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The responses of soil nematode communities to *Bt* maize cultivation at four field sites across Europe

Andrea Čerevková[a,*], Dana Miklisová[a], Márton Szoboszlay[b], Christoph C. Tebbe[b], Ľudovít Cagáň[c]

[a] Institute of Parasitology, Slovak Academy of Science, Košice, Slovakia
[b] Thünen Institute of Biodiversity, Bundesallee 65, 38116 Braunschweig, Germany
[c] Department of Plant Protection, Slovak Agricultural University, Nitra, Slovakia

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ABSTRACT

Transgenic maize expressing the *Bacillus thuringiensis* (*Bt*) insecticidal crystal (Cry1Ab) protein is poisonous to lepidopterans including the European Corn Borer (*Ostrinia nubilalis*). In many European countries, commercial cultivation of *Bt* maize is not allowed. One major reason is the potential variation of the environmental risk across different biogeographical regions. The aim of this study was to collect data about soil nematode communities as bioindicators of unintended effects across geographically diverse maize growing regions in Europe by sampling field sites in Denmark, Slovakia, and Sweden during 2013–2014, and in Spain during 2013. DKC3872YG (*Bt* maize line MON810) and its near-isogenic line DKC3871 were grown at the sites in Slovakia, Denmark, and Sweden and hybrids DKC6451YG (*Bt* maize line MON810) and its near-isogenic line DKC6450 were cultivated at the site in Spain. Dominating nematode genera in the maize fields regardless of the field site or maize variants were bacterial feeders *Rhabditis, Acrobeloides*; root-fungal feeders *Filenchus*; fungal feeders *Aphelenchoides, Aphelenchus*; and omnivores *Eudorylaimus*. A significant effect of the field site location on the total nematode abundance, nematode abundance in trophic groups, diversity of nematode genera, and ecological and functional nematode indices was detected. Significant annual variation was found in the Plant parasite and Structure indices. There were significant differences in the abundances of omnivores and root-fungal feeders and in the Maturity, Channel, and Enrichment indices between *Bt* and non-*Bt* maize plots in Denmark in 2013, and in the abundance of fungal feeders in Sweden (2013). On the other hand, no difference was found between the *Bt* and non-*Bt* plots at the sites in Spain and Slovakia or at any of the sites in 2014. The effect of the field site location and season on the soil nematode community was more pronounced than that of the *Bt* genetic modification. We conclude that *Bt* maize had only a limited or no effect on soil nematode communities.

1. Introduction

Bt maize is genetically modified maize (*Zea mays* L.) containing genes of the bacterium *Bacillus thuringiensis* that codes for insecticidal proteins (*Bt*-toxins; *Bt*). *Bt* maize expressing *Bt*-toxin Cry1Ab is one of the most widely cultivated *Bt* crops worldwide (Benedict and Ring, 2004). It is able to protect itself against feeding by the European corn borer (*Ostrinia nubilalis* Hübner). The fate and effect of insect resistant *Bt* maize in soil ecosystems has intensively been studied (Baumgarte and Tebbe, 2005; Icoz and Stotzky, 2008; Tabashnik et al., 2013), but there is still a lack of information about the importance of different environmental conditions i.e. soil type or agroecosystem, on its ecological impact. In fact, the environmental persistence of *Bt* protein is known to depend on factors such as the type of *Bt* proteins, its expression pattern in plants, soil types, temperature, or precipitation (Tank et al., 2010; Feng et al., 2011; Xue et al., 2014). Saxena et al. (1999) found that Cry1Ab *Bt* toxin was released from corn plants into the rhizosphere soil in root exudates. Baumgarte and Tebbe (2005) reported that *Bt* proteins mainly enter soil by decomposition of roots and can persists during winter until the following growing season, and Tapp and Stotzky (1998) observed that the bound state of the *Bt* toxin persist in soil for up to 234 days. In fact, active *Bt* toxins can persist and remain insecticidal in soil as a result of binding to clay surfaces (Saxena et al., 2002) or humic substances (Crecchioa and Stotzkyb, 1998). Other studies, however, emphasize that *Bt* proteins will not accumulate in soils (Tank et al., 2010; Wang et al., 2013).

Soil nematodes are one of the most abundant groups of soil metazoans with densities reaching up to 50 million per m^2 (Bongers and Bongers, 1998) and with important ecosystem functions (Yeates, 1981). In numerous studies, soil nematodes were successfully used as

* Corresponding author.
E-mail address: cerev@saske.sk (A. Čerevková).

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indicators of environmental conditions and for general ecosystem health (Bongers, 1990; Ettema and Bongers, 1993; Neher, 2001; Ciobanu et al., 2015; Renčo and Baležentienė, 2015).

Nematode communities under maize crops may be influenced by many different factors, including crop species, plant age, and environmental variables (Karuri et al., 2013). Previously, nematode numbers have been shown to be similar in soils planted with *Bt* maize and its isogenic equivalent (Saxena and Stotzky, 2001; Al-Deeb et al., 2003). Höss et al. (2011, 2015) did not find significant differences in the abundance and diversity of field nematodes in soil planted with *Bt* and non-*Bt* maize. Griffiths et al. (2005, 2006) compared *Bt* maize expressing the Cry1Ab to the near-isogenic non-*Bt* cultivar, another conventional maize cultivar, and grasslands in three European sites (Denmark, Eastern France, South-West France). The authors stated that the effect of *Bt* maize on soil nematodes was relatively small compared to the effects of soil type, plant growth stage, and applications of an insecticide. In contrast, another study reported that nematode diversity values were greater in a *Bt* hybrid maize field versus the non-*Bt* isoline maize field treated with insecticide (Neher et al., 2014). Höss et al. (2011, 2013) noted that Cry proteins (Cry1A.105; Cry2Ab2) could potentially also harm free-living nematodes in similar mode of action than the insects. Furthermore, a soil microcosm study using a mixture of the Cry-proteins expressed by MON89034 × MON88017 found significantly deleterious effects on nematode communities at a nominal concentration of 1 μg Cry-proteins per gram of soil (Höss et al., 2014).

While abundant literature is available concerning the effects of *Bt* maize on nematode communities across individual sites, a deficit remains in regards to baseline data on nematode communities and their response to GM maize across multiple geographically distinct regions. Only one study from Europe describes the temporal and spatial impact of *Bt* maize on nematode diversity (Griffiths et al., 2005, 2007), which examined sites in Denmark and France from 2002 to 2005. In this study, the objective was to consider the impact of the biogeographical diversity which exists in Europe by selecting four sites located in distinct regions. This is of special importance for the approval of *Bt* maize for cultivation in Europe due to its diversity of biogeographical regions in which maize is grown, ranging from Spain to Scandinavia. The objective of this study was, therefore, to assess the diversity of nematodes in maize fields from different European regions and analyze the response of the nematode communities to the cultivation of *Bt* maize. The results should enhance our understanding of the importance of the European biogeographical diversity in assessing the effects of GM plants on non-target organisms and thus support the environmental risk assessment of future genetically modified crops in the European Union (Arpaia et al., 2014).

