Genetically modified (GM) corn in the Philippines : Ecological impacts on agroecosystems, effects on the economic status and farmers' experiences

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Invertebrate abundance and species richness in transgenic and non-transgenic cornfields in the Philippines

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To be submitted

Abstract

This study examined the effects of large-scale and long-term use of transgenic corn varieties on the abundance, species richness, and guilds of non-target invertebrates in a wet tropical environment. The study was conducted in 30 fields, including non-transgenic cornfields for comparison, and distributed over three lowland sites in the Philippines. The transgenic corn varieties *Bt* (*Bacillus thuringiensis*) and *Bt*HT (*Bt* Roundup Ready) in this study were introduced to the area in 2002 and 2005. Information on aerial, surface, and soil-dwelling invertebrates were gathered during the mature stage of corn development using sticky-trap, pitfall-trap, and soil-core sampling technologies along 100 m transect lines laid out in the middle of 1.4 to 3.8 hectare fields. A total of 21,639 non-target invertebrates representing 129 different species were identified at the three sites, including surface (69%), aerial (26%), and soil (5%) dwellers. The non-*Bt* cornfields had significantly higher abundance and species richness of all non-target invertebrates than the *Bt* and *BtHT* fields (p-values, 0.001 and 0.020 respectively). Likewise, the abundance and species richness of aerial (p-values: 0.010 and 0.009, respectively) and abundance of soil (p-value: 0.03) dwelling non-target invertebrates were notably higher in the non-*Bt* cornfields. Cornfields' soil chemical properties such as pH, potassium and nitrogen content also influenced the abundance of invertebrates; however corn varieties indicated a stronger influence. Most importantly, the effects of these confounding variables did not take away the effect of corn varieties.

Introduction

One promising way of increasing profits in crop production is the use of genetically modified crop varieties, which possess novel genetic characteristics introduced to protect them against pest infestation or herbicides. Varieties available for corn (*Zea mays* L.) include HT (Herbicide tolerant), *Bt* (*Bacillus thuringiensis*), and *Bt*HT (*Bt* Herbicide tolerant). The first of these is a variety modified to be resistant to the glyphosate-containing herbicides used to control weeds, while the second is a variety able to produce a bacterial toxin with proven efficacy against the Asian Corn Borer (ACB; *Ostrinia furnacalis* Guenée), and the third is a combination of the two. In the Philippines, the Department of Agriculture approved *Bt* corn for direct use in December 2002, making the country the first in Asia to commercialize a transgenic food crop. This was followed by the approval of the *Bt*HT corn variety for commercial technology demonstration in the 2005 dry season planting (Gonzales *et al.,* 2009).

Transgenic corn varieties are believed to offer a number of advantages compared to non-transgenic varieties. They produce higher yields and allow for effective utilization of scarce land because of improved pest resistance and nutrient utilization. Specifically, *Bt* corn has been shown to produce higher yields than a near-isogenic non-*Bt* variety (Qaim and Zilberman, 2003; Dilehay *et al*., 2004; Rice, 2004; Stanger and Lauer, 2006) and to significantly reduce pesticide use (Yorobe & Quicoy, 2006; Dillehay *et al*., 2004). *Bt* corn has delivered important improvements in grain quality through significant reductions in the levels of mycotoxins found in the grain (Hammond *et al*., 2004; Wu, 2007; Folcher *et al*., 2010). Furthermore, *Bt*HT corn is suitable for no-tillage agriculture, a system of planting crops without plowing in order to reduce soil erosion and nutrient loss. Benefits like these directly or indirectly contribute to livelihood improvement and poverty alleviation among farmers.

James (2010) reported a significant increase to one billion hectares in transgenic crop production in 2010. The Philippines was the first Asian country among the 29 mega transgenic crop adopting countries. The adoption of transgenic corn in the Philippines has increased remarkably since its first field testing in 2001, as potential economic benefits made it an attractive alternative to conventional corn varieties. To date, the country ranked 13th, with more than 500,000 hectares planted with transgenic corn by about 270,000 small farmers (James, 2011). Such large-scale use of transgenic corn varieties, however, may considerably change agroecosystems, raising the question of long-term sustainability.

Transgenic crops may affect non-target organisms. For example, *Bt* corn can have adverse effects on non-target invertebrates like Common Swallowtail, *Papilio machaon* L. (Lang and Vojtech, 2006), the larvae of the herbivorous African cotton leaf worm *Spodoptera littoralis* (Meissle *et al*., 2005a), and the generalist predatory ground beetle *Poecilus cupreus* L. (Meissle *et al.,* 2005b), and can exert a sublethal behavioral effect on honey bees (Romero *et al*., 2008). Romero *et al*. (2007) confirmed that the *Bt* Cry1Ab protein has negative effects on non-target insects, including the parasitoids *Cotesia marginiventris* (Hymenoptera). A 200-day feeding experiment by Zwahlen *et al*. (2003) showed that *Bt* corn litter reduced the weight of adult earthworms by 18%, compared to a 4% gain when fed with non-*Bt* corn**.** Further, reduced application of insecticides in areas under *Bt* cotton cultivation may cause an outbreak of non-targeted pests, as happened with the Mirid bug *Creontiades biseratense* in China (Lu *et al.,* 2010).

All the above-mentioned studies refer to impacts of *Bt* corn under temperate conditions. To our knowledge, none of the peer-reviewed articles reflects the potential impact of transgenic corn in a wet tropical environment like the Philippines. Furthermore, quantitative and evidence-based risk analyses are still needed to settle ongoing controversies over the ecological impact of transgenic crops. A meta-analysis of 42 field experiments involving *Bt* corn and insecticide application (Marvier *et al*., 2007) indicated that non-target organisms are more abundant in *Bt* cornfields without insecticide application; but if comparison is made with non-*Bt* cornfields without insecticides, some non-target groups prove significantly less abundant. This means that *Bt* corn is more environment-friendly than non-*Bt* corn, but only under the assumption that non-*Bt* corn always requires insecticides, while *Bt* corn never does. The effects of transgenic corn requires further study in tropical agro-ecosystems, considering that the meta-analysis referred to above was based on controlled field experiments in temperate agroecosystems. The Philippines, with its relatively long-term practice of large-scale transgenic corn cultivation, provides an excellent environment for such studies.

