of $\times 400$ -1,000. They were also placed in different trophic groups according to Yeates *et al.* (1993).

C and N content of plant litter collected over a period of six months each in the first and second season was determined using elemental combustion analysis with a VEP Scientifica Analyzer, USA.

Statistical analysis

The ELISA results were interpreted according to the manufacturer's protocol. In the insect bioassays the median lethal concentration (LC50) and median

growth inhibition concentration (IC50), and their 95% confidence limits were determined through probit analysis (SPSS Inc., 2004, Chicago, IL, USA). When the mortalities of the control were between 5% and 10%, the larval mortalities were corrected using Abbott's formula (Abbott, 1925) before analyses while those $> 10\%$ were excluded. For the soil bioassays, we compared treatment effects on mortality and larval growth inhibition using ANOVA (GenStat 12.1).

Maximum and minimum temperatures and rainfall data during the first and second season were analyzed using ANOVA. We calculated genus richness index d and maturity index (MI) for free-living nematodes following Bongers (1990):

$$d = (S - 1) \log N$$

$$MI = (\sum v_i f_i) / n$$

where S = number of genera, N = total number of nematodes, v_i = colonizer-persister (cp) value for the nematode genus i , f_i = frequency of nematode genus i , n = total number of individual nematodes of the genus i in the sample.

To meet assumptions of normal distribution, abundances of nematodes were log-transformed [$\ln(x + 1)$] and proportions were arcsine transformed prior to analysis. Differences in genus richness, maturity index and proportion of trophic groups among treatments were tested using repeated measures ANOVA (GenStat 12.1). Principal response curves (PRC) analysis, a multivariate technique was used to show treatment effects on specific nematode genera over time (Van den Brink & Ter Braak, 1998). The vegan package of R 2.12.0 software was used for the PRC analysis. Renyi diversity index $HR(\alpha)$ (Tóthmérész, 1995) was used to evaluate diversity of nematode functional groups:

$$HR(\alpha) = \frac{1}{1 - \alpha} \log \sum_{i=1}^s p_i^\alpha$$

where α = scale parameter (with values, 0, 1, 2, 3, 4 and 5), p_i = relative abundance of the species i , s = number of species. Biodiversity-R program was used to generate the diversity profiles (Kindt & Coe, 2005).

The enrichment index (EI), structure index (SI) and channel index (CI) were calculated according to Ferris *et al.* (2001). The EI measures the response of opportunistic bacterivore and fungivore nematodes to organic matter input, SI is an indicator of the structure of the food web and CI describes the dominant decomposition pathways. Analysis of variance was conducted to test the effects of treatment, time and season on EI, SI, CI, % C, % N and C:N ratios in decomposing litter.

Differences at $p < 0.05$ level were considered statistically significant.

Results

Rainfall, maximum and minimum temperature varied significantly ($p < 0.001$) between the two seasons (Table 1). The average minimum temperature was $15.7 \pm 0.10^\circ\text{C}$ and $15.5 \pm 0.07^\circ\text{C}$ while the maximum temperature was $28.9 \pm 0.28^\circ\text{C}$ and $30.2 \pm 0.01^\circ\text{C}$ in the first and second season respectively. The average rainfall was 1.62 ± 0.32 mm in the first and 3.1 ± 0.64 mm in the second season.

No Cry1Ac was detected in the Bt cotton treatment during the entire decomposition period in the first and second season. Cry2Ab2 was detected at 1-MAB and 2-MAB in the first season, while in the second season it was detected in the first 3 months of decomposition. No Cry1Ac or Cry2Ab2 was detected in HART 89M and isoline treatments.

Dose mortality relationship from the probit analysis showed the IC50 of pure Cry1Ac was $0.017 \mu\text{g g}^{-1}$ (95% confidence limit from 0.009 to $0.026 \mu\text{g g}^{-1}$). The LC50 was $0.31 \mu\text{g g}^{-1}$ (95% confidence limit from 0.12 to $0.60 \mu\text{g g}^{-1}$).

In comparison to ELISA that detected Bt protein only in the earlier months of decomposition the insect bioassay detected Bt protein in soil from Bt cotton plots up to 6 months in both seasons as indicated by inhibition of larval growth up to the 3rd instar. There were no significant differences in the number of 3rd

instar larvae between HART 89M, isoline and the artificial diet control.

In the nematode community analysis, there were significant Treatment \times Trophic group ($F = 11.2_{[4,291]}$; $p < 0.001$) and Season \times Time \times Trophic group ($F = 4.83_{[18,291]}$; $p = 0.004$) interactions between the Bt and isoline treatment. Bacterial feeding nematodes were significantly higher but to a smaller extent in the Bt cotton ($53.7 \pm 1.64\%$ and $52 \pm 1.64\%$ in the first and second season respectively) plots than in isoline ($42.8 \pm 1.61\%$ and $45 \pm 1.64\%$ in the first and second season respectively). Predators and plant feeders were the least dominant trophic groups in all treatments. The lesion nematode *Pratylenchus* was the most common plant parasite across the treatments. There were no significant differences in trophic groups composition in the isoline and HART 89M treatments but there was a significant Season \times Time \times Trophic group ($F = 5.04_{[18,302]}$; $p < 0.001$) interaction.

