

# Functional diversity of staphylinid beetles (Coleoptera: Staphylinidae) in maize fields: testing the possible effect of genetically modified, insect resistant maize

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## Abstract

Staphylinid beetles are recommended bioindicators for the pre-market environmental risk assessment of genetically modified (GM) insect protected maize expressing the Cry3Bb1 toxin. Our multiannual study is a unique European analysis of a staphylinid community within a 14 ha maize field. GM maize, its near-isogenic hybrid (with or without insecticide treatment), and two other reference hybrids were each grown in five 0.5 ha plots. The opportunity for exposure to Cry toxin from plant residues ploughed into the soil was shown by the presence of saprophagous dipteran larvae that are common prey of predatory staphylinid species and hosts of the parasitoid species. 2587 individuals belonging to 77 staphylinid species were sampled using pitfall traps. *Lesteva longoelytrata* (31%), *Oxyptoda acuminata* (12%), *Aloconota sulcifrons* (8%) and *Anotylus rugosus* (7%) were the most abundant beetles in the field. Bionomics, food specialization, temperature requirements and size group were assigned for 25 most common species. These traits determine the occurrence of staphylinid beetles in the field, the food sources they could utilize and thus also their likely contact with the Cry3Bb1 toxin. Statistical analysis of activity abundance, Rao indices and multivariate analysis of distribution of particular categories of functional traits in the field showed negligible effects of the experimental treatments, including the GM maize, upon the staphylinid community. Staphylinid beetles represent a considerably diverse part of epigeic field fauna with wide food specialization; these features render them suitable for the assessment of environmental safety of GM insect protected maize. However, the availability of prey and the presence of particular staphylinid species and their abundance are highly variable; this complicates the interpretation of the results.

**Keywords:** GM crops, *Bt* maize, Cry3Bb1, non-target, staphylinid beetles, activity abundance, functional traits, dipteran larvae

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## Introduction

*Bacillus thuringiensis* (*Bt*) formulations have been used in the control of insect pests since 1920s and remain the preferred means of crop protection in organic farming (Lemaux, 2008).

However, owing to the substantial advances in molecular biology it is now possible to replace *Bt* containing sprays with genetically modified (GM) crops that contain *Bt* gene(s) thereby expressing the insecticidal Cry toxins themselves. Accordingly, *Bt* gene expression within the plant eliminates the problem of short Cry toxin persistence on plants exposed to ultraviolet radiation (Fontes *et al.*, 2002) and improves targeting of the toxins to specific herbivores.

The development of transgenic crops containing inserted *Bt* gene(s) affords hope to farmers that must contend with losses caused by various insect pests. More than 100 times increase of global hectareage in 2014 since the introduction of GM crops in 1996 (James, 2014), including *Bt* crops, testifies to the acceptance of this technology by farmers (Hutchison *et al.*, 2010; Brookes & Barfoot, 2012). The International Service for the Acquisition of Agri-biotech Applications (ISAAA) report (James, 2014) points to the benefits of growing GM crops that have been recorded for almost 20 years of their use. It mentions e.g., a reduction in pesticide use by 37%, increased crop yields by 22% and increase in profits of farmers by 68%. However, to date, only *Bt* maize MON 810 has been approved for cultivation in the European Union (EU), although the cultivation of several other GM crops is under active consideration. Consequently, extensive analyses of the possible effects of *Bt* crops on arthropods represents an important part of the pre-market environmental risk assessment (ERA) within the EU.

The *Bt* maize MON 88017 expresses a modified Cry3Bb1 toxin effective against *Diabrotica* spp. (Vaughn *et al.*, 2005) as well as the enzyme CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) that provides tolerance to the non-selective herbicide glyphosate. Considering the current European spread of the serious pest, the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae), it is reasonable to assume that MON 88017 has the potential to be grown in the EU in the future. The use of suitable *Bt* maize events, possibly combined with the application of entomophagous nematodes (Toepfer *et al.*, 2010) could complement the conventional *D. v. virgifera* management strategies, such as crop rotation and chemical protection (Ciobanu *et al.*, 2009); a prescient point given that current control strategies appear to be less and less effective due to the emergence of resistance and behavioural changes in *Diabrotica* spp. (Vaughn *et al.*, 2005). Gassmann *et al.* (2011) have shown that the use of *Bt* maize alone will not solve the problem but should be considered a component of any integrated pest management (IPM) strategy, while also preventing the development of resistance to the *Bt* maize itself and minimizing the outbreaks of secondary pests (Meissle *et al.*, 2011).

Staphylinid beetles (Coleoptera: Staphylinidae), along with ants, spiders, ground beetles and predatory mites, are important predators of insect pests in agroecosystems. Within the agricultural landscape 17.7% of the 1397 staphylinid species occurring in the Czech Republic are a considerable part of the epigeic fauna (Boháč, 1999). Staphylinid beetles have a wide prey spectrum and can suppress numerous herbivores occurring in *Bt* maize fields (Sanvido *et al.*, 2009) including pests. Some staphylinid beetles prey on dipteran larvae, species of the genus *Stenus* feed on springtails and tiny mites, and other species (e.g., genera *Tachyporus* and *Philonthus*) prey on plant-residing aphids and insect larvae (Rose & Dively, 2007; Perumalsamy *et al.*, 2009). However, some groups are saprophagous, mycophagous (genus *Tachyporus*) or algae feeders (genus *Carpelimus*).

Hence, staphylinid beetles are a diversified group with wide food specialization. Owing to their trophic

specialization, they could be used to monitor the environmental status of ecosystems and particularly the effects of human activities on ecosystems (Boháč, 1999). As shown by Boháč (1999), staphylinid beetles are good indicators of the impact of changes in agricultural techniques such as crop rotation, tillage, application of manure, fertilizers and pesticides, and of land use in agricultural settlements because these techniques affect directly the important food sources of staphylinid beetles. If any of this wide spectrum of food sources would be affected either positively or negatively by the *Bt* toxin, staphylinid species depending upon these food sources would be changed in number or in other traceable features.

Additional environmental conditions affect the occurrence and diversity of staphylinid species and determine which food sources they exploit. For example, species with different temperature requirements occur concurrently in the fully-grown maize that provides better balanced microclimates in comparison with the early developmental stages of maize or with a bare field. The stability of field conditions also promotes regular occurrence of species with different bionomics. Most common species in fields are eurytopic, typical for deforested agricultural landscape, but stenotopic species with preference of intermediate habitats affected by human activities, also occur in high abundance. The number of species and their activity abundance within the categories food specialization, bionomics, temperature requirements and size, were determined and separately analyzed to find out any effect of *Bt* maize. If an effect was disclosed on any category within the tested traits, it could be explained accurately in subsequent studies focused on species within a specific category. More attention could be paid to the specified agent mediating negative effect to species of specific category of functional trait/s.

Field trials have not shown any negative effect of Cry3Bb1 toxin expression in *Bt* maize on epigeic predators (Ahmad *et al.*, 2005; Bhatti *et al.*, 2005) and applications of pure Cry3Bb1 toxin also have no effect on ground beetles and spiders (Duan *et al.*, 2006; Meissle & Romeis, 2009). Several studies have also proved no effect of Coleopteran-specific Cry3Bb1 on ladybirds (Li & Romeis, 2010; Álvarez-Alfageme *et al.*, 2011). However, insufficient attention has been paid to the staphylinid beetles, despite the fact that some species are prone to consume Cry toxins within their prey. The presence of Cry3Bb1 within the bodies of the larvae of two saprophagous dipteran families (Knecht & Nentwig, 2010) and in spider mites (Li & Romeis, 2010) has been demonstrated. García *et al.* (2010) had shown prey-mediated Cry1Ab toxin uptake by the staphylinid beetle *Atheta coriaria* and subsequently demonstrated relatively high levels of toxin sequestration in *A. coriaria*, derived from *Tetramesa urticae* fed on Cry3Bb1-expressing maize (García *et al.*, 2012). Indeed, more than 20% of the Cry3Bb1 concentration measured in the *Bt* maize was detected in the staphylinid larvae. Since Cry3Bb1 has been detected in maize root exudates (Icoz & Stotzky, 2008), it has been proposed that small amounts of biologically active toxin may bind to surface-active particles within the soil and persist over long periods of time (Fiorito *et al.*, 2008). Moreover, it remains possible that some toxin may also remain in plant residues left in the field. Residues of Cry toxins in soil are then ingested by the potential prey of the staphylinid beetles. Clearly, pre-market studies of the possible effect of *Bt* crops on staphylinid communities are required to address these concerns. Although this is unlikely due to their role in trophic chain especially as non-specified predators, there is

also probability of negative indirect effects caused by suboptimal prey (target pest, Naranjo, 2009).