There have been only a few, non-reviewed field studies on the impacts of transgenic corn on non-target organisms in the Philippines (Alcantara, 2004; Javier *et al*., 2004; Alcantara *et al*., 2008). While these studies showed no negative effects of *Bt* corn, they mainly focused on aboveground arthropods, particularly the aerial fauna, and not on surface- and soil-dwelling invertebrates. As such, it is imperative to conduct studies covering invertebrate-corn interactions in all layers of the agroecosystem, to improve our ecological understanding of the impact of large-scale application of transgenic corn.

This study aimed to generate solid data on the effects of transgenic corn on invertebrates in a Philippine setting, representing a wet tropical environment. Specifically, this study assessed the impact of long-term cultivation of transgenic corn (*Bt* and *Bt*HT) on non-target organisms in terms of changes in abundance, species richness, and guilds of invertebrates found in different ecosystem layers, including aerial-, surface-, and soil-dwelling species.

Methods

Study area

The study was undertaken in the province of Isabela ($17^0$20'N; $121^0$53'E) in northeast Luzon, the Philippines. Isabela is among the top six yellow corn producing provinces in the country, with a total area of 101,901 hectares used for corn cultivation, comprising 31,190 hectares of river floodplains (frequently flooded), 23,276 hectares of broad plains (occasionally flooded), and 47,436 hectares of hilly land (Anonymous, 2006).

The selected research sites cover the lower floodplain areas along the Cagayan River in the municipalities of Cabagan (near Pilig Abajo village), Tumauini (near Tunggui village), and Ilagan (near Angassian village). All sites have been major corn areas for more than 50 years. White corn was the most cultivated variety up to the mid-1980s, when yellow corn became economically viable due to the rapid increase in demand for animal feed. In recent years, transgenic corn varieties have become widely cultivated in the area.

The Cabagan and Ilagan sites are classified as first-class cornfields because of the favorable moisture and nutrient content of the clayey alluvial soils. While monocropping is the basic practice in Tumauini and Ilagan, multiple cropping with tobacco, legumes or vegetables is common practice in Cabagan (Fig. 1). The corn-based cultivation systems are all rainfed, with yields during both the dry (November to April) and wet (June to October) seasons. Corn growth and development normally take 110-120 days.

Figure 1. Study sites showing examples of mono-cropping system at the Ilagan and Tumauini sites (left photo) and a multiple-cropping system at the Cabagan site, where legumes are planted beside conventional corn (center photo) and tobacco planted near conventional and *Bt* corn (right photo)

Sampling

The research, which was conducted during the dry cropping season from January to May 2008, started with a study of the differences between the various corn growth stages, i.e. the vegetative, tasselling, and maturity stages, from January to March.

Five fields with transgenic corn and five with non-transgenic corn were selected at three sites, totaling 30 cornfields. Since isolated and small sized fields could affect the abundance of aerial invertebrates (Prasifka *et al.,* 2005), which might bias the results, the study included only large fields (ranging from 1.4 to 3.79 hectares, Table 1) that were not isolated. The selected fields were located almost at the center of a large area of continuous corn landscape, so surrounding riparian habitats were assumed to have negligible effects on infield biodiversity.

Sampling of invertebrates from different habitats within the cornfields was accomplished with the same techniques used in a previous study (Afidchao *et al*., Chapter 3). Yellow sticky cards, pitfall traps and soil cores were used for aerial species, aboveground dwellers and belowground dwellers, respectively. Yellow sticky cards were used thrice per cropping season (i.e. during the vegetative, tasselling and maturity stages of the corn). Four sticky traps were placed within each sub-plot and left in the fields for two nights. Pitfall trap samplings were done twice per major corn stage per cropping season. Soil cores were taken with an improvised metal core during the maturity stage of the corn, to prevent damage to the standing crop.

Table 1. Average (±se) field size and soil physico-chemical characteristics of the 30 cornfields surveyed in Isabela province, The Philippines

	Physical Characteristics		Chemical Characteristics					
Corn variety	Field Size (hectare)	Soil Texture	Soil pH	% N	P (ppm Olsen's Mtd)	K (ppm H2SO4 Etxn)	%OC	%OM
Bt	3.79±3.39	Sandy to silt loam	5.76±0.73	0.76±0.17	10.19±8.91	92.78±51.63	0.49±0.25	0.85±0.43
*Bt*HT	1.86±1.60	Clay to silt clay	5.69±0.68	0.71±0.20	11.79±8.23	80.42±18.30	0.50±0.19	0.86±0.33
non-*Bt*	1.40±0.92	Sandy to silt loam	5.79±0.63	0.75±0.25	11.27±8.69	106.83±61.96	0.52±0.17	0.90±0.29

The main physical and chemical characteristics of the soils in the different cornfields are presented in Table 1. All *Bt* fields surveyed had been cultivated with *Bt* corn for a minimum of two consecutive years. Although the *Bt* and *Bt*HT corn varieties in this study were from genetically different corn lines, they were presumed to be similar, both containing the Cry1Ab protein only (Rauschen *et al*., 2009; Rauschen *et al*., 2010). More than half (18 out of 30) of the fields surveyed had been subject to pesticides, viz. insecticides (trade names: Furadan or Cymbush) or herbicides (Round-up Ready or Gramoxone), using a lever-operated knapsack sprayer (Table 2).