Forty two nematode genera composed of predators, omnivores, bacteriovores, fungivores and plant feeders were identified across the treatments (Table 2). There were significant Season \times MAB ($F = 2.94_{[6,81]}$; $p = 0.012$), Season \times Treatment ($F = 10.81_{[1,81]}$; $p = 0.001$) and MAB \times Treatment ($F = 6.42_{[6,81]}$; $p < 0.001$) interactions for genus richness (Bt vs isoline). The highest genus richness in the Bt cotton treatment was recorded at 2-MAB in the first and second season while lowest was at 6-MAB in both seasons (Table 3). There was no significant treatment effect on genus richness between isoline and HART 89M treatment but there was a significant effect of season ($F = 4.60_{[1,81]}$; $p = 0.035$)

Table 1. Rainfall, minimum and maximum temperatures during the first and second season of the Bt cotton decomposition field trial

| Season | Month | Min. temperature (°C) | Max. temperature (°C) | Rainfall (mm) |
|--------|-----------|--------------------------|--------------------------|------------------|
| 1 | July | 14.6 ± 0.23 | 25.8 ± 0.59 | 0.5 ± 0.77 |
| | August | 15.1 ± 0.23 | 26.4 ± 0.59 | 0.7 ± 0.77 |
| | September | 16.2 ± 0.23 | 29.3 ± 0.6 | 0.3 ± 0.78 |
| | October | 16.6 ± 0.43 | 32 ± 1.09 | 2.7 ± 0.77 |
| | November | 16.5 ± 0.18 | 29.4 ± 0.45 | 4.5 ± 0.78 |
| | December | 15.1 ± 0.23 | 30.7 ± 0.59 | 1 ± 0.77 |
| 2 | January | 14.9 ± 0.18 | 31.7 ± 0.26 | 0.1 ± 1.47 |
| | February | 15.1 ± 0.18 | 32 ± 0.27 | 0.8 ± 1.54 |
| | March | 15.3 ± 0.18 | 33.7 ± 0.26 | 1.7 ± 1.47 |
| | April | 16.6 ± 0.18 | 30.2 ± 0.27 | 10.6 ± 1.49 |
| | May | 16.7 ± 0.18 | 27.7 ± 0.26 | 7.7 ± 1.47 |
| | June | 16 ± 0.18 | 28 ± 0.27 | 0.6 ± 1.49 |
| | July | 14 ± 0.31 | 27.8 ± 0.46 | 0.15 ± 2.60 |

Table 2. Nematode genera, colonizer-persister (cp) values, functional guilds and trophic groups in Bt cotton, isoline and HART 89M treatments

| Genus | cp value | Functional guild ¹ | Trophic group |
|-------------------------|----------|-------------------------------|------------------|
| <i>Acrobeles</i> | 2 | Ba2 | Bacterial feeder |
| <i>Acrobeloides</i> | 2 | Ba2 | Bacterial feeder |
| <i>Aphelenchoides</i> | 2 | Fu2 | Fungal feeder |
| <i>Aphelenchus</i> | 2 | Fu2 | Fungal feeder |
| <i>Aporcelaimellus</i> | 5 | Om5 | Omnivore |
| <i>Aporcelaimus</i> | 5 | Om5 | Omnivore |
| <i>Cephalobus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Cervidellus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Chiloplacus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Coarctadera</i> | 1 | Ba1 | Bacterial feeder |
| <i>Discolaimus</i> | 5 | Ca5 | Predator |
| <i>Drilocephalobus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Eucephalobus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Eudorylaimus</i> | 4 | Om4 | Omnivore |
| <i>Filenchus</i> | 3 | H3 | Plant feeder |
| <i>Geomonhystera</i> | 1 | Ba1 | Bacterial feeder |
| <i>Helicotylenchus</i> | 3 | H3 | Plant feeder |
| <i>Heterocephalobus</i> | 2 | Ba3 | Bacterial feeder |
| <i>Hoplolaimus</i> | 3 | H3 | Plant feeder |
| <i>Labronema</i> | 4 | Om4 | Omnivore |
| <i>Longidorous</i> | 5 | H5 | Plant feeder |
| <i>Meloidogyne</i> | 3 | H3 | Plant feeder |
| <i>Mononchus</i> | 4 | Ca4 | Predator |
| <i>Odontolaimus</i> | 3 | Ba3 | Bacterial feeder |
| <i>Paratylenchus</i> | 2 | H2 | Plant feeder |
| <i>Plectus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Pratylenchus</i> | 3 | H3 | Plant feeder |
| <i>Prismatolaimus</i> | 3 | Ba3 | Bacterial feeder |
| <i>Prodorylaimus</i> | 5 | Om5 | Omnivore |
| <i>Pungentus</i> | 4 | Om4 | Omnivore |
| <i>Rhabditis</i> | 1 | Ba1 | Bacterial feeder |
| <i>Rhabdolaimus</i> | 3 | Ba3 | Bacterial feeder |
| <i>Rotylenchulus</i> | 3 | H3 | Plant feeder |
| <i>Rotylenchus</i> | 3 | H3 | Plant feeder |
| <i>Scutellonema</i> | 3 | H3 | Plant feeder |
| <i>Tripyla</i> | 3 | Ca3 | Predator |
| <i>Tylencholaimus</i> | 4 | Fu4 | Fungal feeder |
| <i>Tylenchorhynchus</i> | 2 | H2 | Plant feeder |
| <i>Tylenchus</i> | 2 | H2 | Plant feeder |
| <i>Tylocephalus</i> | 2 | Ba2 | Bacterial feeder |
| <i>Wilsonema</i> | 2 | Ba2 | Bacterial feeder |
| <i>Xiphinema</i> | 5 | H5 | Plant feeder |

¹ Ba: Bacteriovores. Fu: Fungivores. Om: Omnivores. Ca: Carnivores. H: Herbivores. Numbers following the trophic groups' abbreviations represent the colonizer-persister values belonging to each genus.

and MAB ($F=5.07_{[6, 81]}$; $p<0.001$). The highest genus richness in the two seasons was at 4-MAB and 2-MAB in isoline and HART 89M respectively (Table 3).