2. Materials and methods

2.1. Sites, maize variants, and experimental design

The study was carried out in four sites located in different countries during 2013, i.e., in Denmark, Spain, Slovakia, and Sweden. All sites except for Spain were also analysed in the following year 2014.

In Denmark, the field site was located at the Experimental farm of the University of Aarhus Flakkebjerg Research Centre, near the town of Slagelse, on the island of Zealand. Coordinates of the site were 55°19′N, 11°23′E. Altitude was 35 m a.s.l. The soil at the site was classified as an Udoll (USDA soil taxonomy) based on https://soilgrids.org (Hengl et al., 2017) and the European digital archive on soil maps (EuDASM) (Panagos et al., 2011). The preceding crops were barley in 2012 and maize in 2013. Sowing dates were May 15, 2013 and May 20, 2014. Herbicides were used one week before sowing and again three weeks later. Soil for analysis of selected soil chemical parameters and nematode communities was collected on August 26, 2013 and August 13, 2014.

In Spain, the field site was located southeast of Madrid, municipality of Seseña, in the province of Toledo, central Spain. Coordinates of the site were 40°05′N, 3°40′W. Altitude was 495 m a.s.l. The soil was classified as a Fluvent. The preceding crop was maize in 2012. The sowing date was May 9, 2013. Herbicides were used one week before sowing and again three weeks later. Soil for analyses was collected on July 24, 2013.

In Slovakia, the field site was located in Borovce in western Slovakia. Coordinates of the site were 48°34′N, 17°43′E. Altitude was 181 m a.s.l. The soil was classified as an Udoll. The preceding crops were winter wheat in both 2012 and 2013. Sowing dates were May 9, 2013 and April 28, 2014. Herbicides were used one week before sowing and again three weeks later. Soil for analyses was collected on July 30, 2013 and July 21, 2014.

In Sweden, the field site was located northwest of Lund. Coordinates of the site were 55°45′N 13°2′E. Altitude was 10 m a.s.l. The soil was classified as an Ochrept. The preceding crops were winter wheat in both 2012 and 2013. Sowing dates were May 15, 2013 and May 20, 2014. Herbicides were used one week before sowing and again three weeks later. Soil for analyses was collected on August 22, 2013 and August 18, 2014.

In Slovakia, Denmark, and Sweden, the maize *Bt* and isogenic (ISO) hybrids included in the experiment were DKC3872YG (*Bt* maize line MON810) and its near-isogenic line DKC3871. In Spain, hybrid DKC6451YG (*Bt* maize line MON810) and its near-isogenic line DKC6450 were cultivated. At each location, hybrids were sown in 10 replicated plots, each measuring 10 m × 10 m. Each plot was isolated from the adjacent plots by a 5 m wide strip of barley. The location of the respective *Bt* and ISO plots were completely randomized. The experiments were conducted during seasons 2013 and 2014 with the identical plot arrangements.

2.2. Soil sampling and chemical analyses

Soils samples were taken at all sites during the flowering stage of maize with three sub-samples from each field plot. At each plot, three representative plants were uprooted, the roots with the adhering soil were placed into plastic bags and shaken to separate a major part of the soil from the roots. The soil samples from the three plants were pooled, thus both *Bt* and ISO maize cultivation were represented with 10 independent (biological) replicates from each site in each year. All samples were transferred to the laboratory in sealed plastic bags and stored at 5 °C until processing.

To determine the chemical parameters, the soil samples were dried at 105 °C for 17–18 h. Soil pH was measured from 10g soil in 0.01 M $CaCl_2$ with 1:2 (w/v) soil-to-solution ratio using a Professional Meter PP-25 electrode (Sartorius, Germany). Total soil carbon (C %) and nitrogen (N %) content were determined from 3 g grounded soil samples by dry combustion with a LECO TruMac elemental analyzer (Elementar, Germany).

2.3. Analyses of nematodes

Each soil sample was homogenised by gentle hand mixing, and then 50 g of soil was processed by a modified Baermann technique. Nematodes were extracted from the aqueous soil suspensions using a set of two cotton-propylene filters. Sub-samples were then collected after a 24 h extraction at 22 °C. The aqueous suspensions were subsequently examined under a stereomicroscope (40× and 60× magnification), excessive water was removed, and the nematodes were fixed in Ditlevsen´s FAA solution (95% ethanol, 40% formaldehyde, glacial acetic acid, distilled water) (Southey, 1986). The nematodes were then microscopically (100, 200, 400, 600, and 1000 × magnification) identified at genus-level using an Eclipse 90i light microscope (Nikon, Japan).

Identified nematode genera were partitioned to six trophic groups based on their feeding habits: bacterial feeders (B), fungal feeders (F),

predators (P), omnivores (O), root-fungal feeders (RFF), and obligatory plant parasites (PP) as recommended by Wasilewska (1997) and Yeates et al. (1993). The mean nematode abundance, the abundance of nematodes per trophic group, and the Shannon-Weaver index of genus diversity (H'gen) (Shannon and Weaver, 1949) were determined for each country, variant, and year.

Ecological and functional indices were used to assess the status of the soil ecosystems based on the nematode communities. We evaluated the Maturity Index from free-living taxa (MI), Plant Parasite Index (PPI), determined from plant parasite taxa (Bongers, 1990) and the sigma Maturity Index (ΣMI), determined from all taxa (Yeates, 1994). The Enrichment (EI), Structure (SI) (Ferris et al., 2001) and Basal (BI) (Berkelmans et al., 2003) indices were employed to describe food web conditions and the Channel Index (CI) (Ferris et al., 2001) to indicate the predominant decomposition channel in the soil food web. For simple and uniform counting, all ecological (MI, ΣMI and PPI) and functional indices (EI, SI, BI and CI) were calculated using the NINJA online programme (Sieriebriennikov et al., 2014).

2.4. Statistical analysis

The soil parameters were analysed with a linear model in JMP 13.0.0 (SAS Institute) that included 'country', 'maize variant', 'year', and their interactions as factors. Since the site in Spain was only sampled in 2013 and had different maize variants, the samples from Spain were analysed separately with t-tests to compare the soil parameters from the *Bt* and ISO maize plots. The samples were then arranged in seven groups according to countries and years and pairwise comparisons of the soil parameters between the groups were carried out with Tuckey's HSD test in JMP 13.0.0.

The mean nematode abundance, the abundance of nematodes per trophic group (Bacterial feeders, Fungal feeders, Omnivores, Predators, Root-fungal feeders, Plant parasites), and the ecological and functional indices (the Shannon-Weaver index of genus diversity (H'gen), Maturity Index, ΣMaturity Index, Plant Parasite Index, Channel Index, Basal Index, Enrichment Index and Structure Index) were analysed in Statistica software (StatSoft Inc. 2013).

Since the site in Spain was only sampled in 2013 and had different maize variants than the other countries, the samples from Spain were analysed separately with t-tests to compare the abovementioned parameters in the *Bt* and ISO maize plots. The data from the other sites was analysed with repeated, univariate ANOVA with 'country' (DK, SK, S), 'maize variant', 'year' (as repeated measure) and their interactions as factors. To meet the assumptions of these parametric tests, Box-Cox transformation was applied with the maximum likelihood approach and Golden Search iterative procedure. If 'year' was in interaction with the other factors, the data set was split to investigate the effects of 'country' and 'maize variant' with 2-factor ANOVAs separately in the samples from 2013 to 2014. If there was an interaction between 'country' and 'maize variant', t-tests were applied separately for each country to determine the effect of 'maize variant'. If the effect of 'country' was significant, Tuckey´s HSD post hoc tests were carried out to reveal differences between groups.