Table 2. Pesticides used by the farmer respondents (N = 30) on the cornfields surveyed (N = 30) during the first cropping season of 2008 in Isabela Province, The Philippines

Corn variety	Farmers	Insecti-cides	Active Ingredients	Farmers using the insec-ticide	Herbicide[4]	Active Ingredients	Farmers using the herbicide
Bt	9	Furadan Cymbush	Carbofuran[1] Cypermethrin[1]	2 1	HT	Glyphosate[2]	4
*Bt*HT	6	Cymbush	Cypermethrin[1]	1	HT	Glyphosate[2]	4
non-*Bt*	15	Furadan Cymbush	Carbofuran[1] Cypermethrin[1]	3 3	HT Gramoxone	Glyphosate[2] Paraquat[3]	1 1

[1]Source: Snelder *et al*. (2008)
[2]Source: Williams *et al.* (2000)
[3]Source: www.syngenta.com/en/products_brands/gramoxone
[4]Round-up Ready

Confounding variables
Information on confounding variables (i.e., cornfields' size, elevation and location, chemical inputs, plant height, percent weed cover, and soil physical and chemical properties) was gathered and considered in the analysis. Pesticide input was estimated by interviewing 30 farm-owner respondents about the use of pesticides in their fields. Plant height measurements were taken at the cob development stage. Soil analysis used four samples from each cornfield, weighing a minimum of one kilogram per sample. Samples were analyzed at the Regional Soil and Water Laboratory of the Department of Agriculture in Tuguegarao City. Soil texture was determined using the "Texture-by-Feel Method" (Franzmeier & Owens, 2008). Soil chemical properties were analyzed following standard methods, i.e. a 1:5 soil-water ratio to determine soil pH (Mahaney *et al*., 2007), the Walkley-Black method for organic matter (OM), the Kjeldhal method for total nitrogen (N), the Olsen/Bray method for available soil phosphorus (P), and the flame photometric method for exchangeable soil potassium (K).

Statistical design and analysis
Statistical analyses used the Restricted Maximum Likelihood (REML) and Generalized Linear Mixed Model (GLMM). These models were used to be able to include both fixed (corn stage, corn variety, isolines, cornfields physical characteristics, soil physico-chemical properties and chemical applications) and random factors (site, field within site, sampling method) in the analyses (Quinn and Keough, 2007).

For each response variable, we firstly employed a general model including corn stage and corn variety and all the chosen confounding variables, including isoline, as fixed factors. Also, the random factors were included. The general model was then simplified using a stepwise regression analyses. Model simplification involved gradual elimination of the confounding variables. For that a Maximum Likelihood fitting of the models was applied and subsequent models were compared based on AIC. The final model has the lowest AIC value, but may retain confounding variables with low p-values. We will call this the 'best fitting model'. In order to meet the model assumptions, natural log transformations were applied after adding 1 to the abundance or species number. The analysis was performed in R Statistical software version 2.7.3. Bonferroni correction was done by dividing the significance level by the number of models within the specific analysis.

Results

Growth stages
The sampling of non-target invertebrates during three different corn growth stages produced 31,171 individuals, including 8,557 aerial and 22,614 surface dwellers, belonging to 63 aerial and 56 surface species. Invertebrate abundance and species richness were significantly different between corn stages, with aerial dwellers being most abundant during the vegetative stage, while surface dwellers dominated the maturity stage (Fig. 2). However, no significant interaction was found between corn stage and corn variety (*Bt* versus non-*Bt*), neither in terms of abundance nor of species richness (Table 3). These results justify the focus of the study on one growth stage, that of maturity, in the next sections.

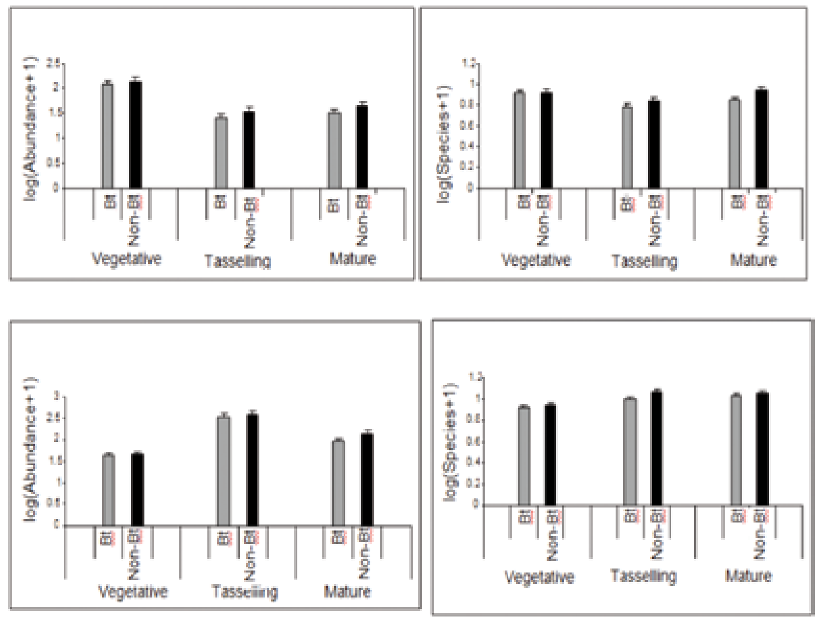

Figure 2. Estimated means (±se) of abundance (left graphs) and species richness (right graphs) of non-target aerial- (upper graphs) and surface- (lower graphs) dwelling invertebrates per corn stage of each corn variety, from the mixed regression analyses (REML, table 3)

Table 3. Results of mixed regression analyses (REML) of abundance and species richness of surface-dwelling and aerial invertebrates, with growth stages and corn variety as fixed factors and field as random factor. (n.d.f.: numerator of degrees of freedom; d.d.f.: denominator of degrees of freedom; Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01; * = equivalent to p<0.05; (*) = equivalent to p<0.10).

	Wald stat.	n.d.f.	F	d.d.f.	p	Sign B
Abundance of aerial species						
Growth stage	244.22	2	122.11	106.0	<0.001	*
Corn variety	1.10	1	1.10	8.0	0.325	ns
Stage*Variety	1.01	2	0.51	106.0	0.604	ns
Abundance of surface species						
Growth stage	412.27	2	206.14	106.0	<0.001	*
Corn variety	1.02	1	1.02	8.0	0.341	ns
Stage*Variety	3.66	2	1.83	106.0	0.166	ns
Species richness of aerial species						
Growth stage	20.15	2	10.08	106.0	<0.001	*
Corn variety	2.57	1	2.57	8.0	0.147	ns
Stage*Variety	3.84	2	1.92	106.0	0.152	ns
Species richness of surface species						
Growth stage	39.46	2	19.73	106.0	<0.001	*
Corn variety	2.70	1	2.70	8.0	0.139	ns
Stage*Variety	0.85	2	0.43	106.0	0.654	ns

Varieties: transgenic vs. non-transgenic corn

At the three research sites (Table 4), 21,639 individual non-target invertebrates were recorded, belonging to 129 different species. Sixty-nine percent or 14,995 individuals were surface dwellers, 26 percent or 5,585 individuals were aerial dwellers and only five percent or 1,059 individuals were soil dwellers.