Due to the abundant occurrence of staphylinid beetles and a good pre-existing knowledge of their ecological requirements, they have been recommended as bioindicators for ERA studies of GM maize expressing Cry3Bb1 toxin (Rose & Dively, 2007). The potential of staphylinid beetles as bioindicators within the Czech Republic was highlighted by Boháč (1999); unfortunately, there are very few studies to date focusing on this family in the agroecosystems of Central Europe (Balog *et al.*, 2009).

Staphylinid beetles were hypothesized as potentially sensitive to *Diabrotica*-resistant *Bt* maize (García *et al.*, 2012). However, the implication of *Bt* toxin exposure on the performance of staphylinid beetles is still not clear in the case of long-term exposure. The aim of this study was to compare the activity abundance within categories of several functional traits and species diversity expressed by using Rao indices and Simpson index of species diversity. We evaluated community of staphylinid beetles in a large field trial that enabled to study community in realistic field conditions minimizing for instance the edge effect.

Comparison of *Bt* maize just with the non-transgenic parental hybrid is insufficient in respect to agricultural practices. Hence the effect of a standard application of the soil insecticide Dursban 10 G (i.e., chlorpyrifos) was tested with the near-isogenic hybrid. Rauschen *et al.* (2009) confirmed, that differences among hybrids are more pronounced than those between the GM and its near-isogenic not-GM hybrids. To verify this hypothesis two non-related reference hybrids were tested.

## Material and methods

### *Site description and experimental field treatments used*

The study was conducted during the vegetation seasons 2009–2011 and in spring 2012. A 14 ha experimental site located in southern Bohemia, Czech Republic (48°59'N, 14°20'E, 423 m a.s.l., average precipitation: 550 mm year<sup>-1</sup>, average annual temperature: 11°C) was selected for the study. The field had a 14% south western slope with a medium, mildly humid clay loam brown soil (Brown, 1990) and was surrounded by forest on the southwest and by fields planted with winter wheat in 2009 and 2010 and with oilseed rape in 2011 on the other three remaining sides. Data regarding precipitation and temperature profiles during the study years are obtainable from the meteorological station in České Budějovice.

The maize was sown on 11 May 2009, 6 May 2011 and 10 June 2010 due to the exceptionally rainy spring. Standard agronomic management operations were applied (see Supplementary Table S1). Maize was harvested at developmental stage BBCH 87 (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, staging according to Lancashire *et al.*, 1991) and immediately shredded into small pieces. In 2009, the shredded maize was ploughed into the soil on the respective experimental plots, whereas in 2010 and 2011 it was ensiled and used for biogas production. Remnants from maize plants in the field were ploughed 25–30 cm deep into the soil in years 2010 and 2011.

### *Field trial design and treatments*

The GM maize MON 88017 YieldGard VT Rootworm/RR2™ (MONSANTO Technology LLC, St. Louis, MO, USA),

a near-isogenic hybrid DK 315 that was either non-treated or treated with the broad-spectrum soil insecticide Dursban 10 G (a.i. 10% chlorpyrifos, 20 kg ha<sup>-1</sup> – applied simultaneously with the seed sowing) and reference hybrids Kipous (KWS SAAT AG, Einbeck, Germany) and PR38N86 (DuPont Pioneer, Johnston, IA, USA) were each grown on five 0.5 ha (63 × 81 m<sup>2</sup>) plots (25 plots in total), randomized across the study site (in a pattern slightly modified due to the presence of drainage wells and hunting hide). All hybrids used in the experiment have the same length of growing season till the maturation of seeds (middle early mature hybrids, FAO 1978–81). A 20 m wide buffer zone was planted with the early hybrid DKC 2870 (MONSANTO Technology LLC) on the field side adjacent to the forest, while a similar 10 m zone was applied on the other three sides.

### *Capture and identification of staphylinid beetles*

Pitfall traps were used for the monitoring of staphylinid beetles. One trap was placed in the centre of each plot and two traps at 40 m intervals were placed in two rows 15 m from the centre on either side (i.e., 5 traps per plot, 125 in total, GPS navigation used). The pitfall traps consisted of doublets of an inner and outer plastic cup: 9 cm in diameter, volume 0.5 litres. Inner cups were filled with 300 ml of 10% NaCl supplemented with 2–3 drops of a detergent and the trap was covered with an aluminium cap. Digging-in effects were reduced by leaving the covered outer plastic cups in the soil for the entire growing season as recommended by Schirmel *et al.* (2010).

The occurrence of staphylinid beetles was monitored for a fortnight prior to sowing, for 1-week intervals at stages of BBCH 09 (sprouting), BBCH 16 (six leaves unfolded, missing in 2009 due to an unusually rainy June when sample collection was not possible), BBCH 65 (full flowering) and BBCH 87 (physiological maturity), and for a fortnight after the harvest and ploughing of manure and digestate. The first sampling in 2009 was used to detect staphylinid preferences or avoidance of plot positions alone and to monitor the colonization process. One fortnight monitoring in spring 2012 was done with the aim to increase the number of observations regarding the possible effect of *Bt* maize residues on the size and composition of the overwintering staphylinid community.

Captured staphylinid beetles were identified to the species level using recent identification keys (Benick, 1974; Assing & Schülke, 2011). Bionomics (Boháč, 1999), food specialization, temperature requirements and size group (Boháč & Růžička, 1990; Assing & Schülke, 2011) were identified for the 25 most common species (table 1); other species listed in Supplementary Table S2 were represented by less than ten individuals.

### *Capture of dipteran larvae*

Soil samples for monitoring dipteran larvae were collected 3 weeks after maize shredding and ploughing in plots planted with MON 88017 and its near-isogenic hybrid (with and without insecticide treatment). Three mixed soil samples (consisting of five subsamples, each of 10 cm diameter and 10 cm depth) were taken along the diagonals of each plot. The cumulative plot samples were processed in Kempson's extractors (Kempson *et al.*, 1963), in which soil is placed on a sieve (5 mm mesh) and the light and heat from overhanging electric bulbs drives all creatures into a solution contained under the

Table 1. The bionomics (Boháč, 1999), food specialization, temperature requirements and size group (Boháč &amp; Růžička, 1990; Assing &amp; Schülke, 2011) of 25 most common staphylinid species.

Subfamily	Species	Bionomics	Food specialization	Temperature requirements	Size group
Aleocharinae	<i>Aleochara bipustulata</i> (Linné, 1760)	E	Parasitic ontogenesis predator	High	II
	<i>Aloconota sulcifrons</i> (Stephens, 1832)	R2	Predator	Low	I
	<i>Amischa analis</i> (Gravenhorst, 1802)	E	Predator	Low	I
	<i>Atheta fungi</i> (Gravenhorst, 1806)	E	Saprophagy, predator	Low	I
	<i>Atheta triangulum</i> (Kraatz, 1856)	E	Predator	Low	I
	<i>Oxypoda acuminata</i> (Stephens, 1832)	R2	Predator	Low	I
	<i>Oxypoda opaca</i> (Gravenhorst, 1802)	E	Predator	High	I
Omaliinae	<i>Lesteva longoelytrata</i> (Goeze, 1777)	E	Predator	Low	III
	<i>Omaliium caesum</i> (Gravenhorst, 1806)	E	Saprophagy	High	II
	<i>Omaliium rivulare</i> (Paykull, 1789)	E	Saprophagy, mycophagy?	High	II
Oxytelinae	<i>Anotylus rugosus</i> (Fabricius, 1775)	E	Saprophagy, mycophagy?	High	III
	<i>Anotylus tetracarinitus</i> (Block, 1799)	E	Saprophagy	High	I
	<i>Carpelimus corticinus</i> (Gravenhorst, 1806)	R2	Phytophagy, algae feeder	High	I
	<i>Carpelimus rivularis</i> (Motschulsky, 1860)	R2	Saprophagy, algae feeder	High	II
Paederinae	<i>Lathrobium fulvipes</i> (Gravenhorst, 1806)	E	Predator	Low	IV
	<i>Lathrobium volgense</i> (Kraatz, 1857)	E	Predator	Low	IV
Staphylininae	<i>Gabrius breviventer</i> (Sperk, 1835)	E	Predator	Low	II
	<i>Philonthus atratus</i> (Stephens, 1829)	E	Predator	High	IV
	<i>Philonthus carbonarius</i> (Gravenhorst, 1802)	E	Predator	High	IV
	<i>Xantholinus linearis</i> (Olivier, 1794)	E	Predator	High	IV
	<i>Xantholinus longiventris</i> (Heer, 1839)	E	Predator	High	IV
Tachyporinae	<i>Mycetoporus brucki</i> (Pandellé, 1869)	R2	Mycophagy	Low	III
	<i>Mycetoporus Lepidus</i> (Gravenhorst, 1802)	R2	Mycophagy	Low	III
	<i>Tachinus fimetarius</i> (Gravenhorst, 1802)	E	Saprophagy, mycophagy?	High	II
	<i>Tachyporus hypnorum</i> (Fabricius, 1775)	E	Saprophagy, mycophagy?	High	II

R2, stenotopic species: intermediate habitats affected by human activities.