There was significant MAB ($F=5.95_{[6, 81]}$; $p<0.001$) and Treatment ($F=7.77_{[1, 81]}$; $p=0.007$) effect on MI between Bt and isoline plots. There was no defined trend in the change in MI in the Bt cotton and isoline treatment in the two seasons (Table 3). There was no treatment effect on MI between isoline and HART 89M treatment but there was a significant effect of MAB ($F=2.65_{[6, 81]}$; $p=0.021$). The lowest MI was in the second season at 4-MAB in the HART 89M treatment (Table 3).

The Renyi diversity profiles of plots in the first season before burying of cotton residues showed lower diversity of nematode functional groups in the Bt cotton treatment. At 6-MAB Bt cotton plots had unequivocally lower diversity than isoline plots, while HART 89M plots had higher diversity than isoline. Bt cotton plots had a higher dominance of the more abundant bacterial feeding functional group (Fig. 1a,b). The diversity of nematode functional groups at the beginning and end of decomposition was not influenced by the richness of functional groups but by the dominance of specific trophic groups. Diversity profiles in the second season were also influenced by abundance of nematodes in specific trophic groups but not by the number of functional groups. Before burying of cotton residues, HART 89M plots had lower diversity of nematode functional groups compared with isoline plots. The low diversity was due to the low number of nematodes in the different trophic groups. At 6-MAB Bt cotton plots were less diverse in terms of nematode trophic groups abundance than HART 89M and isoline plots. At higher values of the scale parameter isoline was more diverse with higher nematode populations in the different functional groups. Bt cotton had a higher abundance of the dominant bacterial feeding functional group (Fig. 1c,d).

The PRC model showed a significant treatment effect on specific nematode genera over time in the first season, with *Rhabditis* and *Acrobeloides* having the greatest contribution to the PRC model ($F=5.3$; $p=0.005$) (Fig. 2a). The change in abundance of the genera with positive weights was similar to the pattern displayed in the PRC diagram while those with negative weights had an opposite response to that displayed in the model. In the second season there was no significant treatment effect on nematode genera abundance (Fig. 2b).

Nematode genera from the different treatments were placed into functional guilds (Table 2) as described by Ferris *et al.* (2001). There was a significant Season \times MAB \times Treatment interaction for CI ($F=9.02_{[6, 81]}$;

Table 3. Comparison of genus richness (*d*) and maturity index (MI) of Bt cotton vs isoline treatments, and of isoline vs HART 89M treatments

| Season | Time (MAR) ¹ | Bt cotton vs Isoline treatments | | | Isoline vs HART 89M treatments | | |
|--------------------------|-------------------------|---------------------------------|----------|-------|--------------------------------|----------|-------|
| | | Treatment | <i>d</i> | MI | Treatment | <i>d</i> | MI |
| 1 | 0 | Bt cotton | 3.1 | 2.1 | Isoline | 2.4 | 2.6 |
| | 1 | | 4.9 | 2.1 | | 3.5 | 2.2 |
| | 2 | | 6.1 | 2.2 | | 3.8 | 2.4 |
| | 3 | | 3.6 | 2.2 | | 3.6 | 2.1 |
| | 4 | | 3.1 | 2.1 | | 4.1 | 2.0 |
| | 5 | | 3.2 | 1.9 | | 3.5 | 2.1 |
| 2 | 6 | 2.8 | 1.9 | 3.6 | 2.2 | | |
| | 0 | Isoline | 4.2 | 2.4 | HART 89M | 3.8 | 2.5 |
| | 1 | | 2.9 | 2.4 | | 3.4 | 2.5 |
| | 2 | | 5.9 | 2.4 | | 4.6 | 2.3 |
| | 3 | | 2.4 | 2.1 | | 4.2 | 2.4 |
| | 4 | | 2.6 | 1.9 | | 5.4 | 2.2 |
| 5 | 2.1 | | 2.1 | 3.7 | | 2.1 | |
| 1 | 6 | 1.2 | 2.0 | 2.8 | 2.1 | | |
| | 0 | Isoline | 2.4 | 2.6 | HART 89M | 2.9 | 2.0 |
| | 1 | | 3.5 | 2.2 | | 4.5 | 2.3 |
| | 2 | | 3.8 | 2.4 | | 5.1 | 2.2 |
| | 3 | | 3.6 | 2.1 | | 3.4 | 2.4 |
| | 4 | | 4.1 | 2.0 | | 3.0 | 2.0 |
| 5 | 3.5 | | 2.1 | 3.2 | | 2.4 | |
| 2 | 6 | 3.6 | 2.2 | 3.6 | 2.2 | | |
| | 0 | Isoline | 3.8 | 2.5 | HART 89M | 3.8 | 2.3 |
| | 1 | | 3.4 | 2.5 | | 4.1 | 2.3 |
| | 2 | | 4.6 | 2.3 | | 6.2 | 2.4 |
| | 3 | | 4.2 | 2.4 | | 4.4 | 2.4 |
| | 4 | | 5.4 | 2.2 | | 4.3 | 1.8 |
| 5 | 3.7 | | 2.1 | 2.8 | | 2.1 | |
| | 6 | 2.8 | 2.1 | 2.9 | 2.1 | | |
| | | SEM | 0.51 | 0.11 | | 0.55 | 0.15 |
| Season | | LSD ² | 0.38 | 0.09 | | 0.41* | 0.12 |
| MAB | | LSD | 0.71* | 0.17* | | 0.77* | 0.21* |
| Treatment | | LSD | 0.38 | 0.09* | | 0.41 | 0.12 |
| Season × MAB | | LSD | 1.00* | 0.24 | | 1.09 | 0.30 |
| Season × Treatment | | LSD | 0.54* | 0.13 | | 0.58 | 0.16 |
| MAB × Treatment | | LSD | 1.00* | 0.24 | | 1.09 | 0.30 |
| Season × MAB × Treatment | | LSD | 1.42 | 0.33 | | 1.55 | 0.43 |

