purchase the appropriate permission via the Mobile Library.

Copyright Terms:
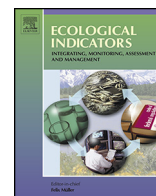
Changes in the dynamics of functional groups in communities of dung beetles in Atlantic forest fragments adjacent to transgenic maize crops

Renata C. Campos *, Malva I.M. Hernández

Department of Ecology and Zoology, Center of Biological Sciences, Federal University of Santa Catarina, Florianópolis, SC 88040-900, Brazil

ABSTRACT

We investigated the composition and structure of dung beetle communities (Coleoptera, Scarabaeidae, Scarabaeinae) inhabiting areas of forest fragments next to either conventional or Bt-transgenic maize crops. The purpose of the study was to examine possible impacts of transgenic plants on non-target organisms associated with mammals through their food chain. In February 2011, we collected a total of 1502 beetles belonging to 33 species in Campos Novos, Santa Catarina state (SC), Brazil. Beetles were captured using 200 pitfall traps distributed among 20 forest fragments, 10 fragments in each site type (adjacent to conventional vs. Bt crops). In the fragments adjacent to conventional maize, 805 dung beetles from 27 species were collected. In the fragments adjacent to Bt-transgenic maize, 697 dung beetles from 27 species were caught. Dung beetle community composition was affected by fragment size and environmental complexity, and by distance between fragments. However, it did not explain the differences related to the two crop types, i.e., the functional group of dwellers was significantly over-represented in the fragments surrounded by transgenic maize. Hence, the dweller species *Eurysternus francinae* and *Eurysternus parallelus* were more frequent and abundant in fragments located near the transgenic maize, while the tunneler species *Onthophagus tristis*, *Uroxys terminalis*, *Ontherus sulcator* and the roller species *Canthon lividus seminitens* were more frequent and abundant in fragments surrounded by conventional maize. This observed impact of transgenic crops on functional group dynamics within dung beetle communities could potentially lead to impaired capacity for feces removal, seed dispersal, edaphic aeration, and incorporation of organic matter in the soil in these areas, as such ecosystem services are not performed by the dominant functional group (i.e., dwellers).

1. Introduction

Insects play a role in many ecological processes and are key dietary components of numerous fauna. They are involved in several trophic interactions in the ecosystem, which makes them important for nutrient cycling within food webs (Miller, 1993; Godfray et al., 1999; Wall and Moore, 1999). Beetles of the subfamily Scarabaeinae (Coleoptera, Scarabaeidae) are extremely important organisms for tropical ecosystem function, since they actively participate in nutrient cycling, with both larvae and adults feeding on decomposing organic matter. Most species feed on feces (coprophagous) or carcasses (necrophagous) (Halffter and Matthews, 1966; Halffter and Edmonds, 1982), both primarily from mammals.

Dung beetles are divided into three functional groups according to their behavior when processing decomposing organic matter.

Rollers, or telecoprid, roll balls of food across the surface of soil some distance away from the initial resource location; tunnelers, or paracoprid, burrow tunnels near or below the food resource in order to carry the food underground; dwellers, or endocoprid, they do not move or store food, but rather only consume it at the initial discovery site (Halffter and Edmonds, 1982; Cambefort, 1991). Dung beetles are detritivores that promote soil removal and incorporation of organic matter in nutrient cycling, helping to clean the environment and to regulate and improve physical and chemical properties of soil (Halffter and Edmonds, 1982; Cambefort and Hanski, 1991; Slade et al., 2007; Simmons and Ridsdill-Smith, 2011). Furthermore, the building of tunnels by some beetles allows soil aeration and hydration, as well as the incorporation of nutrients present in feces, animal carcasses and fruits that are buried in these tunnel spaces (Halffter and Edmonds 1982; Cambefort and Hanski, 1991; Slade et al., 2007; Nichols et al., 2008 and references therein). In neotropical dung beetle communities, tunneler species are found in larger quantities, and contain the greatest diversity of the three functional groups. They are also better resource competitors than other functional groups. Dwellers

* Corresponding author. Tel.: +554837214735.
E-mail address: recalixtocampos@gmail.com (R.C. Campos).

are the least common functional group in most studies (Halffter et al., 1992; Feer, 2000; Scheffler, 2005; Hernández and Vaz-de-Mello, 2009).

Environmental degradation causes changes in dung beetle community structure and composition, resulting in a decrease in species diversity compared to preserved areas (Klein, 1989; Davis et al., 2001; Gardner et al., 2008a). The generally rapid response of dung beetle communities to the effects of destruction, fragmentation and isolation of tropical forests has lead to their recognition as ecological indicators (Favila and Halffter, 1997; Davis et al., 2001; McGeoch et al., 2002; Nichols et al., 2007; Gardner et al., 2008b; Herrnández and Vaz-de-Mello, 2009). In addition to community-level changes, some species tend to have increased or decreased abundance in areas with particular characteristics caused by environmental change (Halffter and Favila, 1993; Viegas et al., 2014). The use of dung beetles to assess the ecological consequences of ecosystem disturbances is both practical and efficient, combining low cost of collection with relative ease of species identification (Gardner et al., 2008a).

Fragmentation is one of the most commonly occurring environmental changes in tropical forests, defined as the process by which a large area of habitat is transformed to a number of smaller patches, isolated from each other by an array of habitat types different than the original type (Wilcove et al., 1986). Continued agricultural expansion, increased fragmentation and subsequent loss of biodiversity is currently a problem in Atlantic forests (Galindo-Leal and Câmara, 2003). The vast majority of Scarabaeinae have highly specific habitats in forest ecosystems (Halffter, 1991; Campos and Hernandez, 2013), and are unable to extend their populations to open areas (Klein, 1989; Spector and Ayzama, 2003). These species are strongly affected by fragmentation and habitat loss, which can both restrict distribution and cause species loss in some locations (Davis and Philips, 2005; Hernández et al., 2014).

Globally, GM transgenic maize was planted on 55.1 million hectares in 2012, single-trait Bt maize occupied 7.5 million hectares. This represents an increase of 1.5 million hectares from 2011, a 25% growth equivalent to 4% of total global biotech. GM maize was grown in 17 countries in 2012, with the largest increase being in Brazil (i.e., nearly three million hectares more than in 2011) (James, 2012).

The nature of the effects of transgenic plant material or plant-expressed Cry1Ab on non-target organisms is highly controversial. A number of articles have reported no effects, while others have described significant negative effects on various invertebrate species (Duan et al., 2010; Hilbeck and Schmidt, 2006; Hilbeck et al., 2008; Obrycki et al., 2001; Wolfsbarger et al., 2008; Zwahlen and Andow, 2005; Harwood et al., 2005; Obrist et al., 2006; Then, 2010). Recently, a heated debate has arisen concerning such effects (e.g., Wickson and Wynne, 2012; Dolezel et al., 2011; Hilbeck et al., 2011; Hilbeck et al., 2012; Bøhn et al., 2012; Romeis et al., 2013). In one example, a meta-analysis of 42 field experiments took into account location, duration, plot sizes, and sample sizes and concluded that the mean abundance of all non-target invertebrate groups, as well as survival and growth, was greater in GM cotton and maize fields than in non-GM fields managed with insecticides. However, if GM crop fields and insecticide-free fields were compared, certain non-target insects were less abundant in GM fields (Marvier et al., 2007).

Most published studies have been based on conspicuous negative parameters such as mortality. More subtle effects, such as aberrations in behavioral or social competence, have not been studied to a comparable extent. Their importance, however, is underscored by evidence of such effects. For example, one study indicated that when honeybees were exposed to a high concentration of Cry1Ab protein the effects were not lethal, but that behavior and vital learning ability was disrupted (Ramírez-Romero et al., 2008). Furthermore, other factors should be considered in the risk analysis of GM plants, such as their effects on pollen dispersal and on vector dispersal of seed or plant debris (e.g., by wind, insects, animals, humans) which can spread materials up to several kilometers away (Emberlin et al., 1999; Heinemann, 2007; Hoyle and Cresswell, 2007; Reuter et al., 2008).

Negative effects of transgenic crop practices on associated fauna via trophic webs are poorly understood (Obrycki et al., 2001; Lovei et al., 2009). When Bt plants were developed and released, scientists postulated that these toxins were highly specific and would not affect organisms outside of the target insect groups (Schuler et al., 1998; Betz et al., 2000). However this did not hold true (Van Frankenhuyzen, 2013), and currently the mode(s) of action of Bt toxins are subject to more controversy than in the early 1990s when Bt plants were first developed and promoted (Vachon et al., 2012).

The use of dung beetles, a taxon with acknowledged importance for the maintenance of ecological processes, can serve as a tool for finding general patterns related to cascade effects of GM crops on wildlife. It is, for instance, understood that when a feces provider species changes its diet, this may have consequences that result in changes in dung beetle community composition and structure via trophic cascade effects. Hence, a trophic link likely exists between GM maize and dung beetles through the mammals that feed on maize, and this link could potentially be utilized in a risk assessment context.

