



**PROJECT NUMBER 289706**

**Collaborative Project**

**Assessing and Monitoring the Impacts of Genetically Modified Plants on Agro-ecosystems**

Work Package (WP) 4	Biological components of soil fertility
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Task 4.3	Impact on soil nematodes: Baselines of their diversity and physiological response to GM plants as potential stressors – Task leader: Dr. Ewen Mullins
Deliverable 4.3	Report on nematode diversity in rhizospheres and soil
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## Summary

Nematode assemblages are considered reliable indicators of the health of a soil ecosystem. In AMIGA the rhizospheres of potato and maize cultivated across different European sites were analysed for their nematode diversity using an array of different methodologies. The overarching goal of this task was to establish baselines for characterizing GM-effects on soil nematode populations by looking at the effect of cultivating GM potato and GM maize. To achieve this, the aim was to sample two sites (The Netherlands and Ireland) for potato and for maize four sites (Denmark, Spain, Slovakia and Sweden). Potato sampling in The Netherlands was completed in the growing season of 2013 and for Ireland it was completed in 2013, 2014 and 2015. For maize, sampling was completed in Slovakia, Denmark, Sweden, Spain in 2013 and Slovakia, Denmark, Sweden in 2014. The GM potato material was modified for resistance against the late blight pathogen *Phytophthora infestans* by the transfer of the *Rpi-vnt1.1* cisgene from the wild potato species *Solanum venturii* into the Desiree cultivar. For the purposes of this study, nematode diversity in the rhizosphere of this cisgenic GM potato line was compared against corresponding soil samples of non-GM Desiree cultivar and an additional cultivar Sarpo Mira. In addition, each potato genotype underwent two treatments: presence/absence of weekly fungicide chemical treatment, which is standard commercial practise for the control of late blight disease in potato crops. The GM maize material used in the experiment was modified to resist larvae of the European Corn Borer. The maize hybrids included in the experiment were DKC3872YG (Bt maize line MON810) and its near-isogenic line DKC3871 in Slovakia, Denmark and in Sweden. While in Spain the hybrid DKC6451YG (Bt maize line MON810) and its near-isogenic line DKC6450 were included. For potato, up to 22 distinct nematode families were associated across all treatments evaluated but no significant difference was noted between the potato cultivars grown. While mild effects were observed on diversity levels relative to the presence/absence of chemical treatments, overall there was no significant difference between the genus and family nematode numbers of cisgenic Desiree and its comparator Desiree genotype, irrespective of the presence/absence of chemical management. Considering potato genotype as an individual factor, no significant difference was recorded in regards to the effect of GM v. non-GM on nematode community structure or on the ecological succession indices studied. Similarly for the analysis of nematode communities from the maize sites, the analysis of this data indicated that the occurrence of nematodes, their abundance, proportion of feeding types and selected ecological indices did not depend on the type of maize hybrid (GM or non-GM). Thus, the cultivation of genetically modified maize did not directly influence nematode populations. Overall, the observed significant higher abundance of nematodes was correlated with soil moisture.

## Introduction

A primary goal of the AMIGA project is to produce new scientific data on GM crop interference with soil fertility by establishing baselines for the diversity of soil biota, which provide important ecosystem functions and beyond that could be indicative for unanticipated effects of GM crops on non-target organisms in general (Arpaia et al., 2014). Nematodes are key agents in important soil processes such as decomposition, mineralisation and nutrient cycling, with alterations in the nematode community structure having the potential to influence ecosystem functioning (Bakyoni et al., 2007). Widespread and highly diverse, nematodes form part of the food web of soil by occupying primary, secondary and tertiary positions in multiple trophic groups: bacterial feeding (BF), fungal feeding (FF), predators (PR), omnivorous (OM) and plant feeding (PF) (Yeates GW et al., 1993), making them excellent indicators of fluctuations in soil composition arising from for example, plant genotype and/or type of soil management and environmental conditions in the rhizosphere. With the ability to provide insightful information on soil food web dynamics (Dupont et al., 2009), it can be hypothesised that nematodes are applicable as possible bio-indicators in monitoring the potential impacts of genetically modified plants on agro-ecosystems.

To date, multiple studies have been carried out using soil nematodes as indicators in different ecosystems evaluating for example the impact of crop management (Neher, 1999, Porazinska, 1999, Briar et al., 2012), fertilizers (Pan K et al., 2015, Zhao & Neher, 2013), water availability (Vandegheuchte et al., 2015), seasonal fluctuations (Vervoort et al., 2012), as well as the application of crop protectants (Zhao J et al., 2013). Previously, nematode numbers have been shown to be similar in soils planted with *Bt* maize or its isogenic equivalent (Al-Deeb et al., 2003). While the effects of *Bt* on soil nematodes were relatively small compared to the effects of soil type, plant growth stage and insecticide application (Griffiths et al., 2005). More recently, it has been reported that nematode diversity values were greater in a *Bt* hybrid versus the non-*Bt* isolate with insecticide (Neher et al., 2014). Overall, the same study concluded that the overall community structure and nematode genera varied more by seasonal phenology than by the actual agronomic treatment applied.

Earlier work indicated the potential of certain *Bt* proteins (Cry5B, Cry14A, Cry21A and Cry6A) to target bacterial-feeding nematode species (Wei et al., 2003). While separate investigations found no effect of Cry3Bb1 in *Bt* maize on *Caenorhabditis elegans* (Al-Deeb et al., 2003, Hoss et al., 2011), follow up studies found that three insecticidal Cry proteins showed dose-dependent inhibitory effects on *Caenorhabditis elegans* reproduction (EC50: 0.12 – 0.38  $\mu\text{mol L}^{-1}$ ), at concentrations that were far above the expected soil concentrations (Hoss et al., 2013).

While a sizable degree of literature is available concerning the effects of *Bt* maize on nematode populations across individual sites of study, there is no published report to date detailing the impact of cultivating a GM potato on non-pathogenic soil nematode populations and their respective community structure. In addition, a deficit remains in regards to generating baseline data on nematode diversity in response to GM maize and potato. Taking this into consideration in this deliverable we report on a baseline of nematode diversity for maize and potato as found at multiple field sites across Europe. In addition, to characterize the nematode community structure for each respective crop rhizosphere, community structures were analysed in terms of basic ecological and functional indices, which in effect provides insight into the physiological status of the soil ecosystems under the particular GM/non-GM maize and potato crops. The presented analyses should be regarded as still being preliminary as they will be further elaborated by additional statistical analyses in advance of peer-reviewed publication.

## **Materials and Methods**

### **Sites of study, potato and maize planted and experimental design**

For potato, the sites studied were Valthermond (The Netherlands) and the Oak Park campus of the Teagasc Crop Research Centre, Carlow, Ireland. Plot sizes were 6 x 6 m in The Netherlands with 6m grass separating each plot. In Ireland, the plot sizes were 3 x 3 m with plots surrounded by 3m grass margins. The Netherlands site was sampled in 2013 and the Irish site was sampled in 2013, 2014 and 2015. In both locations, potato genotypes cultivated were the conventional cultivar Desiree, the modified cisgenic Desiree line and the conventionally bred Sarpo Mira cultivar, with each genotype undergoing two treatments corresponding to a weekly chemical spray regime and a control 'no spray' treatment. Each site contained 54 plots randomised in order across 6 replicating blocks of 9 plots (3 genotypes x 3 treatments). Plots received the same crop management protocols (with the exception of the chemical fungicide treatment) indistinct of the genotype evaluated. The cisgenic line was equipped with a single copy of the *Rpi-vnt1.1* gene of *S. venturii*, which confers resistance to the late blight pathogen *Phytophthora infestans* (Foster et al., 2009, Pel et al., 2009) and was provided to the AMIGA project via the DuRPh programme of Wageningen University.

In regard to maize, the study was carried out at Borovce in Western Slovakia in 2013 and 2014, at Flakkebjerg, Denmark in 2013 and 2014, at Lund, Sweden in 2013 and 2014 and at Madrid, Spain in 2013. The maize *Bt* and isogenic (ISO) hybrids included in the experiment were DKC3872YG (*Bt* maize line MON810) and its near-isogenic line DKC3871 in Slovakia, Denmark and in Sweden, whereas for Spain the hybrids DKC6451YG (*Bt* maize line MON810) and its near-isogenic line

DKC6450 were cultivated. At each location per year, hybrids were sown in 10 repetitions within plots measuring 10 m x 10 m. Each plot was isolated from adjacent plots by a 5 m wide strip of barley. *Bt* and ISO plots were distributed according to a completely randomized design.

### **Soil sampling**

The samples were collected from the potato rhizosphere at the initiation of flowering. A total of 7 plots were randomly sampled/treatment. Once collected, soil samples were stored in individually labelled plastic bags, which were sealed and stored at 4°C before processing/shipping. For maize, sampling was completed from a depth of 10cm across all sites during flowering stages, with sub-samples (n = 5/plot) bulked to produce a representative sample (~0.5 kg) from each plot. A total of 10 representative soil samples were thus collected from the *Bt* and ISO plots in all localities. All samples were transferred to the laboratory in sealed plastic bags and stored at 5 °C until processing.

### **Nematode isolation**

For potato, nematodes were extracted by processing 100g of the homogenized soil/plot (7 samples/treatment) via an Oostenbrink elutriator, followed by passage through a series of sieves (45, 90, 125 and 180 mesh size) and then a cotton wool filter. After a 48 hr incubation period at room temperature, a volume of 50 ml was then recovered from the cotton wool filter, from which nematodes were collected into 10mL following a 40C treatment for 24 hr. Final volumes were subsequently stored at -80°C. For maize, each soil sample was homogenised by gentle hand mixing, and then 50 g of soil (maize) or 100g of soil (potato) was processed by a modified Baermann technique, which saw nematodes extracted from the aqueous soil suspensions using a set of two cotton-propylene filters. Sub-samples were then collected after a 24 h extraction period.

### **Nematode processing**

For DNA sequencing purposes (potato sampled), each 10ml simple was freeze dried overnight before DNA was extracted using the Purelink Genomic DNA kit (Invitrogen/Cat N. 1820-01) and an adapted protocol for nematode DNA. Modifications included: 360 ul of Purelink Genomic buffer plus 40 ul of proteinase K added to each tube which was then agitated at 55°C overnight. The suspension was then centrifuged (13000rpm, 3min) and the resulting supernatant were processed as per kit's recommendations, with the exception that the DNA was eluted from the column using sterile water. All eluted samples were stored at -40°C. For target amplification, the 5' end of the 18 small subunit rDNA gene (~1000bp) was amplified using a set of universal primers (SSU18A and SSU26R(37)). All PCR reactions were completed in a 50ul volume containing 50 ng of DNA template, 5 ul of 10X PCR

buffer, 1 ul of each primer (10mM) and 200 uM dNTP, with cycling conditions of; 95°C – 5min, 30 x (95°C – 30sec, 60°C – 60sec, 72°C – 5min), 72°C – 10min. Five reactions were completed per sample, with these 5 technical replicates/sample then pooled post-PCR before being verified via gel electrophoresis for appropriate amplicon size. Due to staff turnover during the course of the project, attempts to complete T-RFLP in the absence of the requisite expertise proved unsuccessful. In response, a clone-based sequencing strategy was adopted as an alternative to mechanism to capture diversity. For this amplicons were gel purified to obtain a clean composite PCR product which was then cloned into E. coli (p-GEM, Promega). Fifty individual colonies (per treatment) were randomly selected and sent to an external provider for Sanger sequencing. Acquired sequences were analysed against the GenBank database using standard BLAST analysis. Owing to low DNA concentrations attained with some of the 2013 samples taken from Ireland, a nested PCR approach was adopted with the secondary PCR (to that detailed previously) employing the SSU9R and S18 primers (37), which generate a nested fragment ~500bp.

For microscopic analysis (maize samples), the aqueous suspensions were subsequently examined under a stereomicroscope (40 X and 60 X magnification), excessive water was removed, and the nematodes were fixed in a hot solution of 4% formaldehyde: pure glycerol (99:1, v/v). The nematodes were then microscopically (100, 200, 400, 600, and 1000 X magnification) identified to genus using an Eclipse 90i light microscope (Nikon, Japan).

## **Evaluation**

Identified nematode species were partitioned into specific trophic groups based on their feeding habits, as recommended (Yeates et al., 1993, Wasilewska, 1997). Depending on the crop studied some or all of the below basic ecological and functional indices were used to assess the status of the soil ecosystems as per the nematode communities. This included calculating the Maturity Index for free-living taxa ( $MI = \sum[vi \times fi]/n$ , where  $vi$  is the colonizer-persister (c-p) value of taxon  $i$ ,  $fi$  is the frequency of taxon  $i$  in a sample and  $n$  is the total abundance of individuals in a sample). All maturity indices were calculated using a c-p value that represented the life-history characteristics of the nematode taxa associated with r- and K-selection. Species with c-p values of 1 or 2 are r-selected i.e. colonizers. Species with a c-p value of 5 are K-selected, or persisters. Lower c-p values are indicative of more disturbed environments with higher c-p values characteristic of less disturbed environments (Bongers, 1990). In addition, the plant parasite index (PPI), determined similarly as MI but for plant parasitic taxa (Bongers, 1990), as well as the Channel index (CI) indicating the predominant decomposition channel in the soil food web, calculated as  $CI = (0.8 Fu2)/(3.2 Ba1 + 0.8 Fu2)$ , where  $Ba1$  = the abundance of nematodes from the families of Rhabditidae, Panagrolaimidae and

Diplogasteridae in the sample and Fu2 = the abundance of nematodes from the families of Aphelenchidae, Aphelenchoididae and Anguinidae were also calculated as required. A high CI (>50%) indicates a higher proportion of fungal decomposition. A low CI (<50%) suggests bacterial decomposition channels (Ferris, 2010).

The Enrichment (EI), Structure (SI) and Basal (BI) indices were assessed for a food web analysis and were calculated from weighted nematode components as  $EI = 100 \times e / (e+b)$ ;  $SI = 100 \times s / (s+b)$  and  $BI = 100 \times b / (e+s+b)$  where b is basal, e is enrichment and s is structural components and are calculated as  $b = (Ba1+Fu2) \times W2$ ;  $e = (Ba1 \times W1) + (Fu2 \times W2)$  and  $s = (Ban \times Wn + Can \times Wn + Fun \times Wn + Omn \times Wn)$  where  $W1 = 3.2$ ,  $W2 = 0.8$ ,  $W3 = 1$ ,  $n = 3-5$ , Ba are bacterial feeders with c-p 1 and Fu are fungal feeders with c-p 2. The metabolic footprint graphically depicts the intersection of the EI and the SI (Ferris, 2010). Dotted lines on both sides of the rhombus indicate the standard deviation, and solid lines represent the mean values for a treatment. The metabolic footprint is maximal when the rhomboid becomes a square, indicating that the productivity and turnover rates of the enrichment indicators, representative of the prey, are sufficient to maintain the needs of the predators (the structure indicators) so that the system is metabolically balanced. For simple and uniform counting all described ecological indices (MI,  $\Sigma MI$ , and PPI), functional indices (EI, SI, BI), and metabolic footprints were created using the NINJA online programme (Sieriebriennikov et al., 2014).

### **Statistical analyses**

For potato the effect on the nematode community of each factor (location, potato genotypes, chemical treatment, year) was completed as a multivariate analysis. When significant, multiple comparisons were made as per the Tukey test, with differences at probability of  $p < 0.01$  and  $p < 0.05$  considered statistically significant. All analyses were performed using GenStat v10. For maize, three-way ANOVA testing was used for the statistical analysis of data, using 'location (site)', 'variant' and 'year' as factors (Tukey's HSD test,  $p > 0.05$ ), with the analysis completed using Statgraphics™.

## Results

### Potato

For potato, the samples taken from the Netherlands in 2013 had to be omitted from the study due to vandalism of the site, which in turn led to significant admixture of GM and non-GM tubers in the sampled plots. As a result of this and in an effort to capture as much data as possible for AMIGA; with the effective removal of the Dutch site from this study a decision was made to extend the sampling at the Irish site into 2015, in order to provide a 3<sup>rd</sup> year of study.

#### *Nematode Community Structure and Ecological Succession Indices*

Examining the effect of cultivating GM and non-GM potato on nematode community structure and ecological succession indices, the mean MI values (Table 1) obtained for samples taken from untreated plots were higher (2.36 for Desiree; 2.05 for cisgenic Desiree; 2.13 for Sarpo Mira) than those recorded in the presence of fungicide treatment (1.93 for Desiree; 1.81 for cisgenic Desiree; 1.91 for Sarpo Mira). For the PPI index a similar trend was observed for cisgenic Desiree and Desiree only (2.83 v. 1.10, Desiree; 2.63 v 1.83, cisgenic Desiree) and again with the PPI/MI (1.28 v. 0.53, Desiree; 1.28 v. 0.95, cisgenic Desiree) and the MIMO index (2.68 v. 2.54, Desiree; 2.42 v. 2.30, cisgenic Desiree). In contrast, a converse trend was noted with Sarpo Mira.