Non-metric multidimensional scaling (NMDS) ordination was used to seek patterns in the composition of the nematode community. A three-dimensional solution was generated without applying any transformation to the data in the autopilot mode of PC-ORD version 6 (McCune and Mefford, 2011) with the slow and thorough option and Sørensen (Bray-Curtis) distance (appropriate for community data). The significance of the differences between countries, years and maize variants was evaluated by Multi-Response Permutation Procedure (MRPP) done also in PC-ORD version 6.

Redundancy analysis (RDA) was used on the nematode community data from three countries (DK, SK, S) in two years with explanatory variables soil pH, C %, N %, 'country', 'maize variant', and 'year' to reveal relations between the nematode taxa and soil properties. A log-transformation was applied to the data. First, the entire data set was included in the RDA, and then the analysis was repeated with the 24 (out of 45) most abundant genera, which covered 95.5% of the total abundance, to obtain a clear ordination plot. The effects of the explanatory variables were quantified by interactive forward selection. These analyses were performed in Canoco for Windows 5 (Ter Braak and Šmilauer, 2012).

Table 1
Soil properties: average ± standard deviation of soil pH, soil carbon content (C%), soil nitrogen content (N%), and the proportion of carbon and nitrogen (C/N) at the four sites (Denmark, Spain, Sweden, Slovakia) in 2013 and 2014. Statistically significant differences ($p < 0.05$ in Tukey's HSD test) are indicated with capital letters.

Country and year	pH	C%	N%	C/N
Spain 2013	7.55 ± 0.05 A	1.68 ± 0.19 A	0.13 ± 0.01 C	13.11 ± 1.06 A
Denmark 2013	6.47 ± 0.30 B	1.30 ± 0.07 B	0.15 ± 0.01 B	8.68 ± 0.42 D
Denmark 2014	6.07 ± 0.23 C	1.36 ± 0.08 B	0.12 ± 0.01 C	11.08 ± 0.26 B
Slovakia 2013	6.17 ± 0.66 BC	1.24 ± 0.12 B	0.14 ± 0.02 B	8.75 ± 1.32 D
Slovakia 2014	6.12 ± 0.38 BC	1.27 ± 0.14 B	0.13 ± 0.01 C	9.95 ± 0.30 C
Sweden 2013	6.00 ± 0.45 C	1.56 ± 0.16 A	0.17 ± 0.02 A	8.96 ± 0.30 D
Sweden 2014	5.97 ± 0.32 C	1.36 ± 0.15 B	0.13 ± 0.01 C	10.81 ± 0.24 B

3. Results

3.1. Soil physicochemical properties

According to the linear model, maize variants had no significant effect on the soil pH, C %, N %, or on the C/N ratio ($p > 0.58$), neither were there any significant interactions between maize 'maize variant', 'country', and 'year' ($p > 0.15$). Due to the different maize varieties, the samples from Spain were analysed separately. In their case, also no significant differences were detected in the soil parameters from the *Bt* and ISO maize plots ($p > 0.10$).

Soil pH ranged from 5.22 to 7.62 and was significantly higher in the samples from Spain than at the other localities (Table 1). At the site in Denmark, the soil pH significantly decreased from 2013 to 2014. There were no significant differences in soil pH between the plots in Denmark in 2014, and in Slovakia and Sweden in both years. The soil C content of the samples fell between 1.07 and 2.23 %. The samples from Spain and Sweden from 2013 had significantly higher C content than the others (Table 1). Concerning the soil N content, the samples ranged between 0.10 and 0.20 % and the C/N ratio was between 6.39 and 15.37. There was a significant decrease in soil N % from 2013 to 2014 at the sites in Denmark, Slovakia, and Sweden in parallel with a significant increase in the soil C/N ratio (Table 1).

3.2. Effect of the field site location

The average abundances of the identified nematode genera in the soil samples from *Bt* and ISO maize variant cultivated plots at each site in two years are presented in Table 2. The F-values from the ANOVA are given in Table 3. A significant effect of the field site location ('country') was observed in the case of total nematode abundance in both years (both $p < 0.005$) and the diversity index for genera (H'gen) in 2013 ($p < 0.001$) (Table 4). Among the trophic groups, significant differences were observed between the sites in the abundance of bacterial feeders, fungal feeders, and plant parasites ($p < 0.001$), and predators ($p < 0.05$) in 2013; and in the case of fungal feeders, omnivores, predators, root-fungal feeders and plant parasites (all $p < 0.001$) in 2014 (Table 4). The most abundant trophic group in all localities,

Table 2
List of identified Nematoda genera in each trophic group and their mean abundance (individual/50 g soil) with standard errors. Plots with near-isogenic maize hybrid - ISO; plots with *Bt* maize hybrid - *Bt*; Abbr. - Abbreviation used in figures.