The present study was based on the working hypothesis that for dung beetles communities, nearby cultivation of GM (genetically modified) transgenic maize may enhance the negative effects of forest fragmentation. Dung beetle collections were performed in agricultural fields with either transgenic or conventional maize varieties and adjacent to forest fragments. Dung beetles may be exposed to plant materials and toxins derived from Bt-transgenic maize via three different routes: (1) from feces of maize-consuming animals, which has the prerequisite that transgenic DNA and proteins pass through mammalian or avian gastrointestinal tracts either intact or in biologically meaningful fragments (Lutz et al., 2005; Paul et al., 2010; Guertler et al., 2010); (2) from carcasses of maize-consuming animals, which has the prerequisite of uptake and circulation of transgenic DNA and protein in the blood, and presence in the internal organs (Grønsberg et al., 2011); and (3) from exposure to cry1Ab DNA and Cry1Ab proteins present in the soil (Stotzky, 2002; Saxena and Stotzky, 2000), as well as in domestic animal manure (Gruber et al., 2011). The aim of the present study was to evaluate differences in dung beetle community composition and structure in forest fragments next to conventional vs. Bt-transgenic maize crops, and to reveal possible impacts caused by these environmental changes in organisms via trophic cascade interactions.

2. Methods

The study was conducted in Campos Novos, Santa Catarina state, Brazil (27°23′S, 51°12′O). This region contains numerous Atlantic forest fragments, originally Araucaria forest formations (Leite and Klein, 1990), surrounded by soybean and maize crops. The region has a mild mesothermal climate according to the Köeppen classification system (Pandolfo et al., 2002) with altitude ranging from 710 to 950 m.

Twenty sample areas were established in the forest fragments, 10 areas surrounded by a matrix of transgenic maize crops, and 10 areas surrounded by conventional maize crops. Farms were chosen with assistance from the Integrated Agricultural Development Company of Santa Catarina (Companhia Integrada de Desenvolvimento Agrícola de Santa Catarina – CIDASC) and the

Enterprise for Agricultural Research and Rural Extension of Santa Catarina (Empresa de Pesquisa Agropecuária e Extensão Rural – Epagri/Campos Novos), based on the accessibility and degree of isolation of forest fragments in relation to the type of maize cultivation. In terms of crop management, the fragments were chosen to be as similar as possible, with only herbicides (not insecticides) used on crop fields adjacent to the fragments. In areas where transgenic maize was planted, the farmers typically used a 'package' of agricultural inputs sold by the manufacturing company. In areas where conventional maize was planted, farmers used several agricultural inputs, some non-chemical (e.g., animal manure, pork and chicken). Only fragments adjacent to monoculture were chosen. The GM crops were expressing Cry1Ab proteins (DKB 240 YG, AS 1555 YG) and Herculex® Technology (30F53H Pioneer).

The area of each fragment was determined using Google Earth Path (1.4.4a). The distance to the nearest fragment and the distance between fragments were calculated using Google Earth.

To assess the environmental complexity in each fragment to be sampled, we used an adapted quadrat-section method (Brower et al., 1998). Using a cross as a reference, four quadrants (northeast, northwest, southeast, southwest) were marked. In each quadrant, all trees with diameter at breast height (DBH) greater than 15 cm, all shrubs with DBH less than 15 cm and with height greater than 1 m were selected and the distances to the center of the cross, height, crown diameter and trunk diameter were all measured. Trunk diameter was taken at breast height (1.3 m) for the trees and ankle height (DAH = 0.1 m) for shrubs. In each quadrant, the height of leaf litter in 1 m × 1 m marked square (using PVC pipe) was measured with a ruler, and percentages of leaf litter layer, green and exposed soil area (no vegetation or leaf litter) were measured by visual estimation using the following classes, 0–5%, 6–25%, 26–50%, 51–75%, 76–95% and 96–100%. Using these same classes, the percentage of canopy cover in the four quadrats was visually estimated, with the aid of a square paperboard with a hollow area of 10 cm × 10 cm, placed at a distance of 40 cm from the eye of the observer, at an inclination of 20° in relation to the zenith.

Sampling of copro-necrophagous beetles was performed at a distance of 10 m from the edge of the fragment when possible. In smaller-sized fragments (<10 ha), samples were collected near the middle of the fragment. Sampling was intensive during the summer, from February 7th to 20th, 2011, the period before maize harvest. Each fragment was sampled only once during the study. Pitfall traps were used for beetle capture because they are the most common method for sampling active invertebrates on the soil surface (Southwood, 1994). Traps were made using plastic pots (30 cm circumference and 20 cm height) buried in the ground to the top edge, and protected by a cut plastic cap. A detergent/water mixture and 10 g of bait was added to each trap. The bait consisted of both human feces and pieces of decomposing pork meat in order to attract different species according to their feeding habits (i.e., both coprophagous and necrophagous).

The sampling protocol for each fragment consisted of five sampling points spaced 10 m from each other, each sampling point with two traps spaced 5 m apart; total sampling effort consisted of 200 traps (across the 20 forest fragments sampled). After 48 h of trap exposure, captured insects were fixed in 70% alcohol and taken to the Laboratory of Terrestrial Animal Ecology (LECOTA/ECZ/UFSC) for weighing and identification (Vaz-de-Mello et al., 2011), then deposited in either the Entomological Collection of the Center for Biological Sciences, Federal University of Santa Catarina (UFSC) or the Entomological Collection of University of Mato Grosso (UFMT).

Species accumulation curves were built to determine sampling effort, and the Jackknife method and Chao 1 and 2 estimators were used to estimate the possibility of finding other species in the sampled fragments. Both analyses were made using EstimateS v.9 (Colwell, 2013). Ecological measures frequently used in environmental monitoring (i.e., abundance, richness, and Shannon–Weiner diversity index) and the principal components (for PCA) of the environmental variables were calculated using Primer (Clarke and Gorley, 2001). Data were square-root transformed to reduce the influence of common species and differences in total abundance, and a Bray–Curtis similarity matrix was built using communities from different fragments. To examine species similarity with regard to relative abundance and distribution among fragments, a multidimensional scaling (MDS) sorting method was applied using Primer. To test for differences in the distribution of species abundance between the two types of fragments (adjacent to conventional and transgenic maize), an analysis of similarity (ANOSIM) was carried out. Similarity percentage (SIMPER) was used to determine the contributions of each species to similarity and dissimilarity between areas. Both analyses were made using Primer.

To complement the analysis of the relationship between dung beetle communities and environmental variables, we performed a Bio-Env analysis in Primer 6β by calculating the correspondence coefficient between the Euclidean distance matrix of the environmental variables and the Bray–Curtis similarity matrix for dung beetle communities.

A Pearson correlation test was performed using the values of the matrices (spatial distance and similarity) in Statistica (Statsoft, 2001) to determine whether or not distance between fragments was related to dung beetle community similarity. To assess whether fragment size was related to dung beetle community distribution, Spearman correlations were made between fragment size and ecological measures, including Scarabaeinae richness, abundance, and biomass, using Statistica (Statsoft, 2001).

To observe whether or not there were changes in functional groups within the community of dung beetles, Spearman correlations were made between fragment size and ecological measures (richness, abundance, biomass and diversity (H')) of functional groups (dwellers, tunnelers, rollers) using Statistica (Statsoft, 2001). To assess frequency and abundance of functional groups, the classes were divided into octaves.

The single value indicator (IndVal) analysis was performed to determine indicator species using PC-ORD 5.10, which combines the degree of specificity (patterns of relative abundance) of a given species in a given environment, and its fidelity within that environment (patterns of incidence), using randomization to test the significance of each species (Dufrene and Legendre, 1997).

3. Results

3.1. Environmental description, vegetation complexity and fragment size

The principal component analysis (PCA) ordered points in accordance with the environmental characteristics calculated based on the measured variables. There was no pattern for the different farm cultivation practices (GM vs. conventional), indicating that vegetation within the fragments varied for reasons unrelated to the type of cultivation adjacent to them (Fig. 1). The PCA showed that vegetation complexity varied little, regardless of the type of crop adjacent to the fragment. According to the environmental variables, the fragments are homogeneous without separation by farm cultivation practices. None of our environmental characteristics group the fragments adjacent to the two cultivation practices, demonstrating that the randomly selected sample units had no characteristics that could affect the dung beetle communities that inhabit them.

Axis 1 of the PCA analysis (PCA1-"soil") explained 31.8% of the variation in the data, and was strongly influenced by green cover, leaf litter cover, distance to shrub and bare soil (Table 1). Axis 2
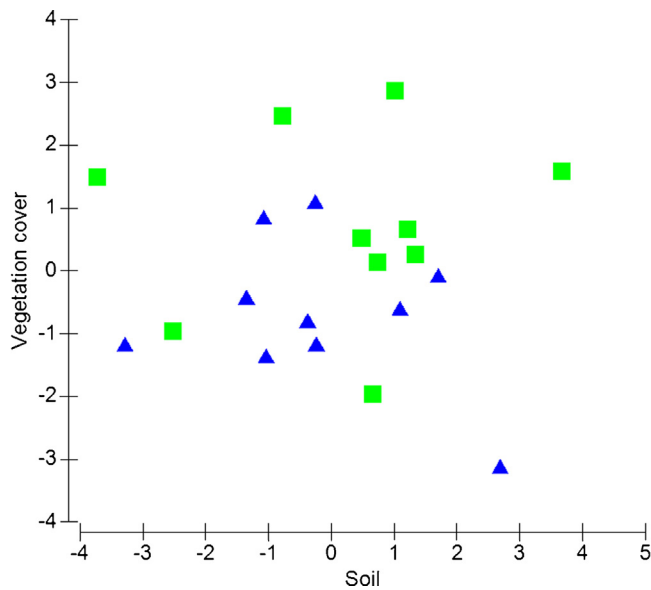
Fig. 1. Principal component analysis (PCA) of environmental variables in 20 forest fragments in the Campos Novos region, Santa Catarina, Brazil. Squares indicate fragments adjacent to conventional maize; triangles indicate fragments adjacent to transgenic maize.