Considering crop genotype as an individual factor, no significant difference was recorded for the indices examined. Cisgenic Desiree derived samples recorded the lowest mean values for the MI (1.93), MIMO (2.36) and  $\Sigma$ MIMO (2.46) indices compared to its direct comparator Desiree and the alternative variety Sarpo Mira, which obtained the higher means values for the PPI (3.01), PPI/MI (1.54), MIMO (2.64) and  $\Sigma$ MIMO (2.79) indices. Examining the impact of crop management (independent of the potato genotype sown), the means values for MI (2.18/1.88), PPI (2.70/2.10) and PPI/MI (1.28/1.10) proportions were larger in the absence of fungicide treatment (Table 1). The opposite was noted for MIMO (2.48/2.51) and  $\Sigma$ MIMO (2.61/2.72). Statistically, weak effects were noted for the effect of chemical treatment on the MI index ( $P < 0.13$ ), the effect of crop genotype on the MIMO index ( $P < 0.20$ ) as well as the interaction of genotype x chemical treatment on the PPI ( $P < 0.20$ ) and the PPI/MI ratio ( $P \leq 0.18$ ). The year sampled had a weak effect on MI ( $P < 0.28$ ) and  $\Sigma$ MIMO ( $P < 0.14$ ) while in the case of the MIMO index 2013 differed significantly from 2014 and 2015 ( $P < 0.05$ , Table 3). Considering all indices reported, potato genotype and chemical treatment (plus their interaction) influenced the values of the indices recorded.



**Table 1.** Impact of potato genotype (Desiree, cisgenic Desiree, Sarpo Mira), crop management (control, chemical treatment) and year (2013, 2014, 2105) on nematode community ecological succession indices in field site in Oak Park (Carlow, Ireland). Indices examined included; Maturity Index (MI), Plant Parasite Index (PPI), and modifications to the MI index including MIMO (removing the family c-p=1) and  $\Sigma$ MIMO (removing the family c-p=1 but with inclusion of the PPI). \*P<0.05

Index	Potato Genotype	Crop Management	2015	2014	2013	Mean	Mean / genotype	Mean / management
MI	Desiree	Control	3.05	1.76	2.27	2.36	2.14	2.18
	Desiree	Chemical	2.10	1.61	2.07	1.93		1.88
	Cisgenic Desiree	Control	2.03	2.15	1.97	2.05	1.93	
	Cisgenic Desiree	Chemical	1.50	2.13	1.79	1.81		
	Sarpo Mira	Control	2.63	1.76	2.00	2.13	2.02	
	Sarpo Mira	Chemical	1.86	1.56	2.30	1.91		
<i>Mean</i>			2.20	1.83	2.07			
PPI	Desiree	Control	3.00	3.50	2.00	2.83	1.97	
	Desiree	Chemical	0.00	0.00	3.31	1.10		2.10
	Cisgenic Desiree	Control	2.50	3.00	2.40	2.63	2.23	
	Cisgenic Desiree	Chemical	0.00	2.67	2.83	1.83		
	Sarpo Mira	Control	3.00	2.71	2.29	2.67	3.01	
	Sarpo Mira	Chemical	3.00	3.67	3.39	3.35		
<i>Mean</i>			1.92	2.59	2.70			
PPI/MI	Desiree	Control	0.98	1.99	0.88	1.28	0.91	
	Desiree	Chemical	0.00	0.00	1.60	0.53		1.10
	Cisgenic Desiree	Control	1.23	1.40	1.22	1.28	1.11	
	Cisgenic Desiree	Chemical	0.00	1.25	1.58	0.95		
	Sarpo Mira	Control	1.14	1.54	1.15	1.28	1.54	
	Sarpo Mira	Chemical	1.61	2.35	1.47	1.81		
<i>Mean</i>			0.83	1.42	1.32			
MIMO	Desiree	Control	3.46	2.47	2.10	2.68	2.61	
	Desiree	Chemical	3.00	2.39	2.23	2.54		2.51
	Cisgenic Desiree	Control	2.33	2.94	2.00	2.42	2.36	
	Cisgenic Desiree	Chemical	2.09	2.80	2.00	2.30		
	Sarpo Mira	Control	2.83	2.63	2.00	2.49	2.64	
	Sarpo Mira	Chemical	3.38	2.71	2.30	2.80		
<i>Mean</i>			2.85 <sup>b</sup>	2.66 <sup>ab</sup>	2.11 <sup>a*</sup>			
$\Sigma$ MIMO	Desiree	Control	3.42	2.89	2.08	2.80	2.74	
	Desiree	Chemical	3.00	2.39	2.64	2.68		2.72
	Cisgenic Desiree	Control	2.34	2.94	2.09	2.46	2.46	
	Cisgenic Desiree	Chemical	2.09	2.79	2.50	2.46		
	Sarpo Mira	Control	2.84	2.68	2.13	2.55	2.79	
	Sarpo Mira	Chemical	3.30	2.88	2.88	3.02		
<i>Mean</i>			2.83	2.76	2.39			

#### *Trophic Groups*

During the three years of the study, seven of the eight feeding groups proposed by Yates (1993) were identified; Bacterial feeding (BF), Plant feeding (PF), Fungal feeding (FF), Omnivorous (OM),

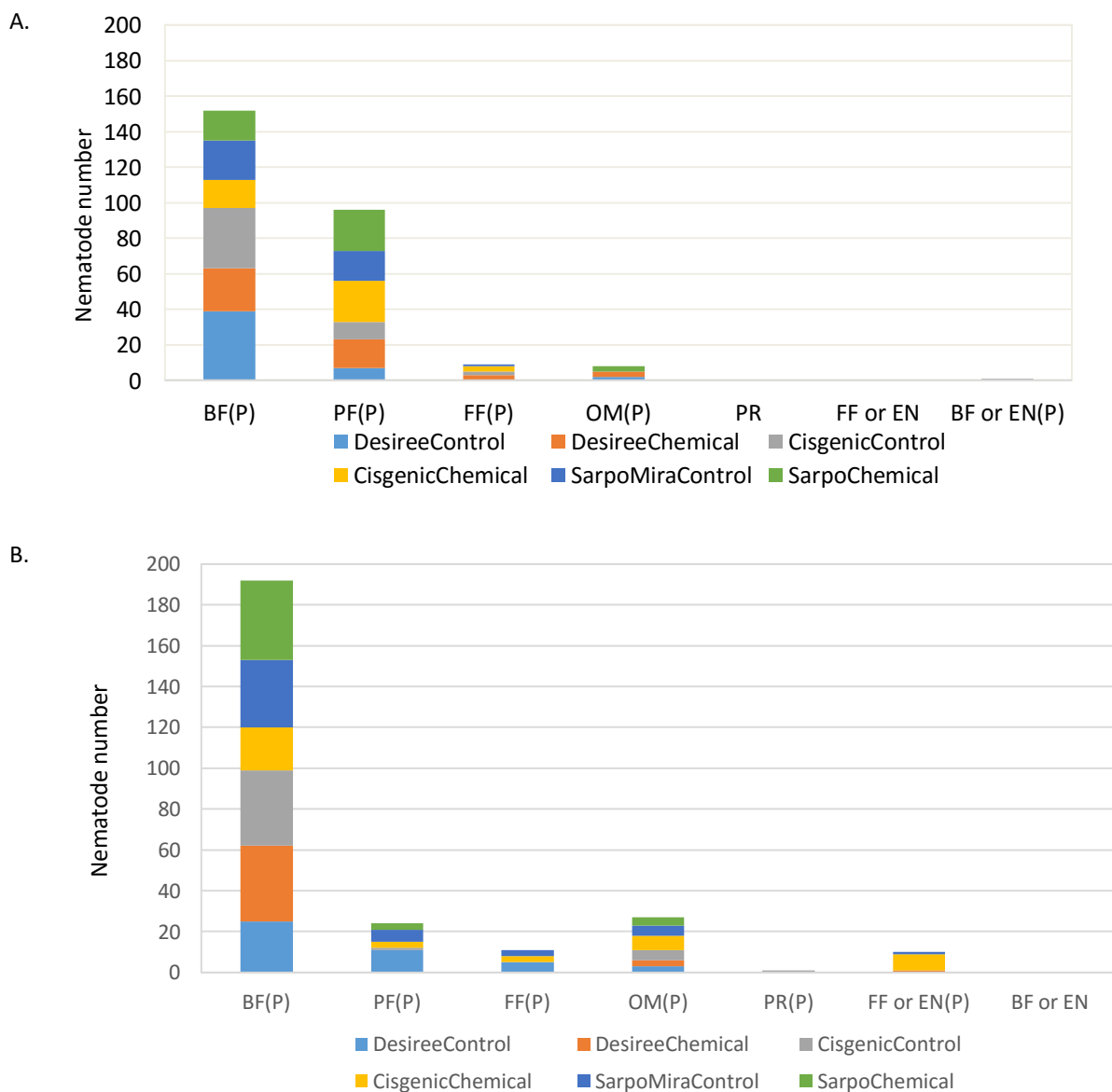
Predacious (PR), Bacterial feeding or Entomopathogenic (BF OR EN) and Fungal feeding or Entomopathogenic (FF OR EN). In the absence of a weekly chemical disease management treatment, up to 5, 6 and 5 trophic groups were present for each respective potato genotype (cisgenic Desiree, Desiree and Sarpo Mira) across the 3 years. In the presence of a weekly chemical disease management application, trophic group numbers were identified at up to 6, 4 and 7 for cisgenic Desiree, Desiree and Sarpo Mira respectively. The variability across the three years of the field study is evident in Fig. 1. In 2013 (Fig. 1a) only five trophic groups (bacterial feeding (BF), plant feeding (PF), omnivorous (OM), fungal feeding (FF) and bacterial feeding or entomopathogens (BF or EN) were identified with BF and PF dominating more than 80% of the total recorded, with PF significantly dominating ( $P<0.01$ ) the chemical treated cultivar over the control samples for each cultivar. Six trophic groups were recorded in 2014 and 2015, with a high population of BF followed by OM in both years. With the exception of the PF group, which was statistically more dominant across the years examined ( $P<0.001$ ) and in regard to year x disease management ( $P<0.01$ ), no significant difference was recorded across the remaining groups for either cultivar/disease management/year studied. Across the study, no predator nematodes were identified in either the Sarpo Mira (control), Desiree (chemical) or cisgenic Desiree (chemical) rhizosphere samples. Across the 3 years examined, weak effects were observed for the impact of disease management on PR ( $P<0.13$ ) and year ( $P<0.07$ ) and the interaction of year x disease management on BF ( $P<0.13$ ) and year for OM ( $P<0.08$ ) but crop genotype had no significant impact on the occurrence of trophic groups observed ( $P<0.05$ ).

#### *Functional Guild Indices*

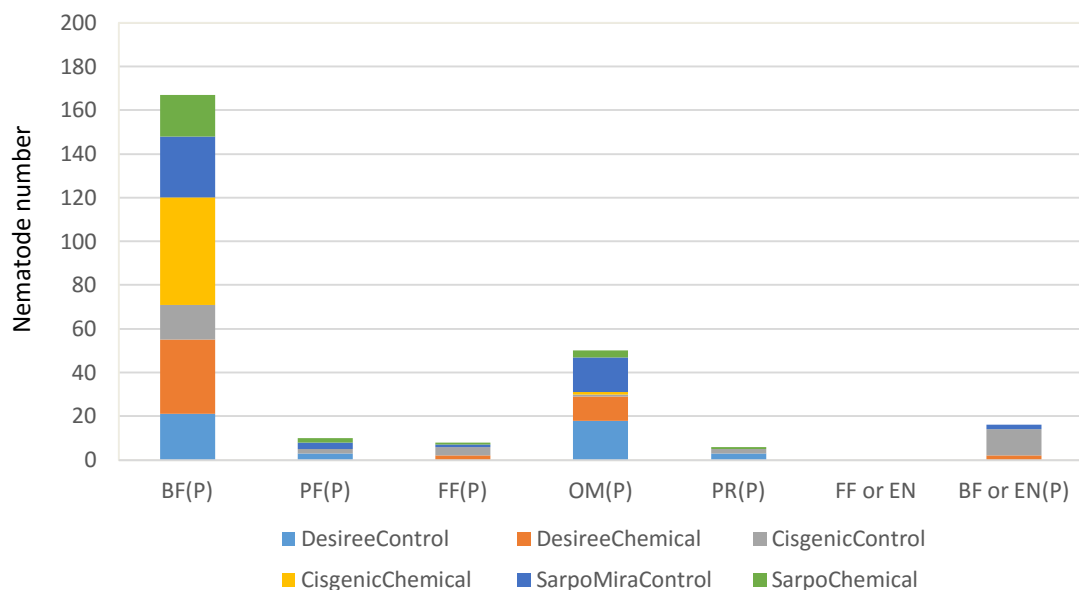
Examining the degree of colonizer-persister across the main trophic groups (BF, PF, FF, OM and PR); BF recorded 1 to 3, FF 2 to 3, PR recorded 1, 3 to 5, OM 4 and 5 and PF 2 to 4. Evaluating the diversity of nematode functional groups and their respective c-p classification, each index recorded a distinct response (Table 2). For EI, the highest mean was associated with chemical treatment (68) versus the absence of chemical fungicides (49), while the inverted trend occurred with BF2 (51/32). In both cases the differential values were significant ( $P<0.05$ ). The influence of chemical applications led to the highest mean values recorded with the EI and BF1 index (EI; 71.61, 70.72, 62.20 and BF1; 99.33, 89.57, 66.67) compared to the respective control values (EI; 49.93, 55.64, 41.39 and BF1; 63.48, 88.89, 65.57). In contrast, for BF2 the highest values were recorded in the absence of chemical management (50.07, 44.36, and 58.61). At a crop genotype level, Sarpo Mira recorded the lowest mean EI value (51.80) but subsequently the highest BF2 mean (48.20) and CH (17.22). The cisgenic Desiree genotype returned the lowest SI mean (35.74) but the highest BF1 (63.18).

Examining the influence of year in more detail, four of the five indices (EI, BF1, BF2 and SI) presented a significant difference ( $P < 0.001$ ,  $P < 0.004$ ,  $P < 0.001$  and  $P < 0.002$  respectively) across the three years of the study (Table 5). Examining the values in more detail, 2013 recorded the lowest mean values for the EI, BF1 and S1 indices (20, 39 and 16 respectively) and highest with the BF2 index (80). No statistical difference was recorded in values compared between 2014 and 2015, with the exception of BF2.

**Figure 1.** Impact of potato genotype (Desiree, cisgenic Desiree, Sarpo Mira), crop management (control, chemical) and year (2013, 2014, 2015) on the prevalence of trophic groups for bacterial feeding (BF), plant feeding (PF), fungal feeding (FF), omnivorous (OM), predacious (PR), fungal feeding or entomopathogens (FF or EN), bacterial feeding or entomopathogens (BF or EN).



C.



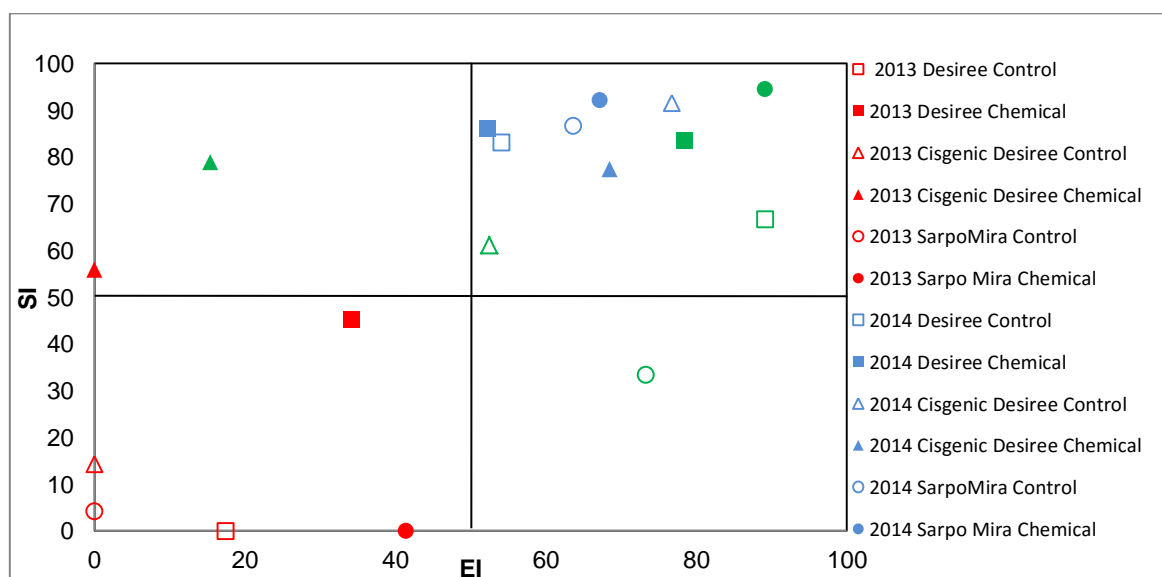
**Table 2.** Effect of potato genotype (Desiree, cisgenic Desiree, Sarpo Mira), crop management (control, chemical treatment) and year (2013, 2014, 2105) on the nematode trophic diversity indices, EI, B2, CH, B1 and SI.