¹ MAB: months after burying. ² LSD: least significant differences at $p < 0.05$. * Significant differences at $p < 0.05$.

$p < 0.001$), EI ($F = 2.94_{[6, 81]}$; $p = 0.012$) and SI ($F = 41.05_{[6, 81]}$; $p < 0.001$) between Bt cotton and isoline treatments. The CI and SI were lower in Bt cotton plots and they decreased towards the end of decomposition period. The CI in isoline plots was higher and did not show great fluctuations during decomposition (Table 4). The EI had moderately low values in all treatments. A comparison of isoline and HART 89M revealed a significant Season × Treatment interaction for CI ($F = 18.9_{[1, 81]}$; $p < 0.001$) and EI ($F = 13.4_{[6, 81]}$; $p < 0.001$) and

a significant Season × MAB × Treatment ($F = 12.6_{[6, 81]}$; $p < 0.001$) interaction for SI. The CI was above 50% in both HART 89M and isoline treatments (Table 4).

The % C generally decreased over time in all treatments but there was no defined trend in the change of % N and C:N ratio. There was a significant MAB × Season × Treatment interaction for % C ($F = 50.6_{[5, 69]}$; $p < 0.001$) and % N ($F = 3.2_{[5, 69]}$; $p = 0.01$) between Bt cotton and isoline treatments. There was a decrease in % C in both treatments with the highest values recor-

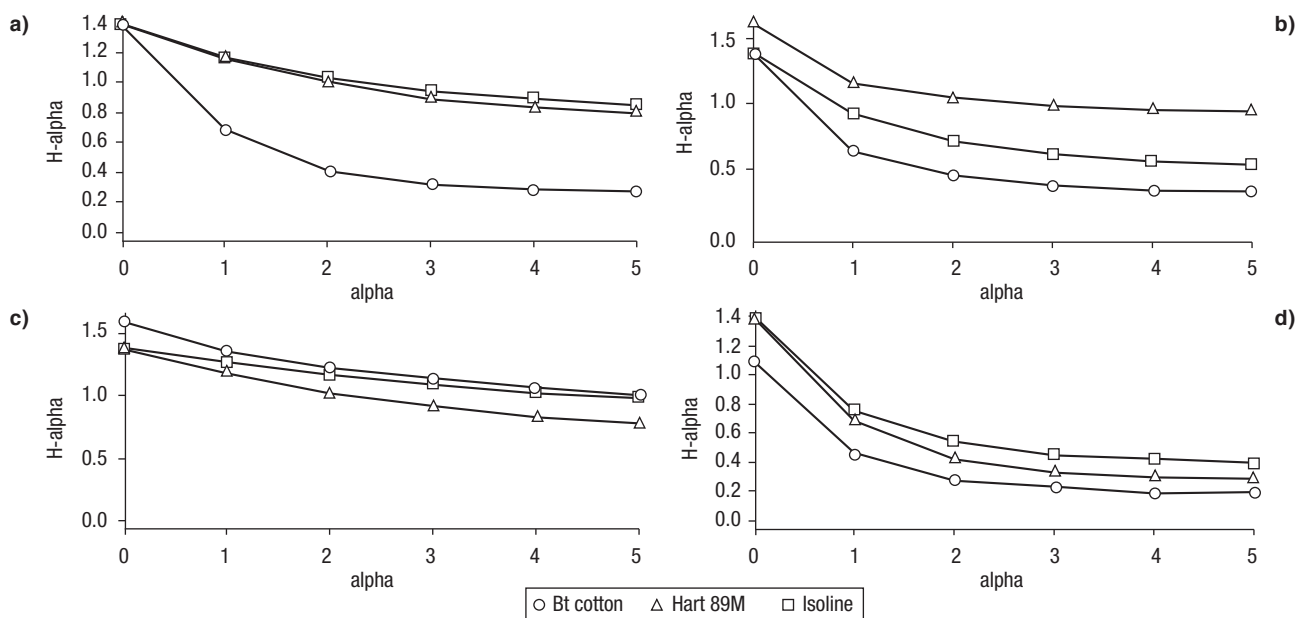


Figure 1. Renyi diversity profiles of nematode communities in Bt cotton, HART 89M and isoline treatments in the first season, July-Dec 2010 (a, b) and in the second season, January-July 2011 (c, d). (a, c) before incorporation of cotton litter and (b, d) at 6 months after burying (MAB). Scale parameter alpha with values 0, 1, 2, 4 and 5 is shown.