Table 4. Total counts of Invertebrate assemblage collected in transgenic and non-transgenic corn fields in Isabela Province, Philippines during the dry cropping season 2008. FG= Functional guild (H=herbivore, O=omnivore, Pa=parasitoid, Pr=predator, De=detritivore); ID= Types of invertebrate dwellers (AD= aerial dweller, SF = soil fauna, SD = surface dweller)

Species	FG	ID	Abundance
Empoasca fabae	*H*	*AD*	2171
Drosophila melanogaster	De	*AD*	844
Sciara sp.	De	*AD*	615
Circulife tenellus	*H*	*AD*	358
Phaenicia sericata	De	*AD*	276
Micraspis discolor Fabr.	*Pr*	*AD*	262
Aphidalestes sp.	*H*	*AD*	179
Dicampus sp.	Pa	*AD*	113
Heppelates sp.	*H*	*AD*	77
Allonemobius fasciatus	O	*AD*	57
Pnyxia sp.	De	*AD*	45
Tetragnatha mandibulata	*Pr*	*AD*	44
Cheilomenes sexmaculatus	*Pr*	*AD*	40
Ephydra sp.	*H*	*AD*	31
Rhysella nitida	Pa	*AD*	30
Draeculacephala mollipes	*H*	*AD*	28
Erythroneura viridis	*H*	*AD*	28
Phytodictus vulgaris	Pa	*AD*	27
Solenopsis invicta	*O*	*AD*	27
ZZ-Species D	*Pr*	*AD*	26
Fannia sp.	De	*AD*	23
Aulacophora sp.	*H*	*AD*	22
Xestocephalus sp.	*H*	*AD*	20
Salticus sp.	*Pr*	*AD*	18
Tettigella viridis	*H*	*AD*	17
Orchesia sp.		*AD*	16
Oxyopes sp.	*Pr*	*AD*	15
Myrmarachne maxillosa	*Pr*	*AD*	14
Chelonus sp.	Pa	*AD*	12
Olibrus sp.	De	*AD*	10
Telleogryllus sp.	*O*	*AD*	10
Isdromus sp.	Pa	*AD*	9
Dolichopus sp.	*Pr*	*AD*	7
Tetragnatha sp.	*Pr*	*AD*	7
Diatraes sp.	Pa	*AD*	6
Andrena sp.	*Pr*	*AD*	5
Phormia regina	De	*AD*	5
Rhagoletis pomonella	*H*	*AD*	5
Scirtes sp.	*H*	*AD*	5
Cocinella sp.	*Pr*	*AD*	4
Gastrolinoides sp.	*H*	*AD*	4
Heterothrips sp.	*H*	*AD*	4
Phobocampe disparis	Pa	*AD*	4
Amerimicromus sp.	*Pr*	*AD*	3
Chortoicetus sp.	*H*	*AD*	3
Labidura truncate	*H*	*AD*	3
Solenopsis globularia	*O*	*AD*	3
Syrphus ribesii	*O*	*AD*	3
ZZ-Species A	*O*	*AD*	3
ZZ-Species B	De	*AD*	3
Balaurara sp.	*O*	*AD*	2
Conocephalus sp.	*H*	*AD*	2
Eurema hecabe	*H*	*AD*	2
Geocoris sp.	*Pr*	*AD*	2
Hesperus sp.	*Pr*	*AD*	2
Labidura sp.	*H*	*AD*	2
Liodontomerus sp.	Pa	*AD*	2
Oncocephalus cenfusus	*Pr*	*AD*	2
Pholcus phalangoides	*Pr*	*AD*	2
Solenopsis pergundei	*O*	*AD*	2
Adelphocoris ropidus	*H*	*AD*	1

Species	FG	ID	Abundance
Agrypnus sp.	*H*	*AD*	1
Apion sp.	*H*	*AD*	1
Aufidus sp.	*H*	*AD*	1
Cercion calamorum	*Pr*	*AD*	1
Cylas formicarius elegantulus	*H*	*AD*	1
Draeculacephala sp.	*H*	*AD*	1
Euphorocera sp.	Pa	*AD*	1
Euxoa excellens	*H*	*AD*	1
Fornax sp.	*H*	*AD*	1
Goelerucella maculicollis	*H*	*AD*	1
Hemiptarsemus sp.	Pa	*AD*	1
Iphiaulax sp.	Pa	*AD*	1
Lucilia ilustris	De	*AD*	1
Meteosus sp.	Pa	*AD*	1
Metoponium sp	*H*	*AD*	1
Ostrinia furnacalis	*H*	*AD*	1
Perkinsiella sp.	*H*	*AD*	1
Scaphisoma sp.	*Pr*	*AD*	1
Sericesthis geminate	*Pr*	*AD*	1
Syrphus torus	*O*	*AD*	1
Tachys sp.	*Pr*	*AD*	1
Trichiohelcon sp.	Pa	*AD*	1
ZZ-Species C	*H*	*AD*	1
Total # of AD Individuals			**5,585**
Total # of AD Species			**84**
Hesperus sp.	Pr	SD	5499
Pselaphus sp.	Pr	SD	2637
Myara sp.	O	SD	1949
Sippuna sp.	Pr	SD	1172
Oxidus gracilis	De	SD	701
Drosophila melanogaster	De	SD	667
Albonemobius fasciatus	O	SD	599
Adelocera sp	H	SD	365
Solenopsis invicta	O	SD	307
Solenopsis globularia	O	SD	197
Cocinella sp.	Pr	SD	137
Labidura truncate	H	SD	131
Cymindis sp.	Pr	SD	93
Sciara sp.	De	SD	93
Hister sp.	Pr	SD	78
Micraspis discolor Fabr.	Pr	SD	70
Lumbricoides sp.	De	SD	46
Monomorium minimum	O	SD	43
Platyzosteria nitidella	O	SD	39
Callibaetis sp.	O	SD	35
Phaenicia sericata	De	SD	35
Adrisa sp.	Pr	SD	19
Draeculacephala mollipes	H	SD	17
Heppelates sp.	H	SD	15
Ctenicera resplendens	H	SD	11
Brachymeria sp.	Pa	SD	8
Euxoa excellens	H	SD	8
Thiara sp.	De	SD	7
Chortoicetus sp.	O	SD	6
Musca domestica	De	SD	4
Anoplolepis longipes	O	SD	1
Onthophagus sp.	H	SD	1
Osorius sp.	Pr	SD	1