Index	Potato Genotype	Crop Management	2015	2014	2013	Mean	Mean / genotype	Mean / management
EI	Desiree	Control	67	83	0	49.93	60.77	49**
	Desiree	Chemical	83	86	45	71.61		68.00
	Cisgenic Desiree	Control	61	92	14	55.64	63.18	
	Cisgenic Desiree	Chemical	79	77	56	70.72		
	Sarpo Mira	Control	33	87	4.2	41.39	51.80	
	Sarpo Mira	Chemical	95	92	0	62.20		
	<i>Mean</i>		70	86	20			
B2	Desiree	Control	33	17	100	50.07	39.23	51**
	Desiree	Chemical	17	14	55	28.39		32.00
	Cisgenic Desiree	Control	39	8	86	44.36	36.82	
	Cisgenic Desiree	Chemical	21	23	44	29.28		
	Sarpo Mira	Control	67	13	96	58.61	48.20	
	Sarpo Mira	Chemical	5	8	100	37.80		
	<i>Mean</i>		30	14	80			
CH	Desiree	Control	2.3	7.2	0.0	3.19	4.91	16.00
	Desiree	Chemical	3.0	1.1	15.8	6.62		6.00
	Cisgenic Desiree	Control	0.0	0.0	33.3	11.11	10.77	
	Cisgenic Desiree	Chemical	0.0	15.5	15.8	10.43		
	Sarpo Mira	Control	0.0	3.3	100.0	34.43	17.22	
	Sarpo Mira	Chemical	0.0	0.0	0.0	0.00		
	<i>Mean</i>		0.9	4.5	27.5			
B1	Desiree	Control	98	93	0	63.48	78.43	73.00
	Desiree	Chemical	97	99	84	93.38		83.00

	Cisgenic Desiree	Control	100	100	67	88.89	89.23	
	Cisgenic Desiree	Chemical	100	85	84	89.57		
	Sarpo Mira	Control	100	97	0	65.57	66.12	
	Sarpo Mira	Chemical	100	100	0	66.67		
	<i>Mean</i>		99	95	39			
SI	Desiree	Control	89	54	17	53.56	54.30	
	Desiree	Chemical	78	52	34	55.05		49.00
	Cisgenic Desiree	Control	53	77	0	43.10	35.74	
	Cisgenic Desiree	Chemical	15	70	0	28.39		
	Sarpo Mira	Control	74	64	0	45.90	54.72	
	Sarpo Mira	Chemical	82	67	41	63.54		
	<i>Mean</i>		65	64	16			

Examining treatment effects on the basal, structural and enrichment components of the soil food web identified a significant difference ( $P < 0.001$ ) between EI and SI over time. The construction of nematode profiles for 2013 revealed that food webs for 5 of the 6 treatments (exception being cisgenic Desiree + chemical) positioned within quadrat D (Fig. 2), indicating a depleted and degraded food web structure. For 2014, all six treatments were plotted to quadrat B, typical of an enrichment condition. In the case of 2015, the final year of the study, all treatments remained in quadrat B, with the exception of the Sarpo Mira control and the cisgenic Desiree + chemical, which positioned in quadrat C and A respectively (Fig. 2).

**Figure 2.** Food web analysis, as per relationship between the Enrichment (EI) and the Structure (SI) index, on the cultivation of potatoes genotypes (Desiree, cisgenic Desiree, Sarpo Mira) treated with different crop management regimes (control, chemical treatment) through the years of 2013, 2014 and 2015.



### *Nematode abundance and diversity indices*

An alternative measure of disturbance considered was the impact of crop genotype and/or disease management treatments on nematode diversity, measured through the abundance of individual nematode family, genus and species and at a species level according to richness (H), evenness (EH) and (D, 1-D and 1/D) dominance indices on a yearly basis through the study. While samples collected from 2014 and the Sarpo Mira control plots recorded higher numbers of nematode species, genera and families than the alternative potato genotypes, there was no significant difference in numbers recorded from Desiree and cisgenic Desiree. Taking into account the rare (less frequent-Shannon index) and abundance (dominant-Simpson index) species per sample, the diversity indices returned similar patterns between treatments (Table 3). The mean H index values were >2 for all treatments, irrespective of year, disease management and potato genotype with no significance recorded between treatment; similarly, no significance was returned between treatments in regards to the evenness distribution (EH) of individuals per species present in samples, which was found to be closer to 1 than to 0 for each combination. The uniformity of the mean H and EH values across treatments is illustrated in Fig 4. In contrast the probability that two nematodes randomly selected from within a sample belonged to the same species (D) was closer to 0 than 1. Lastly, the analyses recorded a statistically similar but high probability (0.84 – 0.89) of nematode diversity (1-D) across genotypes (per treatment per year) with the number of species (1/D) recorded between 7 and 9 per crop.

**Table 3.** Nematode diversity (richness H), evenness EH) and dominance (D, 1-D, 1/D) indices, arising from samples taken from under different potatoes genotypes (Desiree, cisgenic Desiree, Sarpo Mira) treated with different crop management regimes (control, chemical treatment) through the years of 2013, 2014 and 2015 at Oak Park (Carlow, Ireland).

Index	Potato Genotype	Crop Management	2015	2014	2013	Mean	Mean / genotype	Mean / management	
H	Desiree	Control	2.46	2.51	2.54	2.50	2.43	2.43	
	Desiree	Chemical	2.48	2.07	2.54	2.36		2.33	
	Cisgenic Desiree	Control	2.2	2.62	2.27	2.36	2.30		
	Cisgenic Desiree	Chemical	2.58	2.8	1.34	2.24			
	Sarpo Mira	Control	2.26	2.46	2.55	2.42	2.40		
	Sarpo Mira	Chemical	2.47	2.23	2.44	2.38			
	<i>Mean</i>			2.41	2.45	2.28			
EH	Desiree	Control	0.64	0.66	0.67	0.66	0.64		0.64
	Desiree	Chemical	0.64	0.56	0.65	0.62			0.63
	Cisgenic Desiree	Control	0.57	0.69	0.63	0.63	0.61		
	Cisgenic Desiree	Chemical	0.69	0.75	0.34	0.59			
	Sarpo Mira	Control	0.61	0.63	0.65	0.63	0.65		
	Sarpo Mira	Chemical	0.66	0.58	0.76	0.67			
	<i>Mean</i>			0.64	0.65	0.62			

D	Desiree	Control	0.11	0.1	0.1	0.10	0.11	0.12
	Desiree	Chemical	0.09	0.18	0.1	0.12		0.15
	Cisgenic Desiree	Control	0.16	0.09	0.15	0.13	0.16	
	Cisgenic Desiree	Chemical	0.1	0.08	0.38	0.19		
	Sarpo Mira	Control	0.14	0.13	0.12	0.13	0.13	
	Sarpo Mira	Chemical	0.12	0.15	0.11	0.13		
	<i>Mean</i>		0.12	0.12	0.16			
1-D	Desiree	Control	0.89	0.90	0.90	0.90	0.89	0.88
	Desiree	Chemical	0.91	0.82	0.90	0.88		0.85
	Cisgenic Desiree	Control	0.84	0.91	0.85	0.87	0.84	
	Cisgenic Desiree	Chemical	0.90	0.92	0.62	0.81		
	Sarpo Mira	Control	0.86	0.87	0.88	0.87	0.87	
	Sarpo Mira	Chemical	0.88	0.85	0.89	0.87		
	<i>Mean</i>		0.88	0.88	0.84			
1/D	Desiree	Control	9.09	10.00	10.41	9.83	9.33	8.53
	Desiree	Chemical	11.11	5.56	9.80	8.82		8.38
	Cisgenic Desiree	Control	6.25	11.11	6.61	7.99	8.19	
	Cisgenic Desiree	Chemical	10.00	12.50	2.67	8.39		
	Sarpo Mira	Control	7.14	7.69	8.45	7.76	7.85	
	Sarpo Mira	Chemical	8.33	6.67	8.80	7.93		
	<i>Mean</i>		8.65	8.92	7.79			

#### *Nematode families and genus as a bio-indicator of environmental disturbance*

Up to 22 distinct families were identified across all treatments evaluated over the three years (Appendix Table 1). There was no significant difference ( $P>0.05$ ) between the genus and family nematode numbers of cisgenic Desiree and its comparator Desiree genotype, irrespective of the presence/absence of chemical management. For Sarpo Mira, there was a notable decrease in number of families following chemical treatments (Appendix Table 1). Sorensen coefficient values calculated for nematode families within each potato genotype indicated substantial overlap between treatments: 0.63 for Desiree control v. chemical, 0.79 for cisgenic Desiree control v. chemical and 0.67 for Sarpo Mira control v. chemical. Factoring the influence of time, coefficient values were calculated for each respective permutation of genotype and treatment (Appendix Table 2). Examining equivalence at the family level, Sarpo Mira coefficient values were similar through the three years of the study (0.55 – 0.57) compared to the more variable Desiree (0.50 to 0.63) and cisgenic Desiree (0.43 – 0.73). Independent of the management regime deployed, for 2013 a cisgenic Desiree v. Desiree comparison returned a  $CC = 0.53$ , in contrast to 0.34 for cisgenic Desiree v. Sarpo Mira. For 2014, values ranged from 0.37 – 0.47, while from the final year (2015), cisgenic Desiree and Sarpo Mira shared 50% of nematode families sampled.

The distribution of families across 2013 was characterised by 18 families (Appendix Fig. 1A) with the Cephalobidae abundant in all treatments evaluated with more Cephalobidae individuals

noted in the control treatments independent of the crop evaluated (38, 32 and 20 for Desiree control, cisgenic Desiree control and Sarpo Mira control respectively). Only a nominal number of the the Rhabditidae family were recorded while seven families associated with plant feeding (Tylenchulidae, Tylenchidae, Telotylenchidae, Trichodoridae, Merliniinae, Longidoridae, and Pratylenchidae) were counted. For 2014 (Appendix Fig. 1B), twenty three families were detected with an abundance of the Rhabditidae (8 to 21 members) family recorded along with the Cephalobidae family (6 to 14) at the same time and members of a third nematode bacterial feeding, the Panagrolaimidae dominating especially in cisgenic Desiree and SarpoMira derived samples. As with 2013, seven plant feeding families were detected (Heteroderidae, Hoplolaimidae, Merliniidae, Trichodoridae, Pratylenchidae, Tylenchidae, Telotylenchidae). The Heteroderidae and Hoplolaimidae families were present in Desiree control samples and the Hoplolaimidae family was only found associated with the Sarpo Mira control sample. For 2015 (Appendix Fig. 1C), twenty families were listed with a similar ratio of members of the family Rhabditidae (1 to 24) and Cephalobidae (4 to 22) and occurrence of the Panagrolaimidae (1 to 11) family recorded across treatments. The occurrence of two bacterial feeding and entomopathogenic (Neodiplogasteridae/Diplogasterida) nematode families were noted in Desiree chemical and SarpoMira chemical with the Steinernematidae family identified in all three varieties. Five plant feeding families were recorded (Merliniidae, Telotylenchidae, Tylenchulidae, Hoplolaimidae and Trichodoridae) with less plant feeding families recorded in samples derived from chemical managed plots compares with the control management strategies.

Sixty individual genera were identified across the three years of the experiment with 25 to 31 individual genera were identified per crop x management interaction (Appendix Table 1). In relation to the Sorensen coefficient within potato genotypes, 50% of all genera occurred with both chemical and control treatment for Desiree and 63% and 64% for the same treatments with cisgenic Desiree and Sarpo Mira, respectively. As with the nematode family assessment, examining the coefficient values relative to each year of the study identified a broad range from 0.18 to 0.64 when comparing the impact of control v. chemical treatment across the three potato genotypes studied. In addition, the overlap of genera between potato genotypes (irrespective of chemical treatment) ranged from 0.36 to 0.74, with no clear trend evident. Differences on the presence/absence of specific genus were more evident. For example, Clarkus (Dorylaimia/2015) and Pratylenchoides (Tylenchida/2014) were isolated from the Desiree and cisgenic Desiree control plots; though Clarkus (Dorylaimida/2015) was isolated from SarpoMira – chemical treated plots (Appendix Table 1)



## Maize

Based on microscopic examination, the average abundances of nematodes collected from soil samples of *Bt* and ISO maize hybrid cultivated plots across each individual European location (site) as assigned to taxonomic groups are presented in Tables 4 – 18. The highest number of nematodes was assigned to the following genera: *Acrobeloides*, *Cephalobus*, *Chiloplacus*, *Aphelenchoides*, *Aphelenchus*, *Eudorylaimus* and *Filenchus* for Slovakia in 2013; *Acrobeloides*, *Cephalobus*, *Eucephalobus*, *Chiloplacus*, *Rhabditis*, *Aphelenchus* and *Filenchus* for Slovakia in 2014; *Acrobeloides*, *Aphelenchoides*, *Aphelenchus*, *Aglenchus*, *Filenchus*, *Malenchus* and *Geocenamus* for Denmark in 2013; *Acrobeloides*, *Aphelenchoides*, *Aphelenchus*, *Filenchus*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* for Denmark in 2014; *Acrobeloides*, *Rhabditis*, *Aphelenchoides*, *Geocenamus* and *Paratylenchus* for Sweden in 2013, *Acrobeloides*, *Rhabditis*, *Aphelenchoides*, *Paratylenchus* and *Tylenchorhynchus* for Sweden in 2014 and for Spain (2013) *Acrobeloides*, *Eucephalobus*, *Rhabditis*, *Aphelenchoides*, *Eudorylaimus* and *Filenchus*.

**Table 4.** Average abundance of nematodes collected from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Borovce, Slovakia in 2013 (SK2013) (x = average of 10 replications; s = standard deviation)

SK2013 Genera	ISO		BT	
	X	S	x	s
<i>Acrobeloides</i>	33.2	17.5	35.6	36.1
<i>Acrobeles</i>	0	0	0	-
<i>Alaimus</i>	1	0	0	-
<i>Cephalobus</i>	21.5	21.1	8.6	7
<i>Cervidellus</i>	1	-	1	-
<i>Eucephalobus</i>	4.2	3.1	4.2	3.6
<i>Chiloplacus</i>	21.4	14.8	18.1	21.5
<i>Mesorhabditis</i>	0	0	0	-
<i>Panagrolaimus</i>	3	0	0	0
<i>Plectus</i>	1	0	3	1.4
<i>Prismatolaimus</i>	0	0	0	0
<i>Rhabditis</i>	0	0	4.2	2.5
<i>Teratocephalus</i>	0	0	0	0
<i>Wilsonema</i>	2	0	0	0
<i>Aphelenchoides</i>	25.6	7.2	17	23.8
<i>Aphelenchus</i>	33	16.8	47.2	45.2

<i>Ditylenchus</i>	2	1.4	0	0
<i>Tylencholaimus</i>	0	0	0	0
<i>Aporcelaimellus</i>	1	0	2.7	2.0
<i>Axonchium</i>	0	0	0	0
<i>Crassolabium</i>	1	0	0	0
<i>Discolaimus</i>	4	0	0	0
<i>Ecumenicus</i>	4	2.8	5	0
<i>Enchodelus</i>	2.3	2.3	0	0
<i>Eudorylaimus</i>	14.7	10.9	20.2	10.5
<i>Mesodorylaimus</i>	2	1.7	0	0
<i>Anatonchus</i>	0	0	0	0
<i>Clarkus</i>	0	0	0	0
<i>Iotonchus</i>	1.6	0.5	1	0
<i>Mylonchulus</i>	0	0	0	0
<i>Aglenchus</i>	0	0	0	0
<i>Filenchus</i>	21.4	22	28.7	20.7
<i>Malenchus</i>	0	0	0	0
<i>Psilenchus</i>	2	0	0	0
<i>Tylenchus</i>	6.2	8.7	6.1	7.1
<i>Bitylenchus</i>	1	0	3	0
<i>Criconematidae juv.</i>	0	0	0	0
<i>Doryllium</i>	6	6	2	0
<i>Geocenamus</i>	2.5	2.1	1	0
<i>Helicotylenchus</i>	6.2	4.7	15.5	9.1
<i>Heterodera</i>	0	0	0	0
<i>Paratylenchus</i>	2.3	1.5	0	0
<i>Pratylenchus</i>	7.6	7.7	2.3	1.5
<i>Trichodorus</i>	2	2	1	0
<i>Tylenchorhynchus</i>	0	0	0	0
Sum	171.9	67.4	182.7	73.9

**Table 5.** Indexes calculated from nematode analysis from soil samples of Bt maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Borovce, Slovakia in 2013 (SK2013). (x = average of 10 replications; s = standard deviation).

<b>SK2013</b>	<b>ISO</b>		<b>BT</b>	
<b>Index name</b>	<b>x</b>	<b>s</b>	<b>x</b>	<b>s</b>
Total abundance. ind	171.9	67.42148	182.7	73.93999
H'gen	1.96	0.179505	1.751	0.269874
Maturity Index	2.227	0.118514	2.322	0.147784
Maturity Index 205	2.229	0.120227	2.282	0.124971
Sigma Maturity Index	2.262	0.101083	2.36	0.213542
Plant Parasitic Index	2.601	0.353127	2.357	0.376889
Channel Index	98.835	3.684053	92.802	11.07575
Basal Index	48.092	7.036956	43.682	6.394575
Enrichment Index	36.744	2.801302	38.581	7.833629
Structure Index	32.266	15.12594	38.152	12.24401
Total biomass. mg	0.092	0.052239	0.13	0.049216
Composite footprint	28.158	13.83831	36.947	13.34276
Enrichment footprint	6.551	1.989933	10.623	6.299681
Structure footprint	12.707	9.512287	17.72	8.099711
Herbivore footprint	2.123	1.974893	1.708	1.787965
Fungivore footprint	6.841	2.059814	7.286	5.007093
Bacterivore footprint	6.892	3.619705	10.434	6.730574
Predator footprint	0.975	1.8588	0.28	0.590292
Omnivore footprint	11.4	8.473084	17.397	8.13518
Herbivores. % of total	8.05	5.406632	5.45	4.471453
Fungivores. % of total	48.12	7.027375	48.65	16.39771
Fungivores. % free living	52.62	9.343542	51.49	17.50063
Bacterivores. % of total	34.69	7.132157	33.18	14.30639
Bacterivores. % free living	37.61	6.854269	35.02	15.05359
Predators. % of total	0.44	0.980023	0.11	0.242441
Predators. % of free living	0.48	1.070618	0.12	0.269979
Omnivores. % of total	8.71	4.769684	12.62	5.921486
Omnivores. % of free living	9.31	4.950073	13.38	6.337332
Sedentary parasites. % of herbivores	0	0	0	0
Migratory endoparasites. % of herbivores	30.42	39.4851	14.66	13.48507
Semi-endoparasites. % of herbivores	14.41	24.58403	15.09	31.81322
Ectoparasites. % of herbivores	17.11	20.02334	4.19	8.144862
Epidermal/root hair feeders. % of herbivores	0.53	1.676007	0	0
Algal/lichen/moss feeders. % of herbivores	37.55	33.25662	66.06	34.54206

CP 1. % of free living	0.14	0.442719	1.19	1.857388
CP 2. % of free living	88.58	5.977513	85.18	5.884971
CP 3. % of free living	0	0	0	0
CP 4. % of free living	10.97	5.559586	13.03	6.090621
CP 5. % of free living	0.31	0.721803	0.61	1.018114
PP 2. % of herbivores	45.76	33.54255	67.06	35.02568
PP 3. % of herbivores	48.43	34.58005	30.09	33.9856
PP 4. % of herbivores	5.82	9.875762	2.84	7.859206
PP 5. % of herbivores	0	0	0	0

**Table 6.** Average abundance of nematodes collected from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Borovce, Slovakia in 2014 (SK2014) ( $\bar{x}$  = average of 10 replications;  $s$  = standard deviation).