		2013		2013						2014					
		Spain		Denmark		Slovakia		Sweden		Denmark		Slovakia		Sweden	
Nematode genera	Abbr.	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO
Bacterial feeders															
Acrobeloides	*Acro*	25.3 ± 21.5	15.3 ± 13.8	16.8 ± 10.6	26.7 ± 17.4	35.6 ± 36.2	33.2 ± 17.6	54.7 ± 40.4	60.9 ± 28.0	44.0 ± 19.5	38.9 ± 36.4	50.6 ± 30.1	60.9 ± 28.0	26.2 ± 9.3	19.7 ± 9.6
Acrobeles	*Acrb*	-	-	-	-	-	-	0.3 ± 0.7	0.6 ± 1.9	-	-	-	0.6 ± 1.9	0.7 ± 1.1	1.1 ± 1.4
Alaimus	*Alai*	-	-	0.5 ± 1.6	0.2 ± 0.6	-	0.2 ± 0.4	0.4 ± 1.3	0.1 ± 0.3	0.4 ± 0.7	0.5 ± 0.8	0.7 ± 1.2	0.1 ± 0.3	0.4 ± 0.7	0.2 ± 0.6
Cephalobus	*Ceph*	5.7 ± 6.3	12.1 ± 9.5	-	2.3 ± 4.5	2.6 ± 5.3	8.6 ± 16.5	7.7 ± 6.7	12.9 ± 10.9	5.7 ± 8.8	3.6 ± 2.5	29.1 ± 18.6	12.9 ± 10.9	1.3 ± 2.0	2.4 ± 1.6
Cervidellus	*Cerv*	1.3 ± 2.8	2.4 ± 7.6	-	0.6 ± 1.9	0.1 ± 0.3	0.1 ± 0.3	0.7 ± 1.6	1.2 ± 2.9	0.8 ± 2.5	0.1 ± 0.3	-	1.2 ± 2.9	0.6 ± 1.3	1.9 ± 2.5
Eucephalobus	*Euce*	7.3 ± 7.6	13.1 ± 10.6	-	1.8 ± 2.7	3.0 ± 3.6	3.4 ± 3.3	3.5 ± 4.6	8.2 ± 5.8	6.0 ± 6.9	6.4 ± 4.9	16.0 ± 9.5	8.2 ± 5.8	10.9 ± 8.3	10.9 ± 7.7
Chiloplacus	*Chil*	0.1 ± 0.3	1.5 ± 3.6	9.0 ± 5.4	10.3 ± 8.3	18.1 ± 21.5	15.0 ± 15.9	6.4 ± 6.3	10.2 ± 7.1	-	1.2 ± 2.1	1.2 ± 2.6	10.2 ± 7.1	0.4 ± 0.7	1.3 ± 2.0
Mesorhabditis	*Meso*	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1 ± 0.3
Panagrolaimus	*Pana*	3.0 ± 9.5	-	-	8.8 ± 20.5	-	0.3 ± 0.9	-	-	4.7 ± 11.6	0.1 ± 0.3	-	-	0.3 ± 0.9	-
Plectus	*Plec*	25.2 ± 25.9	39.2 ± 39.0	2.0 ± 3.4	4.4 ± 4.2	0.6 ± 1.3	0.1 ± 0.3	2.1 ± 2.6	2.6 ± 2.3	6.6 ± 4.3	5.4 ± 4.6	5.0 ± 4.7	2.6 ± 2.3	5.8 ± 6.6	9.4 ± 6.8
Prismatolaimus	*Pris*	0.9 ± 1.5	0.9 ± 2.8	0.3 ± 0.9	0.1 ± 0.3	-	-	-	-	-	0.9 ± 1.7	2.5 ± 3.0	-	1.5 ± 3.1	3.5 ± 6.8
Rhabditis	*Rhab*	70.1 ± 62.5	67.5 ± 40.6	30.0 ± 14.4	6.1 ± 9.3	1.7 ± 2.6	-	20.7 ± 8.3	36 ± 18.8	48.5 ± 29.9	93.6 ± 48.7	38.0 ± 21.7	36.0 ± 18.8	68.0 ± 46.7	96.2 ± 88.4
Teratocephalus	*Tera*	-	-	-	-	-	-	-	0.2 ± 0.6	-	-	-	0.2 ± 0.6	-	0.7 ± 1.6
Wilsonema	*Wils*	-	-	-	-	-	0.2 ± 0.6	-	-	-	-	-	-	0.3 ± 0.7	0.2 ± 0.4
Fungal feeders															
Aphelenchoides	*Aphe*	12.9 ± 10.4	15.0 ± 10.8	13.9 ± 17.9	17.2 ± 8.0	17.0 ± 23.9	25.6 ± 7.2	22.6 ± 11.2	34.6 ± 14.8	10.1 ± 6.0	15.3 ± 12.6	10.5 ± 10.7	34.6 ± 14.8	10.9 ± 8.3	11.0 ± 3.8
Aphelenchus	*Aphu*	0.3 ± 0.7	1.0 ± 1.9	8.1 ± 6.2	12.5 ± 16	42.5 ± 45.2	33.0 ± 16.8	4.1 ± 3.9	5.9 ± 7.3	29.1 ± 19.6	20.2 ± 15.6	23.0 ± 11.9	5.9 ± 7.3	1.7 ± 2.2	2.5 ± 2.1
Ditylenchus	*Dity*	-	-	-	0.4 ± 1.3	-	0.4 ± 1.0	-	-	-	-	-	-	-	-
Tylencholaimus	*Tyle*	1.0 ± 2.5	-	-	-	-	-	-	-	6.1 ± 19.3	-	6.2 ± 11.2	-	0.5 ± 1.6	-
Omnivores															
Aporcelaimellus	*Apor*	-	-	0.8 ± 1.1	4.0 ± 4.1	1.1 ± 1.9	0.1 ± 0.3	0.4 ± 1.0	-	-	-	1.0 ± 1.5	-	-	-
Axonchium	*Axon*	6.4 ± 5.9	1.5 ± 2.7	-	-	-	-	-	-	-	-	-	-	-	0.2 ± 0.6
Crassolabium	*Cras*	-	-	0.1 ± 0.3	-	-	0.1 ± 0.3	0.6 ± 1.9	1.0 ± 3.2	-	-	-	1.0 ± 3.2	-	-
Discolaimus	*Disc*	-	-	-	-	-	0.4 ± 1.3	0.5 ± 1.6	-	-	-	-	-	-	-
Ecumenicus	*Ecum*	-	1.1 ± 1.9	-	-	0.5 ± 1.6	0.8 ± 1.9	-	-	-	-	-	-	-	-
Enchodelus	*Ench*	-	0.5 ± 1.1	-	-	-	0.7 ± 1.6	-	-	-	-	0.1 ± 0.3	-	-	0.2 ± 0.6
Eudorylaimus	*Eudo*	10.4 ± 13.1	17.5 ± 12.5	0.7 ± 1.3	8.9 ± 10.6	20.2 ± 10.5	13.3 ± 11.3	3.5 ± 3.8	5.0 ± 6.4	2.6 ± 3.6	4.4 ± 3.9	5.1 ± 4.9	5.0 ± 6.4	1.6 ± 2.2	0.8 ± 1.0
Mesodorylaimus	*Meso*	-	-	0.2 ± 0.6	0.7 ± 1.6	-	0.6 ± 1.3	-	-	-	0.2 ± 0.6	-	-	0.3 ± 0.7	-
Predators															
Anatonchus	*Anat*	-	-	-	-	-	-	-	-	0.5 ± 0.8	1.3 ± 1.7	2.3 ± 5.7	-	-	-
Clarkus	*Clar*	-	-	-	-	-	-	-	-	-	-	1.4 ± 2.1	-	-	-
Iotonchus	*Ioto*	0.1 ± 0.3	-	1.2 ± 1.7	1.5 ± 1.7	0.2 ± 0.4	0.5 ± 0.8	-	-	1.7 ± 2.1	0.3 ± 0.5	-	-	-	-
Mylonchulus	*Mylo*	2.3 ± 2.8	1.0 ± 1.6	0.2 ± 0.4	-	-	-	0.6 ± 1.1	0.7 ± 1.9	0.6 ± 1.1	1.6 ± 3.9	2.6 ± 5.7	0.7 ± 1.9	0.6 ± 0.8	1.4 ± 2.2
Root-fungal feeders															
Aglenchus	*Agle*	0.7 ± 2.2	2.5 ± 4.0	5.5 ± 11.2	12.4 ± 25.5	-	-	0.7 ± 1.3	3.6 ± 8.4	0.4 ± 0.7	0.3 ± 0.7	0.5 ± 1.1	3.6 ± 8.4	0.9 ± 1.5	0.1 ± 0.3
Filenchus	*Files*	21.1 ± 16.1	26.2 ± 13.6	13.2 ± 11.8	29.8 ± 18.1	28.7 ± 20.8	19.3 ± 21.8	5.9 ± 5.8	5.8 ± 6.2	39.7 ± 24.5	26.9 ± 16.3	26.9 ± 14.3	5.8 ± 6.2	9.8 ± 8.4	6.3 ± 6.4
Malenchus	*Male*	-	0.4 ± 1.3	6.1 ± 12.2	6.9 ± 9.9	-	-	-	2.3 ± 6.3	0.2 ± 0.6	0.6 ± 1.9	-	2.3 ± 6.3	-	-
Psilenchus	*Psil*	-	-	-	1.3 ± 4.1	-	0.2 ± 0.6	-	-	-	-	0.5 ± 1.6	-	-	-
Tylenchus	*Tylu*	2.2 ± 7.0	1.9 ± 6.0	1.6 ± 3.7	10.3 ± 10.5	5.5 ± 7.0	5.6 ± 8.5	0.9 ± 2.2	1.5 ± 4.7	0.1 ± 0.3	-	0.4 ± 0.8	1.5 ± 4.7	0.3 ± 0.9	-