E, eurytopic species: deforested agricultural landscape.

Size groups: I: 2.1–3.0 mm, II: 3.1–4.5 mm, III: 4.6–7.0 mm, IV: 7.1–11.0 mm.

sieve. Accordingly, samples of 3.925 dm<sup>3</sup> soil were exposed to light for 7 days and the organisms were collected in 500 ml of water containing 2 ml of 2% formaldehyde. Larvae were identified to the family level (Brauns, 1954) and the numbers of individuals observed were used to calculate the abundance per square metre (individual m<sup>-2</sup>) to a depth of 10 cm.

#### Data analysis

Activity abundance within each category of functional traits (bionomics, food specialization, temperature requirements and size group) was compared among treatments using repeated measures analysis of variance (RM ANOVA) with sample date as replicative unit within analysis (StatSoft Statistica 8, Statsoft, Inc., Tulsa 74104, OK, USA). Interaction between treatment and sample date was tested (sample collections with no individuals of particular category had to be excluded from analysis).

The Rao indices for given functional traits and Simpson index of species diversity (Botta-Dukát, 2005) were calculated according to de Bello & Lepš (2006) using a macro excel file provided by Dr J. Lepš (Lepš *et al.*, 2006). Given traits were

coded as categorical with food specialization as fuzzy coded trait in case of multiple food sources. Rao indices were calculated for each plot (sum of individuals of 25 most common species per plot during season). Repeated measures ANOVA was employed to evaluate variation among treatments in functional traits and Simpson index of species diversity with year as replicative unit within analysis. Interaction between treatment and year (2009–2012) was tested. Repeated measures ANOVA was used to analyse the dipteran abundance per square metre (data for each family were summed per plot, abundance per square metre was calculated and averaged per treatment, calculated average abundance per treatment for each family was log transformed and statistically analyzed) with year as replicative unit within analysis. Interaction between treatment and year (2009–2012) was tested.

The results of *F*-tests were accompanied by degrees of freedom and degrees of freedom of the error (within-groups degrees of freedom). Tests of the homogeneity of variances confirmed the normal distribution of the dataset (data were not transformed) with the exception of dipteran larvae. A 5% significance level was applied in all statistical tests. Illustrative statistics were compiled using the Graph Pad



Prism 4.5 (GraphPad Software, Inc., San Diego 92037, CA, USA).

The correlation (Pearson's linear correlation test), for which data remained divided according to the experimental treatment, between abundance of dipteran larvae and activity abundance of parasitoid and predatory staphylinid beetles (food specialization in table 1) was processed in Statistica 8. The occurrence of parasitoid staphylinid beetles (*Aleochara bipustulata* and *Aleochara curtula*) in the second sample data (BBCH 09) and the occurrence of predatory staphylinid beetles in the first sample data (before sowing) were correlated with the abundance of dipteran larvae in previous autumns.

Distribution of particular categories of functional traits in the field was identified using multivariate analysis (Lepš & Šmilauer, 2003) using the Canoco software for Windows 4.5 (Plant Research International, Wageningen 6700 AA, The Netherlands). The effect of environmental variables that could modify the effect of our experimental treatments, namely year, sample date (time series: number of days since the sowing day marked as number 1, sample date before sowing was marked with zero) and spatial arrangement (column: owing to the distance from adjacent fields; row: owing to the distance from the forest, the slight field inclination and moisture; distance from the unevenly distributed grassy area around drainage wells and hunting hide) were identified. Canonical correspondence analysis (CCA) (0.001 attributed to each value, CCA: log transformation:  $y = \log(100x + 1)$ , downweight rare functional traits, biplot scaling) was employed. Monte Carlo permutation tests (MCPT: 999 permutations, split-plot according treatment, forward selection) within CCA were used to find out that spatial arrangement of experimental plots did not affect activity abundance of staphylinid beetles in experimental area. Significant effect of time variables (years, sample date) was eliminated using them as covariates because the theoretical principle for permutation based on time is less certain than permutation based on places (Lepš & Šmilauer, 2003). The resulting design of CCA (0.001 attributed to each value, CCA: log transformation:  $y = \log(100x + 1)$ , downweight rare functional traits, biplot scaling, covariates: years, sample date, MCPT: 999 unrestricted permutations, forward selection) was used to evaluate effect of treatments on distribution of particular categories of functional traits.

In regard to assaying for any effect of spatial arrangement prior to the start of the experiment, one sampling was carried out before sowing in 2009 (beginning of experiment). Activity abundance within each category of functional traits was compared among treatments using one-way ANOVA (data given per plot). One-way ANOVA (data given per plot) was employed to evaluate variation among treatments in functional traits and Simpson index of species diversity. Multivariate analysis (0.001 attributed to each value, CCA: log transformation:  $y = \log(100x + 1)$ , downweight rare functional traits, biplot scaling, MCPT: 999 unrestricted permutations, forward selection) was used for identifying distribution of particular categories of functional traits in the field.

## Results

### *Staphylinid community in particular years*

2587 individuals (2009: 77, 2010: 1 490, 2011: 892, 2012: 128) belonging to 77 species (2009: 36, 2010: 53, 2011: 44, 2012: 20) of 33 genera (Supplementary Table S2) were identified in this

study. In 2009, the lowest number of individuals were trapped (fig. 1). Two-thirds of the captured species were eurytopic (e.g., *Amischa analis*). Several typical stenotopic species were also identified (e.g., *Aloconota sulcifrons*). The trapped species represented different feeding specializations: non-specific predators attacking larger prey (larger species of the genera *Philonthus* and *Xantholinus*), smaller species such as *Tachyporus hypnorum* that chase aphids and mites on vegetation, saprophagous species (genera *Anotylus* and *Oxytelops*), algophagous species (genus *Carpelimus*) and genus *Aleochara* with parasitic ontogenesis in dipteran puparia.

The number of trapped beetles increased in 2010. Activity abundance changed similarly during the season in all plots. Fig. 1 represents the activity of predatory staphylinid beetles as a category with most individuals. Overall, the biodiversity of staphylinid beetles was higher than in 2009. The first, pre-season sample of 2010 was species-rich (29) but most individuals were recorded at the end of the season and after harvest. *A. bipustulata*, a staphylinid beetle with parasitic ontogenesis, made up 33% of individuals caught after maize sowing (BBCH 09). Frequently occurring species included eurytopic *Lesteva longolytrata* (36% of individuals), *Anotylus rugosus* (6%) and *Tachinus fimetarius* (6%), as well as an assembly of stenotopic species (41% of species), dominated by *Oxytoda acuminata* (16%). The occurrence of species typical for wet agrocoenosis and littoral habitats (*Carpelimus rivularis*, *Gabrieus breviventer*, *Philonthus quisquiliarius*, *Stenus ater*) contrasted with their absence in the very wet 2009 (total precipitation 628 mm). However, only June was exceptionally rainy in 2009, whereas 2010 was characterized by regular intervals of rainfall (total precipitation 517 mm). Therefore, it appears that such weather patterns obviously promoted the establishment of a stable community of staphylinid beetles that preferred wet agrocoenosis in 2010.

The number of individuals trapped in 2011 was lower than in 2010 (fig. 1). *L. longolytrata* dominated (29% of individuals) as in 2010, followed by *A. sulcifrons* (13%) and *A. rugosus* (9%). The sample data of the bare field in 2012 was dominated by *A. sulcifrons* (31%) and by wet agrocoenosis species *G. breviventer* (27%).