(PCA2-"vegetation cover") explained 20.3% of variation in the data, and was influenced primarily by shrub and tree height and tree basal area.

Some fragments, such as those adjacent to conventional maize 3, 5, 6 and 10 showed strong vegetation cover, with large shrubs and few trees. In others, such as transgenic fragment 1, the shrubs were closer to each other, and the soil had a large extent of leaf litter cover and minimal green cover.

The 20 forest fragments ranged from 1141 m^2 to 355,938 m^2 (Table 1). Fragment size variation was similar for fragments adjacent to both cultivation types, although those adjacent to GM maize were slightly larger. There was no relationship between

Table 1
Area, altitude, distance to the nearest fragment, first principal component (PCA1), second principal component (PCA2) of the 20 fragments where the beetles were collected, in Campos Novos, SC. C, fragments adjacent to conventional maize, T, fragments adjacent to transgenic maize.

Fragments	Area (m^2)	Altitude (m.s.n. m.)	Distance to next fragment (m)	PCA1 (soil)	PCA$_2$ (vegetation cover)
1C	186,929	860	232	0.49	0.53
2C	11,691	900	235	1.21	0.66
3C	5664	900	233	−0.77	2.47
4C	1141	860	40	−2.53	−0.96
5C	1905	860	40	−3.73	1.49
6C	2616	900	81	1.02	2.88
7C	2501	905	223	0.66	−1.96
8C	2891	730	125	0.74	0.13
9C	11,971	740	25	1.34	0.26
10C	25,053	760	25	3.68	1.58
1T	21,017	720	87	2.69	−3.15
2T	1770	710	88	−0.37	−0.82
3T	2727	720	284	1.71	−0.11
4T	9300	890	140	−1.35	−0.45
5T	23,873	890	273	−1.04	−1.39
6T	3768	900	175	−0.25	1.06
7T	355,938	900	126	−0.24	−1.20
8T	4248	900	140	−3.28	−1.21
9T	35,467	920	36	1.10	−0.63
10T	13,815	950	134	−1.07	0.81

fragment size and environmental complexity, and measures of Spearman correlation between fragment size and values of PC1 (soil characteristics) and PC2 (vegetation cover) did not reach significance for conventional maize fragments ($r = 0.61$ Size × PC1, $r = 0.26$ Size × PC2), or transgenic maize fragments ($r = 0.18$ Size × PC1, $r = −0.41$ Size × PC2).

The distance between fragments ranged from 25 m to 2 km across a total area of approximately 400 km^2, and both fragment types (adjacent to GM vs. conventional (lacked spatial concentration). Inter-fragment distance ranged from 25 to 284 m (Table 1). The geographic distance between the fragments did not affect the distribution of either conventional or transgenic maize fields (see Fig. 1).

Altitude ranged from 710–950 m above sea level. Fragments 9T and 10T were the upmost regions, and fragments 1T, 2T and 3T were the lowest regions (Table 1).

3.2. Dung beetle community structure and composition

A total of 1502 beetles were collected (805 in fragments adjacent to conventional maize and 697 in fragments adjacent to GM maize), belonging to six tribes, 12 genera and 33 species (Table 2). We collected 27 species in conventional maize fragments and 28 species in transgenic maize fragments.

The dominant species in conventional maize fragments were *Onthophagus tristis* Harold, 1873, *Uroxys terminalis* Waterhouse, 1891, *Uroxys* sp.; in transgenic maize fragments the dominant species were *Eurysternus francinae* Génier, 2009, *Canthon rutilans cyanescens* Blanchard, e *Eurysternus parallelus* Castelnau, 1840. *Canthidium aff. breve* (Germar, 1824), *Dichotomius luctuosus* (Harold, 1869), *Deltochilum riehli* Harold, 1868, *Eurysternus calligrammus* Dalman, 1824 and *Eurysternus caribaeus* (Herbst, 1789) were only collected in conventional maize fragments, whereas *Dichotomius fissus* (Harold, 1867), *Ontherus azteca* Harold, 1869, *Onthophagus catharinensis* Paulian, 1936, *Onthophagus aff. hirculus* Mannerheim, 1829 and *Malagoniella virens* (Harold, 1869) were only collected in transgenic maize fragments (Table 2).

For species that were found in both fragment types, some had strong differences in abundance between fragment types. For *Dichotomius sericeus* (Harold, 1867), 75% of the total individuals were in the fragments near conventional maize crops; for *E. francinae* 98% were found in fragments near transgenic maize; for *O. tristis*, 82% were found in fragments near conventional maize; and for *U. terminalis*, 75% were found in fragments near conventional maize (Table 2).

Mean dry weights of the three most abundant species in each fragment type indicated lower biomass in conventional fragments (12 mg for *O. tristis*, 8 mg for *U. terminalis* and 7 mg for *Uroxys* sp.) compared to transgenic fragments (75 mg for *E. francinae*, 44 mg for *C. rutilans cyanescens* and 32 mg for *E. parallelus*) (Table 2).

Feeding behavior functional groups were distributed as follows: in conventional maize fragments, 15 tunneler species, eight roller species and four dweller species were found; in transgenic maize fragments, 17 tunneler species, nine roller species, and two dweller species were found (Table 2).

Species accumulation curves for beetle communities in both fragment types indicate sampling sufficiency (Fig. 2). The number of species observed was at least 80% of the species richness values generated by Chao 1 and 2 estimators, and Jackknife 1 (Table 3). No estimator showed any significant difference in richness between the communities of dung beetles in fragments near conventional and transgenic maize (Table 3).

Species abundance distribution curves between fragment types were similar, with few highly abundant species and many species of intermediate abundance (Fig. 3A and B). The species that most contributed to biomass (i.e., biomass distribution)

Table 2

Scarabaeinae species collected in 20 fragments (Feb/2011) in Campos Novos/SC, Brazil. (*) Asterisk indicates species found only in one fragment. S, Number of species, N, number of individuals, T, fragments adjacent to transgenic maize, C, fragments adjacent to conventional maize, Mean weight of individuals, GF, functional guild, E, endocoprid, P, paracoprid, T, telecoprid.

Tribe/species	Fragments		Total	Mean weight (mg)	FG
	C	T			
Ateuchini					
Uroxys terminalis Waterhouse, 1891	51	153	204	8	P
Uroxys sp.	5	97	102	7	P
Coprini					
Canthidium aff. *breve* (Germar, 1824)	0	2	2	5	P
Canthidium cavifrons Balthasar, 1939	15	16	31	7	P
Canthidium aff. *dispar* Harold, 1867	6	11	17	28	P
Canthidium moestum Harold, 1867	2	2	4	22	P
Canthidium aff. *trinodosum* (Boheman, 1858)	16	17	33	8	P
Dichotomius bicuspis Germar,1824	14	10	24	137	P
Dichotomius fissus (Harold, 1867)	2	0	2	437	P
Dichotomius sericeus (Harold, 1867)	25	75	100	171	P
*Dichotomius luctuosus** (Harold, 1869)	0	1	1	201	P
Homocopris sp.	18	6	24	118	P
Ontherus azteca Harold, 1869	3	0	3	44	P
Ontherus sulcator (Fabricius, 1775)	1	25	26	82	P
Deltochilini					
Canthon lividus seminitens Harold, 1868	23	55	81	32	T
Canthon ibarragrassoi Martinez,1952	4	0	4	8	R
Canthon rutilans cyanescens Harold, 1868	119	93	212	44	T
Canthon auricollis Redtenbacher,1867	10	24	34	30	T
Canthon luctuosus Harold, 1868	9	3	12	13	T
Canthon aff. *oliverioi* Pereira and Martínez,1956	1	1	2	14	T
Deltochilum brasiliense (Castelnau, 1840)	27	6	33	362	T
Deltochilum cristatum Paulian, 1938	18	6	24	58	T
*Deltochilum riehli** Harold, 1868	0	1	1	27	T
*Malagoniella virens** (Harold, 1869)	1	0	1	249	T
Oniticellini					
*Eurysternus calligrammus** Dalman, 1824	0	1	1	40	E
*Eurysternus caribaeus** (Herbst, 1789)	0	1	1	109	E
Eurysternus francinae Génier, 2009	165	4	169	76	E
Eurysternus parallelus Castelnau, 1840	83	25	108	32	E
Onthophagini					
Onthophagus catharinensis Paulian, 1936	17	0	17	6	P
Onthophagus aff. *hirculus* Mannerheim, 1829	8	0	8	6	P
Onthophagus tristis Harold, 1873	37	167	204	12	P
Phanaeini					
Coprophanaeus saphirinus (Sturm, 1828)	9	1	10	361	P
Sulcophanaeus menelas (Castelnau, 1840)	8	2	10	200	P
Abundance	697	805	1502		
Richness	28	27	33		

were different between conventional maize fragments (*D. sericeus*) and GM maize fragments (*E. francinae* and *D. brasiliense*) (Fig. 3A and B).

When analyzed based on species abundance, community similarity of beetles in conventional maize fragments was 47%, with *O. tristis*, *Canthon lividus seminitens* and *C. rutilans cyanescens* most contributing to the similarity (60% of total). Community similarity of beetles in transgenic maize fragments was 44%, with *C. rutilans cyanescens*, *E. francinae*, *E. parallelus* and *C. lividus seminitens* most contributing to the similarity (59%).