(*continued on next page*)

Table 2 (*continued*)

		2013		2013						2014					
		Spain		Denmark		Slovakia		Sweden		Denmark		Slovakia		Sweden	
Nematode genera	Abbr.	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO
Plant parasites															
Bitylenchus	*Bity*	0.7 ± 2.2	-	9.2 ± 10.2	5.1 ± 7.1	0.3 ± 0.9	0.1 ± 0.3	-	-	-	-	-	-	-	-
Criconematidae juv.	*Cric*	-	-	0.1 ± 0.3	-	-	-	-	-	-	-	-	-	-	-
Dorylium	*Dory*	-	-	-	-	0.2 ± 0.6	1.8 ± 4.1	-	-	-	-	-	-	-	-
Geocenamus	*Geoc*	7.1 ± 22.5	2.0 ± 6.3	15.1 ± 9.9	10.6 ± 12.7	0.1 ± 0.3	0.5 ± 1.3	37.5 ± 30.4	36.3 ± 35.9	-	2.9 ± 6.0	-	36.3 ± 35.9	6.4 ± 9.7	0.2 ± 0.6
Helicoylenchus	*Heli*	5.1 ± 4.1	17.2 ± 11.0	-	-	3.1 ± 7.2	2.5 ± 4.2	3.2 ± 4.0	4.6 ± 3.6	-	-	-	4.6 ± 3.6	0.1 ± 0.3	0.7 ± 1.1
Heterodera juv.	*Hete*	-	-	-	-	-	-	-	0.5 ± 1.3	-	-	-	0.5 ± 1.3	0.2 ± 0.4	-
Paratylenchus	*Para*	8.3 ± 7.5	1.1 ± 2.3	9.4 ± 7.0	8.3 ± 7.5	-	0.7 ± 1.3	40.2 ± 39.6	34 ± 25.2	14.1 ± 12.8	11.8 ± 6.8	0.6 ± 1.3	34 ± 25.2	11.3 ± 15.9	25.1 ± 27.4
Pratylenchus	*Prau*	17.2 ± 18.5	-	10.1 ± 6.2	17.2 ± 18.5	1.4 ± 1.6	3.8 ± 6.5	0.4 ± 1.0	2.1 ± 3.7	18.9 ± 10.0	33.3 ± 25.7	4.3 ± 5.2	2.1 ± 3.7	5.1 ± 8.8	8.1 ± 12.7
Trichodorus	*Tric*	0.4 ± 1.3	-	-	0.4 ± 1.3	0.2 ± 0.4	0.8 ± 1.5	-	-	-	-	-	-	-	-
Tylenchorhynchus	*Tyls*	-	-	-	-	-	-	-	-	33.7 ± 19.4	27.6 ± 18.1	-	-	21.7 ± 6.9	24.3 ± 15.6

variants, and years was the group of bacterial feeders (Table 4). Plant parasites were the second most abundant group observed in soils from the Danish (2014) and Swedish (2013, 2014) sites (Table 4). At the site in Denmark, they were represented mostly by genera *Pratylenchus* and *Tylenchorhynchus*, while in Sweden, they were represented mostly by genera *Paratylenchus* and *Geocenamus* (Table 2). Root-fungal feeders were abundant in Denmark (2013) and Spain 2013 (Table 4) mostly due to the high abundance of *Aglenchus, Filenchus*, and *Tylenchus* in Denmark and *Filenchus* in Spain (Table 2). Fungal feeders were the second most abundant group in Slovakia in both years as a result of the high abundance of *Aphelenchoides* and *Aphelenchus* (Table 2). The Maturity Index (MI) and the sigma Maturity Index (ΣMI) were significantly different between the field site locations ($p < 0.001$, $p < 0.05$) in 2014 (Table 4). Differences between the sites in the Basal Index were found in both 2013 ($p < 0.001$) and 2014 ($p < 0.01$), and in the Channel and Enrichment indices in 2014 ($p < 0.001$) (Table 4).

3.3. Effect of annual variability

The RDA ordination of the selected genera (containing 95.5 % of total nematode abundance) (Fig. 1) shows the two values of the nominal variable 'year' relatively far from each other. The effect of 'year' (explained 34.4 % of variance, p (adjusted) = 0.02) 'countries' (27.9 %, p (adjusted) = 0.02) and N % (2 %, p (adjusted) = 0.02) was found significant by interactive forward selection method. Monte Carlo permutation tests confirmed the statistical significance of all constrained axes (pseudo F = 12.0, p = 0.002).

The NMDS analysis showed that the sampling year had a greater impact on the composition of the nematode community than 'country' or the maize variant. The samples from 2013 (black symbols) and 2014 (red ones) clearly separate on the NMDS plot (Fig. 2). The effect of 'year' on the nematode community composition was found significant in MRPP (A = 0.078, $p < 0.001$). On the NMDS plot, the samples from 2014 grouped according to the countries, with especially pronounced difference between the samples from Slovakia and Sweden. Similar patterns were apparent among the samples from 2013, except for the samples from the sites in Denmark as the Danish ISO samples are closer to the Slovakian samples and the Danish *Bt* samples to the Swedish samples (Fig. 2). The effect of 'year' was found significant (all $p < 0.001$) in the case of the Plant Parasite Index (2.47 ± 0.27 in 2013 vs 2.69 ± 0.32 in 2014) and the Structure Index (27.60 ± 18.10 vs 26.02 ± 17.71).

3.4. Effect of the genetic modification

The RDA ordination diagram does not show much difference between the two maize variants with *Bt* and ISO plotted next to each other (Fig. 1). The 'maize variant' could not explain a significant part of the variance in the data set (less than 2 %). However, three nematode genera with low abundance were observed in the ISO variants but not detected in the *Bt* variants. These were *Mesorhabditis* observed in the samples from Sweden (2014); *Teratocephalus* in the samples from Sweden in both years and Slovakia in 2014; and *Ditylenchus* in the samples from Denmark (2013) and Slovakia 2013 (Table 2).