SK2014	ISO		BT	
	$\bar{x}$	$s$	$\bar{x}$	$s$
<i>Acrobeloides</i>	53.2	32.5	50.6	30.1
<i>Acrobeles</i>	0	0	0	0
<i>Alaimus</i>	1.5	0.7	2.3	0.5
<i>Cephalobus</i>	36.6	23.4	29.1	18.5
<i>Cervidellus</i>	0	0	0	0
<i>Eucephalobus</i>	20.3	15.5	16	9.5
<i>Chiloplacus</i>	21.6	14.7	6	1.4
<i>Mesorhabditis</i>	0	0	0	0
<i>Panagrolaimus</i>	0	0	0	0
<i>Plectus</i>	3.7	3.4	5.5	4.6
<i>Prismatolaimus</i>	4	1	5	2.1
<i>Rhabditis</i>	47.4	37.8	38	21.7
<i>Teratocephalus</i>	0	0	0	0
<i>Wilsonema</i>	0	0	0	0
<i>Aphelenchoides</i>	8.3	8.4	11.6	10.6
<i>Aphelenchus</i>	17.2	13.4	23	11.8
<i>Ditylenchus</i>	0	0	0	0
<i>Tylencholaimus</i>	11	3.3	15.5	13.6
<i>Aporcelaimellus</i>	3.8	3.4	2.5	1.2
<i>Axonchium</i>	0	0	0	0
<i>Crassolabium</i>	0	0	0	0

<i>Discolaimus</i>	0	0	0	0
<i>Ecumenicus</i>	0	0	0	0
<i>Enchodelus</i>	8	0	1	0
<i>Eudorylaimus</i>	8	5	6.3	4.6
<i>Mesodorylaimus</i>	0	0	0	0
<i>Anatonchus</i>	5	0	7.6	9
<i>Clarkus</i>	25.2	31.8	3.5	1.7
<i>Iotonchus</i>	0	0	0	0
<i>Mylonchulus</i>	7.5	7	8.6	8.3
<i>Aglenchus</i>	1	0	2.5	0.7
<i>Filenchus</i>	38.7	23.7	26.9	14.3
<i>Malenchus</i>	0	0	0	0
<i>Psilenchus</i>	0	0	5	0
<i>Tylenchus</i>	2	0	2	0
<i>Bitylenchus</i>	0	0	0	0
<i>Criconematidae juv.</i>	1	0	0	0
<i>Doryllium</i>	0	0	0	0
<i>Geocenamus</i>	0	0	0	0
<i>Helicotylenchus</i>	0	0	0	0
<i>Heterodera</i>	0	0	0	0
<i>Paratylenchus</i>	0	0	3	1.4
<i>Pratylenchus</i>	4.1	1.8	7.1	4.9
<i>Trichodorus</i>	0	0	0	0
<i>Tylenchorhynchus</i>	0	0	0	0
Sum	268	85.8	228.5	80.2

**Table 7.** Indexes calculated from nematode analysis from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Borovce, Slovakia in 2014 (SK2014). (x = average of 10 replications; s = standard deviation).

SK2014	ISO		BT	
	x	s	x	s
Total abundance. ind	268	85.8008	228.5	80.26242
H'gen	2.04	0.144914	2.135	0.084886
Maturity Index	2.251	0.279661	2.072	0.180665

Maturity Index 205	2.423	0.443573	2.315	0.216961
Sigma Maturity Index	2.268	0.275552	2.151	0.258648
Plant Parasitic Index	2.65	0.474342	2.526	0.4645
Channel Index	29.321	11.84068	31.046	11.96559
Basal Index	33.756	12.826	35.931	9.244998
Enrichment Index	55.615	13.41994	56.139	9.273662
Structure Index	42.861	19.02438	34.044	14.37339
Total biomass. mg	0.519	0.32566	0.384	0.179951
Composite footprint	138.244	83.73893	104.994	47.61936
Enrichment footprint	98.408	75.20907	80.105	43.57017
Structure footprint	26.547	21.13011	13.743	10.86217
Herbivore footprint	0.336	0.126245	0.506	0.359357
Fungivore footprint	5.453	2.995296	5.688	2.462938
Bacterivore footprint	107.408	75.42726	86.807	45.21732
Predator footprint	14.056	22.47255	6.423	9.257234
Omnivore footprint	11.063	10.65813	5.571	4.724029
Herbivores. % of total	1.4	0.579272	2.75	2.094039
Fungivores. % of total	25.39	7.998118	29.37	2.868236
Fungivores. % free living	25.74	8.133907	30.2	2.874022
Bacterivores. % of total	62.12	10.81807	62.1	3.501746
Bacterivores. % free living	63	10.91462	63.9	3.930507
Predators. % of total	6.81	11.34239	2.95	3.28642
Predators. % free living	6.93	11.58755	3.01	3.310404
Omnivores. % of total	4.28	3.959742	2.84	2.015606
Omnivores. % free living	4.35	3.97974	2.89	2.021798
Sedentary parasites. % herbivores	0	0	0	0
Migratory endoparasites. % herbivores	63.33	46.34652	52.6	46.44997
Semi0endoparasites. % herbivores	0	0	0	0
Ectoparasites. % herbivores	1.67	5.281004	11.9	31.52583
Epidermal/root hair feeders % herbivores	5	15.81139	15.5	31.83726
Algal/lichen/moss feeders. % herbivores	30	48.30459	20	42.1637
CP 1. % of free living	16.96	10.45298	17.03	6.645307
CP 2. % of free living	68.67	17.26905	72.64	10.26907
CP 3. % of free living	0.54	0.893433	1.35	1.624979
CP 4. % of free living	12.59	11.58432	8.54	5.033046
CP 5. % of free living	1.23	1.274581	0.45	0.646787

PP 2. % of free living	35	47.43416	47.4	46.44997
PP 3. % of free living	65	47.43416	52.6	46.44997
PP 4. % of free living	0	0	0	0
PP 5. % of free living	0	0	0	0

**Table 8.** Average abundance of nematodes collected from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Flakkebjerg, Denmark in 2013 (DK2013), (x = average of 10 replications; s = standard deviation).

DK2013 Genera	ISO		BT	
	x	s	x	s
<i>Acrobeloides</i>	26.7	17.3	16.8	10.61236
<i>Acrobeles</i>	0	0	0	0
<i>Alaimus</i>	2	0	5	0
<i>Cephalobus</i>	7.6	5.5	0	0
<i>Cervidellus</i>	6	0	0	0
<i>Eucephalobus</i>	4.5	2.5	0	0
<i>Chiloplacus</i>	11.4	7.8	10	4.6
<i>Mesorhabditis</i>	0	0	0	0
<i>Panagrolaimus</i>	29.3	31.5	0	0
<i>Plectus</i>	6.2	3.5	4	3.9
<i>Prismatolaimus</i>	1	0	3	0
<i>Rhabditis</i>	12.2	9.9	30	14.3
<i>Teratocephalus</i>	0	0	0	0
<i>Wilsonema</i>	0	0	0	0
<i>Aphelenchoides</i>	17.2	8.0	13.9	17.9
<i>Aphelenchus</i>	15.6	16.5	8.1	6.2
<i>Ditylenchus</i>	4	0	0	0
<i>Tylencholaimus</i>	0	0	0	0
<i>Aporcelaimellus</i>	6.6	3	2	0.8
<i>Axonchium</i>	0	0	0	0
<i>Crassolabium</i>	0	0	1	0
<i>Discolaimus</i>	0	0	0	0
<i>Ecumenicus</i>	0	0	0	0
<i>Enchodelus</i>	0	0	0	0
<i>Eudorylaimus</i>	14.8	9.8	2.3	1.1
<i>Mesodorylaimus</i>	3.5	2.1	2	0

<i>Anatonchus</i>	0	0	0	0
<i>Clarkus</i>	0	0	0	0
<i>Iotonchus</i>	2.5	1.5	2.4	1.6
<i>Mylonchulus</i>	0	0	1	0
<i>Aglenchus</i>	31	34.2	13.75	14.9
<i>Filenchus</i>	33.1	15.6	18.8	9.1
<i>Malenchus</i>	11.5	10.5	15.25	16.1
<i>Psilenchus</i>	13	0	0	0
<i>Tylenchus</i>	14.7	9.4	8	4.2
<i>Bitylenchus</i>	10.2	6.8	15.3	8.5
<i>Criconematidae juv.</i>	0	0	1	0
<i>Doryllium</i>	0	0	0	0
<i>Geocenamus</i>	15.1	12.6	15.1	9.9
<i>Helicotylenchus</i>	0	0	0	0
<i>Heterodera</i>	0	0	0	0
<i>Paratylenchus</i>	10.3	6.8	10.4	6.5
<i>Pratylenchus</i>	21.5	18.2	10.1	6.1
<i>Trichodorus</i>	4	0	0	0
<i>Tylenchorhynchus</i>	0	0	0	0
Sum	208.8	97.4	154.1	46.2

**Table 9.** Indexes calculated from nematode analysis from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Flakkebjerg, Denmark in 2013 (DK2013). Ten repetitions were used in each variant.

DK2013 Index name	ISO		BT	
	x	s	x	s
Total abundance. ind	208.8	97.42553	154.1	46.28283
H'gen	2.312	0.147934	2.159	0.148133
Maturity Index	2.303	0.307139	1.816	0.204733
Maturity Index 205	2.36	0.216128	2.277	0.214323
Sigma Maturity Index	2.305	0.135749	2.031	0.193474
Plant Parasitic Index	2.357	0.180373	2.516	0.188043
Channel Index	63.626	27.90173	25.53	11.35239



Basal Index	37.496	10.09618	27.266	7.864397
Enrichment Index	47.62	14.04841	69.872	8.495758
Structure Index	38.452	19.08466	21.652	21.45457
Total biomass. mg	0.171	0.065735	0.277	0.10144
Composite footprint	47.79	18.63081	75.984	27.6866
Enrichment footprint	18.691	18.951	62.222	29.03582
Structure footprint	15.659	10.31225	3.842	3.951846
Herbivore footprint	7.814	4.101775	6.288	3.644962
Fungivore footprint	4.285	2.875495	2.644	1.437353
Bacterivore footprint	20.365	18.67768	63.398	29.59387
Predator footprint	2.11	2.425077	1.783	2.412919
Omnivore footprint	13.503	9.749683	1.941	2.426829
Herbivores. % of total	32.77	9.748852	36.07	15.91268
Fungivores. % of total	27.6	7.429222	22.44	5.841271
Fungivores. % free living	42.42	14.19881	36.49	10.69844
Bacterivores. % of total	30.73	8.253491	39.11	15.93078
Bacterivores. % free living	45.25	7.958957	59.24	12.46356
Predators. % of total	0.92	1.21271	1.06	1.396981
Predators. % of free living	1.41	1.834515	1.85	2.47622
Omnivores. % of total	7.97	7.449541	1.32	1.589409
Omnivores. % of free living	10.93	9.6455	2.4	3.22249
Sedentary parasites. % of herbivores	0	0	0	0
Migratory endoparasites.% herbivores	19.96	19.01439	24.28	21.88611
Semi-endoparasites. % herbivores	0	0	0	0
Ectoparasites. % herbivores	33.34	26.24357	55.67	20.99026
Epidermal/root hair feeders. % herbivores	23.48	18.08178	15.99	15.34662
Algal/lichen/moss feeders. % herbivores	23.24	23.92582	4.07	11.53372
CP 1. % of free living	9.21	9.420126	29.92	10.87063
CP 2. % of free living	78.22	12.3833	64.58	11.06012
CP 3. % of free living	0.09	0.284605	0.35	1.106797
CP 4. % of free living	8.94	8.715784	4.17	6.65433
CP 5. % of free living	3.56	3.764513	0.96	1.396185
PP 2. % of herbivores	65.81	18.49057	48.33	18.88621
PP 3. % of herbivores	32.86	19.79333	51.67	18.88621
PP 4. % of herbivores	1.33	4.205829	0	0
PP 5. % of herbivores	0	0	0	0

**Table 10.** Average abundance of nematodes collected from soil samples of Bt maize hybrid (DKC 3872 YG) and its isoline (DKC 3871 grown at Flakkebjerg, Denmark in 2014 (DK2014), (x = average of 10 replications; s = standard deviation).

DK2014	ISO		BT	
	x	s	x	s
<i>Acrobelloides</i>	38.9	36.4	44	19.5
<i>Acrobeles</i>	0	0	0	0
<i>Alaimus</i>	1.6	0.5	1.3	0.5
<i>Cephalobus</i>	4.5	1.7	6.3	9
<i>Cervidellus</i>	1	0	8	0
<i>Eucephalobus</i>	8	4	6	6.8
<i>Chiloplacus</i>	2.4	2.6	0	0
<i>Mesorhabditis</i>	0	0	0	0
<i>Panagrolaimus</i>	1	0	15.6	18.5
<i>Plectus</i>	6.7	4.1	6.6	4.2
<i>Prismatolaimus</i>	3	1.7	0	0
<i>Rhabditis</i>	93.6	48.6	48.5	29.9
<i>Teratocephalus</i>	0	0	0	0
<i>Wilsonema</i>	0	0	0	0
<i>Aphelenchoides</i>	17	12	11.2	5
<i>Aphelenchus</i>	20.2	15.6	29.1	19.5928
<i>Ditylenchus</i>	0	0	0	0
<i>Tylencholaimus</i>	0	0	61	0
<i>Aporcelaimellus</i>	0	0	0	0
<i>Axonchium</i>	0	0	0	0
<i>Crassolabium</i>	0	0	0	0
<i>Discolaimus</i>	0	0	0	0
<i>Ecumenicus</i>	0	0	0	0
<i>Enchodelus</i>	0	0	0	0
<i>Eudorylaimus</i>	5.5	3.6	4.3	3.7
<i>Mesodorylaimus</i>	2	0	0	0
<i>Anatonchus</i>	2.6	1.5	1.6	0.5
<i>Clarkus</i>	0	0	0	0
<i>Iotonchus</i>	1	0	2.8	1.9
<i>Mylonchulus</i>	8	5.6	2	1

<i>Aglenchus</i>	1.5	0.7	1.3	0.5
<i>Filenchus</i>	29.8	14.1	39.7	24.5
<i>Malenchus</i>	6	0	2	0
<i>Psilenchus</i>	0	0	0	0
<i>Tylenchus</i>	0	0	1	0
<i>Bitylenchus</i>	0	0	0	0
<i>Criconematidae juv.</i>	0	0	0	0
<i>Doryllium</i>	0	0	0	0
<i>Geocenamus</i>	9.6	8	0	0
<i>Helicotylenchus</i>	0	0	0	0
<i>Heterodera</i>	0	0	0	0
<i>Paratylenchus</i>	11.8	6.7	15.6	12.5
<i>Pratylenchus</i>	33.3	25.7	18.9	10
<i>Trichodorus</i>	0	0	0	0
<i>Tylenchorhynchus</i>	27.6	18	37.4	16.2
Sum	297.4	70.1	274.5	78.9

**Table 11.** Indexes calculated from nematode analysis from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown at Flakkebjerg, Denmark in 2014 (DK2014). Ten repetitions were used in each variant.