Beetle communities differed among fragment types (ANOSIM $r = 0.4$, $p = 0.002$). Dissimilarity between the two types of fragments (conventional and transgenic) was 63%, and seven species were responsible for 50% of such dissimilarity: *E. francinae*, *O. tristis*, *Uroxys* sp., *E. parallelus*, *C. rutilans cyanescens*, *D. sericeus* and *U. terminalis*.

Community similarity on conventional maize fragments with regard to individual species biomass was 38%; *C. rutilans cyanescens*, *O. tristis*, *C. lividus seminitens* and *Ontherus sulcator* (Fabricius, 1775) most contributed to such similarity (62%). The similarity in biomass distribution among fragments near transgenic maize was 39%, where *C. rutilans cyanescens*, *E. francinae*, *E. parallelus* and *D. brasiliense* were the species that most contributed to similarity (67%).

The distribution pattern of species biomass was different in the two types of fragments (ANOSIM $r = 0.286$, $p = 0.009$). Dissimilarity between conventional and transgenic fragments was 66%, and seven species were responsible for 55% of such dissimilarity, including *E. francinae*, *D. sericeus*, *D. brasiliense*, *C. rutilans cyanescens*, *E. parallelus*, *Dichotomius bicuspis* Germar, 1824 and *O. sulcator*.

Multidimensional scaling of species relative abundance from the similarity analysis of dung beetles communities of all fragments showed that most of the transgenic fragments were clumped, with about 40% similarity (Fig. 4), i.e., when the same species occurred in both types of fragments, their abundance differed between fragment types.

The BioEnv analysis showed no significant differences between the dung beetle similarity matrix and the environmental variables matrix ($r = 0.238$, $p = 0.09$), demonstrating that the environmental variables of fragments do not explain the differences in dung beetles communities between the fragments.
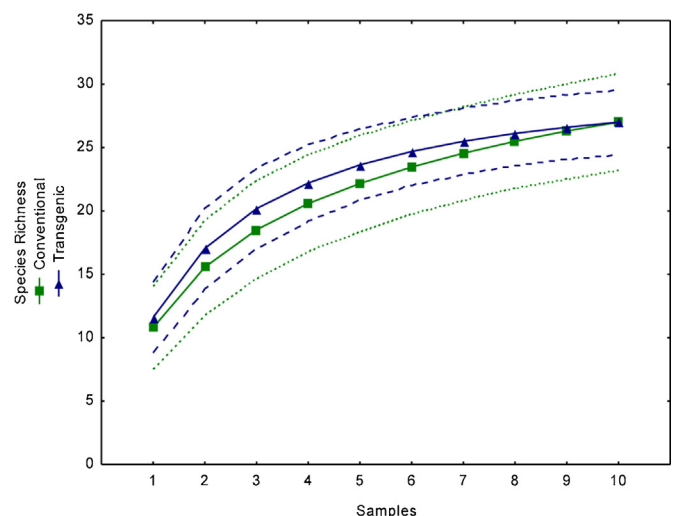

Fig. 2. Species accumulation curves for dung beetles in 20 sampled forest fragments in February 2011, in the Campos Novos region, Santa Catarina, Brazil.

Table 3

Abundance, observed richness, richness estimators Chao 1, Chao 2 and Jackknife 1 (with confidence intervals of 95%), mean biomass of species per fragment and total biomass, calculated for the communities of Scarabaeinae beetles in fragments adjacent to transgenic and adjacent to conventional maize in the region of Campos Novos, SC.

Ecological measures of Scarabaeinae community	Fragments adjacent to transgenic maize	Fragments adjacent to conventional maize
Abundance (N)	697	805
Richness (S)	28	27
Variation of richness/fragments	10–16	8–15
Estimated richness, Chao 1 Chao 2 Jackknife 1	29.00 30.25 32.5 (11.5–31.98)	30.75 30.15 33.3 (11.8–30.6)
Shannon (H′)	2.583	2.395
Total biomass	47.60 g	33.75 g
Mean biomass per fragment	0.0723 g	0.0428 g

The Pearson correlation between the values of the similarity matrix and the geographical distance matrix showed a weak negative correlation ($r_S = -0.366$), indicating that the distances between fragments do not explain the differences between communities in conventional vs. transgenic maize fragments.

The correlation between the ecological measures of the Scarabaeinae community and fragment size showed a significant correlation between species richness and fragment size only for conventional maize fragments ($r_S = 0.72$), and no correlation between fragment size and either abundance, biomass, or diversity
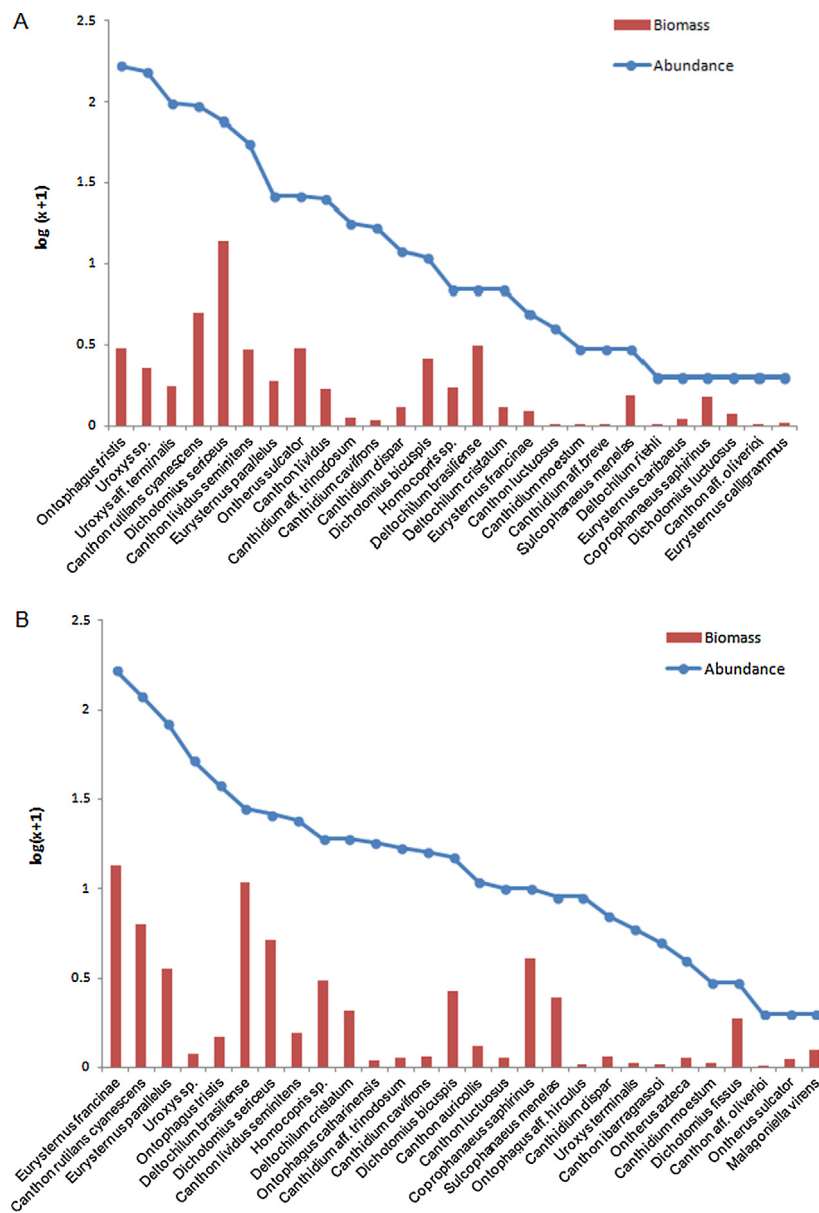

Fig. 3. Distribution of abundance (line) and biomass (bar), in log ($X + 1$) of copro-necrophagous Scarabaeinae collected in February 2011 from forest fragments adjacent to conventional (A) vs. transgenic (B) maize crops in Campos Novos, Santa Catarina, Brazil.
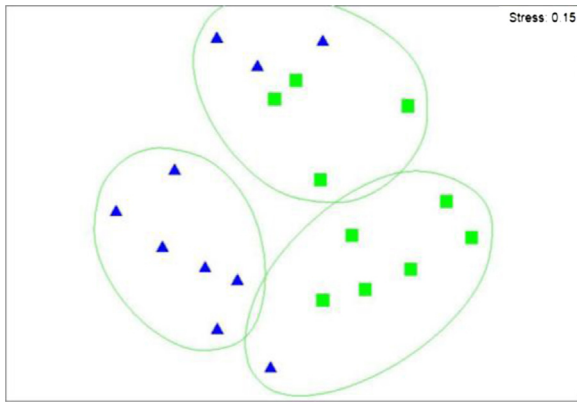
Fig. 4. Multidimensional scaling (MDS) of Scarabaeinae communities in 20 forest fragments in Campos Novos, SC. The circles group with a Bray–Curtis similarity of 40%. represents fragments adjacent to conventional maize, ▲ represents fragments adjacent to transgenic maize.

Table 4
Values of the Spearman correlation between fragment size (log) and the ecological measures of dung beetle community functional groups collected in the region of Campos Novos, SC. T, fragments adjacent to transgenic maize, C, fragments adjacent to conventional maize.

	Dwellers		Tunnelers		Rollers		All dung beetles	
	C	T	C	T	C	T	C	T
Richness	0.43	0.85*	0.41	0.15	0.47	0.26	0.72*	0.18
Abundance	0.71*	0.71*	0.40	0.16	0.60	0.33	0.48	0.03
Biomass	0.71*	0.70*	0.50	0.19	0.64*	0.37	0.48	0.62
Shannon	0.24	0.86*	0.09	0.02	0.05	0.41	0.26	0.01

* Significant at $p < 0.05$.