Species	FG	ID	Abundance
Pachycondyla sp.	O	SD	1
Ploiaria regina	Pr	SD	1
Tetragnatha mandibulata	Pr	SD	1
white grub	H	SD	1
Total # of SD Individuals			**14,995**
Total # of SD Species			**36**
Anomalomyrma taylori	*O*	*SF*	292
Lumbricoides sp.	*De*	*SF*	222
Solenopsis globularia	*O*	*SF*	67
Nabis ferus	*Pr*	*SF*	60
Thiara sp.	*De*	*SF*	53
Oxidus gracilis	*De*	*SF*	47
Adrisa sp.	*Pr*	*SF*	42
Oligomyrmex nitidulus	*O*	*SF*	37
Anoplolepis longipes	*O*	*SF*	36
white grub	*H*	*SF*	31
Xylion sp.	*Pr*	*SF*	31
Cerapachys augustae	*O*	*SF*	25
Pheidole megacephala	*O*	*SF*	16
Monomorium minimum	*O*	*SF*	12
Oxyopes sp.	*Pr*	*SF*	12
Sericesthis geminate	*Pr*	*SF*	12
Titanolabis colossea(Dohrn)	*Pr*	*SF*	11
Paratrechina longicormis	*O*	*SF*	9
Cylisticus convexus	*De*	*SF*	6
Blatella sp.	*O*	*SF*	5
Nomadacris gutturosa	*H*	*SF*	5
Pselaphus sp.	*Pr*	*SF*	4
Geophilomorpha sp.	*Pr*	*SF*	3
Hesperus sp.	*Pr*	*SF*	3
Platyzosteria nitidella	*O*	*SF*	3
Coniontis sp.	*H*	*SF*	2
Dermestes sp.	*H*	*SF*	2
Geophilus electricus	*Pr*	*SF*	2
Pinophilus sp.	*Pr*	*SF*	2
Cryptopone mjobergi	*O*	*SF*	1
Cymindis sp.	*Pr*	*SF*	1
Nomius pygmaeus	*Pr*	*SF*	1
Onthophagus declivis	*H*	*SF*	1
Stegobium panicerum	*Pr*	*SF*	1
Talia sp.	*O*	*SF*	1
Trogoderma sp.	*H*	*SF*	1
Total # of SF Individuals			**1,059**
Total # of SF Species			**36**

*GRAND TOTAL OF INVERBRATE INDIVIDUALS ACCOUNTED=**21,639***

The non-*Bt* corn plots had the highest non-target invertebrate abundance and species richness, while the *Bt* corn plots had the lowest abundance and species richness (Table 5). This same pattern emerged for the aerial, surface, and soil dwellers. The apparent significance of the difference for the aerial dwellers did not hold under Bonferroni correction for multiple models (Table 6), but may indicate relatively large differences in this group. The lowest abundance of aerial dwellers was found for *Bt*HT corn (Table 6).

Our best fitting models show that soil pH, potassium and nitrogen have significant influence on invertebrate abundance and that nitrogen also has a slight effect on species richness. However, the effects of soil chemical characteristics did not take away the effect of corn variety. Non-*Bt* corn has highest abundance and species richness (Table 5).

Regarding the effects of confounding variables on different invertebrate dwellers (Table 6), the best fitting models show a high effect of soil pH and a slight effect of soil organic matter content on the aerial abundance but greater effect was manifested of corn variety. For aerial species richness, herbicides manifested a slight influence but greater effect is manifested of corn variety.

The pesticide application rate was highest in *Bt* cornfields, with herbicides applied most frequently in *Bt*HT and insecticides most frequently in non-*Bt* cornfields. However, application of pesticides did not differ significantly between the corn varieties (Table 7, Fig. 3).

Table 5. Results of mixed regression analyses (REML) of abundance and species richness of all invertebrates, with corn variety, soil pH, soil nitrogen (N), and soil potassium (K) contents, as confounding variable/fixed factors and field within site within sampling method as random factor. Mean abundance per dweller was ln(x+1) transformed. SD = standard deviation. P-values in italics are of contrasts. Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01; * = equivalent to p<0.05; (*) = equivalent to p<0.10).

	Mean± SD	F value	p-value	Sign B
I. Total Abundance				
Variate				
Corn variety		7.384	0.0011	*
- Non-Bt corn (Intercept)	3.490 ± 1.405			
- *Bt* corn	3.146 ± 1.491		*0.0019*	*
- *Bt*HT corn	3.162 ± 1.382		*0.3553*	ns
Covariates				
Soil pH		6.311	0.0126	*
N		6.080	0.0143	*
lnK		7.333	0.0072	*
II. Total Species Richness				
Variate				
Corn variety		4.129	0.0197	*
- Non-Bt corn (Intercept)	7.511 ± 4.129			
- *Bt* corn	6.824 ± 3.266		*0.0069*	*
- *Bt*HT corn	7.028 ± 3.113		*0.2455*	ns
Covariates				
N		4.616	0.0326	(*)

Table 6. next Page (page 85):

Table 6. Results of mixed regression analyses (REML) of abundance and species richness of aerial, surface and soil invertebrates, with corn variety, corn isolines, weed cover (WC), plant height (PH), soil texture, insecticide, herbicide, ln(x+1) field size (lnAr), ln(x+1) field elevation (lnElev), field longitude (Longi) and latitude (Lat), soil organic matter (OM), soil pH, soil nitrogen (N), soil phosphorus (P) and soil potassium (K) contents as confounding variable/fixed factors and field within site as random factor. Only the best fitted models are given. Mean abundance per dweller was ln(x+1) transformed. SD = standard deviation. P-values in italics are of contrasts. Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01; * = equivalent to p<0.05; (*) = equivalent to p<0.10).