ded at 1-MAB in the two seasons (Table 5). Isoline had higher % C than Bt cotton at the beginning of decomposition in both seasons. The C:N ratio showed a significant MAB \times Season ($F = 44.3_{[5, 69]}$; $p < 0.001$), MAB \times Treatment ($F = 12.8_{[5, 69]}$; $p < 0.001$) and Season \times Treatment ($F = 31.9_{[1, 69]}$; $p < 0.001$) interaction. A comparison of isoline and HART 89M revealed a similar trend in the change in % C, % N and C:N ratio during decomposition in both seasons (Table 5). A significant MAB \times Season \times Treatment interaction was observed for all the parameters ($F = 102.9_{[5, 69]}$; $p < 0.001$, $F = 6.1_{[5, 69]}$; $p < 0.001$ and $F = 10.3_{[5, 69]}$; $p < 0.001$ for % C, % N and C:N ratio respectively).

Discussion

The insect bioassay through inhibition of larval growth was able to detect Bt protein in soil throughout the decomposition period compared with ELISA which detected Cry1Ac and Cry2Ab2 in the earlier months only. The lack of protein detection by ELISA in later months of decomposition may have been caused by protein extraction inefficiencies due to the presence of soil particles. This may have been as a result of binding of the protein to clay particles at the experimental site (77% clay) or reduced microbial degradation (Sims & Holden, 1996). Cry2Ab2 protein level in

soil was high in the first month of decomposition in both seasons which may have been due to carry over from the previous months of vegetative growth, and then it decreased in the second month probably due to binding to clay particles and degradation by ultra violet radiation. The presence of Bt protein during the entire decomposition period as detected by the insect bioassay is in agreement with other studies. Sims & Ream (1997) detected the Cry2Ab protein up to 120 days in decomposing Bt cotton litter. Gupta & Watson (2004) also found high levels of Cry1Ac in decomposing Bt cotton stubbles. Although Bt protein was present in soil during the 6 months in both seasons, it was present at low concentrations that would not have caused the observed differences in nematode community composition between the Bt cotton and isoline treatment.

Genetic transformation in plants has been shown to cause changes in N content, C:N ratio (Masoero *et al.*, 1999; Escher *et al.*, 2000), lignin (Saxena & Stotzky, 2001), fructose and carbohydrate content. Some Bt crops decompose more slowly than their isogenic counterparts due to high lignin content (Saxena & Stotzky, 2001) while others decompose faster. The change in lignin content in Bt crops may affect the rate of organic matter decomposition and this may affect biogeochemical cycles (Mina *et al.*, 2008). In the current study, there was a general decrease in % C and no defined trend in change of % N and C:N ratio in all