(Fig. 5, see squares). There was no significant correlation between transgenic fragment size and abundance, richness, biomass, and diversity (H′) (Fig. 5, see triangles).

Functional group incidence and abundance differed between the two types of fragments. Dweller species were significantly correlated with richness, abundance, biomass and diversity (H′) in the fragments adjacent to transgenic maize. In the fragments located adjacent to conventional maize, we found a correlation between fragment size and the abundance and total biomass of dwellers (Table 4). For roller species, there was correlation between fragment size and biomass only for conventional maize fragments. For tunneler species, no correlations were found (Table 4).
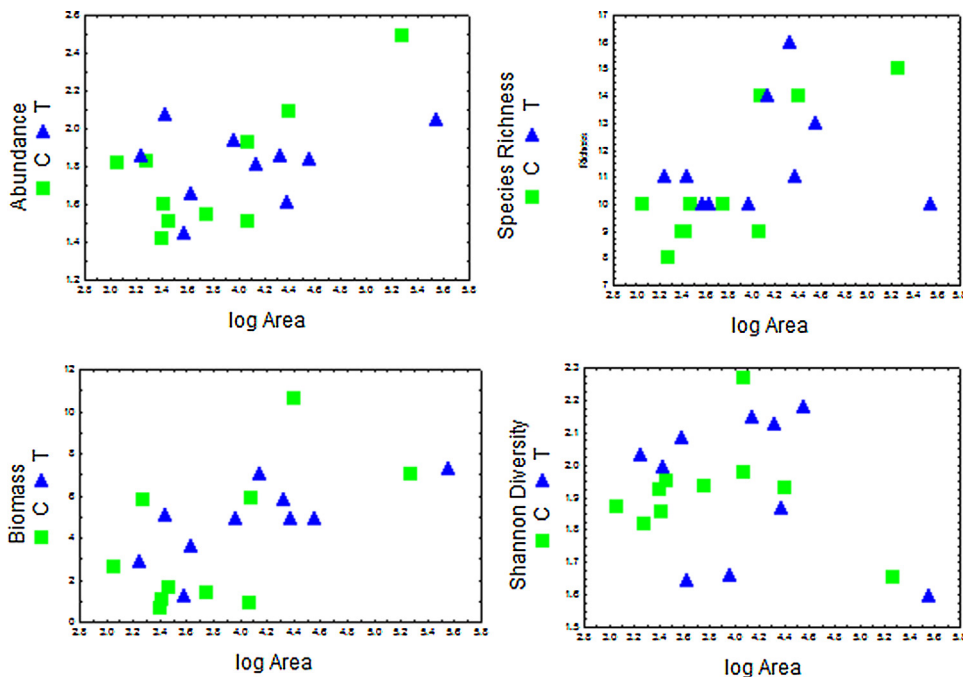
Scarabaeinae abundance distribution diagrams show differences in abundance of functional groups between the two types of fragments (Fig. 6). The dwellers were more abundant in fragments transgenic maize fragments, whereas tunnelers were more abundant in the conventional maize fragments. While in the fragments located in the adjacent to conventional maize many tunneler individuals were collected, in the fragments near transgenic maize a larger number of dwellers was collected.

The abundance distribution diagrams for the contribution of biomass per functional group in fragments near conventional and transgenic maize crops differed (Fig. 7). Tunnelers most contributed to biomass in conventional maize fragments, and dwellers in the fragments adjacent to transgenic maize.

The results of the single value test (IndVal) showed that five species had a significant preference for one fragment type (Table 5). *O. tristis* (81.9%), *C. lividus seminitens* (67.9%), *O. sulcator* (67.3%) and *U. terminalis* (66.6%) are indicators of forest fragments adjacent to conventional maize crops; *E. francinae* (78.1%) and *E. parallelus* (61.5%) are indicators of forest fragments adjacent to transgenic maize crops (Table 5).
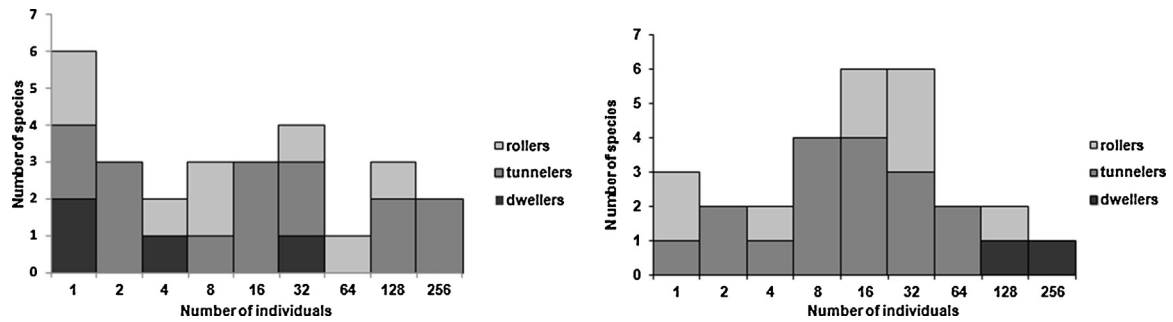

Fig. 5. Distribution of dung beetle community ecological measures in relation to fragment size (log area in m²), individual abundance log ($x + 1$), richness (S), total biomass (in g) and diversity (Shannon). Squares indicate fragments adjacent to conventional maize; triangles indicate fragments adjacent to transgenic maize.

Fig. 6. Abundance distribution (in octaves) of the functional groups of copro-necrophagous Scarabaeinae collected in February 2011 in fragments adjacent to conventional (A) and transgenic (B) maize crops in the Campos Novos region, Santa Catarina, Brazil.
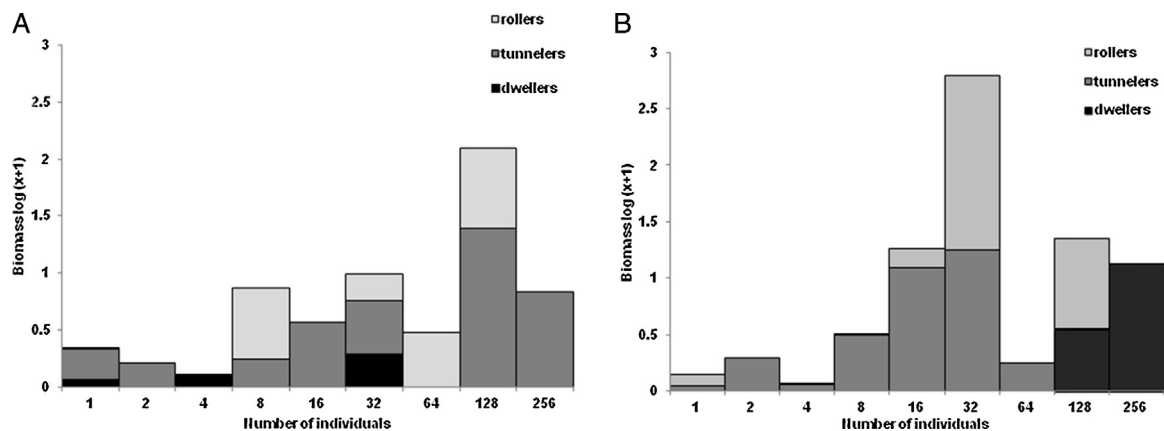

Fig. 7. Biomass distribution (in octaves) of the functional groups of copro-necrophagous Scarabaeinae collected in February 2011 in fragments located in fragments adjacent to conventional (A) and transgenic (B) maize crops in the Campos Novos region, Santa Catarina, Brazil.

The IndVal results agree with our previous result that most dwellers are found in transgenic maize fragment, and that rollers and tunnelers are most found in conventional maize fragments.

The number of individuals of *C. lividus seminitens* and *O. sulcator* within the community is represented in the MDS based on Bray–Curtis similarity (Fig. 8A and D), and the same for *U. terminalis* and *O. tristis* is depicted in Fig. 8C and B. Both figures show that these species were found mostly in fragments near conventional maize crops. *E. francinae* and *E. parallelus* had a greater number of individuals in forest fragments near transgenic maize crops (Fig. 9).

Table 5
Single value (IndVal) test results of five dung beetle species with significant preference (p^*) for some type of fragment, adjacent to conventional and transgenic maize crops. Species were ordered according to the value of IndVal.

Species	Cultivation	IndVal value	p^*
Onthophagus tristis	Conventional	81.9	0.0014
Eurysternus francinae	Transgenic	78.1	0.0030
Canthon lividus seminitens	Conventional	67.9	0.0272
Ontherus sulcator	Conventional	67.3	0.0134
Uroxys terminalis	Conventional	66.6	0.0252
Eurysternus parallelus	Transgenic	61.5	0.0638

4. Discussion

The pattern observed in the studied community indicates few abundant species, and many with a small number of individuals; this is common in communities of this taxon (Nichols et al., 2007; Gardner et al., 2008b). However, it is known that such differences in abundance between dominant and non-dominant species increase in areas with higher levels of alteration (Nichols et al., 2007).