Tabel 6.

	Mean± SD	F-value	p-value	Sign B
I. ABUNDANCE				
1. Aerial Dweller				
Variate				
Corn variety		5.635	0.0099	*
- Non-Bt corn (Intercept)	3.885 ± 0.568			
- *Bt* corn	3.500 ± 0.540		*0.0050*	*
- *Bt*HT corn	3.360 ± 0.380		*0.0412*	ns
Covariates				
OM		4.132	0.0533	ns
pH		5.796	0.0181	(*)
2. Surface Dweller				
Variate				
Corn variety		0.931	0.4123	ns
- Non-Bt corn (Intercept)	4.688 ± 0.625			
- *Bt* corn	4.619 ± 0.515		*0.2635*	ns
- *Bt*HT corn	4.500 ± 0.486		*0.0614*	ns
Covariates				
Isolines		3.648	0.0126	(*)
pH		8.573	0.0043	*
lnElev		12.594	0.0023	*
Longi		6.622	0.0191	(*)
3. Soil Dweller				
Variate				
Corn variety		4.161	0.0275	ns
- Non-Bt corn (Intercept)	1.897 ± 1.035			
- *Bt* corn	1.320 ± 0.672		*0.0365*	ns
- *Bt*HT corn	1.625 ± 1.068		*0.0144*	(*)
Covariates				
lnK		6.237	0.0144	(*)
II. SPECIES RICHNESS				
1. Aerial Dweller				
Variate				
Corn variety		5.787	0.0089	*
- Non-Bt corn (Intercept)	7.967 ± 1.965			
- *Bt* corn	6.556 ± 2.335		*0.3061*	ns
- *Bt*HT corn	7.250 ± 1.700		*0.6839*	ns
Covariates				
Herbicides		7.512	0.0114	(*)
2. Surface Dweller				
Variate				
Corn variety		1.366	0.2921	ns
- Non-Bt corn (Intercept)	10.450 ± 2.134			
- *Bt* corn	10.528 ± 2.360		*0.2635*	ns
- *Bt*HT corn	9.792 ± 2.519		*0.0614*	ns
Covariates				
Isolines		5.324	0.0058	*
ST		4.520	0.0242	(*)
pH		11.716	0.0009	*
lnElev		3.049	0.1063	ns
Longi		2.767	0.1221	ns
Lat		9.519	0.0094	(*)
3. Soil Dweller				
Variate				
Corn variety		2.034	0.1731	ns
- Non-Bt corn (Intercept)	4.117 ± 1.795			
- *Bt* corn	3.389 ± 1.572		*0.4525*	ns
- *Bt*HT corn	4.042 ± 1.829		*0.7277*	ns
Covariates				
Isolines		2.194	0.0981	ns
PH		2.034	0.1731	ns
N		1.295	0.2582	ns
Insecticides		1.310	0.2692	ns
lnAr		3.360	0.0855	ns

Table 7. Results of mixed logistic regression analyses (GLMM) of pesticide use, with corn variety as fixed factor and site as random factor. (n.d.f.: numerator of degrees of freedom; d.d.f.: denominator of degrees of freedom.

Pesticide use models	Wald stat.	n.d.f.	F	d.d.f.	p
All pesticides ~ Corn variety	2.07	2	1.03	27.0	0.369
Herbicides ~ Corn variety	6.26	2	3.11	24.1	0.063
Insecticides ~ Corn variety	0.89	2	0.44	27.0	0.646

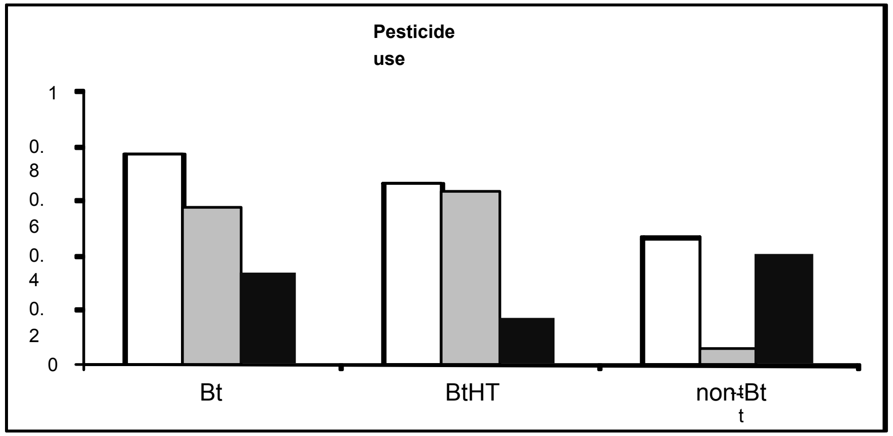

Figure 3. Estimated frequency of pesticide use per corn variety (GLMM, table 6). Open bars: all pesticides; grey bars: herbicides; black bars: insecticides

Functional guilds

For all functional guilds except parasitoids species, the highest abundance and species richness were recorded in non-*Bt* cornfields. Parasitoids were found to have higher species richness in *Bt* corn though not significantly different from other corn variety. Omnivores and detritivores were shown to have relatively large differences, although the differences were not statistically significant under Bonferroni correction for multiple testing (Table 8).

Table 8. Results of mixed regression analyses (REML) of abundance and species richness of all herbivore, detrivore, omnivore, predator, and parasitic invertebrates, with corn variety, corn isolines, weed cover (WC), plant height (PH), soil texture, herbicide, ln(x+1) field elevation (lnElev), soil organic matter (OM), soil pH, soil nitrogen (N), soil phosphorus (P) and soil potassium (K) contents, as confounding variable/fixed factors and field within site within sampling method as random factor. P values: *** = p<0.001, ** = p<0.01, * = <0.05, (*) = <0.10. Abundance and species richness of all the functional guilds were ln(x+1) transformed. SD = standard deviation. P-values in italics are of contrasts. Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01; * = equivalent to p<0.05; (*) = equivalent to p<0.10).