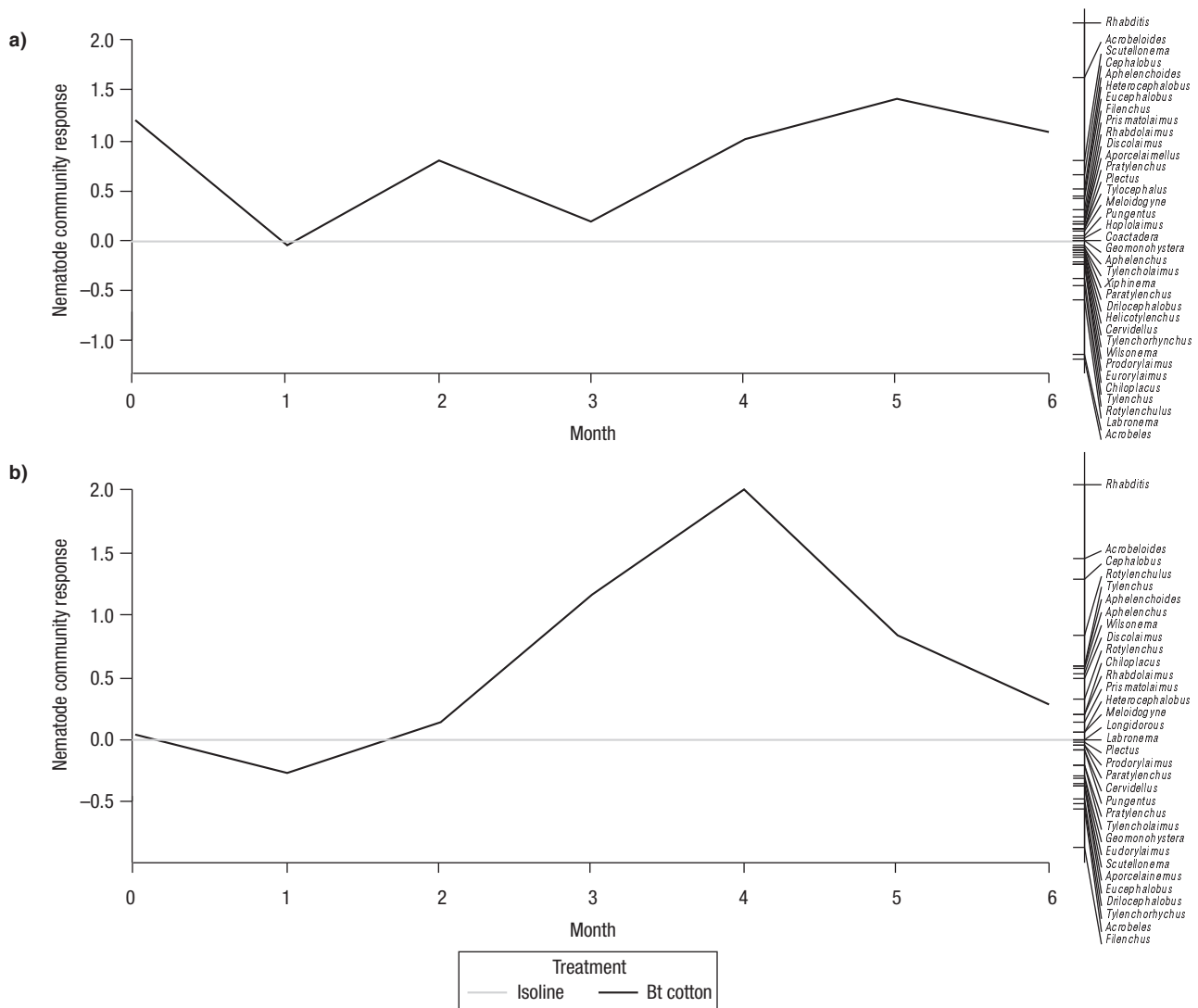


Figure 2. Principal response curves of the nematode genera showing the effects of Bt cotton compared to the isogenic non-Bt cotton. The ordinate axis represents the first principal component of the variance explained by treatment. The abscissa axis represents sampling months in the first season (a) and in the second season (b). All taxa identified in Bt cotton and isoline plots are shown on the right. The horizontal line at 0 shows the response of the isoline nematode community. In (a) PRC model was statistically significant ($F = 5.3$; $p = 0.005$). In (b) PRC model was not statistically significant.

treatments. This result is in agreement with Coviella *et al.* (2000) who reported an undefined trend in the change of N content in Bt and non-Bt cotton. On the other hand, Lachnicht (2004) reported a decrease in % C as Bt cotton litter decomposed while % N remained unchanged; this was also observed by Donegan *et al.* (1997). A decrease in % C has also been reported in decomposition studies of transgenic rice (Kimura *et al.*, 2004). Decomposition experiments of Bt cotton in Australia showed that decomposition rates were similar for Bt and non-Bt litter but Bt stubble had a higher N content and lower C:N ratio (Gupta & Watson, 2004).

In addition to the observed differences in % C, % N and C:N ratio between decomposing Bt cotton and isoline, there may have been variations in other chemical and structural components in lignin, carbohydrates or other compounds. The differences in % C, % N and C:N ratio between Bt cotton and isoline and their interactions with other environmental factors may also have influenced decomposition and nematode communities in the two treatments.

Changes in nematode trophic group composition may affect ecosystem functions due to their role in decomposition and nutrient cycling (Ingham *et al.*, 1985).

Table 4. Comparison of enrichment index (EI), structure index (SI) and channel index (CI) in Bt cotton vs isoline treatments, and in isoline vs HART 89M treatments, in the first and second season