A significant effect of 'maize variant' in 2013 was observed on H'gen ($p < 0.001$) and fungal feeders ($p < 0.05$). In both cases, greater values were reached with the ISO variant (H'gen: 2.09 ± 0.23 with ISO vs 1.93 ± 0.29 with *Bt*; Fungal feeders: 36.40 ± 21.77 with ISO vs 30.60 ± 35.67 with *Bt*). The 'maize variant' affected some of the trophic groups in the samples from Denmark and Sweden (2013) (Table 5). In Denmark (2013), significantly more omnivores, caused by high abundance of *Eudorylaimus*, and root fungal feeders, related to high abundance of *Aglenchus, Filenchus*, and *Tylenchus* (Table 2), were detected in the samples from the ISO than from the *Bt* variant. In Sweden (2013), significantly more fungal feeders were present, due to the high abundance of *Aphelenchoides* (Table 2), in the samples from the

Table 3
F values from repeated ANOVA with factors country (Denmark, Slovakia and Sweden), maize variant (near-isogenic maize hybrid - ISO; *Bt* maize hybrid - *Bt*) as factors and year (2013, 2014) as repeated measure from analysis of trophic groups of nematode community structure and nematode community with associated probabilities (P) and degree of freedom are reported.

Factors and its combinations	Country	Maize Variant	Country x Maize Variant	Year	Year x Country	Year x Maize Variant	Year x Country x Maize Variant
degree of freedom	(2, 54)	(1, 54)	(2, 54)	(1, 54)	(2, 54)	(1, 54)	(2, 54)
Abundance	2.6	4.6*	0.1	1436.3***	9***	0.7	0.4
Diversity index (H'gen)	9.3***	1.2	0.2	4.7*	7.8**	7.5**	0.3
Bacterial feeders	12.7***	3.5	0.8	199***	13.5***	0.6	0.8
Fungal feeders	11.8***	0.1	0.7	1.9	15.3***	7.3**	0.5
Omnivores	20.6***	3.4	5.5**	64.7***	4.3*	0.8	7**
Predators	7.9**	1.1	0.5	48.3***	7.2**	0.8	0.3
Root-fungal feeders	33.1***	0	0.1	15.4***	2.7	2	6.7**
Plant parasites	166.7***	0.7	0.4	3.7	9.8***	0.1	1.7
Maturity Index (MI)	35.2***	0.4	0.3	8.7**	11.6***	1.1	8.1***
Σ Maturity Index(ΣMI)	5*	0	0.6	240.2***	4.3*	0.1	1.5
Plant Parasite Index (PPI)	0.7	0	1	273.5***	0.7	0	0.9
Channel Index (CI)	115.2***	10.3**	8.6***	710.4***	88.8***	13.9***	11.7***
Basal Index (BI)	12.1***	2.6	1.9	601.9***	9.9***	3.6	2.3
Enrichment Index (EI)	13.6***	0.9	1.4	670***	13.3***	0.9	1.5
Structure Index (SI)	9.7***	0.8	2	29.6***	2.7	0.1	3.1

P: *0.05, **0.01, ***0.001.

ISO than from the *Bt* variant. The ecological and functional indices MI, ΣMI, CI, and EI also differed significantly between maize variants but only in the samples from Denmark (2013) with MI, ΣMI, and CI being higher with the ISO variant while EI with *Bt* maize (Table 5). The ANOVA models indicated no significant effect of 'maize variant' on the trophic groups and ecological and functional indices in the data from 2014.

Plotting the Enrichment Index and the Structure Index against each other, showed most samples to fall within quadrat “A” (Enrichment Index > 50, Structure Index < 50) without a clear separation between maize variants, sites, or years (Fig. 3). Quadrat A characterises an environment as highly disturbed, N-enriched, with bacterial decomposition channels, and with low C:N ratio; all of which is typical for managed agricultural soils like the ones included in this study.

4. Discussion

Nematodes have numerous interactions with soil organisms, play important roles in soil nutrient cycling and are generally considered to be suitable indicators of soil condition, quality and soil health (Bongers and Ferris, 1999; Neher, 2001; Ferris et al., 2012). To assess potential unintended harm to nematode communities triggered by direct or indirect effects of maize with the genetically modified Cry1Ab protein, field studies are indispensable (Arpaia et al., 2017). To characterize the nematode community, parameters such as nematode abundance, diversity, dominance of taxa, representation of nematodes as assigned to trophic groups, as well as functional and ecological indices are highly useful.

Soil collected from fields in which the same genetic event, i.e., *Bt* maize Mon810 was cultivated, affected the reproduction of the nematode *Caenorhabditis elegans* compared to soil from plots with near-isogenic maize (Höss et al., 2008). The experimental study of Höss et al. (2011) on nematode *C. elegans* mentioned that the risk to free-living soil nematodes posed by Mon88017 cultivation can be regarded as low, as long as the coleopteran specific Cry3Bb1 concentrations in soil remain four orders of magnitude below the toxicity threshold. However, in field conditions, the authors reported that neither the nematode abundance nor the feeding-type composition differed between *Bt*- and non-*Bt* plots, the concentration of toxin in the soil being too low to induce any toxic effect (Höss et al., 2011).

Our results indicate that both the field site and the year of cultivation had a larger effect on the nematode community composition than the genetic modification (comparison of *Bt* and ISO variants). This is in line with another study where the nematode composition varied

Table 4
The average values ± standard errors of the indices in each country in years 2013 and 2014
Capital letters (A, B, C within 2013 (Spain not included) and X, Y, Z within 2014) indicate significant differences between sites within a sampling year. The ‘year’ column indicates if the difference between years was significant.

	2013	2013			2014			
Indices	Spain	Denmark	Slovakia	Sweden	Denmark	Slovakia	Sweden	Year
Abundance	227.0 ± 71.7	181.0 ± 77.4**B**	177.0 ± 67.3**B**	244.0 ± 63.0**A**	286.0 ± 71.8**X**	248.0 ± 81.3**XY**	208.0 ± 84.3**Y**	
Diversity index (H'gen)	1.9 ± 0.3	2.2 ± 0.2**A**	1.9 ± 0.2**C**	2.0 ± 0.2**B**	2.1 ± 0.2	2.1 ± 0.1	1.9 ± 0.3	
Bacterial feeders	145.0 ± 65.9	59.9 ± 22.4**B**	61.4 ± 34.4**B**	114.0 ± 45.7**A**	133.0 ± 48.7	156.0 ± 60.6	132.0 ± 70.7	
Fungal feeders	15.1 ± 10.0	26.0 ± 20.8**B**	59.2 ± 41.4**A**	33.6 ± 14.9**AB**	40.4 ± 19.8**X**	34.6 ± 19.9**X**	13.3 ± 6.3**Y**	
Omnivores	18.7 ± 12.4	7.7 ± 9.3	18.9 ± 10.5	5.5 ± 5.3	3.6 ± 3.8**Y**	8.7 ± 8.2**X**	1.6 ± 1.7**Y**	
Predators	1.7 ± 2.4	1.5 ± 1.7**A**	0.4 ± 0.7**B**	0.7 ± 1.5**AB**	3.0 ± 3.5**XY**	11.2 ± 17.3**X**	1.0 ± 1.6**Y**	
Root-fungal feeders	27.5 ± 13.1	43.5 ± 33.2	29.6 ± 22.4	10.3 ± 13.4	34.1 ± 20.9**X**	33.8 ± 19.2**X**	8.7 ± 7.4**Y**	
Plant parasites	18.8 ± 20.9	42.7 ± 29.1**B**	7.8 ± 8.4**C**	79.4 ± 49.8**A**	71.1 ± 31.7**X**	4.0 ± 4.6**Y**	51.6 ± 27.1**X**	
Maturity Index (MI)	1.9 ± 0.3	2.1 ± 0.4	2.3 ± 0.1	1.9 ± 0.2	1.8 ± 0.3**Y**	2.2 ± 0.2**X**	1.6 ± 0.2**Y**	
Σ Maturity Index (ΣMI)	2.1 ± 0.3	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.3	2.1 ± 0.3**XY**	2.2 ± 0.3**X**	1.9 ± 0.3**Y**	
Plant Parasite Index (PPI)	3.4 ± 0.8	2.4 ± 0.2	2.5 ± 0.4	2.5 ± 0.2	2.8 ± 0.2	2.6 ± 0.5	2.7 ± 0.2	sign.
Channel Index (CI)	13.8 ± 7.1	44.6 ± 27.8	95.8 ± 8.4	28.3 ± 11.3	24.7 ± 15.1**Y**	30.2 ± 11.3**Y**	13.3 ± 20.9**X**	
Basal Index (BI)	23.5 ± 9.8	32.4 ± 10.0**B**	45.9 ± 6.8**A**	41.5 ± 10.7**A**	26.4 ± 12.6**XY**	34.8 ± 10.7**X**	22.1 ± 15.3**Y**	
Enrichment Index (EI)	71.9 ± 10.3	58.7 ± 15.6	37.7 ± 5.7	54.3 ± 11.5	71.2 ± 12.7**X**	55.9 ± 10.9**Y**	76.7 ± 15.9**X**	
Structure Index (SI)	36.4 ± 21.0	30.0 ± 21.0	35.2 ± 13.4	17.5 ± 14.0	20.2 ± 20.1	38.4 ± 16.6	19.4 ± 14.2	sign.