DK2014	ISO		BT	
	x	s	x	s
Total abundance. ind	297.4	70.17312	274.5	78.98699
H'gen	1.988	0.298842	2.102	0.159778
Maturity Index	1.67	0.259015	1.864	0.296693
Maturity Index 205	2.287	0.236692	2.3	0.255778
Sigma Maturity Index	1.995	0.305987	2.139	0.218909
Plant Parasitic Index	2.784	0.152403	2.794	0.172059
Channel Index	17.997	14.77979	31.453	13.86772
Basal Index	21.482	12.49439	31.384	11.82954
Enrichment Index	76.711	13.41225	65.758	10.65004
Structure Index	22.142	18.6461	18.303	23.29247
Total biomass. mg	0.835	0.446897	0.437	0.227647
Composite footprint	209.53	91.04648	121.767	60.14348
Enrichment footprint	190.704	96.10419	103.456	59.29028
Structure footprint	7.139	6.393093	6.653	7.704399

Herbivore footprint	5.772	3.293829	4.952	1.965298
Fungivore footprint	4.751	2.638326	6.939	2.876551
Bacterivore footprint	192.252	95.56909	104.422	59.8883
Predator footprint	3.45	3.466827	3.509	3.964412
Omnivore footprint	3.459	3.10141	1.997	2.737152
Herbivores. % of total	25.57	10.80875	24.22	5.160491
Fungivores. % of total	20.64	10.30471	31.89	9.337791
Fungivores. % free living	27.53	13.68235	42.03	11.34666
Bacterivores. % of total	50.91	13.0242	42.05	8.994844
Bacterivores. % free living	68.35	15.01764	55.57	11.41471
Predators. % of total	1.1	1.294433	0.99	1.031127
Predators. % of free living	1.62	2.029669	1.31	1.330372
Omnivores. % of total	1.75	1.80447	0.83	1.088373
Omnivores. % free living	2.48	2.578458	1.09	1.444876
Sedentary parasites. % herbivores	0	0	0	0
Migratory endoparasites. % herbivores	41.33	20.76771	28.59	12.62911
Semi-endoparasites. % herbivores	0	0	0	0
Ectoparasites. % herbivores	57.11	19.39871	70.63	12.25815
Epidermal/root hair feeders. % herbivores	1.56	3.809404	0.71	1.047165
Algal/lichen/moss feeders. % herbivores	0	0	0.08	0.252982
CP 1. % of free living	42.77	20.03747	25.46	14.41444
CP 2. % of free living	52.53	19.03628	68.58	16.88805
CP 3. % of free living	0.41	0.768042	0	0
CP 4. % of free living	4.28	3.966471	5.93	11.72908
CP 5. % of free living	0	0	0	0
PP 2. % of herbivores	21.55	15.22865	20.71	17.2903
PP 3. % of herbivores	78.45	15.22865	79.29	17.2903
PP 4. % of herbivores	0	0	0	0
PP 5. % of herbivores	0	0	0	0

**Table 12.** Average abundance of nematodes collected from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown in Lund, Sweden in 2013 (S2103), ( $x$  = average of 10 replications;  $s$  = standard deviation).

S2013	ISO		BT	
	$x$	$s$	$x$	$s$
<i>Acrobeloides</i>	60.9	28	54.7	40.4

<i>Acrobeles</i>	6	0	1.5	0.7
<i>Alaimus</i>	1	0	4	0
<i>Cephalobus</i>	12.9	10.9	9.625	5.9
<i>Cervidellus</i>	6	4.2	3.5	2.1
<i>Eucephalobus</i>	9.1	5.3	7	4.1
<i>Chiloplacus</i>	11.3	6.4	8	6
<i>Mesorhabditis</i>	0	0	0	0
<i>Panagrolaimus</i>	0	0	0	0
<i>Plectus</i>	3.7	1.7	3.5	2.5
<i>Prismatolaimus</i>	0	0	0	0
<i>Rhabditis</i>	36	18.8	20.7	8.2
<i>Teratocephalus</i>	2	0	0	0
<i>Wilsonema</i>	0	0	0	0
<i>Aphelenchoides</i>	34.6	14.7	22.6	11.2
<i>Aphelenchus</i>	7.3	7.4	5.1	3.6
<i>Ditylenchus</i>	0	0	0	0
<i>Tylencholaimus</i>	0	0	0	0
<i>Aporcelaimellus</i>	0	0	2	1.4
<i>Axonchium</i>	0	0	0	0
<i>Crassolabium</i>	10	0	6	0
<i>Discolaimus</i>	0	0	5	0
<i>Ecumenicus</i>	0	0	0	0
<i>Enchodelus</i>	0	0	0	0
<i>Eudorylaimus</i>	8.3	6.3	5	3.5
<i>Mesodorylaimus</i>	0	0	0	0
<i>Anatonchus</i>	0	0	0	0
<i>Clarkus</i>	0	0	0	0
<i>Iotonchus</i>	0	0	0	0
<i>Mylonchulus</i>	3.5	3.5	2	1
<i>Aglenchus</i>	9	12	2.3	1.1
<i>Filenchus</i>	6.4	6.2	7.3	5.5
<i>Malenchus</i>	11.5	12	0	0
<i>Psilenchus</i>	0	0	0	0
<i>Tylenchus</i>	15	0	4.5	3.5
<i>Bitylenchus</i>	0	0	0	0
<i>Criconematidae juv.</i>	0	0	0	0

<i>Doryllium</i>	0	0	0	0
<i>Geocenamus</i>	36.3	35.9	37.5	30.4
<i>Helicotylenchus</i>	5.7	3	4.5	4
<i>Heterodera</i>	2.5	2.1	0	0
<i>Paratylenchus</i>	34	25.1	40.2	39.6
<i>Pratylenchus</i>	5.2	4.3	2	1.4
<i>Trichodorus</i>	0	0	0	0
<i>Tylenchorhynchus</i>	0	0	0	0
Sum	270.8	56.8	217.6	63.4

**Table 13.** Indexes calculated from nematode analysis from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isolate (DKC 3871) grown in Lund, Sweden in 2013. Ten repetitions were used in each variant.

S2013	ISO		BT	
	x	s	x	s
Total abundance. ind	270.8	56.82292	217.6	63.41959
H'gen	2.112	0.1924	1.953	0.220457
Maturity Index	1.9	0.16	1.95	0.148623
Maturity Index 205	2.245	0.245142	2.251	0.265139
Sigma Maturity Index	2.369	0.320882	2.231	0.220829
Plant Parasitic Index	2.503	0.229978	2.524	0.188809
Channel Index	27.076	12.40959	29.609	11.32897
Basal Index	40.555	11.17375	42.436	11.32811
Enrichment Index	56.568	11.2082	52.058	12.455
Structure Index	14.86	13.67145	20.181	15.3454
Total biomass. mg	0.346	0.161809	0.22	0.077316
Composite footprint	99.157	43.00228	64.499	22.0653
Enrichment footprint	75.137	37.37061	43.648	16.5969
Structure footprint	4.554	4.725515	4.231	3.190125
Herbivore footprint	9.532	6.95357	9.483	6.585506
Fungivore footprint	3.597	1.183479	2.487	1.087648
Bacterivore footprint	81.5	36.95053	48.537	16.51265
Predator footprint	0.325	0.878095	0.621	1.101367
Omnivore footprint	4.203	4.736572	3.536	3.130631

Herbivores. % of total	30.36	15.96379	35.63	18.08462
Fungivores. % of total	18.01	6.93052	16.23	8.68358
Fungivores. % free living	25.87	8.017211	25.28	10.63619
Bacterivores. % total	49.16	12.36269	45.42	15.82444
Bacterivores. % free living	70.7	7.137226	69.94	9.7956
Predators. % of total	0.34	0.913114	0.61	1.043977
Predators. % of free living	0.39	1.039711	0.92	1.756132
Omnivores. % of total	2.14	2.08284	2.1	1.914274
Omnivores. % of free living	3.03	2.981815	3.87	3.482352
Sedentary parasites. % herbivores	0	0	0	0
Migratory endoparasites. % herbivores	2.6	4.431955	0.6	1.449138
Semi endoparasites. % herbivores	6.17	4.600495	6.9	7.544829
Ectoparasites. % herbivores	86.34	13.48095	89.88	8.121549
Epidermal/root hair feeders. % herbivores	4.12	7.524744	1.45	2.350532
Algal/lichen/moss feeders. % of herbivores	0.78	2.466577	1.19	2.868391
CP 1. % of free living	19.93	9.364359	16.75	10.09469
CP 2. % of free living	76.51	10.91059	78.28	11.32772
CP 3. % of free living	0.07	0.221359	0	0
CP 4. % of free living	3.48	3.322583	4.16	3.3334
CP 5. % of free living	0	0	0.83	1.802498
PP 2. % of herbivores	49.69	23.01354	47.5	18.66059
PP 3. % of herbivores	50.31	23.01354	52.5	18.66059
PP 4. % of herbivores	0	0	0	0
PP 5. % of herbivores	0	0	0	0

**Table 14.** Average abundance of nematodes collected from soil samples of Bt maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown in Lund, Sweden in 2014 (S2014), (x = average of 10 replications; s = standard deviation).

S2014	ISO		BT	
	x	s	x	s
<i>Acrobelloides</i>	19.7	9.5	26.2	9.2
<i>Acrobeles</i>	2.2	1.3	1.7	0.9
<i>Alaimus</i>	2	0	1.3	0.5
<i>Cephalobus</i>	3	1.1	3.25	1.8
<i>Cervidellus</i>	3.8	2.1	2	1.7
<i>Eucephalobus</i>	12.1	7	12.1	7.7

<i>Chiloplacus</i>	3.2	1.8	1.3	0.5
<i>Mesorhabditis</i>	1	0	0	0
<i>Panagrolaimus</i>	0	0	3	0
<i>Plectus</i>	9.4	6.7	7.25	6.6
<i>Prismatolaimus</i>	7	8.6	5	4
<i>Rhabditis</i>	106.8	86.5	68	46.6
<i>Teratocephalus</i>	2.3	2.3	0	0
<i>Wilsonema</i>	1	0	1.5	0.7
<i>Aphelenchoides</i>	11	3.8	10.9	8.2
<i>Aphelenchus</i>	3.5	1.5	2.4	2.2
<i>Ditylenchus</i>	0	0	0	0
<i>Tylencholaimus</i>	0	0	5	0
<i>Aporcelaimellus</i>	0	0	0	0
<i>Axonchium</i>	2	0	0	0
<i>Crassolabium</i>	0	0	0	0
<i>Discolaimus</i>	0	0	0	0
<i>Ecumenicus</i>	0	0	0	0
<i>Enchodelus</i>	2	0	0	0
<i>Eudorylaimus</i>	1.6	0.8	3.2	2.1
<i>Mesodorylaimus</i>	0	0	1.5	0.7
<i>Anatonchus</i>	0	0	0	0
<i>Clarkus</i>	0	0	0	0
<i>Iotonchus</i>	0	0	0	0
<i>Mylonchulus</i>	2.8	2.4	1.5	0.5
<i>Aglenchus</i>	1	0	3	1
<i>Filenchus</i>	7.8	6.2	10.8	8
<i>Malenchus</i>	0	0	0	0
<i>Psilenchus</i>	0	0	0	0
<i>Tylenchus</i>	0	0	3	0
<i>Bitylenchus</i>	0	0	0	0
<i>Criconematidae juv.</i>	0	0	0	0
<i>Doryllium</i>	0	0	0	0
<i>Geocenamus</i>	2	0	12.8	10.4
<i>Helicotylenchus</i>	1.7	0.9	1	0
<i>Heterodera</i>	0	0	1	0
<i>Paratylenchus</i>	25.1	27.4	12.5	16.3



<i>Pratylenchus</i>	9	13.1	17	7
<i>Trichodorus</i>	0	0	0	0
<i>Tylenchorhynchus</i>	24.3	15.6	21.7	6.9
Sum	228.5	101.3	187.8	67.8

**Table 15.** Indexes calculated from nematode analysis from soil samples of *Bt* maize hybrid (DKC 3872 YG) and its isoline (DKC 3871) grown in Lund, Sweden in 2014 (S2014). Ten repetitions were used in each variant.

S2014	ISO		BT	
	x	s	x	s
Total abundance. ind	228.5	101.3807	187.8	67.88356
H'gen	1.88	0.351315	1.915	0.244824
Maturity Index	1.617	0.31861	1.593	0.160212
Maturity Index 205	2.239	0.094452	2.555	0.338862
Sigma Maturity Index	1.946	0.395789	1.919	0.244243
Plant Parasitic Index	2.629	0.271598	2.76	0.209709
Channel Index	17.06	29.93668	9.634	6.807659
Basal Index	23.122	20.89428	21.067	8.91566
Enrichment Index	75.352	21.75188	78.139	9.448264
Structure Index	22.398	17.35737	16.378	11.22975
Total biomass. mg	0.785	0.721515	0.545	0.355035
Composite footprint	205.417	174.5092	148.368	93.94568
Enrichment footprint	192.633	175.4246	136.745	92.70927
Structure footprint	2.112	1.557004	2.052	2.113185
Herbivore footprint	3.99	2.020341	4.077	1.909933
Fungivore footprint	1.451	0.68434	1.627	0.873385
Bacterivore footprint	198.572	174.6219	141.037	93.48072
Predator footprint	0.649	1.031218	0.278	0.391601
Omnivore footprint	0.778	0.819509	1.35	1.627295
Herbivores. % of total	27.11	11.82309	25.11	7.894506
Fungivores. % of total	9.86	5.872951	12.41	7.077107
Fungivores. % free living	14.26	9.354642	16.88	9.896329
Bacterivores. % of total	61.56	14.81892	61.28	11.54237
Bacterivores. % free living	83.69	10.00405	81.46	10.00313
Predators. % of total	0.97	1.676007	0.27	0.386005
Predators. % of free living	1.34	2.313343	0.39	0.580134

Omnivores. % of total	0.5	0.592546	0.95	0.86442
Omnivores. % of free living	0.72	0.89169	1.3	1.229273
Sedentary parasites. % herbivores	0	0	0	0
Migratory endoparasites. % herbivores	16.32	21.46448	9.5	16.51154
SemiOendoparasites. % herbivores	1.58	2.293372	0.18	0.56921
Ectoparasites. % of herbivores	81.93	22.46632	87.61	16.75519
Epidermal/root hair feeders. % herbivores	0.17	0.537587	2.2	3.684502
Algal/lichen/moss feeders. % herbivores	0	0	0.52	1.644384
CP 1. % of free living	46.82	26.69714	45.69	15.31234
CP 2. % of free living	48.63	26.23446	51.43	15.29045
CP 3. % of free living	2.42	2.833843	0.84	1.58899
CP 4. % of free living	2.12	2.772404	2.06	1.648029
CP 5. % of free living	0	0	0	0
PP 2. % of herbivores	37.8	27.06404	23.97	20.94437
PP 3. % of herbivores	61.83	27.16207	76.03	20.94437
PP 4. % of herbivores	0	0	0	0
PP 5. % of herbivores	0.37	1.170043	0	0

**Table 16.** Average abundance of nematodes collected from soil samples of *Bt* maize hybrid and its isoline grown in Madrid, Spain in 2013 (ESP2013), (x = average of 10 replications; s = standard deviation).

ESP2013	ISO		BT	
	x	s	x	s
<i>Acrobeloides</i>	17	13.4	28.1	20.7
<i>Acrobeles</i>	0	0	0	0
<i>Alaimus</i>	0	0	0	0
<i>Cephalobus</i>	15.1	7.9	8.1	6
<i>Cervidellus</i>	24	0	4.3	4
<i>Eucephalobus</i>	14.5	10.1	10.4	7
<i>Chiloplacus</i>	7.5	4.9	1	0
<i>Mesorhabditis</i>	0	0	0	0
<i>Panagrolaimus</i>	0	0	30	0
<i>Plectus</i>	49	37.5	28	25.8
<i>Prismatolaimus</i>	9	0	3	1

<i>Rhabditis</i>	67.5	40.6	77.8	60.8
<i>Teratocephalus</i>	0	0	0	0
<i>Wilsonema</i>	0	0	0	0
<i>Aphelenchoides</i>	16.6	9.9	12.9	10.3
<i>Aphelenchus</i>	3.3	2	1.5	0.7
<i>Ditylenchus</i>	0	0	0	0
<i>Tylencholaimus</i>	0	0	5	4.2
<i>Aporcelaimellus</i>	0	0	0	0
<i>Axonchium</i>	5	2.6	9.1	4.7
<i>Crassolabium</i>	0	0	0	0
<i>Discolaimus</i>	0	0	0	0
<i>Ecumenicus</i>	3.6	1.5	0	0
<i>Enchodelus</i>	2.5	0.7	0	0
<i>Eudorylaimus</i>	17.5	12.5	13	13.5
<i>Mesodorylaimus</i>	0	0	0	0
<i>Anatonchus</i>	0	0	0	0
<i>Clarkus</i>	0	0	0	0
<i>Iotonchus</i>	0	0	1	0
<i>Mylonchulus</i>	3.3	0.5	4.6	2.1
<i>Aglenchus</i>	6.2	4	7	0
<i>Filenchus</i>	29.1	10.6	23.4	15.1
<i>Malenchus</i>	4	0	0	0
<i>Psilenchus</i>	0	0	0	0
<i>Tylenchus</i>	19	0	22	0
<i>Bitylenchus</i>	0	0	7	0
<i>Criconeematidae juv.</i>	0	0	0	0
<i>Doryllium</i>	0	0	0	0
<i>Geocenamus</i>	20	0	71	0
<i>Helicotylenchus</i>	19.1	9.7	7.2	2.6
<i>Heterodera</i>	0	0	0	0
<i>Paratylenchus</i>	5.5	0.7	4	2
<i>Pratylenchus</i>	0	0	5	0
<i>Trichodorus</i>	0	0	15	0
<i>Tylenchorhynchus</i>	0	0	0	0
Sum	240.9	89.1	213.6	55.2

**Table 17.** Indexes calculated from nematode analysis from soil samples of *Bt* maize hybrid and its isoline, grown in Madrid, Spain in 2013 (ESP2013). Ten repetitions were used in each variant.