	Mean± SD	F value	p-value	Sign B
I. ABUNDANCE				
1. Predator				
Variate				
Corn variety		1.182	0.3126	ns
- *Non-Bt corn (Intercept)*	1.725 ± 1.322			
- *Bt* corn	1.566 ± 1.354		*0.0671*	ns
- *Bt*HT corn	1.601 ± 1.367		*0.8956*	ns
Covariates				
Isolines		2.264	0.0468	ns
PH		3.882	0.0527	ns
lnElev		2.863	0.0950	ns
2. Herbivores				
Variate				
Corn variety		1.636	0.2013	ns
- *Non-Bt corn (Intercept)*	1.400 ± 1.181			
- *Bt* corn	1.255 ± 1.322		*0.0796*	ns
- *Bt*HT corn	1.175 ± 1.104		*0.9088*	ns
Covariates				
pH		2.102	0.1483	ns
lnP		2.422	0.1208	ns
3. Omnivores				
Variate				
Corn variety		3.170	0.0478	ns
- *Non-Bt corn (Intercept)*	1.426 ± 1.376			
- *Bt* corn	1.198 ± 1.209		*0.0026*	(*)
- *Bt*HT corn	1.152 ± 1.192		*0.2586*	ns
Covariates				
ST		2.911	0.0400	ns
OM		2.634	0.1088	ns
pH		4.019	0.0460	ns
lnK		6.417	0.0119	ns
Herbicide		9.639	0.0027	*
4. Detrivores				
Variate				
Corn variety		2.494	0.0892	ns
- *Non-Bt corn (Intercept)*	1.982 ± 1.256			
- *Bt* corn	1.786 ± 1.194		*0.0336*	ns
- *Bt*HT corn	1.725 ± 1.187		*0.1064*	ns
Covariates				
lnElev		3.687	0.0585	ns
5. Parasitoids				
Variate				
Corn variety		1.327	0.2711	ns
- *Non-Bt corn (Intercept)*	0.114 ± 0.348			
- *Bt* corn	0.102 ± 0.296		*0.5362*	ns
- *Bt*HT corn	0.044 ± 0.188		*0.0818*	ns
Covariates				
N		4.335	0.0383	ns
lnK		2.613	0.1072	ns

	Mean± SD	F value	p-value	Sign B
II. SPECIES RICHNESS				
1. Predator				
Variate				
Corn variety		1.811	0.1708	ns
- Non-Bt corn (Intercept)	0.960 ± 0.580			
- *Bt* corn	0.879 ± 0.681		*0.4368*	ns
- *Bt*HT corn	0.874 ± 0.597		*0.6065*	ns
Covariates				
Isolines		2.111	0.0622	ns
2. Herbivores				
Variate				
Corn variety		2.713	0.0725	ns
- Non-Bt corn (Intercept)	0.750 ± 0.513			
- *Bt* corn	0.632 ± 0.509		*0.0105*	ns
- *Bt*HT corn	0.703 ± 0.540		*0.6550*	ns
Covariates				
pH		8.047	0.0049	*
N		2.432	0.1201	ns
3. Omnivores				
Variate				
Corn variety		2.043	0.1365	ns
- Non-Bt corn (Intercept)	0.695 ± 0.621			
- *Bt* corn	0.616 ± 0.616		*0.0147*	ns
- *Bt*HT corn	0.661 ± 0.604		*0.3720*	ns
Covariates				
Herbicide		2.272	0.1358	ns
4. Detritivores				
Variate				
Corn variety		3.020	0.0545	ns
- Non-Bt corn (Intercept)	1.076 ± 0.458			
- *Bt* corn	0.961 ± 0.478		*0.0375*	ns
- *Bt*HT corn	0.961 ± 0.538		*0.0663*	ns
5. Parasitoids				
Variate				
Corn variety		1.419	0.2480	ns
- Non-Bt corn (Intercept)	0.085 ± 0.239			
- *Bt* corn	0.087 ± 0.240		*0.8632*	ns
- *Bt*HT corn	0.038 ± 0.160		*0.1061*	ns
Covariates				
N		4.857	0.0284	ns

Discussion

Growth stages

The high abundance of non-target aerial invertebrates in the vegetative stage and the low abundance in the tasselling stage can be attributed to plant height and density of canopy. These corn plant characteristics limit the flight of aerial dwellers only in the inner parts of the cornfields, whereas aerial dwellers can move freely in a field with an open and lower density of canopy field. This result supports the study by Alston *et al*. (1991) which found larger Corn Earworm, *Helicoverpa zea* (Boddi) and larval populations in more open canopies. Similarly, the within-plant distribution of Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) was highest in the pre-tasselling stage, mostly in the wrapped leaves of the whorl (Labate 1993). The findings of the survey by Hagen *et al.* (2010) using fly traps also coincides with our result, with more abundant aerial arthropods recorded in both native and invaded forests with lower canopies.

Abundance and species richness

This study demonstrates that the abundance and species richness of all non-target invertebrates are slightly lower in *Bt* cornfields than in non-*Bt* cornfields. Pesticide input did not confound these results, i.e., the abundance and species richness remained lowest in *Bt* cornfields, whether or not pesticides were used. These results confirm the study by Marvier *et al*. (2007), albeit that in their case the adverse effects on non-target invertebrates were only demonstrated for experimental *Bt* fields under equal management as the non-*Bt* control fields, i.e. involving no pesticide use. It should be noted however that the adverse effects of *Bt* corn in our study were weak. In addition, increasing or decreasing pH and nitrogen compositions of the soil directly or indirectly favored invertebrate abundance which is probably due to the response of species to the presence of these chemicals or pH state supportive to their physiological needs. A study by Fischer and Führer (1990) showed that soil acidity plays a major role in the nematode's ability to parasitize Cephalcia nymphs and soil with pH levels below 4.0 may limit the nematode's host-finding. Moreover, invertebrates can tolerate soil acidity at different ranges like termites which are most tolerant to acidity with maximum abundance at pH 4 to 6 and coleopteran larvae are abundant only in soils with high pH whilst ants are not affected by soil acidity (Lavelle *et al*., 1995). Also, as stated in Lavelle *et al*. (1995), invertebrates are abundant and active population may exist in soil with pH 3.8 to 4.0.