| Season | Time (MAB) ¹ | Bt cotton vs Isoline treatments | | | | Isoline vs HART 89M treatments | | | | |
|--------|-------------------------|---------------------------------|------------------|--------|--------|--------------------------------|-------|--------|-------|------|
| | | Treatment | EI | SI | CI | Treatment | EI | SI | CI | |
| 1 | 0 | Bt cotton | 27.0 | 33.5 | 31.5 | Isoline | 28.0 | 67.8 | 100.0 | |
| | 1 | | 34.0 | 44.0 | 85.8 | | 27.5 | 41.3 | 90.0 | |
| | 2 | | 46.3 | 53.8 | 82.5 | | 32.5 | 34.0 | 90.0 | |
| | 3 | | 34.8 | 51.5 | 98.0 | | 35.8 | 16.0 | 99.2 | |
| | 4 | | 44.3 | 8.8 | 93.8 | | 37.3 | 42.0 | 99.2 | |
| | 5 | | 48.0 | 6.8 | 18.2 | | 39.0 | 42.0 | 98.5 | |
| 2 | 0 | Isoline | 42.5 | 53.0 | 55.5 | HART 89M | 39.3 | 26.0 | 95.0 | |
| | 1 | | 33.5 | 42.5 | 89.2 | | 44.3 | 60.3 | 81.5 | |
| | 2 | | 25.0 | 47.3 | 86.2 | | 32.5 | 33.8 | 88.2 | |
| | 3 | | 36.8 | 2.0 | 97.8 | | 41.5 | 33.8 | 87.2 | |
| | 4 | | 45.0 | 14.0 | 11.3 | | 28.5 | 25.5 | 87.2 | |
| | 5 | | 33.8 | 12.0 | 31.5 | | 32.0 | 19.0 | 86.5 | |
| 1 | 0 | Isoline | 28.0 | 67.8 | 100.0 | HART 89M | 30.8 | 38.8 | 91.7 | |
| | 1 | | 27.5 | 41.3 | 90.0 | | 36.3 | 52.0 | 63.2 | |
| | 2 | | 32.5 | 34.0 | 90.0 | | 37.0 | 33.8 | 97.2 | |
| | 3 | | 35.8 | 16.0 | 99.2 | | 35.3 | 36.0 | 79.0 | |
| | 4 | | 37.3 | 42.0 | 99.2 | | 45.8 | 9.8 | 80.5 | |
| | 5 | | 39.0 | 42.0 | 98.5 | | 46.5 | 52.3 | 80.5 | |
| 2 | 0 | Isoline | 23.5 | 55.3 | 95.5 | HART 89M | 40.8 | 61.0 | 79.2 | |
| | 1 | | 39.3 | 26.0 | 95.0 | | 44.0 | 49.5 | 85.5 | |
| | 2 | | 44.3 | 60.3 | 81.5 | | 40.3 | 48.8 | 87.8 | |
| | 3 | | 32.5 | 33.8 | 88.2 | | 27.5 | 54.3 | 97.5 | |
| | 4 | | 41.5 | 33.8 | 87.2 | | 32.8 | 44.3 | 98.8 | |
| | 5 | | 28.5 | 25.5 | 87.2 | | 29.3 | 17.3 | 98.2 | |
| 1 | 0 | Isoline | 32.0 | 19.0 | 86.5 | HART 89M | 29.3 | 13.5 | 98.2 | |
| | 1 | | 28.8 | 17.8 | 74.5 | | 27.0 | 29.5 | 88.0 | |
| | 2 | | SEM | 3.77 | 3.33 | | 5.63 | 3.77 | 3.72 | 6.73 |
| | 3 | | LSD ² | 2.84* | 2.50* | | 4.23* | 2.54 | 2.80* | 5.06 |
| | 4 | | LSD | 5.31* | 4.69* | | 7.91* | 4.75* | 5.24* | 9.48 |
| | 5 | | LSD | 2.84* | 2.51* | | 4.23* | 2.54 | 2.80 | 5.06 |
| 2 | 0 | Isoline | 7.51* | 6.63* | 11.19* | 6.72* | 7.41* | 13.40 | | |
| | 1 | | LSD | 4.01* | 3.55* | 5.98 | 3.59* | 3.96* | 7.16* | |
| | 2 | | LSD | 7.51 | 6.63* | 11.19* | 6.72 | 7.41* | 13.40 | |
| | 3 | | LSD | 10.61* | 9.38* | 15.83* | 9.50 | 10.48* | 18.95 | |
| | 4 | | LSD | 3.77 | 3.33 | 5.63 | 3.77 | 3.72 | 6.73 | |
| | 5 | | LSD ² | 2.84* | 2.50* | 4.23* | 2.54 | 2.80* | 5.06 | |

¹ MAB: months after burying. ² LSD: least significant differences at $p < 0.05$. * Significant differences at $p < 0.05$.

There were variations in trophic groups between Bt cotton and isoline as decomposition progressed in both seasons and this may be an indication of differences in litter characteristics. Predators which are sensitive to physical and chemical disturbances were less common in all treatments. The decrease in plant parasitic nematodes at later stages of decomposition in all treatments is expected and has been reported in other studies (Briar, 2007). The Renyi diversity profile in the first

season at the end of decomposition showed that Bt cotton plots had a lower diversity of functional groups than isoline and this was due to the differences in the abundance of the trophic groups. HART 89M plots were more diverse than isoline plots on the lower and higher levels of the scale parameter, an indication of differences in dominant functional groups. In the second season at 6-MAB, Bt cotton plots were less diverse in terms of abundance of different functional groups

Table 5. Comparison of carbon (% C) and nitrogen (% N) percentages and C:N ratio of Bt cotton vs isoline litter, and of isoline vs HART 89M litter in the first and second season