<b>ESP2013</b>	<b>ISO</b>		<b>BT</b>	
<b>Index name</b>	<b>x</b>	<b>s</b>	<b>x</b>	<b>s</b>
Total abundance. ind	240.9	89.19448	213.6	55.21312
H'gen	1.981	0.242782	1.851	0.34145
Maturity Index	1.918	0.258276	1.828	0.34794
Maturity Index 205	2.338	0.198315	2.391	0.368343
Sigma Maturity Index	2.05	0.268162	2.084	0.401807
Plant Parasitic Index	3.144	0.690929	3.705	0.874824
Channel Index	15.517	8.127767	12.139	6.247711
Basal Index	24.472	9.968244	22.594	10.61557
Enrichment Index	69.775	10.48169	73.969	10.75529
Structure Index	39.42	20.13323	33.365	23.52391
Total biomass. mg	0.694	0.430354	0.639	0.444408
Composite footprint	173.298	87.57992	171.897	117.7645
Enrichment footprint	136.787	80.83361	142.226	123.6637
Structure footprint	14.78	9.925811	9.526	10.47168
Herbivore footprint	4.507	2.394025	8.59	7.08698
Fungivore footprint	2.705	1.15322	2.324	1.108965
Bacterivore footprint	151.609	88.95625	151.931	123.3039
Predator footprint	0.464	0.757983	1.208	1.472185
Omnivore footprint	14.095	9.587557	7.984	10.08121
Herbivores. % of total	12.1	8.740074	12.17	15.30098
Fungivores. % of total	17.95	6.62273	16.98	8.952566
Fungivores. % of free living	20.2	6.968182	18.87	9.235325
Bacterivores. % of total	60.6	11.56864	64.74	18.7993
Bacterivores. % of free living	68.68	9.156152	72.25	14.18655
Predators. % of total	0.41	0.75196	1.28	1.676504
Predators. % of free living	0.44	0.78202	1.64	2.113028
Omnivores. % of total	8.92	6.719094	4.8	5.790605
Omnivores. % of free living	10.71	9.091687	7.24	12.05047
Sedentary parasites. % herbivores	0	0	0	0
Migratory endoparasites. % herbivores	0	0	0.39	1.233288
Semi endoparasites. % herbivores	61.26	38.41658	32.01	24.17728

Ectoparasites. % herbivores	23.61	31.33502	65.31	21.58072
Epidermal/root hair feeders. % herbivores	11.11	15.01551	0.55	1.739253
Algal/lichen/moss feeders. % herbivores	4.04	12.7756	1.73	5.47074
CP 1. % of free living	30.89	12.23378	36.77	16.46957
CP 2. % of free living	57.45	11.81085	53.24	17.8032
CP 3. % of free living	0.49	1.549516	0.41	0.76369
CP 4. % of free living	11.15	9.022349	9.57	13.25167
CP 5. % of free living	0	0	0	0
PP 2. % of herbivores	18.2	17.75519	19.53	18.60998
PP 3. % of herbivores	65.52	35.1897	37.99	22.73252
PP 4. % of herbivores	0	0	1.18	3.731488
PP 5. % of herbivores	16.29	32.43929	41.28	34.19177

**Table 18.** Average total abundance of nematodes collected from soil samples of *Bt* maize hybrid and its isoline hybrid across sites in Slovakia (SK), Denmark (DK), Sweden (S) and Spain (ESP) in 2013, 2014. Analysis of variance compared *Bt* maize and its isoline hybrid, with  $x$  = average of 10 replications;  $s$  = standard deviation.

Site	Year	ISO		BT		F-ratio	P-value
		$x$	$S$	$x$	$s$		
SK	2013	171.9	67.4	182.7	73.9	0.03	0.857
SK	2014	268	85.8	228.5	80.3	1.13	0.302
DK	2013	208.8	97.4	154.1	46.3	2.57	0.126
DK	2014	297.4	70.2	274.5	79.0	0.47	0.502
S	2013	270.8	56.8	217.6	63.4	3.90	0.064
S	2014	228.5	101.4	187.8	67.9	1.11	0.305
ESP	2013	240.9	89.2	213.6	55.2	0.68	0.421

The basic data generated to evaluate the influence of locality, variant and year on the abundance of nematodes in soil samples taken and the abundance of nematodes from selected genera is presented in Appendix A. Taken together analysis of this data indicated the abundance of nematodes was significantly influenced by the year in which the samples were collected and to a lesser degree by the hybrid line sampled. Surprisingly, location was not found to induce a significant effect on the abundance of nematodes in soil (Table 19).

**Table 19.** Influence of locality (location (site)location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of *Bt* maize hybrid; BT – *Bt* maize hybrid) and year (2013, 2014) to the

abundance of nematodes (sum) in soil samples taken; with accompanying figure for illustrative purposes.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>*F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site)location (site)	8987.22	2	4493.61	0.78	0.4590
B:variant	33400.0	1	33400.0	5.83	0.0174
C:year	64774.5	1	64774.5	11.31	0.0011
Interactions:					
AB	5756.62	2	2878.31	0.50	0.6065
AC	107763.	2	53881.5	9.40	0.0002
BC	30.0	1	30.0	0.01	0.9424
ABC	9213.95	2	4606.98	0.80	0.4501
Residual:	618757.	108	5729.23		
Total (corrected):	848682.	119			

\*F-ratios are calculated based on the residual mean square error

Owing to the lower abundance of nematodes in the variant with *Bt* maize coupled with the fact that the abundance of nematodes was significantly influenced by the year of sampling, the sensitivity of the analysis was increased by calculating the impact of location (site)location (site), year and variant on the nine most abundant genera (*Acrobelloides*, *Cephalobus*, *Eucephalobus*, *Chiloplacus*, *Rhabditis*, *Aphelenchoides*, *Aphelenchus*, *Eudorylaimus* and *Filenchus*) as identified from the fundamental datasets (Appendix Table 3). For *Acrobelloides* (Table 20), no main effects were observed across location (site)location (site), variant or year sampled. For *Cephalobus* (Table 21), *Eucephalobus* (Table 22), *Chiloplacus* (Table 23), *Rhabditis* (Table 24) a significant effect was observed for location (site)location (site) sampled and between the years of the sampling. In the case of *Aphelenchoides* only years sampled was found to have a significant effect on nematode abundance (Table 25), while location (site)location (site) was a significant factor that affected the abundance of *Aphelenchus* genera (Table 26). For *Eudorylaimus*, both location (site)location (site) and year affected nematode abundances significantly (Table 27) as was also the case for the *Filenchus* genera (Table 28).

**Table 20.** Influence of locality (location (site)location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Acrobelloides* in soil samples taken.

<b>Source</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>*F-Ratio</b>	<b>P-Value</b>
Main effects:					
A:location (site)location (site)	2908.05	2	1454.03	2.10	0.1272
B:variant	18.4083	1	18.4083	0.03	0.8707
C:year	18.4083	1	18.4083	0.03	0.8707
Interactions:					
AB	39.5167	2	19.7583	0.03	0.9718
AC	19070.2	2	9535.11	13.79	0.0000
BC	429.408	1	429.408	0.62	0.4325
ABC	598.817	2	299.408	0.43	0.6498
Residual:	74699.3	108	691.66		
Total (corrected):	97782.1	119			

\*F-ratios are calculated based on the residual mean square error

**Table 21.** Influence of locality (location (site)location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Cephalobus* in soil samples taken.

<b>Source</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-Ratio</b>	<b>P-Value</b>
Main effects:					
A:location (site)location (site)	5307.65	2	2653.83	20.64	0.0000
B:variant	224.133	1	224.133	1.74	0.1895
C:year	1400.83	1	1400.83	10.90	0.0013
Interactions:					
AB	120.217	2	60.1083	0.47	0.6278
AC	5912.72	2	2956.36	22.99	0.0000
BC	93.6333	1	93.6333	0.73	0.3953
ABC	7.81667	2	3.90833	0.03	0.9701
Residual:	13885.8	108	128.572		
Total (corrected):	26952.8	119			

\*F-ratios are calculated based on the residual mean square error

**Table 22.** Influence of locality (location (site)|location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Eucephalobus* in soil samples taken.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site) location (site)	1057.82	2	528.908	10.27	0.0001
B:variant	112.133	1	112.133	2.18	0.1430
C:year	2133.63	1	2133.63	41.43	0.0000
Interactions:					
AB	10.4167	2	5.20833	0.10	0.9039
AC	637.317	2	318.658	6.19	0.0029
BC	4.03333	1	4.03333	0.08	0.7801
ABC	94.1167	2	47.0583	0.91	0.4041
Residual:	5562.4	108	51.5037		
Total (corrected):	9611.87	119			

\*F-ratios are calculated based on the residual mean square error

**Table 23.** Influence of locality (location (site)|location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Chiloplacus* in soil samples taken.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site) location (site)	1106.87	2	553.433	5.79	0.0041
B:variant	156.408	1	156.408	1.64	0.2036
C:year	2439.01	1	2439.01	25.52	0.0000
Interactions:					
AB	20.0667	2	10.0333	0.10	0.9004
AC	48.0667	2	24.0333	0.25	0.7781
BC	78.4083	1	78.4083	0.82	0.3671
ABC	345.867	2	172.933	1.81	0.1687
Residual:	10322.3	108	95.5769		
Total (corrected):	14517.0	119			

\*F-ratios are calculated based on the residual mean square error



**Table 24.** Influence of locality (location (site)|location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Rhabditis* in soil samples taken.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site) location (site)	23354.1	2	11677.1	8.82	0.0003
B:variant	4368.13	1	4368.13	3.30	0.0720
C:year	73606.5	1	73606.5	55.62	0.0000
Interactions:					
AB	1634.32	2	817.158	0.62	0.5412
AC	888.317	2	444.158	0.34	0.7157
BC	7207.5	1	7207.5	5.45	0.0215
ABC	5419.05	2	2709.53	2.05	0.1341
Residual:	142936.	108	1323.48		
Total (corrected):	259414.	119			

\*F-ratios are calculated based on the residual mean square error

**Table 25.** Influence of locality (location (site)|location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Aphelenchoides* in soil samples taken.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site) location (site)	698.015	2	349.008	2.30	0.1048
B:variant	517.432	1	517.432	3.42	0.0673
C:year	3427.97	1	3427.97	22.63	0.0000
Interactions:					
AB	72.52	2	36.26	0.24	0.7875
AC	1109.28	2	554.64	3.66	0.0290
BC	427.972	1	427.972	2.83	0.0957
ABC	336.757	2	168.379	1.11	0.3328
Residual:	16206.1	107	151.459		
Total (corrected):	22817.7	118			

\*F-ratios are calculated based on the residual mean square error

**Table 26.** Influence of locality (location (site)location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Aphelenchus* in soil samples taken.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site)location (site)	12506.1	2	6253.06	20.52	0.0000
B:variant	297.675	1	297.675	0.98	0.3251
C:year	165.675	1	165.675	0.54	0.4625
Interactions:					
AB	492.35	2	246.175	0.81	0.4484
AC	5400.15	2	2700.08	8.86	0.0003
BC	126.075	1	126.075	0.41	0.5214
ABC	328.65	2	164.325	0.54	0.5847
Residual:	32905.3	108	304.679		
Total (corrected):	52222.0	119			

\*F-ratios are calculated based on the residual mean square error

**Table 27.** Influence of locality (location (site)location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Eudorylaimus* in soil samples taken.

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Main effects:					
A:location (site)location (site)	1703.73	2	851.863	20.52	0.0000
B:variant	34.4099	1	34.4099	0.83	0.3647
C:year	765.859	1	765.859	18.45	0.0000
Interactions:					
AB	276.758	2	138.379	3.33	0.0394
AC	477.457	2	238.728	5.75	0.0042
BC	0.0539246	1	0.0539246	0.00	0.9713
ABC	324.708	2	162.354	3.91	0.0230
Residual:	4442.72	107	41.5208		

Total (corrected):	8045.97	118			
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\*F-ratios are calculated based on the residual mean square error

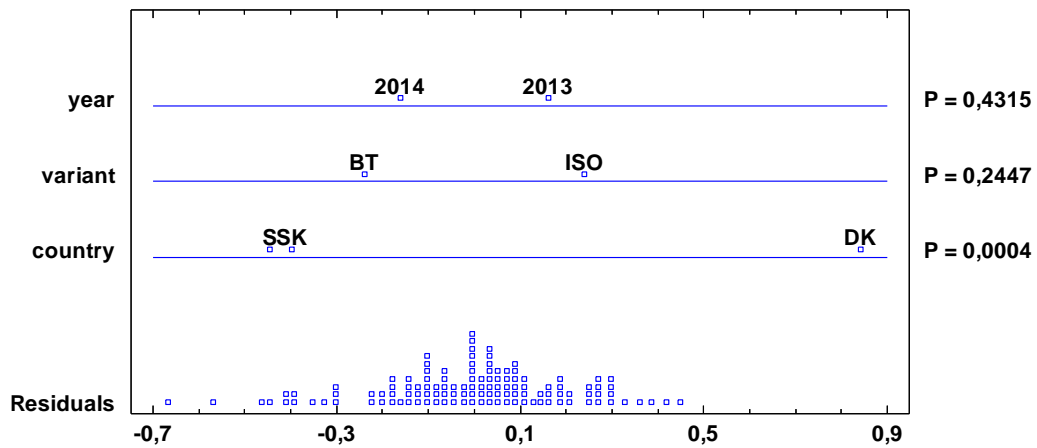
**Table 28.** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isolate of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the abundance of nematodes from the genus *Filenchus* in soil samples taken.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects:					
A:location (site)	11724.1	2	5862.03	22.02	0.0000
B:variant	5.63333	1	5.63333	0.02	0.8846
C:year	1732.8	1	1732.8	6.51	0.0121
Interactions:					
AB	77.2667	2	38.6333	0.15	0.8651
AC	482.4	2	241.2	0.91	0.4071
BC	112.133	1	112.133	0.42	0.5177
ABC	3201.27	2	1600.63	6.01	0.0033
Residual:	28747.6	108	266.181		
Total (corrected):	46083.2	119			

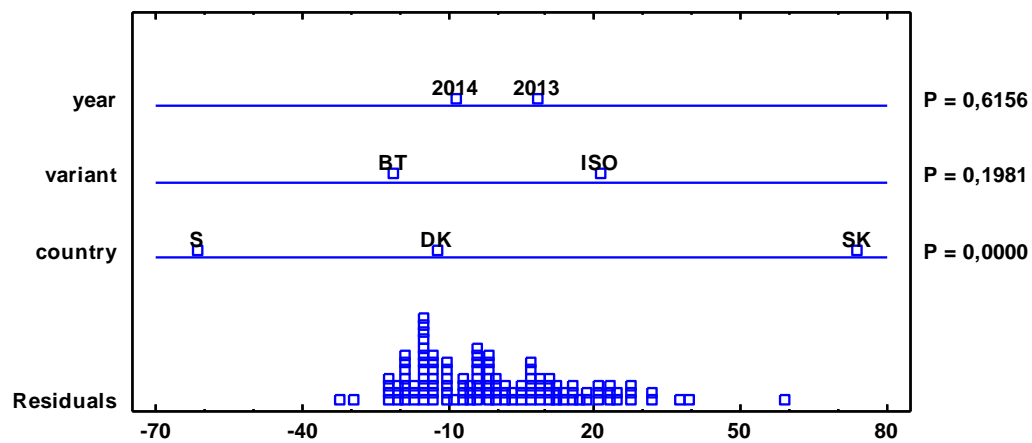
\*F-ratios are calculated based on the residual mean square error

The crop lines investigated were found to exert no significant influence on any of the diversity indices (Genera diversity (H'gen), Maturity index (MI), Plant parasite index (PPI), Enrichment (EI), Structure (SI) and Basal (BI)) studied, across all countries and years examined. In the case of the H'gen index (P=0.0004, Fig. 3) and Structure Index (P=0.0000, Fig. 4) only the influence of location (site) imposed a significant effect. For the Plant Parasite Index, year of sampling had a significant effect (P=0.0001, Fig. 5). While for the Maturity (Fig. 6), Enrichment (Fig. 7) and Basal Index (Fig. 8), both location (site) and year had a significant effect.

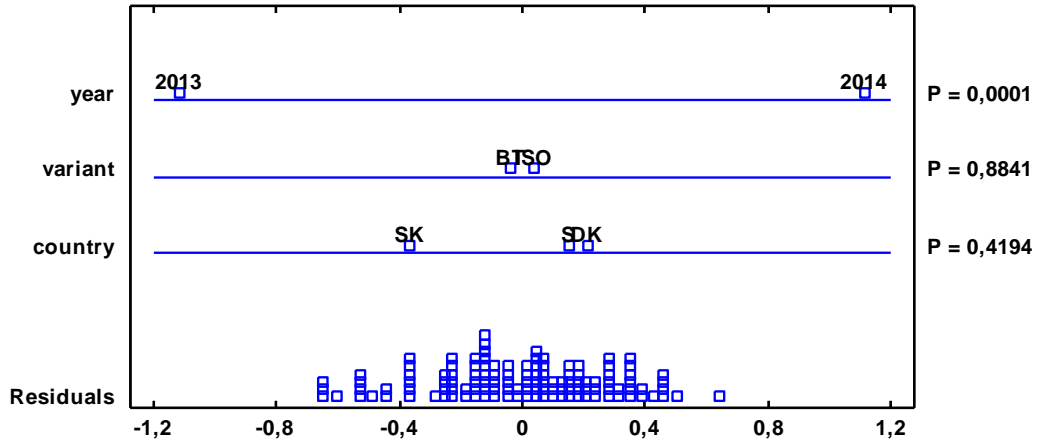
**Fig. 3.** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isolate of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the H'gen (genera diversity) calculated for nematode populations in soil samples (for details please see material and methods). Each variant included 10 repetitions.