The size of the fragments influenced Scarabaeinae community composition mainly in the fragments surrounded by conventional maize, but the two kinds of fragments showed no differences in community structure. The hypothesis that smaller fragments have fewer species of Scarabaeinae beetles is strongly supported in the literature (Klein, 1989; Nichols et al., 2007; Gardner et al., 2008b; Filgueiras et al., 2011). Additionally, the loss of large mammals caused by the reduction and fragmentation of forests may change the pattern of resource availability for copro-necrophagous beetles (Estrada et al., 1999; Nichols et al., 2009), resulting in lower species richness when compared to less degraded areas.

Another hypothesis that could differentiate community structure among fragments is that fragments with less environmental complexity have lower richness and abundance of dung beetles (Halffter et al., 1992; Halffter and Arellano, 2002; Hernández and Vaz-de-Mello, 2009). In this study, it was shown that dung beetle communities did not differ between fragment types when the
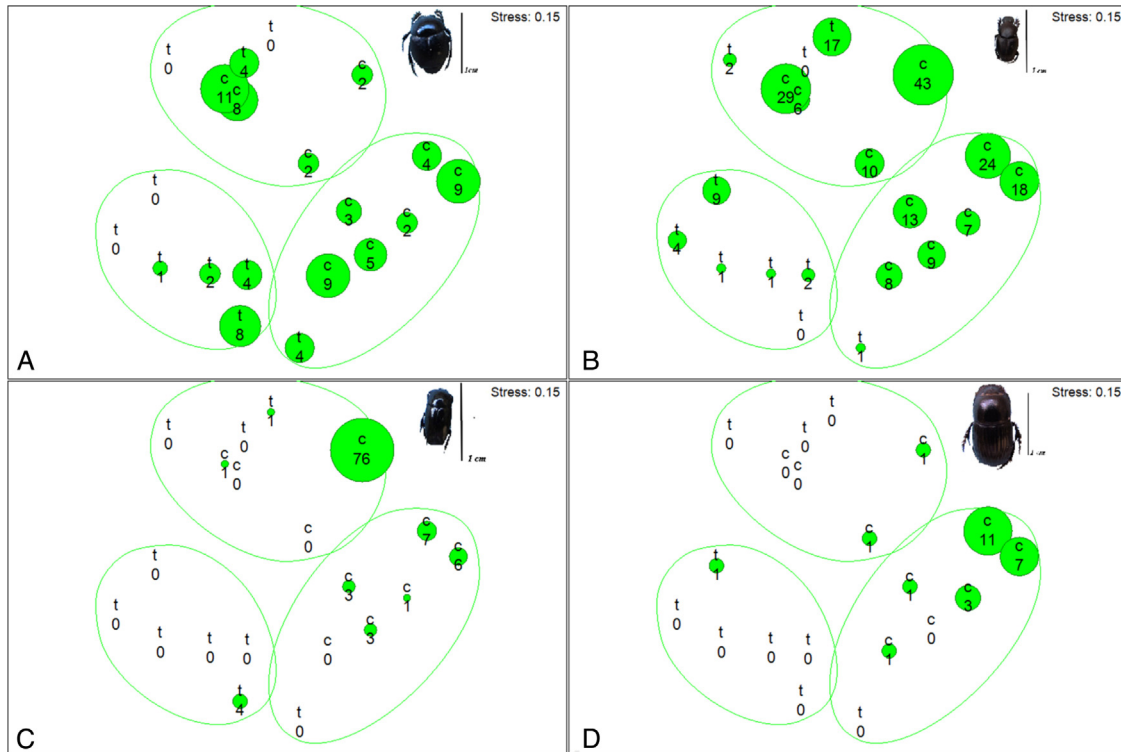
Fig. 8. Multidimensional Scaling (MDS) showing a Bray-Curtis similarity of 40% amongst the dung beetle community of sampled fragments. The solid circles represent the abundance of (A) *Canthon lividus seminitens*, (B) *Onthophagus tristis*, (C) *Uroxys terminalis* and (D) *Ontherus sulcator* in each fragment.

relationship between dung beetle diversity and environmental complexity was tested.

When the descriptors of the dung beetle community were analyzed, there were differences, in both the abundance and biomass, of communities in fragments near conventional and transgenic maize. Differences in crop management (agricultural inputs), specifically in areas planted with transgenic maize, could also explain the difference found in the community of dung beetles, but these results suggest that the dung beetles communities may be being affected by the Bt toxin chronic exposure and/or ingestion.

Hilbeck et al. (1998) showed in laboratory experiments that 57% of *C. carnea* larvae, a neuroptera predator that acts in the ecosystem as a biological control agent, died when feeding on diet containing *Bacillus thuringiensis*. In another study, Ramírez-Romero et al. (2008) showed that bees' behavior was affected when exposed to high concentrations of Cry1Ab protein. Rosi-Marshall et al. (2007)

observed that decrease in growth rate and even mortality of aquatic species are potential ecological effects of toxins produced by transgenic maize. Futher, Bøhn et al. (2010) demonstrated that feed derived from Bt-transgenic maize in aquatic environments decreased the adaptive value of the crustacean *D. magna*, a non-target arthropod that is at the base of the fresh water food chain. Although Glare and O'Callagham (2000) emphasize that the harmful effects of expressed Bt toxin on natural enemies are minimal and/or significantly lower than those of pesticides, through the studies and outcomes listed before makes clear that they cannot be neglected.

Marvier et al. (2007) in a meta-analysis of 42 studies showed that non-target invertebrates are generally more abundant in transgenic cotton and maize than in crops with insecticides. However, when compared with non-transgenic and insecticide-free crops, non-target invertebrates in transgenic crops are less abundant.
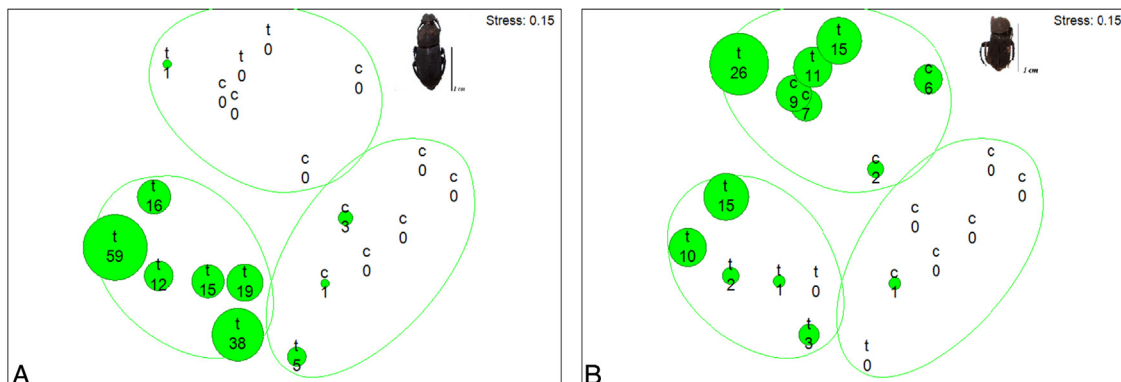

Fig. 9. Multidimensional Scaling (MDS) showing a Bray–Curtis similarity of 40% amongst the beetle community of sampled fragments. The solid circles represent the abundance of (A) *Eurysternus francinae* and (B) *Eurysternus parallelus* in each fragment.

Differences in the distribution of functional groups amid fragments in transgenic and conventional maize were also found. Davis et al. (2001), when working with Scarabaeinae beetles in Borneo, observed that the distribution of species across different environmental characteristics may show discrete associations typical to particular biotypes within the landscape. Indicator species that emerge from these associations are able to identify levels of responses to anthropogenic disturbances in forest ecosystems.

In this study, the 'dweller' functional group was more representative in fragments near transgenic maize, which is perhaps a reflection of a decrease in other functional groups, especially tunnelers. The reduction in competition for resources due to the decrease in the abundance of tunnelers, may result in increase resource availability and allocation by dwellers, resulting in higher reproductive rates (Hanski and Cambefort, 1991). Thus, *E. francinae* and *E. parallelus* (both dwellers), though belonging to a functional group that generally is less frequent and abundant in the community of dung beetles, were indicator species of forest fragments near transgenic maize. It is known that species within *Eurysternus* have a preference for forested areas of open meadows, as their habit of remaining within the food resource above ground makes them more susceptible to adverse environmental conditions (Doube, 1991).

Differences in soil type (e.g., due to parent rocks) can explain much of the difference in species composition, but if, on the other hand, there is a great number of tunnelers and rollers, dwellers may have no chance to reproduce (Hanski and Cambefort, 1991). In this way, inclusion of all functional groups maximizes ecosystem function (Slade et al., 2007).

Tunnelers are good competitors for resources because they utilize the resource upon discovery (Hanski and Cambefort, 1991; Halffter et al., 1992) and it is possible that the decrease in their proportion in the communities adjacent to transgenic crops was caused by Bt toxin exposure. The worst competitors (dwellers) may also be affected when they reach the resource, if they do not have physiological differences compared to other functional groups. The loss of tunneler and roller species causes reduction in the removal of feces, seed dispersal, edaphic aeration, and incorporation of organic matter in the soil. Furthermore tunneler loss causes the feces, and consequently the seeds, to be exposed longer, attracting potential seed predators (Estrada and Coates-Estrada, 1991; Andresen, 2002; Slade et al., 2007). Therefore, the functional group of dwellers seems unable to compensate in the short term for reduction in ecosystem function due to reduction of tunneler species.