| Season | Time (MAB) ¹ | Bt cotton vs Isoline litter | | | | Isoline vs HART 89M litter | | | |
|--------------------------|-------------------------|-----------------------------|-------|-------|-------|----------------------------|-------|-------|------|
| | | Treatment | C | N | C:N | Treatment | C | N | C:N |
| 1 | 1 | Bt cotton | 24.5 | 1.3 | 18.5 | Isoline | 30.2 | 1.3 | 24.0 |
| | 2 | | 12.9 | 2.2 | 6.0 | | 15.4 | 2.2 | 7.1 |
| | 3 | | 11.4 | 1.1 | 10.1 | | 11.2 | 1.3 | 9.1 |
| | 4 | | 9.1 | 1.1 | 8.2 | | 11.3 | 1.4 | 7.9 |
| | 5 | | 8.6 | 1.2 | 7.6 | | 8.7 | 1.5 | 5.9 |
| | 6 | | 4.5 | 2.3 | 1.9 | | 3.7 | 2.3 | 1.6 |
| 2 | 1 | Isoline | 24.4 | 1.0 | 24.4 | HART 89M | 24.7 | 1.0 | 24.7 |
| | 2 | | 18.4 | 1.2 | 15.3 | | 17.6 | 1.4 | 12.6 |
| | 3 | | 15.6 | 1.4 | 11.2 | | 14.3 | 1.7 | 8.3 |
| | 4 | | 9.2 | 1.3 | 7.0 | | 7.3 | 1.4 | 5.2 |
| | 5 | | 8.4 | 1.2 | 7.3 | | 7.9 | 2.1 | 3.8 |
| | 6 | | 7.5 | 1.2 | 6.2 | | 7.1 | 1.4 | 5.2 |
| 1 | 1 | Isoline | 30.2 | 1.3 | 24.0 | HART 89M | 22.4 | 1.4 | 16.4 |
| | 2 | | 15.4 | 2.2 | 7.1 | | 15.3 | 1.5 | 11.3 |
| | 3 | | 11.2 | 1.3 | 9.1 | | 11.3 | 1.2 | 10.1 |
| | 4 | | 11.3 | 1.4 | 7.9 | | 10.4 | 1.1 | 9.5 |
| | 5 | | 8.7 | 1.5 | 5.9 | | 8.6 | 1.3 | 7.0 |
| | 6 | | 3.7 | 2.3 | 1.6 | | 4.8 | 1.9 | 3.1 |
| 2 | 1 | Isoline | 24.7 | 1.0 | 24.7 | HART 89M | 23.2 | 1.0 | 23.2 |
| | 2 | | 17.6 | 1.4 | 12.6 | | 16.5 | 1.0 | 16.4 |
| | 3 | | 14.3 | 1.7 | 8.3 | | 15.3 | 1.8 | 8.3 |
| | 4 | | 7.3 | 1.4 | 5.2 | | 8.5 | 1.4 | 6.2 |
| | 5 | | 7.9 | 2.1 | 3.8 | | 8.3 | 0.7 | 11.9 |
| | 6 | | 7.1 | 1.4 | 5.2 | | 7.3 | 1.7 | 4.2 |
| | | SEM | 0.16 | 0.07 | 0.53 | 0.20 | 0.13 | 0.60 | |
| MAB | | LSD ² | 0.22* | 0.11* | 0.76* | 0.19* | 0.18* | 0.88* | |
| Season | | LSD | 0.13* | 0.06* | 0.44* | 0.11* | 0.11* | 0.51* | |
| Treatment | | LSD | 0.13* | 0.06* | 0.43* | 0.11* | 0.11* | 0.51* | |
| MAB × Season | | LSD | 0.31* | 0.15* | 1.07* | 0.26* | 0.26* | 1.25* | |
| MAB × Treatment | | LSD | 0.32* | 0.15* | 1.07* | 0.26* | 0.26* | 1.25* | |
| Season × Treatment | | LSD | 0.18* | 0.09* | 0.62* | 0.15* | 0.20 | 0.72* | |
| MAB × Season × Treatment | | LSD | 0.45* | 0.21* | 1.51 | 0.37* | 0.37* | 1.76* | |

¹ MAB: months after burying. ² LSD: least significant differences at $p < 0.05$. * Significant differences at $p < 0.05$.

compared to isoline plots. The high abundance of bacterial feeders at 6-MAB in the Bt cotton plots in the first and second season may have contributed to its lower ranking in the diversity profiles. Investigating the effect of transgenic crops on specific nematode genera can show small changes that may not be evident at the trophic level. The PRC model was significant in the first season with *Rhabditis* and *Acrobeloides* showing positive contribution with an increase in activity from the fourth to sixth month. The lack of significance of the PRC model in the second season may have been an indication of lack of treatment effects on specific genera.

The maturity index is dependent on the composition of nematode groups and it provides an insight into the state of an ecosystem (Bongers, 1990). Low values of MI were recorded across all treatments but Bt cotton showed a decrease in MI towards the end of decomposition probably due to the change in nematode composition. The EI, which indicates the response of opportunists to organic matter, was low in all the treatments, mainly because the Ba2 guild made up of members of Cephalobidae was dominant and they are insensitive to disturbances. The SI was also low in all treatments probably due to the low numbers of nematodes from higher trophic groups. At 5-MAB and 6-MAB the CI

was significantly lower in Bt cotton treatment than in isoline, this was due to the high population of enrichment opportunists in cp1 category and general opportunists in cp2 and it reflected a decomposition pathway that was mainly bacterial dominated (Ferris & Matute, 2003). The increase in bacterivores towards the end of decomposition in Bt cotton treatment could be due to an increase in microbial biomass as a result of increase in nutrients from the decomposing material.

There were differences in the temperature and rainfall between the two seasons and this may also have influenced the % C, %N, C:N ratio and nematode diversity. Seasonal changes in weather conditions have been reported to affect soil communities under transgenic crops (Griffiths *et al.*, 2007; Icoz & Stotzky, 2008). Decomposition processes are also influenced by climatic conditions. Temperature influences decomposition due to its association with microbial activities. Cortet *et al.* (2006) and Icoz & Stotzky (2008) showed that decomposition in Bt maize fields was influenced more by climatic factors than the Bt gene. A comparison of HART 89M and isoline has highlighted differences in various parameters that may have been due to varietal effects. Variations in nematode communities between the two cotton cultivars were evident and in some instances these differences were greater than in the Bt cotton vs isoline comparison. The ecological significance of the observed differences due to varietal differences and genetic modification of cotton is not clear and requires further study. It would be important to assess if the observed shifts in nematode communities would occur in other soil types in different cotton growing regions of Kenya over multiple seasons.

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