### *Assumption of the initial similarity of experimental plots*

Only 55 individuals belonging to 16 species were found prior to the start of the experiment in 2009. Consequently, the assumption of the initial similarity of experimental plots was confirmed by the Simpson index of species diversity and Rao indices (results not shown). However, analysis of activity abundance within each category of defined functional traits revealed significantly higher activity abundance of saprophagous and mycophagous species in plots suggested for sowing with near-isogenic hybrid than in plots planned for all other treatments. Activity abundance of species with requirements on high temperature was significantly higher in plots designed for sowing near-isogenic hybrid in comparison with plots planned to sow with *Bt* maize. Individuals of second and third size groups were significantly more abundant in plots suggested for sowing with near-isogenic hybrid than in plots designed for insecticide treatment and the third size group also than in plots stated for reference hybrid Kipous (results not shown). Multivariate analysis confirmed significant proportion of variability explained with plots designed for near-isogenic hybrid with and without insecticide treatment.

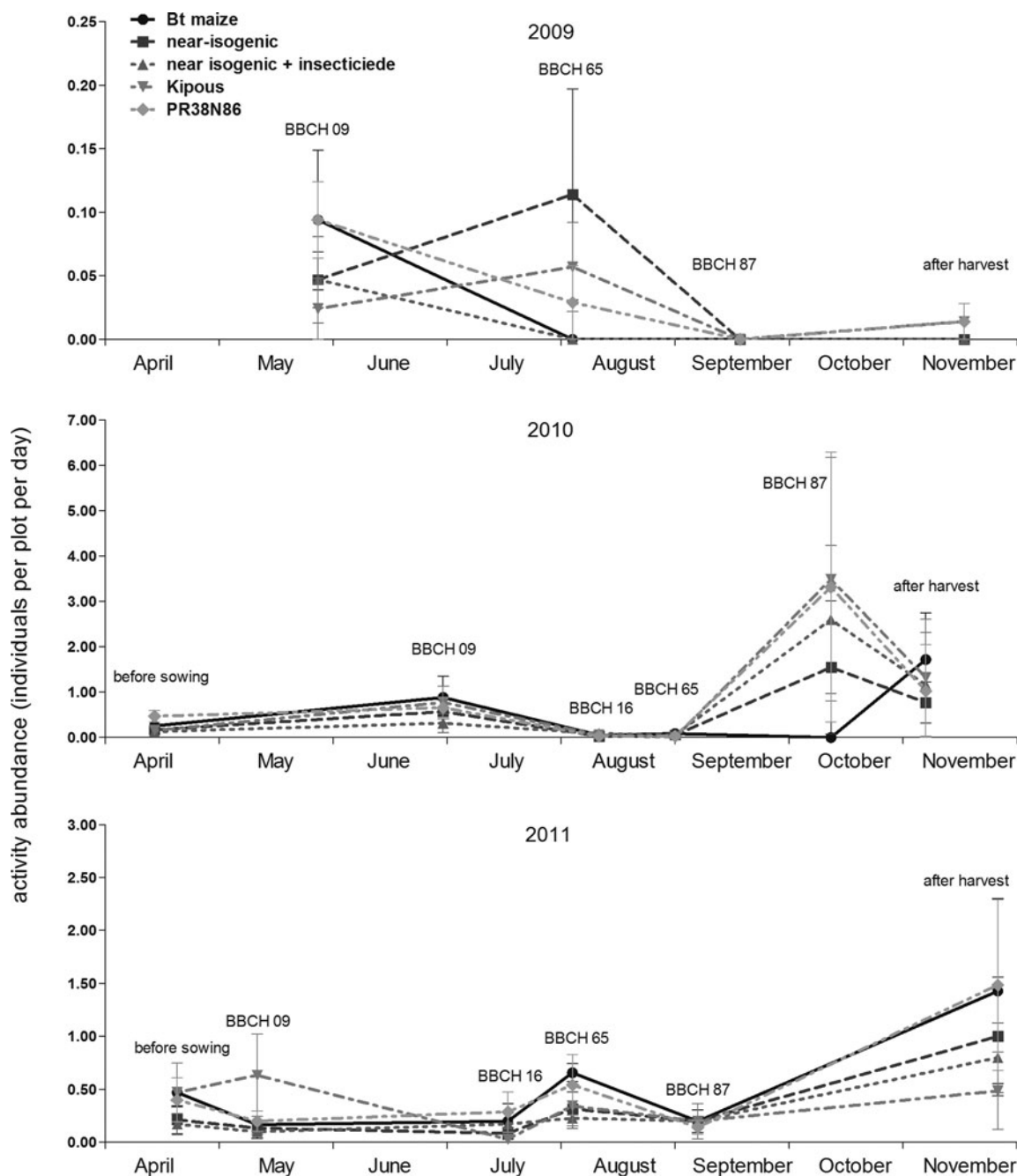


Fig. 1. Seasonal changes in predatory staphylinid beetles activity abundance in 2009–2011 showed as average numbers ( $\pm$ SE) of individuals caught per 0.5 ha (one plot, five pitfall traps per plot) per day.

Hunting hide had also significant effect on species distribution on field before sowing (results not shown).

To sum up, there were indications of differences of plots planned for near-isogenic hybrid with and without insecticide treatment regarding activity abundance within several categories of functional traits and in multivariate analysis. However, with respect to the results shown below, it is possible to conclude that differences in activity abundance during colonization of bare soil before sowing in 2009 did not affect subsequent population after sowing. In multivariate analysis,

suggestion of the highest activity abundance in plots with near-isogenic hybrid and absence of some categories in plots with near-isogenic hybrid with insecticide treatment were not confirmed.

#### *Analysis of the staphylinid community variation among treatments*

The highest total activity abundance was detected in plots with the reference hybrid PR38N86 following by plots with *Bt*

maize, reference hybrid Kipous, untreated near-isogenic hybrid and lastly by plots with near-isogenic hybrid treated with the insecticide. The seasonal changes observed in the number of individuals and the number of species displayed similar trends across the experimental treatments (fig. 1, species richness not shown) but lower activity abundance was recorded at the stage of physiological maturity (BBCH 87) in those planted with *Bt* maize in 2010. Activity abundance increased later in the season but species richness was also high at the beginning of the season. The 1 month delay in sowing in 2010 had a negligible effect on the above trends (fig. 1).

*L. longolytrata* (31%), *O. acuminata* (12%), *A. sulcifrons* (8%) and *A. rugosus* (7%) proved to be the most abundant species. Another 21 species were represented by more than ten trapped individuals and 28 species by less than ten individuals of which 24 were single records (Supplementary Table S2). Eurytopic species were more common than the stenotopic species, from which 14 were caught only once.

In general, activity abundance was highest in 2010 followed by 2011 which was in some categories, such as predators, very similar to 2012. Activity abundance in 2009 was very low for all functional traits. However, variability among treatments within 1 year was low. The effects of the experimental treatments on the activity abundance within categories of functional traits was statistically insignificant as well as the interaction between treatment and sample date (table 2).

The diversity of the staphylinid community characterized by Simpson index of species diversity (table 3) ranged 0.30–0.83. It revealed the lowest recorded diversity (Simpson index) in the first year of study. The Simpson index of species diversity was similar for years 2010 and 2011. Rao index for bionomics was generally low but higher in 2010 and 2011 when more individuals of stenotopic species were recorded. From the perspective of food specialization, the community was least diversified in 2009 when half of the collected individuals were predators. Generally, predators prevailed in all years. Less species but more individuals with low temperature requirements were recorded in comparison with species with high temperature requirements. Most individuals were from the third size group followed by the second size group. Rao indices for size groups are higher than for other traits because it consists of four different categories, none of which dominates so distinctly as predators among the categories for food specialization. Generally, Rao indices revealed a similarity of the staphylinid communities in 2010 and 2011. On the contrary, Rao indices were different for the first and last year of experiment (not verified by statistics). The values of all Rao indices and the Simpson index of species diversity were very similar in different treatments that did not significantly affect structure of community of staphylinid beetles from the perspective of species diversity, bionomics, food specialization, temperature requirements and size groups (table 3). Interaction between treatment and year was not significant for any of the traits (table 3) suggesting that no effect of treatment was observed in any of experimental season.