On invertebrate dwellers

Abundance and species richness of non-target surface-dwelling invertebrates were not affected by transgenic corn varieties. This finding supports previous studies (Toschki *et al*., 2007; Peterson *et al*., 2011) in temperate regions, which concluded that some non-target species such as carabid beetles and spiders were not affected by *Bt* corn. The results of the current study further indicate that aerial and soil-dwelling species are more susceptible to *Bt* corn. The transgenic *Bt* and *Bt*HT corn had lower abundance and species richness of aerial species, and we also found a nearly significantly lower abundance of soil-dwelling species, though significance was lost after Bonferroni correction.

The unintended effects of *Bt* toxin on the abundance and species richness of the aerial- and soil dwelling invertebrates other than the target pest species (ACB) may be caused by numerous factors. One conceivable reason is the accumulation of *Bt* pollen in the axils of the leaves and its deposition within the cornfield (Hansen-Jesse and Obrycki, 2000; Li *et al.,* 2005). Furthermore, the ability of aerial dwellers to move from one plant to another may expose them to high concentration of *Bt* toxin in wind-dispersed pollen even outside fields (Koziel *et al*., 1993; Fearing *et al*., 1997). Although Obrist *et al*. (2006) found no or negligible amounts of Cry1Ab protein in some predators (hemerobiids*, Nabis sp*., *Hippodamia sp., Demetrias sp*.), the dilution of protein in the animal body may vary between species and groups of organisms. *Bt* toxin has been detected in aphids, a herbivore, with a mean concentration of 2.0±0.8 ppb (Burgio *et al*., 2007).

Cry1Ab delta-endotoxin, the active component of *Bt* corn seeds in the Philippines, persists in the soil, and can still be detected after 240 days in tillage and 200 days in no tillage cornfields (Zwahlen *et al*., 2003). After four consecutive years of *Bt* corn cultivation, the Cry1Ab protein can be detected even in the rhizosphere soil (Icoz *et al*., 2008). Soil properties of cornfields may also play a critical role in Cry1Ab protein absorption. An increase in the amount of surface clay particles and a decrease in organic carbon content will lead to an increased absorption capacity (Nguyen and Jehle, 2007). The fields we surveyed consist of clayey soils, and the organic carbon contents in the *Bt*HT and *Bt* cornfields are lower than in those in the non-*Bt* cornfields (Table 2). The persistence and absorption of Cry1Ab protein in the field soils (Saxena and Stotzky, 2000; Saxena *et al*., 2002) may have been one of the factors causing the decreased abundance of non-target aerial- and soil- dwelling invertebrates in the *Bt*HT and *Bt* cornfields.

Lastly, as noted from our analysis on confounding variables, some soil chemical properties of the surveyed cornfield could potentially affect the aerial or foliage dwelling invertebrates. Since foliage dwellers, mostly herbivores, are not feeding on the soil but feed on plant parts, hence the effect is considered to be indirect. Our result was supported by previous studies done by Prudic *et al*. (2005) and Kinney *et al*. (1997). Their studies showed that alteration of soil chemical properties can have indirect effects to the insect's performance. The alteration of soil chemistry can modify plant chemistry as well as the performances of insects particularly the herbivory insects (Prudic *et al*., 2005; Kinney *et al*., 1997). In particular, Prudic *et al*. (2005) showed that fertilized fields increased the availability of soil nutrients which in turn caused the host-plant's foliar nitrogen to increase and its chemical defense against pests to decrease. Also, Kinney *et al*. (1997) found out that the feeding performance of the Penultimate gypsy moth larvae (*Lymantria dispar*) can be affected by elevated CO_2 and NO_3 in the soil. The increased soil CO_2 and/or low NO_3 caused the plants to increase the concentrations of starch, condensed tannins and ellagitannins increased which can affect larvae feeding performance.

Guilds

Among the functional guilds recorded in the *Bt* cornfields, the omnivores seem to be affected the most in terms of abundance and species richness. This may be explained by the way *Bt* toxin spreads through the food web. Groot and Dicke (2002), for example, refer to direct effects of *Bt* corn when non-target invertebrates feed on plant parts containing the toxin, or indirect effects when they prey on herbivores containing the toxin. These effects also vary between species, depending on differences in *Bt* toxin ingestion (Head *et al*., 2001; Raps *et al*., 2001; Obrist *et al.,* 2005). In addition, ingestion is not the only way in which non-target species can be affected. Experiments by Prasifka *et al.* (2007), for instance, attributed the decreased weight and feeding habits of monarch butterfly larvae to the avoidance behavior of larvae when exposed to *Bt* expressing anthers. In the meta-analysis by Wolfenbarger et *al.* (2008), omnivores were more abundant in insecticide-sprayed non-*Bt* corn than in non-sprayed *Bt* corn, and the high abundance of omnivores, mostly soil-dwelling, was associated with a reduction in the population of predators, greatly affected by insecticide spraying. Overall, they found that the pesticide effect was stronger than the *Bt* corn effect. In the current study, we found no difference in the influence of pesticides between corn types. Hence, the low abundance of omnivores in *Bt* corn indicates that other causal factors must be taken into account. Omnivores are phytophagous as well as entomophagous invertebrates. Their ability to change prey and feed on plant materials allows them to survive in an environment inimical to specialized entomophagous invertebrates (Coll and Guershon, 2002). However, this also makes them more susceptible to toxin exposure. Nonetheless, further research is needed to uncover the mechanisms.

Conclusion

The study shows that long-term and continuous cultivation of transgenic corn has an impact on humid tropical corn-based agro-ecosystems, in terms of reducing the abundance and species richness of non-target invertebrates. Our results seem to contradict earlier studies in temperate regions, where endotoxin from *Bt* and *Bt*HT corn affected only the targeted pest species (ACB) (Candolfi *et al.,* 2004). As large-scale monocropping of transgenic corn is currently highly prevalent in the Philippines, precautionary measures or effective refuge strategies should be considered to abate serious implications for the biodiversity and sustainability of corn agro-ecosystems. Nonetheless, this study suggests that more research is needed to enable continuous monitoring and to address some emanating ecological issues about recently released *Bt*, *Bt*HT and HT corn lines.

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