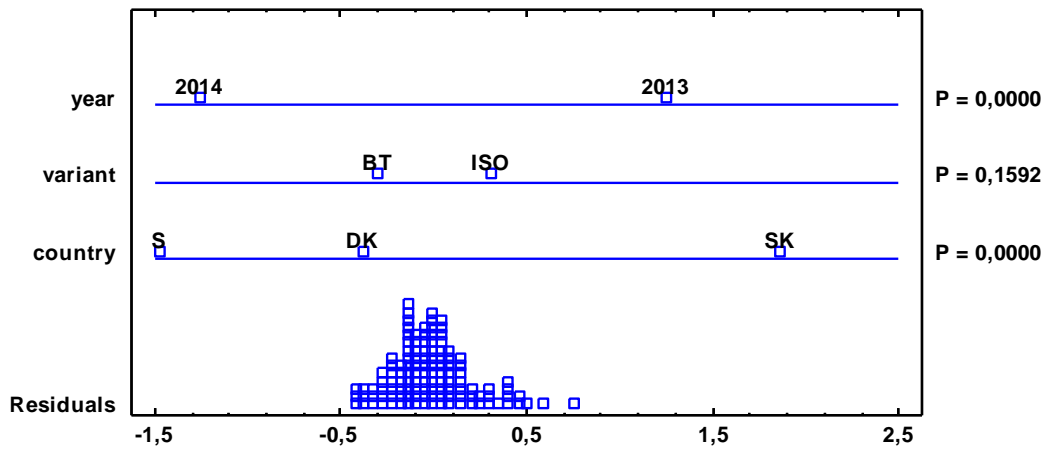
**Fig. 4** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the Structure Index as calculated for nematode populations in soil samples (for details please see material and methods). Each variant included 10 repetitions.



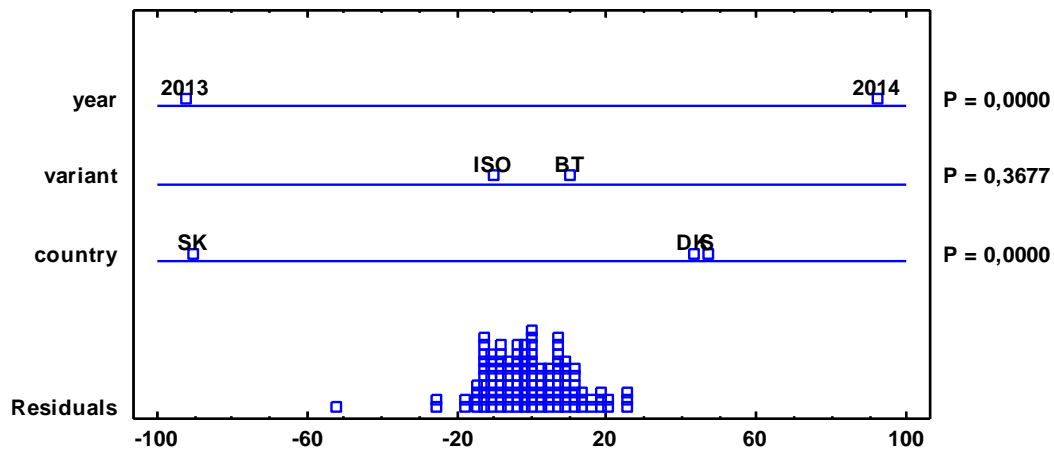
**Fig. 5.** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the Plant Parasite Index calculated for nematode populations in soil samples (for details please see material and methods). Each variant included 10 repetitions.



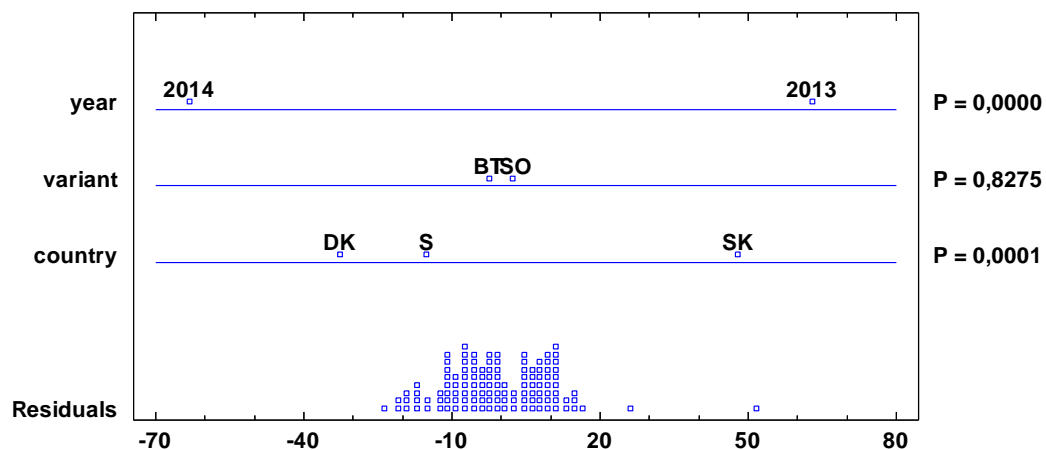
**Fig. 6.** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the Maturity Index calculated for nematode populations in soil samples (for details please see material and methods). Each variant included 10 repetitions.



**Fig. 7.** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the Enrichment Index calculated for nematode populations in soil samples (for details please see material and methods). Each variant included 10 repetitions.

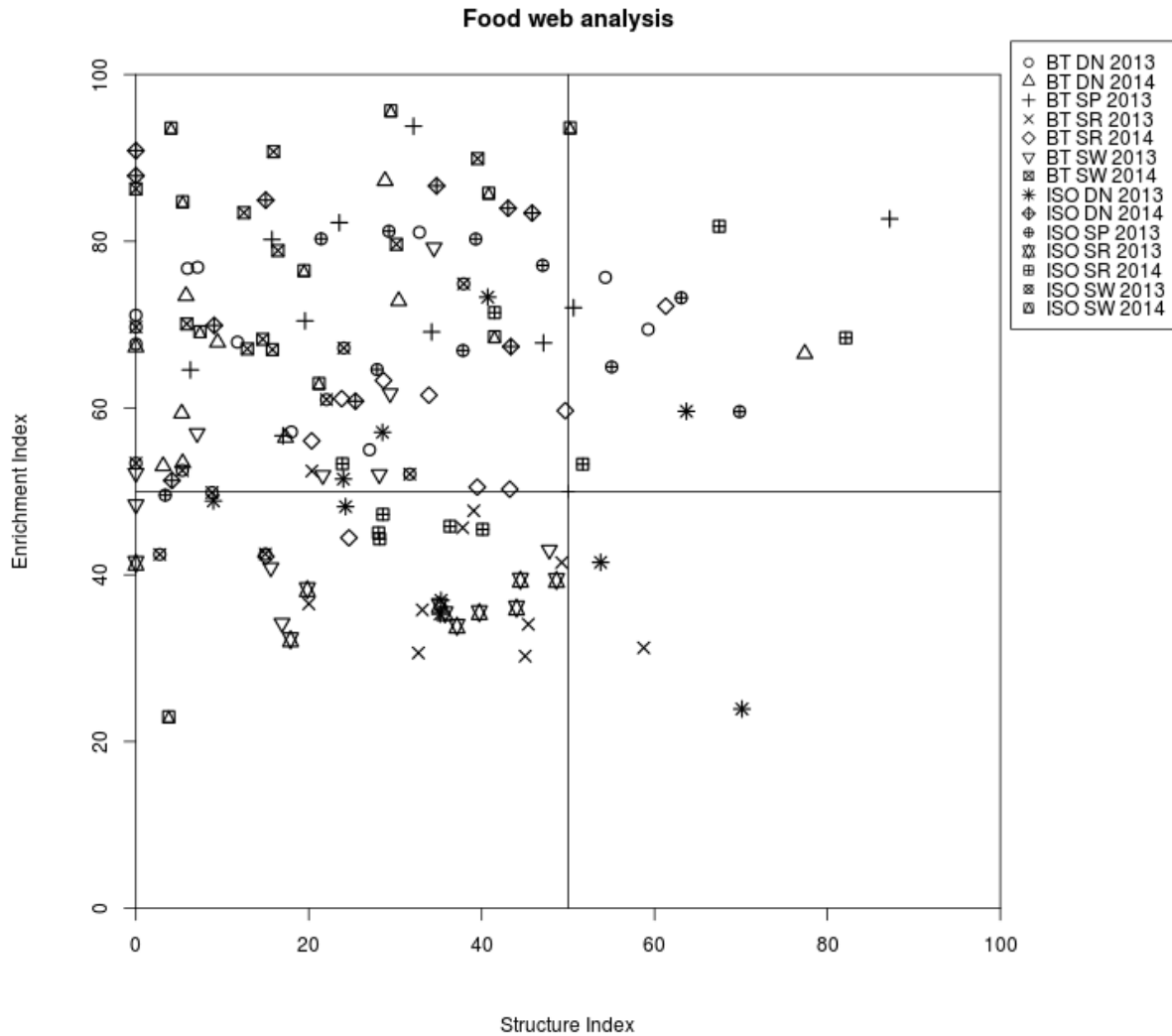


**Fig. 8.** Influence of locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) to the Basal Index as calculated for nematode populations in soil samples (for details please see material and methods). Each variant included 10 repetitions.

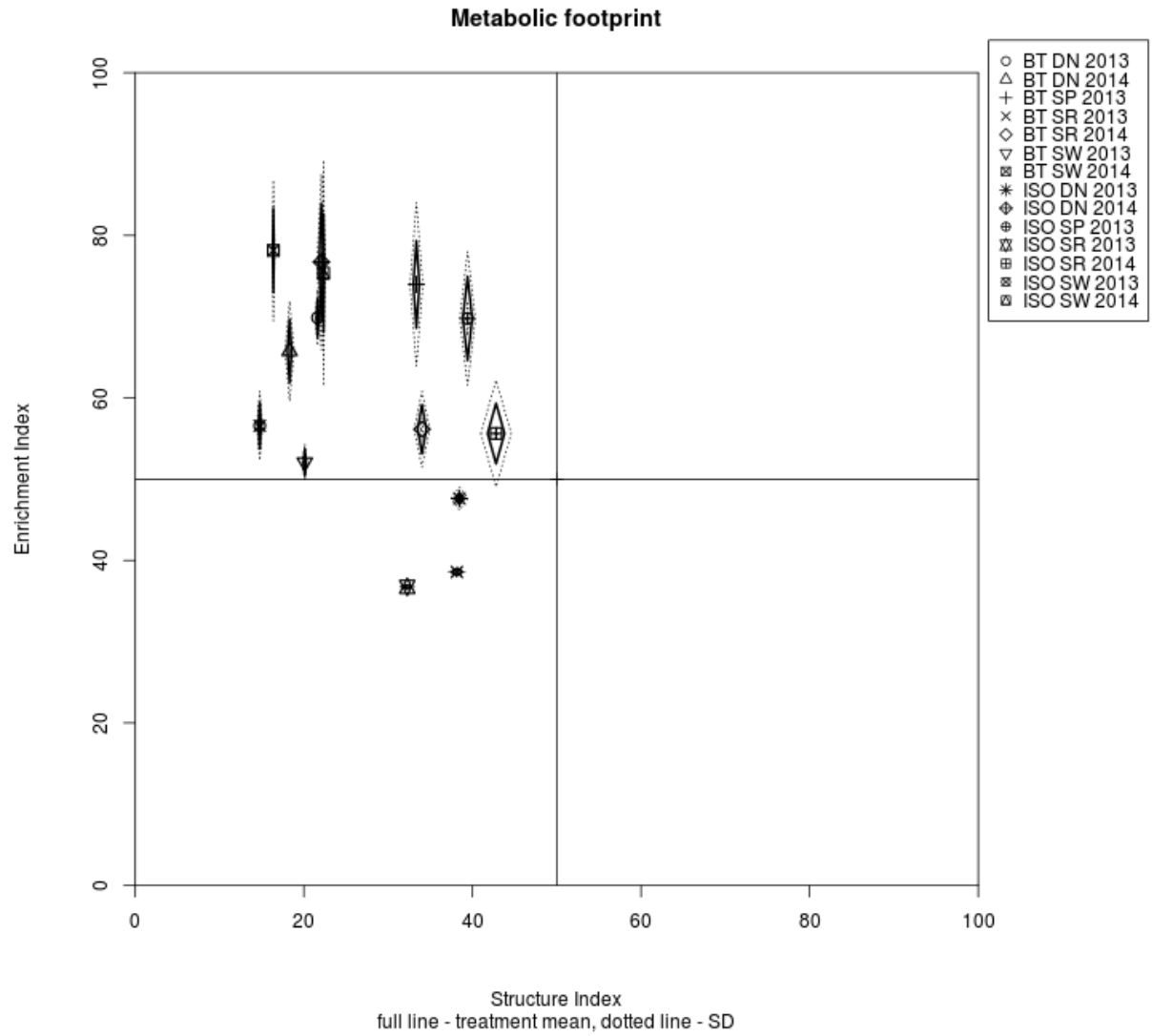


The Enrichment Index and the Structure Index, which correlates with the degree of maturity of ecosystems, delineated most samples from all plots to within quadrat “A” without a clear difference identified between hybrid, locality or year (Fig. 9). Quadrat A characterises an environment that is highly disturbed, N-enriched, with bacterial decomposition channels and with low C:N ratio; all of which is typical for managed agricultural soil within which the plots were located. This finding corresponded with the values from the analyses of the metabolic footprints (Fig. 10) for all nematode functional guilds (herbivore, fungivore, bacterivore, predators and omnivore footprints) and the enrichment, structure and composite footprints, where no statistical differences between the *Bt* and ISO-line hybrid were recorded.

**Fig. 9.** Relationship between Enrichment and Structure Indexes calculated as per location (site) (SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of *Bt* maize hybrid; BT – *Bt* maize hybrid) and year (2013, 2014) (n = 10).



**Fig. 10.** Metabolic footprint calculated separately for the locality (location (site): SK – Slovakia; DK – Denmark, S – Sweden), variant (ISO – isoline of Bt maize hybrid; BT – Bt maize hybrid) and year (2013, 2014) (n = 10).





## Conclusions

The overarching goal of Task 4.3 was to establish baseline data for soil nematodes at field sites selected for environmental risk assessment of GM crops. Using two crops, potato and maize, several geographical locations were analysed. Overall, the nematode analyses demonstrated differences of nematode diversity between sites, but no significant differences were detected between the cultivation of GM cisgenic Desiree potato line and the non-GM potato cultivar Desiree or between the GM and non-GM maize varieties tested.

For potato, the ecological indices revealed the rhizospheric nematode community did not register any significant affect with the cultivation of the cisgenic Desiree line compared to its comparator, cv. Desiree in the presence or absence of fungicide management. Neither was there a significant difference between crop cultivars (cv. Desiree and cv. Sarpo Mira) or crop management. A qualitative analysis of the maturity indices did indicate differences between the potato genetic background and their interaction with the disease management strategies (the no spray control v. weekly chemical applications). For example, while for the MI, which encapsulates all free living nematodes, the three genotypes showed a similar tendency, when the members with a c-p=1 are removed (MIMO) or included the PPI and the PPI/MI ratio, both Desiree and cisgenic Desiree reported comparable tendencies in contrast to Sarpo Mira, which has a different genetic background to that of cv. Desiree. This would indicate that both the Desiree genotype and the cisgenic Desiree genotype studied here interact with and regulate their respective rhizobiomes (likely via root exudates) in the same manner and the variability being recorded is inter- as opposed to intra-cultivar specific. In the context of the prevalence of trophic groups that describe bacterial feeding (BF), plant feeding (PF), fungal feeding (FF), omnivorous (OM), predacious (PR), fungal feeding or entomopathogens (FF or EN), bacterial feeding or entomopathogens (BF or EN) nematodes, no statistical difference was identified in quantitative values between the cisgenic Desiree and either its genetic comparator, cv. Desiree or the alternative genotype Sarpo Mira, plus/minus chemical treatment. Based on the analysis completed in this study, the presence/absence of PR was more strongly influenced by the application of chemical fungicides in the crop management regimes and the weather patterns than by the potato genotype. For example, the absence of PR and FF and the increase of BF and PF in all treatments (chemical and control) in 2013 in comparison to 2014 and 2015 could be associated more with limited resources (stress conditions) due to the scarcity of precipitation and the high air and soil temperatures, which occurred through July 2013 and would have biased those nematodes less sensitive to environmental disturbance. In contrast, the weather conditions of 2014 and 2015 were more supportive of an enrichment condition, which can be linked with the reduction of PF (and increase of BF and OM).

Comparing the disease management regimes independently of potato genotype showed a significant difference existed for the EI and BF2 indices in regard to presence/absence of fungicide applications. As shown previously with the trophic groups, fungicide applications also altered the structure of the soil food web. In this study, the weekly chemical fungicide treatments generated an enrichment condition given per an increment of BF1 and reduction in CH. In contrast, the corresponding control treatment highlighted a more basal condition which included recovery from a moderate disturbance, through tillage and fertilizer operations as part of the standard management of the site.