The CCA revealed low percentage of explained variability by experimental treatments (fig. 2). Whole model with experimental treatments as environmental variables explained 0.95% of variability in distribution of staphylinid beetles within experimental area. Each treatment explained 0–0.48% of variability (fig. 2). All points for categories within four functional traits are around intersection of ordination space indicating similarity of activity abundance in all experimental

treatments. Points for stenotopic species, phytophagous species and fourth size group are a little bit far from points for other categories indicating lower activity abundance of these categories in the most distant experimental treatment. The proximity of centroids for reference hybrid Kipous and near-isogenic hybrid suggests higher mutual similarity of these plots than with other three treatments that are in different part of the ordination diagram. Activity abundance of all categories of functional traits was average in plots with the reference hybrid Kipous that explains zero per cent of variability explained by this treatment (fig. 2). On the contrary, activity abundance in *Bt* maize was highest for four categories, in reference hybrid PR38N86 for seven categories including predators and lowest in near-isogenic hybrid treated with insecticide for six categories within functional traits. However, these differences were not significant (MCPT: *Bt* maize:  $F = 2.12$ ,  $P = 0.06$ , near-isogenic:  $F = 0.51$ ,  $P = 0.79$ , near-isogenic + insecticide:  $F = 1.15$ ,  $P = 0.31$ , Kipous:  $F = 0.32$ ,  $P = 0.92$ , PR38N86:  $F = 1.04$ ,  $P = 0.37$ ). The effect of the experimental treatments upon the staphylinid community was negligible (fig. 2).

#### Presence of dipteran larvae in soil samples

The abundance of dipteran larvae per square metre was highest in the autumn of 2009 (table 4). In 2009 and 2010, most dipteran larvae were found in the plots sown with the *Bt* maize, and in 2011 in plots with its near-isogenic hybrid treated with insecticide. However, no effect on the abundance of dipteran larvae was observed among the experimental treatments ( $F_{2,39} = 0.10$ ,  $P = 0.90$ ) nor in particular years (interaction between treatment and year:  $F_{4,78} = 0.34$ ,  $P = 0.85$ ).

The occurrence of staphylinid beetles with parasitic ontogenesis in dipteran puparia was highest in the late spring of 2010. The decline of activity abundance of parasitic staphylinid beetles in the following year was correlated with a reduced number of dipteran larvae ( $r = 0.81$ ,  $F_{1,4} = 7.62$ ,  $P = 0.05$ ). By contrast, the occurrence of predators attacking dipteran larvae did not match ( $r = 0.18$ ,  $F_{1,7} = 0.24$ ,  $P = 0.64$ ) the significant decrease of dipteran larvae in the autumns of 2010 and 2011.

## Discussion

This study describes the staphylinid community of a maize field in the Czech Republic. Plots planted with the *Bt* maize MON 88017 or its near-isogenic hybrid (either treated with or without standard applications of a soil insecticide) were compared with plots sown with two unrelated maize hybrids. Saprophagous dipteran larvae consuming plant residues in soil were monitored as a common prey of predatory staphylinid species and hosts of the parasitoid species.

The staphylinid beetles identified in the present study were typical for the fields of Central Europe (Boháč, 1999). Despite the fact that the literature describes staphylinid beetles as an abundant group in agroecosystems, they were recorded in lower numbers than the ground beetles and spiders monitored in our previous study (Svobodová *et al.*, 2012, 2013). Relatively low numbers of staphylinid beetles were also detected in a field trial that was carried out about 10 km away from the former site (Skoková Habušťová *et al.*, 2015). Balog *et al.* (2010, 2011) reported similar staphylinid beetle activity abundance but lower species richness in the field trials carried out in Hungary. Two-thirds of species listed in their first publication

Table 2. Activity abundance (average  $\pm$  SE,  $N = 5$ , data from five traps were pooled) of individuals within each category of defined traits (bionomics, food specialization, temperature requirements and size group) per plot (0.5 ha) during season (four sample data in 2009, six sample data in each 2010 and 2011, one sample data in 2012, degrees of freedom and degrees of freedom of the error for interaction are variable because sample collections with no individuals of a particular category were excluded from analysis).