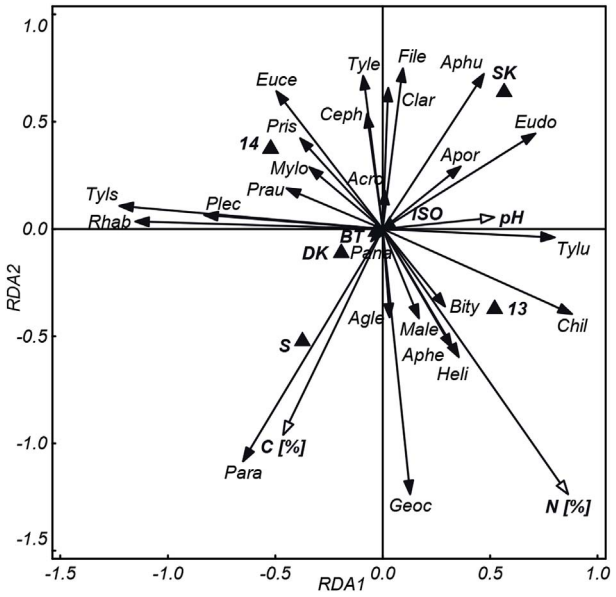

Fig. 1. RDA ordination diagram of the nematode communities in the samples from 2013 to 2014 with explanatory variables: soil pH, C %, N %, country, maize variant, and year. Sites: DK – Denmark, SK – Slovakia, and S – Sweden. Maize variants: BT - *Bt* maize hybrid (line MON 810), ISO - near-isogenic hybrid line. Years: 13–2013, 14–2014. Quantitative variables are plotted as arrows with white heads, nominal variables as triangles. Nematode genera are plotted as arrows with black heads (for abbreviations see Table 2). The eigenvalues of the first two axes are 0.19 and 0.14 and they explain 45.3% and 32.1% of the variation, respectively.

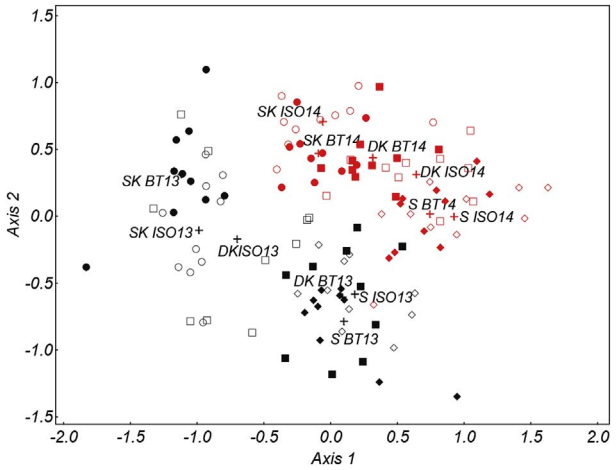

Fig. 2. NMS ordination from the genus-level composition of the soil nematode communities in the samples from 2013 to 2014 from three sites: DK – Denmark (squares), SK – Slovakia (circles) and S – Sweden (diamonds). BT - plots with *Bt* maize hybrid (line MON 810) (full symbols); ISO - plots with near-isogenic hybrid line (empty symbols), in 2013 (black symbols) and 2014 (red symbols). The centroids of groups are represented by plus symbols. The variance explained by axes 1 and 2 is 41.0% and 22.6%, respectively. Stress = 17.58.

among maize varieties, whether they contained *Bt* proteins or not (Griffiths et al., 2005, 2007). In our results, the field site location ('country') significantly affected nematode abundance in most trophic groups. An effect of genetic modification of maize on nematode trophic groups was, however, only observed in 2013 at the site in Denmark, where omnivores and root-fungal feeders were significantly more abundant in plots with the ISO compared to plots with the *Bt* variant, and at the site in Sweden, where fungal feeders had higher abundance with the ISO maize variant. It is noteworthy, that whether the genetic modification of maize had a detectable effect on the soil nematode community did not only depend on the site but was also inconsistent between years as we were not able to find any differences between the *Bt* and ISO samples from 2014. Neher et al. (2014) observed that during the growing season of a coleopteran-active *Bt* maize (event MON863) the relative abundance of fungivores was greater in the *Bt* hybrid than in the non-*Bt* isoline with or without insecticide. The isoline with insecticide had greater non-target effects on nematode communities than the *Bt* hybrid (Neher et al., 2014). Manachini and Lozzia (2002) noted that fungivores were dominant in soil from *Bt* maize (event MON810) fields compared to non-*Bt*, but our results did not confirm these findings. For the bacterial feeders, the most abundant trophic group in arable soil, we did not detect significant differences between maize variants. This is similar to a previous study that found no differences between the bacterial communities in the rhizospheres of MON88017 (Cry3Bb1) and three other non-*Bt* cultivars grown on 32 field plots in Germany (Miethling-Graff et al., 2010).

A significant level of disturbance in the nematode community caused by *Bt* maize cultivation was confirmed by the low value of the maturity indices MI and ΣMI only in Denmark (2013). The lowest MI was observed in soils from Sweden and the highest in soils from Slovakia. Höss et al. (2011) confirmed lower MI values in the Cry3Bb maize variant compared to its isoline variant during maize flowering stage, but they did not confirm it during planting or harvesting. In contrast, Neher et al. (2014) mentioned that the value of MI was not significantly different in *Bt* and non-*Bt* isoline variants and both were greater than it was in the variant with non-*Bt* isoline with insecticide.