Feces removal and seed dispersion are more dependent on the functional properties and combinations of species than on solely species richness (Slade et al., 2007). Although it may seem that different species' roles in the environment are redundant within functional groups, their responses within that group to environmental changes may differ (Hooper et al., 2002; Slade et al., 2007). This fact has important implications for the understanding of ecosystem functioning, and how it can be affected by environmental changes. In degraded areas, the greater the number of remaining species of the original ecosystem, the more likely there will be greater compensation for ecosystem services (Yachi and Loreau, 1999). The reduction of tunnelers, the most important group in maintaining the ecosystem function of dung and seed removal (Estrada and Coates-Estrada, 1991; Andresen, 2002; Slade et al., 2007) in the face of environmental alterations in general, as well as changes in the relative proportion of functional groups, suggest that the role of dung beetles in ecosystems may be in the process of changing.

References

Andresen, E., 2002. Dung beetles in a Central Amazonian rainforest and their ecological role as secondary seed dispersers. Ecol. Entomol. 27, 257–270.

Bøhn, T., Traavik, T., Primicerio, R., 2010. Demographic responses of *Daphnia magna* fed transgenic Bt-maize. Ecotoxicol 19, 419–430.

Bøhn, T., Primicerio, R., Traavik, T., 2012. The German ban on GM maize MON810, scientifically justified or unjustified? Environ. Sci. Eur. 24, 1–7.

Betz, F.S., Hammond, B.G., Fuchs, R.L., 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regul. Toxicol. Pharm. 32, 156–173.

Brower, J.E., Zar, J.H., Von Ende, C., 1998. Field and Laboratory Methods for General Ecology, 4th ed. WCB. McGraw-Hill, Boston, pp. 237.

Cambefort, Y., 1991. Biogeography and Evolution, p. 51–67. In: Hanski, I., Cambefort, Y. (Eds.), Dung Beetle Ecology. Priceton University Press, Princeton, pp. 481.

Cambefort, Y., Hanski, I., 1991. Dung beetle population biology, p. 36–50. In: Hanski, I., Cambefort, Y. (Eds.), Dung Beetle Ecology. Priceton Univerity Press, Princeton, pp. 481.

Campos, R.C., Hernandez, M.I.M., 2013. Dung beetle assemblages (Coleoptera, Scarabaeinae) in Atlantic forest fragments in southern Brazil. Rev. Bras. Entomol. 57, 47–54.

Clarke, K.R., Gorley, R.N., 2001. Primer v5, User Manual/Tutorial. Plymouth, UK, pp. 91.

Colwell, R.K., 2013. EstimateS Statistical Estimation of Species Richness and Shared Species from Samples. Version 9 and earlier. User's Guide and Application. University of Connecticut.

Davis, A.L.V., Philips, T.K., 2005. Effect of deforestation on a southwest Ghana dung beetle assemblage (Coleoptera, Scarabaeidae) at the periphery of Ankasa conservation area. Environ. Entomol. 34, 1081–1088.

Davis, A.J., Holloway, J.D., Huijbregts, H., Krikken, J., Kirk-Spriggs, A.H., Sutton, S.L., 2001. Dung beetles as indicators of change in the forests of northern Borneo. J. Appl. Ecol. 38, 593–616.

Dolezel, M., Miklau, M., Hilbeck, A., Otto, M., Eckerstorfer, M., Heisenberger, A., Tappeser, B., Gaugitsch, H., 2011. Scrutinizing the current practice of environmental risk assessment of GM maize applications for cultivation in the EU. Environ. Sci. Eur. 23, 1–15.

Doube, B.M., 1991. Dung beetles of Southern Africa.. In: Hanski, I., Cambefort, Y. (Eds.), Dung Beetle Ecology. University Press, Princeton, pp. 133–155.

Duan, J.J., Lundgren, J.G., Naranjo, S., Marvier, M., 2010. Extrapolating non-target risks of Bt crops from laboratory to field. Biol. Lett. 6, 74–77.

Dufrene, M., Legendre, P., 1997. Species assemblages and indicator species, the need for a flexible asymmetrical approach. Ecol. Monogr. 67 (3), 345–366.

Emberlin, J., Adams-Groom, B., Tidmarsh, J., 1999. The Dispersal of Maize Pollen *Zea mays* – A Report Based on Evidence Available from Publications and Internet SitesNational Pollen Research Unit, University College, Worcester. . acessed 7.07.14 www.mindfully.org/GE/Dispersal-Maize-Pollen-UK.htm.

Estrada, A., Coates-Estrada, R., 1991. Howler monkeys (*Alouatta palliata*), dung beetles (Scarabaeidae) and seed dispersal, ecological interactions in the tropical rain forest of Los Tuxtlas, Mexico. J. Trop. Ecol. 7, 459–474.

Estrada, A., Anzures, A.D., Coates-Estrada, R., 1999. Tropical rain forest fragmentation, howler monkeys (*Alouatta palliata*) and dung beetles at Los Tuxtlas, Mexico. Am. J. Primatol. 48, 253–262.

Favila, M.E., Halffter, G., 1997. The use of indicator groups for measuring biodiversity as related to community structure and function. Acta Zool. Mex. 72, 1–25.

Feer, F., 2000. Les Coléoptères coprophages et nécrophages (Scarabaeidae *s. str.* et Aphodiidae) de la forêt de Guyane Française, composition spécifique et structure des peuplements. Annal. Sociét. Entomol. Fr. 36, 29–43.

Filgueiras, B.K.C., Ianuzzi, L., Leal, I.R., 2011. Habitat fragmentation alters the structure of dung beetle communities in the Atlantic Forest. Biol. Conserv. 144, 362–369.

Galindo-Leal, C., Câmara, I.G., 2003. Atlantic forest hotspots status, an overview.. In: Galindo-Leal, C., Câmara, I.G. (Eds.), The Atlantic Forest of South America, Biodiversity Status, Threats, and Outlook. Center for Applied Biodiversity Science Island Press, Washington, D.C, pp. 3–11.

Gardner, T.A., Barlow, J., Araujo, I.S., Ávila-Pires, T.C., Bonaldo, A.B., Costa, J.E., Esposito, M.C., Ferreira, L.V., Hawes, J., Hernández, M.I.M., Hoogmooed, M.S.,

Leite, R.N., Lo-Man-Hung, N.F., Malcom, J.R., Martins, M.B., Mestre, L.A.B., Miranda-Santos, R., Nunes-Gutjahr, A.L., Overal, W.L., Parry, L., Peters, S.L., Ribeiro-Junior, M.A., Da Silva, M.N.F., Motta, C., Peres, C.A., 2008a. The cost-effectiveness of biodiversity surveys in tropical forests. Ecol. Lett 11, 139–150.

Gardner, T.A., Hernández, M.I.M., Barlow, J., Peres, C.A., 2008b. Understanding the biodiversity consequences of habitat change, the value of secondary and plantation forests for neotropical dung beetles. J. Appl. Ecol. 45, 883–893.

Glare, T.R., O'Callagham, M., 2000. Bacillus thuringiensis, Biology, Ecology and Safety. John Wiley & Sons, Chichester, pp. 350.

Godfray, H.C.J., Lewis, O.T., Memmott, J., 1999. Studying insect diversity in the tropics. Philos. Trans. R. Soc. 354, 1811–1824.

Grønsberg, I.M., Nordgård, L., Fenton, K., Hegge, B., Nielsen, K.M., Bardocz, S., Pusztai, A., Traavik, T., 2011. Uptake and organ distribution of feed introduced plasmid DNA in growing or pregnant rats. Food Nutr. Sci. 2, 377–386.

Gruber, H., Paul, V., Guertler, P., Spiekers, H., Tichopad, A., Meyer, H., Muller, M., 2011. Fate of Cry1Ab protein in agricultural systems under slurry management of cows fed genetically modified maize (Zea mays L.) MON810, a quantitative assessment. J. Agric. Food Chem. 59, 7135–7144.

Guertler, P., Paul, V., Steinke, K., Wiedemann, S., Preißinger, W., Albrecht, C., 2010. Long-term feeding of genetically modified maize (MON810) – fate of cry1Ab DNA and novel protein during the metabolism of the dairy cow. Livest. Sci. 131, 250–259.

Halffter, G., Arellano, L., 2002. Response of dung beetle diversity to human-induced changes in a tropical landscape. Biotropica 34 (1), 144–154.

Halffter, G., Edmonds, W.D., 1982. The Nesting Behavior of Dung Beetles (Scarabaeinae): An Ecological and Evolutive Approach. Man and the Biosphere Program UNESCO, México D.F, pp. 177.

Halffter, G., Favila, M., 1993. The Scarabaeinae (Insecta, Coleoptera) an animal group for analysing, inventorying and monitoring biodiversity in tropical rain forest and modified landscapes. Biol. Int. 27, 15–21.

Halffter, G., Matthews, E.G., 1966. The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera, Scarabaeidae). Folia Entomol. Mex. 12 (14), 1–312.

Halffter, G., Favila, E., Halffter, V., 1992. A comparative study of the structure of the scarab guild in Mexican tropical rain forests and derived ecosystems. Folia Entomol. Mex. 84, 131–156.

Halffter, G., 1991. Historical and ecological factors determining the geographical distribution of beetles (Coleoptera, Scarabaeidae, Scarabaeinae). Folia Entomol. Mex. 82, 195–238.

Hanski, I., Cambefort, Y., 1991. Competition in Dung Beetles. In: Hanski, I., Cambefort, Y. (Eds.), Dung Beetle Ecology. Princeton University Press, Princeton, pp. 305–329.

Harwood, J.D., Wallin, W.G., Obrycki, J.J., 2005. Uptake of Bt endotoxins by non target herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol. Ecol. 14, 2815–2823.