Trait	Category	Year	Treatment					RM ANOVA		
			<i>Bt</i> maize	Near-isogenic	Near-isogenic + insecticide	Kipous	PR38N86	Treatment	Interaction: sample date $\times$ treatment	
Bionomics	R2	2009	0.10 $\pm$ 0.06	0.05 $\pm$ 0.05	0.05 $\pm$ 0.05	0.05 $\pm$ 0.05	0.15 $\pm$ 0.06	$F_{4,20} = 0.94, P = 0.46$	$F_{52,260} = 0.51, P > 0.99$	
		2010	2.30 $\pm$ 0.91	2.23 $\pm$ 0.63	1.70 $\pm$ 0.59	3.37 $\pm$ 1.75	3.73 $\pm$ 1.66			
		2011	2.93 $\pm$ 0.83	1.13 $\pm$ 0.48	0.73 $\pm$ 0.11	1.17 $\pm$ 0.53	1.03 $\pm$ 0.24			
		2012	1.80 $\pm$ 1.11	1.80 $\pm$ 0.92	1.40 $\pm$ 0.87	1.60 $\pm$ 0.93	2.00 $\pm$ 1.52			
	E	2009	0.75 $\pm$ 0.19	0.60 $\pm$ 0.22	0.30 $\pm$ 0.12	0.35 $\pm$ 0.17	0.65 $\pm$ 0.13	$F_{4,20} = 0.53, P = 0.72$	$F_{60,300} = 0.42, P > 0.99$	
		2010	6.67 $\pm$ 2.13	4.87 $\pm$ 1.73	7.07 $\pm$ 2.55	8.30 $\pm$ 2.62	7.27 $\pm$ 3.03			
		2011	4.47 $\pm$ 1.10	3.80 $\pm$ 1.13	3.57 $\pm$ 0.85	3.40 $\pm$ 1.04	6.13 $\pm$ 1.41			
		2012	4.00 $\pm$ 1.95	2.60 $\pm$ 1.44	1.80 $\pm$ 1.36	3.60 $\pm$ 2.16	3.80 $\pm$ 1.69			
Food specialization	Predatory	2009	0.40 $\pm$ 0.23	0.40 $\pm$ 0.20	0.20 $\pm$ 0.15	0.25 $\pm$ 0.19	0.50 $\pm$ 0.11	$F_{4,20} = 0.63, P = 0.65$	$F_{60,300} = 0.32, P > 0.99$	
		2010	6.10 $\pm$ 2.49	4.90 $\pm$ 1.80	6.77 $\pm$ 2.63	8.70 $\pm$ 3.80	8.37 $\pm$ 4.04			
		2011	5.83 $\pm$ 1.88	3.67 $\pm$ 1.25	3.07 $\pm$ 0.62	3.53 $\pm$ 1.05	5.73 $\pm$ 1.66			
		2012	4.60 $\pm$ 2.46	3.00 $\pm$ 1.10	3.00 $\pm$ 1.90	3.60 $\pm$ 2.20	4.20 $\pm$ 2.62			
	Saprophagous	2009	0.45 $\pm$ 0.05	0.25 $\pm$ 0.14	0.15 $\pm$ 0.10	0.15 $\pm$ 0.10	0.25 $\pm$ 0.11	$F_{4,20} = 0.54, P = 0.71$	$F_{60,300} = 0.74, P = 0.92$	
		2010	2.80 $\pm$ 0.69	2.20 $\pm$ 0.59	2.13 $\pm$ 0.61	3.10 $\pm$ 0.70	2.53 $\pm$ 0.73			
		2011	1.70 $\pm$ 0.32	1.37 $\pm$ 0.19	1.40 $\pm$ 0.33	1.27 $\pm$ 0.20	1.90 $\pm$ 0.40			
		2012	1.20 $\pm$ 0.58	1.20 $\pm$ 0.58	0.20 $\pm$ 0.20	1.60 $\pm$ 0.93	1.60 $\pm$ 0.68			
	Mycophagous	2009	0.30 $\pm$ 0.09	0.15 $\pm$ 0.10	0.15 $\pm$ 0.10	0.10 $\pm$ 0.10	0.15 $\pm$ 0.10	$F_{4,20} = 1.15, P = 0.36$	$F_{60,300} = 0.71, P = 0.95$	
		2010	1.63 $\pm$ 0.47	0.87 $\pm$ 0.15	0.77 $\pm$ 0.22	1.60 $\pm$ 0.70	1.67 $\pm$ 0.57			
		2011	0.57 $\pm$ 0.16	0.27 $\pm$ 0.08	0.53 $\pm$ 0.12	0.60 $\pm$ 0.11	0.80 $\pm$ 0.27			
		2012	0.80 $\pm$ 0.37	0.60 $\pm$ 0.40	0	0.20 $\pm$ 0.20	1.20 $\pm$ 0.73			
	Phytophagous	2009	0.10 $\pm$ 0.06	0.05 $\pm$ 0.05	0	0	0	$F_{4,20} = 0.70, P = 0.60$	$F_{48,240} = 0.96, P = 0.55$	
		2010	0.57 $\pm$ 0.22	0.90 $\pm$ 0.52	0.43 $\pm$ 0.23	0.67 $\pm$ 0.17	0.33 $\pm$ 0.11			
		2011	0.37 $\pm$ 0.18	0.07 $\pm$ 0.07	0.10 $\pm$ 0.07	0.03 $\pm$ 0.03	0.13 $\pm$ 0.06			
		2012	0	0	0	0.20 $\pm$ 0.20	0.20 $\pm$ 0.20			
	Temperature requirements	High	2009	0.75 $\pm$ 0.19	0.45 $\pm$ 0.15	0.25 $\pm$ 0.11	0.25 $\pm$ 0.11	0.55 $\pm$ 0.09	$F_{4,20} = 0.88, P = 0.49$	$F_{60,300} = 0.80, P = 0.85$
			2010	4.00 $\pm$ 1.03	2.87 $\pm$ 0.70	2.50 $\pm$ 0.74	4.07 $\pm$ 0.69	3.70 $\pm$ 1.14		
			2011	2.13 $\pm$ 0.48	1.43 $\pm$ 0.15	1.57 $\pm$ 0.15	1.97 $\pm$ 0.61	2.13 $\pm$ 0.46		
			2012	1.60 $\pm$ 0.68	1.60 $\pm$ 0.81	0.20 $\pm$ 0.20	1.60 $\pm$ 0.93	1.60 $\pm$ 0.81		
		Low	2009	0.10 $\pm$ 0.06	0.20 $\pm$ 0.09	0.10 $\pm$ 0.10	0.15 $\pm$ 0.10	0.25 $\pm$ 0.11	$F_{4,20} = 0.40, P = 0.80$	$F_{60,300} = 0.31, P > 0.99$
2010			4.97 $\pm$ 2.32	4.23 $\pm$ 1.62	6.27 $\pm$ 2.44	7.60 $\pm$ 3.93	7.30 $\pm$ 3.76			
2011			5.27 $\pm$ 1.89	3.50 $\pm$ 1.29	2.73 $\pm$ 0.65	2.60 $\pm$ 0.73	5.03 $\pm$ 1.72			
2012			4.20 $\pm$ 2.40	2.80 $\pm$ 1.07	3.00 $\pm$ 1.90	3.60 $\pm$ 2.20	4.20 $\pm$ 2.62			
Size group	I	2009	0.05 $\pm$ 0.05	0.20 $\pm$ 0.09	0.10 $\pm$ 0.10	0.15 $\pm$ 0.06	0.25 $\pm$ 0.14	$F_{4,20} = 1.34, P = 0.29$	$F_{60,300} = 0.54, P > 0.99$	
		2010	1.83 $\pm$ 0.68	1.60 $\pm$ 0.38	1.67 $\pm$ 0.42	3.13 $\pm$ 1.80	3.83 $\pm$ 1.78			
		2011	3.77 $\pm$ 0.76	1.67 $\pm$ 0.46	1.27 $\pm$ 0.10	1.53 $\pm$ 0.58	2.00 $\pm$ 0.23			
		2012	2.60 $\pm$ 1.60	2.20 $\pm$ 0.58	1.80 $\pm$ 1.11	3.40 $\pm$ 1.96	2.40 $\pm$ 1.91			
	II	2009	0.50 $\pm$ 0.08	0.20 $\pm$ 0.09	0.15 $\pm$ 0.10	0.10 $\pm$ 0.10	0.20 $\pm$ 0.05	$F_{4,20} = 0.65, P = 0.63$	$F_{56,280} = 0.64, P = 0.98$	
		2010	2.43 $\pm$ 0.66	1.97 $\pm$ 0.59	1.70 $\pm$ 0.53	3.00 $\pm$ 0.43	2.10 $\pm$ 0.70			
		2011	0.63 $\pm$ 0.24	0.67 $\pm$ 0.16	0.53 $\pm$ 0.21	0.53 $\pm$ 0.22	0.67 $\pm$ 0.19			
		2012	2.40 $\pm$ 1.12	1.40 $\pm$ 0.87	1.20 $\pm$ 0.97	1.40 $\pm$ 0.93	2.80 $\pm$ 1.20			

Staphylinid beetles as bioindicators of environmental safety



Table 2. (Cont.)

Trait	Category	Year	Treatment				RM ANOVA			Interaction: sample date × treatment
			Bt maize	Near-isogenic	Near-isogenic + insecticide	Kipous	PR38N86	Treatment	Treatment	
	III	2009	0.05 ± 0.05	0.15 ± 0.10	0.05 ± 0.05	0.05 ± 0.05	0.10 ± 0.06	0.19	0.94	$F_{60,300} = 0.35, P > 0.96$
		2010	4.03 ± 1.62	2.93 ± 1.39	4.87 ± 2.12	5.03 ± 2.65	4.47 ± 2.33			
		2011	2.30 ± 1.06	2.17 ± 1.19	2.10 ± 0.61	1.57 ± 0.76	3.47 ± 1.39			
	IV	2012	0.40 ± 0.24	0.40 ± 0.40	0	0.20 ± 0.20	0			
		2009	0.25 ± 0.16	0.10 ± 0.06	0.05 ± 0.10	0.10 ± 0.10	0.25 ± 0	0.56	0.69	$F_{56,280} = 0.67, P = 0.96$
		2010	0.67 ± 0.32	0.60 ± 0.22	0.53 ± 0.23	0.50 ± 0.14	0.60 ± 0.30			
		2011	0.70 ± 0.30	0.43 ± 0.12	0.40 ± 0.11	0.93 ± 0.46	1.03 ± 0.31			
		2012	0.40 ± 0.24	0.40 ± 0.24	0.20 ± 0.20	0.20 ± 0.20	0.60 ± 0.40			

R2: stenotopic species; intermediate habitats affected by human activities.

E: eurytopic species; deforested agricultural landscape.

Size groups: I: 2.1–3.0 mm, II: 3.1–4.5 mm, III: 4.6–7.0 mm, IV: 7.1–11.0 mm.

and half of those registered in the second one were also found in our field study. Surprisingly, only a few individuals found in Germany by Rauschen *et al.* (2010) belonged to the same genera as we discovered. However, the authors discontinued activity monitoring of staphylinid beetles when they found their low incidence in the first year of their experiments. A study from the Northeastern Iberian Peninsula reported high variability among years and plots in addition to a generally low number of staphylinid beetles (Albajes *et al.*, 2013).

Pitfall traps can have certain effects on the number and species composition of trapped individuals. Species with high activity are preferentially trapped, while less mobile species or those requiring specific microclimate or food sources may fall into the traps less often. Since only actively moving staphylinid beetles can be caught in the pitfall traps, this type of monitoring does not provide estimates of staphylinid density (Toschki *et al.*, 2007). Accordingly, we appropriately use the alternative term ‘activity abundance’ in regard to quantifications based on beetles captured in the pitfall traps. We used maximally effective 1 and 2 weeks’ exposure intervals (Schirmel *et al.*, 2010) and trust that our data faithfully reflect the status of the actively moving staphylinid beetles within the plots. In spite of such uncertainties, the use of pitfall traps is the standard and most widely used method for the monitoring of epigeic arthropods.

Large fluctuations in the number of trapped individuals and in species composition throughout the growing season are a commonly described phenomenon (e.g., Hawes *et al.*, 2003; Farinós *et al.*, 2008) that is more evident than human effects on the ecosystem (Boháč, 1999). Additionally, the structure of staphylinid communities in the agroecosystems of Central Europe is strongly affected by the soil type and the surrounding habitats (Dennis & Fry, 1992; Frank & Reichhart, 2004; Perumalsamy *et al.*, 2009). However, in our study the effect of adjacent forest and fields, moisture gradient, drainage wells and the hunting hide within the field appeared to be insignificant.