Low values of the Channel Index CI (< 50%) indicates decomposition pathways dominated by bacteria. High CI (> 50%) indicates a higher proportion of fungal decomposition (Ferris et al., 2001). Comparing maize variants, we found significantly lower level of CI only in the samples from *Bt* Denmark (2013). Höss et al. (2011) also found low values of CI, but no differences between *Bt* maize and non-*Bt* maize variants.

According to Ferris et al. (2001) the Structure Index (SI) is an indicator of the state of the food web affected by stress or disturbance and the Enrichment Index (EI) is a measure of opportunistic bacterial and fungal feeder nematodes. We found relatively high EI and relatively low SI values characteristic to an N-enriched, highly disturbed environment with low C:N ratio that is typical for conventional annual crop production (Ferris et al., 2001) but with confirmed differences between maize variants only in Denmark (2013). Similarly, no differences between *Bt* maize and non-*Bt* maize variants in EI and SI were confirmed by Neher et al. (2014). According to EI and SI, most of our samples fell within quadrat A without a clear difference between maize variants, countries, or years (Fig. 3). As opposed to this, Neher et al. (2014) obtained lower EI and higher SI values and their samples were placed in quadrat C. Höss et al. (2011) found higher EI and SI values and their samples fell into quadrat B.

5. Conclusions

In summary, our findings confirmed that the cultivation of *Bt* maize expressing Cry1Ab proteins is not of substantial risk to nematode communities. However, some indicator metrics, such as the trophic groups of fungal feeders, root-fungal feeders, and omnivores were affected by the maize variants at one of the sites in 2013. We also noticed differences in the Maturity, Enrichment and Channel indices between the maize variants but only in Denmark (2013). Studying four sites in two years, we found that the impact of the field site location and year-to-year variation on the composition of the soil nematode community was larger than the effects of *Bt* maize cultivation which were not consistent between sites and only detectable in one of the sampling years.

Acknowledgements

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Table 5
The average values ± standard errors of the indices in each sites and maize variant (near-isogenic maize hybrid - ISO; *Bt* maize hybrid - *Bt*) in year 2013. Capital letters (A, B) indicate significant differences between maize variants within a sites.

	Spain		Denmark		Slovakia		Sweden	
Indices	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO	*Bt*	ISO
Abundance	213.0 ± 52.4	241.0 ± 84.6	154.0 ± 43.9	209.0 ± 92.4	183.0 ± 70.1	172.0 ± 64.0	218.0 ± 60.2	271.0 ± 53.9
Diversity index (H'gen)	1.9 ± 0.3	2.0 ± 0.2	2.2 ± 0.1**B**	2.3 ± 0.1**A**	1.8 ± 0.3	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.2
Bacterial feeders	139.0 ± 57.0	152.0 ± 73.2	58.6. ± 23.3	61.3 ± 21.3	61.7 ± 39.3	61.1 ± 28.8	96.5 ± 39.1	133.0 ± 44.6
Fungal feeders	14.2 ± 9.7	16.0 ± 10.2	22.0 ± 20.3	30.1 ± 20.5	59.5 ± 57.1	59.0 ± 12.8	26.7 ± 11.4**B**	40.5 ± 14.9**A**
Omnivores	16.8 ± 10.4	20.6 ± 13.9	1.8 ± 2.1**B**	13.6 ± 9.9**A**	21.8 ± 9.7	16.0 ± 10.4	5.0 ± 4.5	6.0 ± 6.0
Predators	2.4 ± 2.8	1.0 ± 1.6	1.4 ± 1.7	1.5 ± 1.6	0.2 ± 0.4	0.5 ± 0.8	0.6 ± 1.0	0.7 ± 1.8
Root-fungal feeders	24.0 ± 13.6	31.0 ± 11.4	26.4 ± 13.6**B**	60.7 ± 37.9**A**	34.2 ± 17.6	25.1 ± 25.5	7.5 ± 6.7	13.2 ± 17.2
Plant parasites	17.3 ± 27.2	20.3 ± 11.3	43.9 ± 22.6	41.6 ± 34.3	5.3 ± 7.5	10.2 ± 8.5	81.3 ± 60.0	77.5 ± 36.8
Maturity Index (MI)	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.2**B**	2.3 ± 0.3**A**	2.3 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.2
Σ Maturity Index (ΣMI)	2.1 ± 0.4	2.1 ± 0.3	2.0 ± 0.2**B**	2.3 ± 0.1**A**	2.4 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	2.4 ± 0.3
Plant Parasite Index (PPI)	3.7 ± 0.8	3.1 ± 0.7	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.4	2.6 ± 0.3	2.5 ± 0.2	2.5 ± 0.2
Channel Index (CI)	12.1 ± 5.9	15.5 ± 7.7	25.5 ± 10.8**B**	63.6 ± 26.5**A**	92.8 ± 10.5	98.8 ± 3.5	29.6 ± 10.7	27.1 ± 11.8
Basal Index (BI)	22.6 ± 10.1	24.5 ± 9.5	27.3 ± 7.5	37.5 ± 9.6	43.7 ± 6.1	48.1 ± 6.7	42.4 ± 10.7	40.6 ± 10.6
Enrichment Index (EI)	74.0 ± 10.2	69.8 ± 9.9	69.9 ± 8.1**A**	47.6 ± 13.3**B**	38.6 ± 7.4	36.7 ± 2.7	52.1 ± 11.8	56.6 ± 10.6
Structure Index (SI)	33.4 ± 22.3	39.4 ± 19.1	21.6 ± 20.3	38.4 ± 18.1	38.1 ± 11.6	32.3 ± 14.3	20.2 ± 14.6	14.8 ± 13.0

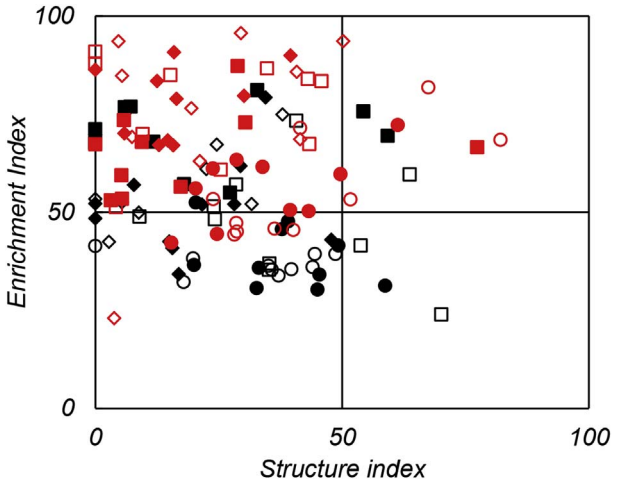

Fig. 3. Relationship between the Enrichment and Structure indices in the samples from 2013 (black) and 2014 (red) from three countries: DK – Denmark (squares), SK – Slovakia (circles), S – Sweden (diamonds). BT - plots with *Bt* maize hybrid (line MON 810) (full symbols); ISO - plots with near-isogenic line (empty symbols).

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