Heinemann, J.A., 2007. A Typology of the Effects of (Trans) Gene Flow on the Conservation and Sustainable Use of Genetic Resources. Roma, FAO, pp. 94.

Hernández, M.I.M., Vaz-de-Mello, F.Z., 2009. Seasonal and spatial species richness variation of dung beetles (Coleoptera, Scarabaeinae s. str.) in the Atlantic forest of southeastern Brazil. Rev. Bras. Entomol. 53, 607–613.

Hernández, M.I.M., Barreto, P.S.C.S., Costa, V.H., Creão-Duarte, A.J., Favila, M.E., 2014. Response of a dung beetle assemblage along a reforestation gradient in Restinga Forest. J. Insect. Conserv. 18, 539–546.

Hilbeck, A., Schmidt, J.E.U., 2006. Another view on Bt proteins, how specific are they and what else might they do? Biopest. Int. 2 (1), 1–50.

Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filipini, A., Bigler, F., 1998. Toxicity of Bacillus thuringiensis Cry1Ab toxin to the predator Chrysoperla carnea (Neuroptera, Chrysopidae). Environ Entomol. 27 (5), 1255–1263.

Hilbeck, A., Meier, M., Benzler, A., 2008. Identifying indicator species for post-release monitoring of genetically modified, herbicide resistant crops. Euphytica 164, 903–912.

Hilbeck, A., Meier, M., Römbke, J., Jänsch, S., Teichmann, H., Tappeser, B., 2011. Environmental risk assessment of genetically modified plants – concepts and controversies. Environ. Sci. Eur. 23, 13.

Hillbeck, A., Meier, M., Trtikova, M., 2012. Underlying reasons of the controversy over adverse effects of Bt toxins on lady beetle and lacewing larvae. Environ. Sci. Eur. 24, 9.

Hooper, D.U., Solan, M., Symstad, A., Díaz, S., Gessner, M.O., Buchmann, N., Degrange, V., Grime, P., Hulot, F., Mermillod-Blondin, F., Roy, J., Spehn, E., Van Peer, L., 2002. Species diversity, functional diversity and ecosystem functioning.. In: Inchausti, P., Loreau, M., Naeem, S. (Eds.), Biodiversity and Ecosystem Functioning, Synthesis and Perspectives. Oxford University Press, Oxford, UK, pp. 195–208.

Hoyle, M., Cresswell, J.E., 2007. The effect of wind direction on cross-pollination in wind-pollinated GM crops. Ecol. Appl. 17, 1234–1243.

James, C., 2012. Global Status of Commercialized Biotech/GM crops. Brief No. 44, 2012. ISAAA. ISAAA, Ithaca, New York.

Klein, B.C., 1989. Effects of forest fragmentation on dung and carrion beetle communities in Central Amazonia. Ecology 70 (6), 1715–1725.

Leite, P.F., Klein, R.M., 1990. Geografia do Brasil. Fundação Instituto Brasileiro de Geografia e Estatística, Diretoria de Geociência, Rio de Janeiro, IBGE, pp. 420.

Lovei, G.L., Andow, D.A., Arpaia, S., 2009. Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environ. Entomol. 38, 293–306.

Lutz, B., Wiedemann, S., Einspanier, R., Mayer, J., Albrecht, C., 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. J. Agric. Food Chem. 53, 1453–1456.

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

McGeoch, M.A., van Rensburg, B.J., Botes, A., 2002. The verification and application of bioindicators: a case study of dung beetles in a savanna ecosystem. J. Appl. Ecol. 39, 661–672.

Miller, J.C., 1993. Insect natural history, multi-species interactions and biodiversity in ecosystems. Biodivers. Conserv. 2, 233–241.

Nichols, E., Larsen, T., Spector, S., Davis, A.L., Escobar, F., Favila, M., Vulinec, K., 2007. Global dung beetle response to tropical forest modification and fragmentation, a quantitative literature review and meta-analysis. Biol. Conserv. 137, 1–19.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biol. Conserv. 141, 1461–1474.

Nichols, E., Gardner, T.A., Peres, C.A., Spector, S., 2009. Co-declining mammals and dung beetles, an impending ecological cascade. Oikos 118, 481–487.

Obrist, L.B., Dutton, A., Albajes, R., Bigler, F., 2006. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecol. Entomol. 31, 143–154.

Obrycki, J.J., Losey, J.E., Taylor, O.R., Jesse, L.C.H., 2001. Transgenic insecticidal maize, beyond insecticidal toxicity to ecological complexity. BioScience 51, 353–361.

Pandolfo, C., Braga, H.J., Silva Júnior, V.P., Massignam, A.M., Pereira, E.S., Thomé, V.M.R., 2002. Atlas Climático digital do estado de Santa Catarina (CD-ROM). Epagri, Florianópolis.

Paul, V., Guertler, P., Wiedemann, S., Meyer, H.H., 2010. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Res. 19 (4), 683–689.

Ramírez-Romero, R., Desneux, N., Decourtye, A., Chaffiol, A., Pham-Delégue, M.H., 2008. Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? Ecotoxicol. Environ. Saf. 70, 327–333.

Reuter, H., Böckmann, S., Breckling, B., 2008. Analysing cross-pollination studies in maize. In: Breckling, B., Reuter, H., Verhoeven, R. (Eds.), Implications of GM-Crop Cultivation at Large Spatial Scales. Theorie in der Ökologie 14. Peter Lang, Frankfurt, pp. 47–53.

Romeis, J., McLean, M.A., Shelton, A.M., 2013. When bad science makes good headlines, Bt maize and regulatory bans. Nat. Biotechnol. 31, 386–387.

Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokelsek, J., Stephen, M.L., 2007. Toxins in transgenic crop by products may affect headwater stream ecosystems. Proc. Natl. Acad. Sci. U. S. A. 104 (41), 16204–16208.

Saxena, D., Stotzky, G., 2000. Insecticidal toxin from Bacillus thuringiensis is released from roots of transgenic Bt corn in vitro and in situ. FEMS Microbiol. Ecol. 33, 35–39.

Scheffler, P.Y., 2005. Dung beetle (Coleoptera, Scarabaeidae) diversity and community structure across three disturbance regimes in eastern Amazonia. J. Trop. Ecol. 21, 9–19.

Schuler, T.H., Poppy, G.M., Kerry, B.R., Denholm, I., 1998. Insect-resistant transgenic plants. Trends Biotechnol. 16, 168–174.

Simmons, L.W., Ridsdill-Smith, T.J., 2011. Ecology and Evolution of Dung Beetles. Blackwell Publishing, Oxford, pp. 368.

Slade, E.M., Mann, D.J., Villanueva, J.F., Lewis, O.T., 2007. Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem function in a tropical forest. J. Anim. Ecol. 76, 1094–1104.

Southwood, T.R.E., 1994. Ecological Methods, with Particular Reference to the Study of Insect Populations. Chapman & Hall, London, pp. 524.

Spector, S., Ayzama, S., 2003. Rapid turnover and edge effects in dung beetle assemblages (Scarabaeidae) at a Bolivian neotropical forest-savanna ecotone. Biotropica 35 (3), 394–404.

Statsoft, 2001. Statistica for Windows (Data Analysis Software System), version 6.0. Tulsa, Oklahoma (USA).

Stotzky, G., 2002. Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis. In: Letourneau, D.K. and Burrows, B.E. (Eds.), Genetically Engineered Organisms. Assessing Environmental and Human Health Effects, pp. 187–222.

Then, C., 2010. Risk assessment of toxins derived from Bacillus thuringiensis synergism, efficacy, and selectivity. Environ. Sci. Pollut. Res. 17, 791–797.

Vachon, V., Laprade, R., Schwartz, J.L., 2012. Current models of the mode of action of Bacillus thuringiensis insecticidal crystal proteins: a critical review. J. Invertebr. Pathol. 111, 1–12.

Van Frankenhuyzen, K., 2013. Cross-order and cross phylum activity of Bacillus thuringiensis pesticidal proteins. J. Invertebr. Pathol. 114, 76–85.

Vaz-de-Mello, F.Z., Edmonds, W.D., Ocampo, F., Schoolmeesters, P., 2011. A multilingual key to the genera and subgenera of the subfamily Scarabaeinae of the new world. Zootaxa 2854, 1–73.

Viegas, G., Stenert, C., Schulz, U.H., Maltchik, L., 2014. Dung beetle communities as biological indicators of riparian forest widths in southern Brazil. Ecol. Indic. 36, 703–710.

Wall, D.H., Moore, J.C., 1999. Interactions underground soil biodiversity, mutualism, and ecosystem processes. BioScience 49, 109–117.

Wickson, F., Wynne, B., 2012. Ethics of science for policy in the environmental governance of biotechnology, MON810 maize in Europe. Ethics Policy Environ. 15, 321–340.

Wilcove, D.S., Mclellan, C.H., Dobson, A.P., 1986. Habitat fragmentation in the temperate zone. In: Soulé, M.E. (Ed.), Conservation Biology, the Science of Scarcity and Diversity. Sinauer, Sunderland, pp. 237–256.

Wolfsbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Wartrud, L.S., 2008. Bt crop effects on functional guilds of non-target arthropods, a meta-analysis. PLoS One 3, 1–4.

Yachi, S., Loreau, M., 1999. Biodiversity and ecosystem functioning in a fluctuating environment, the insurance hypothesis. Proc. Natl. Acad. Sci. U. S. A. 96, 1463–1468.

Zwahlen, C., Andow, D.A., 2005. Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. Environ. Biosaf. Res. 4, 113–117.