Activity abundance of staphylinid beetles can be stimulated by manure application (Boháč, 1999). This could explain the increase in activity abundance observed in the last sample data of 2009 by comparison with the previous sample. The second highest activity abundance was observed in 2010. Interestingly, the last sample of 2011 contained more individuals than the sum of all samples collected during the growing season. Hence, it is advisable to focus on the post-harvest collections when more staphylinid beetles are exposed to the biomass of *Bt* maize left on field.

Staphylinid beetles can tolerate various agro-technical operations (e.g., tillage, organic fertilization, NPK fertilizers and pesticides) better than other environmental factors such as the structure of the ground surface, soil humidity and crop rotation (Boháč, 1991; Leslie *et al.*, 2007). It is possible that the low beetle incidence in 2009 was a consequence of maize plantation after wheat. Changes of staphylinid communities after switching from wheat to maize have been described by Boháč & Pospíšil (1984). Thus, crop history of the fields must be considered in the post-market monitoring of GM crops.

The abundance of dipteran larvae was maximal in the autumn of 2009, obviously in response to the availability of plant biomass that was ploughed into the soil. Staphylinid beetles with parasitic ontogenesis showed a similar activity abundance increase with a delay and emerged as adults in the subsequent spring. Correlation was tested and verified in plots planted with MON 88017 and its near-isogenic hybrid with

Table 3. Average Simpson index of species diversity and Rao indices for bionomics, food specialization, temperature requirements and size group per plot (0.5 ha) during season ( $N = 3-5$  because no individuals on some plots were found some years)  $\pm$  SE (four sample data in 2009, six sample data in each 2010 and 2011, one sample data in 2012).

	Treatment					RM ANOVA		
	Year	Bt maize	Near-isogenic	Near-isogenic + insecticide	Kipous	PR38N86	Treatment	Interaction: year $\times$ treatment
Simpson index								
2009	0.51 $\pm$ 0.14	0.50 $\pm$ 0.17	0.56 $\pm$ 0.06	0.36 $\pm$ 0.19	0.56 $\pm$ 0.14	$F_{4,10} = 0.14,$	$F_{12,30} = 0.68, P = 0.75$	
2010	0.75 $\pm$ 0.04	0.78 $\pm$ 0.03	0.68 $\pm$ 0.08	0.72 $\pm$ 0.07	0.80 $\pm$ 0.04	$P = 0.96$		
2011	0.83 $\pm$ 0.04	0.76 $\pm$ 0.10	0.79 $\pm$ 0.02	0.70 $\pm$ 0.08	0.73 $\pm$ 0.06			
2012	0.46 $\pm$ 0.16	0.30 $\pm$ 0.18	0.57 $\pm$ 0.07	0.65 $\pm$ 0.06	0.69 $\pm$ 0.03			
Bionomics								
2009	0.12 $\pm$ 0.08	0.09 $\pm$ 0.09	0.15 $\pm$ 0.15	0.13 $\pm$ 0.13	0.23 $\pm$ 0.10	$F_{4,10} = 0.35,$	$F_{12,30} = 1.08, P = 0.41$	
2010	0.30 $\pm$ 0.09	0.44 $\pm$ 0.02	0.26 $\pm$ 0.07	0.36 $\pm$ 0.04	0.43 $\pm$ 0.03	$P = 0.84$		
2011	0.47 $\pm$ 0.01	0.33 $\pm$ 0.07	0.30 $\pm$ 0.07	0.37 $\pm$ 0.09	0.25 $\pm$ 0.05			
2012	0.22 $\pm$ 0.13	0.10 $\pm$ 0.10	0.43 $\pm$ 0.01	0.35 $\pm$ 0.12	0.34 $\pm$ 0.09			
Food specialization								
2009	0.30 $\pm$ 0.12	0.20 $\pm$ 0.12	0.17 $\pm$ 0.17	0.15 $\pm$ 0.15	0.35 $\pm$ 0.09	$F_{4,10} = 0.16,$	$F_{12,30} = 0.53, P = 0.87$	
2010	0.47 $\pm$ 0.05	0.47 $\pm$ 0.04	0.37 $\pm$ 0.07	0.41 $\pm$ 0.07	0.45 $\pm$ 0.06	$P = 0.95$		
2011	0.42 $\pm$ 0.07	0.45 $\pm$ 0.07	0.46 $\pm$ 0.03	0.41 $\pm$ 0.03	0.36 $\pm$ 0.08			
2012	0.24 $\pm$ 0.10	0.20 $\pm$ 0.12	0.09 $\pm$ 0.09	0.37 $\pm$ 0.13	0.41 $\pm$ 0.12			
Temperature requirements								
2009	0.12 $\pm$ 0.08	0.32 $\pm$ 0.11	0.15 $\pm$ 0.15	0.31 $\pm$ 0.16	0.28 $\pm$ 0.12	$F_{4,10} = 0.79,$	$F_{12,30} = 0.77, P = 0.68$	
2010	0.38 $\pm$ 0.06	0.45 $\pm$ 0.03	0.43 $\pm$ 0.04	0.30 $\pm$ 0.03	0.43 $\pm$ 0.03	$P = 0.56$		
2011	0.40 $\pm$ 0.05	0.41 $\pm$ 0.06	0.46 $\pm$ 0.02	0.48 $\pm$ 0.01	0.37 $\pm$ 0.07			
2012	0.25 $\pm$ 0.10	0.19 $\pm$ 0.11	0.09 $\pm$ 0.09	0.34 $\pm$ 0.11	0.37 $\pm$ 0.12			
Size group								
2009	0.32 $\pm$ 0.13	0.47 $\pm$ 0.16	0.31 $\pm$ 0.16	0.36 $\pm$ 0.19	0.53 $\pm$ 0.14	$F_{4,10} = 0.45,$	$F_{12,30} = 0.47, P = 0.92$	
2010	0.63 $\pm$ 0.04	0.67 $\pm$ 0.02	0.59 $\pm$ 0.04	0.56 $\pm$ 0.05	0.62 $\pm$ 0.04	$P = 0.77$		
2011	0.59 $\pm$ 0.03	0.60 $\pm$ 0.06	0.63 $\pm$ 0.02	0.58 $\pm$ 0.05	0.61 $\pm$ 0.04			
2012	0.29 $\pm$ 0.17	0.26 $\pm$ 0.16	0.50 $\pm$ 0	0.38 $\pm$ 0.14	0.43 $\pm$ 0.06			

and without insecticide treatment. It suggests that the correlation was not affected by *Bt* maize planting. *A. bipustulata*, a species with parasitic ontogenesis, was the tenth most common species in our study. Larvae of such species spend their whole development inside dipteran larvae in which the presence of Cry3Bb1 has been demonstrated (Knecht & Nentwig, 2010). Considering proved prey-mediated Cry toxin uptake by the staphylinid beetle, it would thus be interesting to investigate the uptake of the Cry toxin by the parasitic species during its development.

No correlation was found between the high occurrence of dipteran larvae and the activity abundance of predatory staphylinid beetles in the subsequent spring. This indicates that their prey on bare soil is not limited to the overwintering dipteran larvae. Other food sources are exploited by the predators and it is highly probable that various saprophagous arthropods consuming maize detritus on bare soil are important prey of the predatory staphylinid beetles.

Few studies have compared the effects of *Bt* maize and conventional insecticides applied in soil on staphylinid beetles. Bhatti *et al.* (2005) reported significant reduction in the numbers of staphylinid beetles monitored with pitfall traps for the tefluthrin-treated near-isogenic hybrids in comparison with *Bt* maize (Cry3Bb1) in one of three monitored years. No differences were detected in the number of individuals collected with the pan traps (extraction from soil samples). No effect of chlorpyrifos on staphylinid beetles was detected by Al-Deeb & Wilde (2003). Similarly, we did not detect any insecticide effects on staphylinid beetles belonging to different functional groups.

Our conclusion that the tested GM maize exerted no detrimental effect on the staphylinid community and the dipteran larvae is consistent with the results of other investigations employing *Bt* maize and utilizing similar collection techniques. Al-Deeb & Wilde (2003) did not find any negative effect of *Bt* maize expressing Cry3Bb1 on staphylinid beetles activity abundance in three field trials in 2 consecutive years. Similarly, Ahmad *et al.* (2005) showed an absence of any negative effect of maize expressing Cry3Bb1 on staphylinid beetles activity abundance in three field trials. Bhatti *et al.* (2005) did not detect any effect of maize expressing Cry3Bb1 on staphylinid beetles assemblage monitored by the pitfall and pan traps. Similarly, species richness was not different between maize expressing Cry1Ab and its near-isogenic non-*Bt* hybrid in a field trial conducted by Leslie *et al.* (2007); they evaluated the associations between species and treatment variables using multivariate analysis and found insignificantly higher activity abundance in the *Bt* maize as we found for four categories within the functional traits. *Bt* maize had highest explanatory power in our model (fig. 2). Rose & Dively (2007) also did not detect any negative effect of the Cry1Ab expression in maize on the staphylinid beetles. Farinós *et al.* (2008) compared species richness and the Shannon index of staphylinid beetle communities in maize expressing Cry1Ab and came to a conclusion consistent with our data.

It is interesting that Balog *et al.* (2010) describing the staphylinid beetle community of a field with *Bt* maize in considerable detail showed that information on the prey preference could explain some of the significant changes and trends in their community structure. They did not find differences in

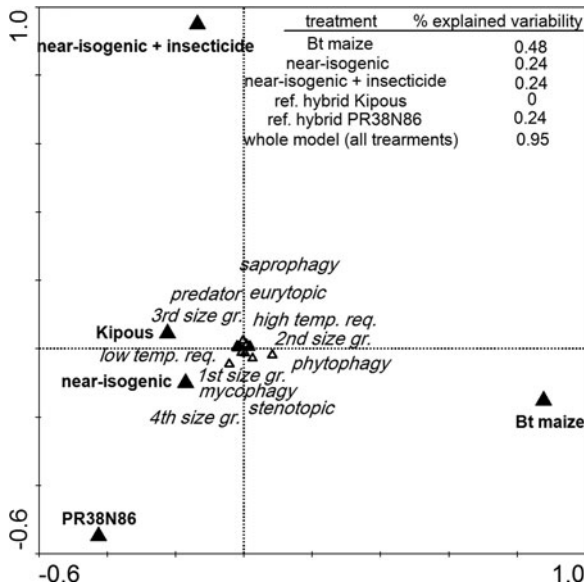


Fig. 2. Canonical correspondence analysis (CCA) of the spatial distribution of functional traits of staphylinid beetles among experimental treatments. Species represented by more than ten individuals were included. Functional traits: bionomics: stenotopic species, eurytopic species; food specialization: predator, saprophagy, phytophagy, mycophagy; temperature requirements: high, low; size group: I: 2.1–3.0 mm, II: 3.1–4.5 mm, III: 4.6–7.0 mm, IV: 7.1–11.0 mm; List of species with classification to the traits' groups is provided in table 1.

the non-aphidophagous predators and parasitoids but higher activity abundance for predators with aphids in their diet was found in the near-isogenic hybrid in 2 from 3 years of study. However, difference in aphid abundance was significant only in one sample date in 1 year and insignificant correlation was found between aphids and predators with aphids in their diet recorded within the same year. In a subsequent study, Balog et al. (2011) found a higher larval density of staphylinid beetles in the beetle-resistant (Cry34/35Ab1) maize than in plots with the stacked event (Cry34/35Ab1, Cry1F) and in the near-isogenic control treated with the soil insecticide tefluthrin. In contrast to the adults, whose activity abundance may be affected by migration, the larvae of staphylinid beetles obviously migrate only for very short distances. On the other hand, Higgins et al. (2009) reported no differences in the abundance of adults and larvae with the use of litterbags. In our trial, insignificant preference of plots with the Bt maize or the reference hybrid PR38N86 was detected for four and seven categories within functional traits, respectively. Preference of hybrid PR38N86 by predatory species may be causally related to the presence of *Ostrinia nubilalis* larvae that caused evident damage in these plots (Svobodová et al., 2012).

No effect of maize expressing Cry3Bb1 on the abundance of dipteran larvae has been shown by Bhatti et al. (2005). This observation is in accordance with successful rearing of several generations of *Drosophila melanogaster* and *Megaselia scalaris* (Diptera: Drosophilidae, Phoridae) on a diet containing decaying leaves of maize expressing Cry1Ab or Cry3Bb1 (Knecht & Nentwig, 2010). Jensen et al. (2010) showed significant reduction of the growth rate in the larvae of *Tipula abdominalis*

Table 4. The average abundance of dipteran larvae per square metre (individuals m<sup>-2</sup>) per plot (0.5 ha, N = 5, data from five traps were pooled) ± SE. Soil samples were taken in the autumn to a depth of 10 cm (one sample data per year).

Family	Individuals m <sup>-2</sup>								
	2009	2010			2011				
	Bt maize	Near-isogenic	Near-isogenic + insecticide	Bt maize	Near-isogenic	Near-isogenic + insecticide	Bt maize	Near-isogenic	Near-isogenic + insecticide
Bibionidae	3.40 ± 2.08	0	0	0	0	0	0	0	0
Calliphoridae	1.70 ± 1.70	3.40 ± 2.08	0	0	0	0	1.70 ± 1.70	3.40 ± 2.08	0
Cecidomyiidae	69.64 ± 34.22	18.68 ± 6.24	56.05 ± 21.42	5.10 ± 5.10	0	0	22.08 ± 10.26	5.10 ± 3.40	54.35 ± 38.97
Chironomidae	621.66 ± 242.56	676.01 ± 308.37	412.74 ± 147.26	168.15 ± 52.52	105.31 ± 27.20	113.80 ± 54.42	8.49 ± 3.80	16.99 ± 8.91	6.79 ± 4.95
Dolichopodidae	3.40 ± 2.08	1.70 ± 1.70	0	1.70 ± 1.70	0	0	30.57 ± 8.74	15.29 ± 7.31	13.59 ± 6.90
Drosophilidae	0	1.70 ± 1.70	0	0	0	0	0	0	0
Ephydriidae	1.70 ± 1.70	0	1.70 ± 1.70	0	0	0	0	0	0
Limoniidae	3.40 ± 3.40	0	0	0	0	0	0	0	1.70 ± 1.70
Muscidae	0	0	1.70 ± 1.70	0	0	0	0	0	0
Psychodidae	0	0	1.70 ± 1.70	0	0	0	0	0	0
Rhagionidae	0	0	1.70 ± 1.70	0	0	0	0	0	0
Sciariidae	71.34 ± 33.93	40.76 ± 17.28	33.97 ± 19.37	1.70 ± 1.70	5.10 ± 3.40	1.70 ± 1.70	3.40 ± 2.08	3.40 ± 2.08	1.70 ± 1.70
Tipulidae	1.70 ± 1.70	0	0	1.70 ± 1.70	5.10 ± 3.40	0	1.70 ± 1.70	5.10 ± 3.40	6.79 ± 4.95
Trichoceridae	1.70 ± 1.70	0	0	0	0	0	0	0	0
Total (N = 5)	779.62 ± 273.58	742.25 ± 316.72	512.95 ± 176.20	178.34 ± 54.58	115.50 ± 27.60	115.50 ± 54.02	67.94 ± 16.34	49.26 ± 12.99	84.93 ± 50.31

(Diptera: Tipulidae) fed on the leaves of maize expressing Cry1Ab and Cry3Bb1. However, growth reduction was obviously caused by other factors than the toxins because the senescent maize tissue failed to act on the toxin-sensitive target species, *O. nubilalis*.

In conclusion, staphylinid beetles are a considerable part of the epigeic fauna in maize fields with a wide food specialization. Staphylinid beetles are thus valuable indicators of changes in agricultural landscapes. However, their importance is currently limited because of difficulties in their identification and high variability of their abundance. Long-term monitoring with precise timing of sampling is necessary to catch representative assemblages. Accordingly, species-level community analyses of staphylinid beetles are rare. Here, we identified staphylinid beetles to the species level, and analyzed them in relation to the main functional traits that determine their occurrence in the field as well as the food sources they could utilize. We described the staphylinid community in a maize field trial in relatively large plots subjected to different treatments. The number and size of plots (5 × 0.5 ha) minimized any artefacts in our experiment. Statistical analyses did not disclose any significant negative effect of the GM insect protected maize, application of the soil insecticide and the maize hybrids. Our data and numerous published reports (reviewed in Devos *et al.*, 2012) unambiguously demonstrate that GM maize MON 88017 expressing Cry3Bb1 may be safely included in the IPM system as a highly specific tool that can contribute to the suppression of *D. v. virgifera* in Europe.

### Supplementary material

The supplementary material for this article can be found at <http://dx.doi.org/10.1017/S000748531500111X>

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