For maize, the goal of this study was to determine the effects of *Bt* maize hybrid cultivation on soil nematode communities in field trials in Slovakia, Denmark, Sweden and Spain. The dominant nematode genera identified were *Acrobelloides*, *Cephalobus*, *Eucephalobus*, *Chiloplacus*, *Rhabditis*, *Aphelenchoides*, *Aphelenchus*, *Eudorylaimus* and *Filenchus*. These genera belong to different trophic groups and the different trophic groups reacted to the influence of location, variety location (site) and year in a similar way. For example, in the group of bacterial feeders (*Acrobelloides*, *Cephalobus*, *Eucephalobus*, *Chiloplacus*, *Rhabditis*) only the populations of the genus *Acrobelloides* did not react to the influence of site location (site) or year. The analysis of nematode communities from the maize sites indicated that the occurrence of nematodes, their abundance, proportion of feeding types and selected ecological indices did not depend on the type of maize hybrid (GM or non-GM). Thus, the cultivation of genetically modified maize did not directly influence nematode populations. Surprisingly, location was usually not found to induce a significant effect on the abundance of nematodes in soil. Calculation and analysis of the maturity index, plant parasitic index, enrichment index, structure index and basal index did not confirm any clear influence of maize hybrid type on soil nematode communities, irrespective of the location or year sampled. Influence of location (site) or year to these indexes needs deeper analysis, but it was not the aim of this study. The Enrichment Index and the Structure Index, which correlates with the degree of maturity of ecosystems, indicated most samples from all plots, without a clear difference identified between hybrid, locality or year, were positioned within quadrat A, indicating environments that were highly disturbed, N-enriched, with bacterial decomposition channels and with low C:N ratio; all of which is typical for managed agricultural soil within which the plots were located. These findings corresponded with the values from the analyses of the metabolic footprints for all nematode functional guilds (herbivore, fungivore, bacterivore, predators and omnivore footprints) and the enrichment, structure and composite footprints, where no statistical differences between the *Bt* and ISO-line hybrid were

recorded. Thus it can be concluded based on the analysis completed in this study that *Bt* maize had no significant effect on the nematode populations surveyed.

## References

- Al-Deeb MA, Wilde GE, Blair JM, Todd TC, 2003. Effect of Bt corn for corn rootworm control on nontarget soil microarthropods and nematodes. *Environ. Entomol.* **32**, 859 - 65.
- Arpaia S, A. Messéan, N.A. Birch, *et al.*, 2014. Assessing and monitoring impacts of genetically modified plants on agro-ecosystems: the approach of AMIGA project. *Entomologia* **2**, 79-86.
- Bakyoni G, Nag P, Kovacs-Lang E, *et al.*, 2007. Soil nematode community structure as affected by temperature and moisture in a temperate semiarid shrubland. *Appl. Soil Ecol* **37**, 31 - 40.
- Bongers T, 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* **83**, 14 - 9.
- Briar SS, Barker C, Tenuta M, Entz MH, 2012. Soil Nematode Responses to Crop Management and Conversion to Native Grasses. *J. Nematology* **44**, 245-54.
- Dupont ST, Ferris H, Van Horn M, 2009. Effects of cover crop quality and quantity on nematode-based soil food webs and nutrient cycling. *Appl. Soil Ecol.* **41** 157 - 67.
- Foster SJ, Park T-H, Pel M, *et al.*, 2009. Rpi-vnt1.1, a Tm-22 Homolog from *Solanum venturii*, Confers Resistance to Potato Late Blight. *Mol. Pl. Micr. Inter.* **22**, 589-600.
- Griffiths BS, Caul S, Thompson J, *et al.*, 2005. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* CryIAb toxin. *Pl. Soil*, **275**, 135 - 46.
- Höss S, Menza R, Gessle F, Nguyen HT, Jehle JA, Traunspurger W, 2013. Effects of insecticidal crystal proteins (Cry proteins) produced by genetically modified maize (Bt maize) on the nematode *Caenorhabditis elegans*. *Environ. Pollut.* **178**, 147 - 51.
- Höss S, Nguyen HT, Menzel. R., *et al.*, 2011. Assessing the risk posed to free-living soil nematodes by a genetically modified maize expressing the insecticidal Cry3Bb1 protein. *Sci. Total. Environ.* **409**, 2674 - 84.
- Neher DA, 1999. Soil community composition and ecosystem process. *Agroforestry Sys.* **45**, 185-99.
- Neher DA, Muthumbi AWN, Dively GP, 2014. Impact of coleopteran-active Bt corn on non-target nematode communities in soil and decomposing corn roots. *Soil Biol. and Biochem.* **76**, 127-35.
- Pan K, Gong P, Wang J, *et al.*, 2015. Application of nitrate and ammonium fertilizers alter soil nematode food webs in a continuous cropping system in South-western Sichuan China. *Eurasian J. Soil Sci.e* **4**, 287-300.
- Pel MA, Foster SJ, Park T-H, *et al.*, 2009. Mapping and Cloning of Late Blight Resistance Genes from *Solanum venturii* Using an Interspecific Candidate Gene Approach. *Mol. Pl. Micr. Inter.* **22**, 601-15.
- Porazinska EA, 1999. Nematodes communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. *App. Soil Ecol.* **13**, 69-86.
- Vandegheuchte M, Sylvain Za, Reichmann Lg, *et al.*, 2015. Responses of a desert nematode community to changes in water availability. *Ecosphere* **6**, 1-15.
- Vervoort MTW, Vonk JA, Mooijman PJW, *et al.*, 2012. SSU Ribosomal DNA-Based Monitoring of Nematode Assemblages Reveals Distinct Seasonal Fluctuations within Evolutionary Heterogeneous Feeding Guilds. *PLOS One* **7**, e47555.

- Wasilewska L, 1997. Soil invertebrates as bioindicators, with special reference to soil-inhabiting nematodes. *Rus. J. Nematol.* **5**, 113-26.
- Wei JZ, Hale K, Carta L, *et al.*, 2003. *Bacillus thuringiensis* crystal proteins that target nematodes. . *Proc. Natl. Acad. Sci* **100**, 2760 - 5.
- Yeates Gw, Bongers T, De Goede Rgm, Dw F, Ss G, 1993. Feeding habitats in soil families and genera- An outline for soil ecologist. *J. Nematol.* **25**, 315-31.
- Yeates GW, Bongers T, De Goede RGM, Freckman DW, Georgieva SS, 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. *J. Nematol.* **25**, 315 - 31.
- Zhao J, Neher Da, Fu S, Li Z, K W, 2013. Non-target effect of herbicides on nematode soil assemblages. *Pest Man. Sci.* **69**, 679-84.
- Zhao J, Neher D, 2013. Soil nematode genera that predict specific types of disturbance. *App. Soil Ecol.* **64**, 135-41.

## Appendix of supporting tables and figures

**Appendix Table 1.** Listing of nematode families and genus isolated from under different potatoes genotypes (Desiree, cisgenic Desiree, Sarpo Mira) treated with different crop management regimes (control, chemical treatment) across the three years of the study. Sorenson coefficient values to identify similarity across treatments within potato genotypes are entered at the end of respective columns.

Desiree Control	Desiree Chemical	Cisgenic Des. Control	Cisgenic Des. Chemical	Sarpomira Control	Sarpomira Chemical
<b>Family</b>					
<i>Aphelenchidae</i>	<i>Aphelenchoididae</i>	<i>Anquiniidae</i>	<i>Anguinidae</i>	<i>Aphelenchoididae</i>	<i>Cephalobidae</i>
<i>Aporcelaimidae</i>	<i>Aporcelaimidae</i>	<i>Aphelenchidae</i>	<i>Aphelenchoididae</i>	<i>Aporcelaimidae</i>	<i>Aporcelaimidae</i>
<i>Cephalobidae</i>	<i>Cephalobidae</i>	<i>Aphelenchoididae</i>	<i>Aporcelaimidae</i>	<i>Cephalobidae</i>	<i>Dorylaimidae</i>
<i>Dorylaimidae</i>	<i>Dorylaimidae</i>	<i>Aporcelaimidae</i>	<i>Cephalobidae</i>	<i>Diphtherophoridae</i>	<i>Merliniidae</i>
<i>Heteroderidae</i>	<i>Merliniinae</i>	<i>Cephalobidae</i>	<i>Diphtherophoridae</i>	<i>Diploscapteridae</i>	<i>Mononchidae</i>
<i>Hoplolaimidae</i>	<i>Neodiplogasteridae</i>	<i>Diphtherophoridae</i>	<i>Diploscapteridae</i>	<i>Dorylaimidae</i>	<i>Mydonomidae</i>
<i>Merliniidae</i>	<i>Neotylenchidae</i>	<i>Dorylaimidae</i>	<i>Dorylaimidae</i>	<i>Hoplolaimidae</i>	<i>Neodiplogasteridae</i>
<i>Mononchidae</i>	<i>Panagrolaimidae</i>	<i>Merliniidae</i>	<i>Longidoridae</i>	<i>Merliniinae</i>	<i>Panagrolaimidae</i>
<i>Mydonomidae</i>	<i>Plectidae</i>	<i>Mononchidae</i>	<i>Merliniidae</i>	<i>Mydonomidae</i>	<i>Plectidae</i>
<i>Panagrolaimidae</i>	<i>Qudsianematidae</i>	<i>Mydonomidae</i>	<i>Mydonomidae</i>	<i>Neotylenchidae</i>	<i>Quadsianematidae</i>
<i>Plectidae</i>	<i>Rhabditidae</i>	<i>Panagrolaimidae</i>	<i>Neotylenchidae</i>	<i>Nordiidae</i>	<i>Rhabditidae</i>
<i>Qudsianematidae</i>	<i>Steinernematidae</i>	<i>Rhabditidae</i>	<i>Panagrolaimidae</i>	<i>Panagrolaimidae</i>	<i>Telotylenchidae</i>
<i>Rhabditidae</i>	<i>Telotylenchidae</i>	<i>Steinernematidae</i>	<i>Pratylenchidae</i>	<i>Plectidae</i>	<i>Trichodoridae</i>
<i>Trichodoridae</i>	<i>Trichodoridae</i>	<i>Telotylenchidae</i>	<i>Rhabditidae</i>	<i>Pratylenchidae</i>	<i>Tylenchidae</i>
<i>Tripylidae</i>	<i>Tylenchulidae</i>	<i>Tylenchidae</i>	<i>Telotylenchidae</i>	<i>Qudsianematidae</i>	
<i>Tylenchidae</i>		<i>Tylenchulidae</i>	<i>Tylenchidae</i>	<i>Rhabditidae</i>	
<i>Tylenchulidae</i>			<i>Tylenchulidae</i>	<i>Shaerulariidae</i>	
				<i>Steinernematidae</i>	
				<i>Telotylenchidae</i>	
				<i>Trichodoridae</i>	
				<i>Tylenchidae</i>	
				<i>Tylenchulidae</i>	
17	15	16	17	22	14
	CC(0.63)*		CC(0.79)*		CC(0.67)*
<b>Genus</b>					
<i>Acrobelooides</i>	<i>Acrobelooides</i>	<i>Acrobelooides</i>	<i>Acrobelooides</i>	<i>Acrobelooides</i>	<i>Acrobelooides</i>
<i>Aglenchus</i>	<i>Allodorylaimus</i>	<i>Aglenchus</i>	<i>Aglenchus</i>	<i>Aglenchus</i>	<i>Aglenchus</i>
<i>Allodorylaimus</i>	<i>Amplimerlinius</i>	<i>Aphelenchoides</i>	<i>Amplimerlinius</i>	<i>Amplimerlinius</i>	<i>Allodorylaimus</i>
<i>Aphelenchus</i>	<i>Anaplectus</i>	<i>Aphelenchus</i>	<i>Aphelenchoides</i>	<i>Anaplectus</i>	<i>Amblydorylaimus</i>
<i>Aporcelaimellus</i>	<i>Aphelenchoides</i>	<i>Aporcelaimellus</i>	<i>Aporcelaimellus</i>	<i>Aphelenchoides</i>	<i>Amplimerlinius</i>

<i>Calcaridorylaimus</i>	<i>Aporcelaimellus</i>	<i>Bitylenchus</i>	<i>Bitylenchus</i>	<i>Aporcelaimellus</i>	<i>Anaplectus</i>
<i>Cervidellus</i>	<i>Bitylenchus</i>	<i>Calcaridorylaimus</i>	<i>Calcaridorylaimus</i>	<i>Calcaridorylaimus</i>	<i>Aporcelaimellus</i>
<i>Chiloplacus</i>	<i>Calcaridorylaimus</i>	<i>Cephalobus</i>	<i>Cervidellus</i>	<i>Cephalenchus</i>	<i>Bitylenchus</i>
<i>Clarkus</i>	<i>Cephaloboides</i>	<i>Cervidellus</i>	<i>Chiloplacus</i>	<i>Cervidellus</i>	<i>Calcaridorylaimus</i>
<i>Dorylaimoides</i>	<i>Cervidellus</i>	<i>Chiloplacus</i>	<i>Diphtherophora</i>	<i>Chiloplacus</i>	<i>Cervidellus</i>
<i>Ecumenicus</i>	<i>Chiloplacus</i>	<i>Clarkus</i>	<i>Diploscapter</i>	<i>Deladenus</i>	<i>Chiloplacus</i>
<i>Eucephalobus</i>	<i>Ecumenicus</i>	<i>Coslenchus</i>	<i>Ditylenchus</i>	<i>Diphtherophora</i>	<i>Clarkus</i>
<i>Helicotylenchus</i>	<i>Eucephalobus</i>	<i>Diphtherophora</i>	<i>Dorylaimoides</i>	<i>Diploscapter</i>	<i>Dorylaimoides</i>
<i>Heterocephalobus</i>	<i>Heterocephalobus</i>	<i>Ditylenchus</i>	<i>Eucephalobus</i>	<i>Dorylaimoides</i>	<i>Eucephalobus</i>
<i>Heterodera</i>	<i>Mesodorylaimus</i>	<i>Dorylaimoides</i>	<i>Heterocephalobus</i>	<i>Ecumenicus</i>	<i>Heterocephalobus</i>
<i>Mesorhabditis</i>	<i>Mesorhabditis</i>	<i>Eucephalobus</i>	<i>Irantylenchus</i>	<i>Eucephalobus</i>	<i>Merlinius</i>
<i>Neopsilenchus</i>	<i>Panagrolaimus</i>	<i>Filenchus</i>	<i>Longidorus</i>	<i>Geocenamus</i>	<i>Miculenchus</i>
<i>Opisthodorylaimus</i>	<i>Paratylenchus</i>	<i>Heterocephalobus</i>	<i>Merlinius</i>	<i>Helicotylenchus</i>	<i>Panagrolaimus</i>
<i>Panagrolaimus</i>	<i>Pristionchus</i>	<i>Mesorhabditis</i>	<i>Mesodorylaimus</i>	<i>Heterocephalobus</i>	<i>Plectidae</i>
<i>Plectus</i>	<i>Rhabditis</i>	<i>Panagrolaimus</i>	<i>Panagrolaimus</i>	<i>Miculenchus</i>	<i>Pristionchus</i>
<i>Pratylenchoides</i>	<i>Rubzovinema</i>	<i>Paratylenchus</i>	<i>Pelodera</i>	<i>Panagrolaimus</i>	<i>Rhabditis</i>
<i>Pseudacrobeles</i>	<i>Sectonema</i>	<i>Pratylenchoides</i>	<i>Pratylenchus</i>	<i>Paractinolaimus</i>	<i>Sectonema</i>
<i>Rhabditis</i>	<i>Steinernema</i>	<i>Rhabditis</i>	<i>Rhabditis</i>	<i>Paratylenchus</i>	<i>Trichodorus</i>
<i>Thonus sp.</i>	<i>Trichodorus</i>	<i>Sectonema</i>	<i>Rubzovinema</i>	<i>Prodorylaimus</i>	<i>Tylencholaimus</i>
<i>Trichodorus</i>	<i>Tylenchorhynchus</i>	<i>Steinernema</i>	<i>Sectonema</i>	<i>Pungentus</i>	<i>Tylenchorhynchus</i>
<i>Tripyla</i>		<i>Tylenchorhynchus</i>	<i>Tylenchorhynchus</i>	<i>Rhabditis</i>	<i>Tylenchus</i>
<i>Tylenchulus</i>			<i>Tylenchulus</i>	<i>Rubzovinema</i>	
				<i>Steinernema</i>	
				<i>Trichodorus</i>	
				<i>Tylenchorhynchus</i>	
				<i>Tylenchus</i>	
27	25	26	27	31	26
	CC(0.50)*		CC(0.63)*		CC(0.64)*

1 **Appendix Table 2.** Sorensen coefficients calculated on comparisons within<sup>1</sup> potato genotypes (control v. chemical) and between<sup>2</sup> potato genotypes for each  
 2 individual year (2013, 2014 and 2015) for nematode families and genus identified from study completed at Oak Park (Carlow, Ireland).

3

	Comparison <sup>1</sup>	Year			Comparison <sup>2</sup>	Year			Comparison	Year		
		2015	2014	2013		2015	2014	2013		2015	2014	2013
<b>Family</b>	Desiree control v. chemical	0.63	0.50	0.53	Desiree v. Cisgenic Desiree	0.48	0.37	0.53	Control v. chemical	0.36	0.54	0.41
	Cisgenic Desiree control v. chemical	0.43	0.73	0.43	Desiree v. SarpoMira	0.49	0.47	0.62				
	Sarpo Mira control v. chemical	0.55	0.57	0.55	Cisgenic Desiree v. Sarpo Mira	0.50	0.36	0.34				
<b>Genus</b>	Desiree control v. chemical	0.46	0.58	0.18	Desiree v. Cisgenic Desiree	0.48	0.36	0.69	Control v. chemical	0.32	0.48	0.31
	Cisgenic Desiree control v. chemical	0.40	0.58	0.38	Desiree v. SarpoMira	0.47	0.40	0.74				
	Sarpo Mira control v. chemical	0.28	0.52	0.64	Cisgenic Desiree v. Sarpo Mira	0.49	0.41	0.64				

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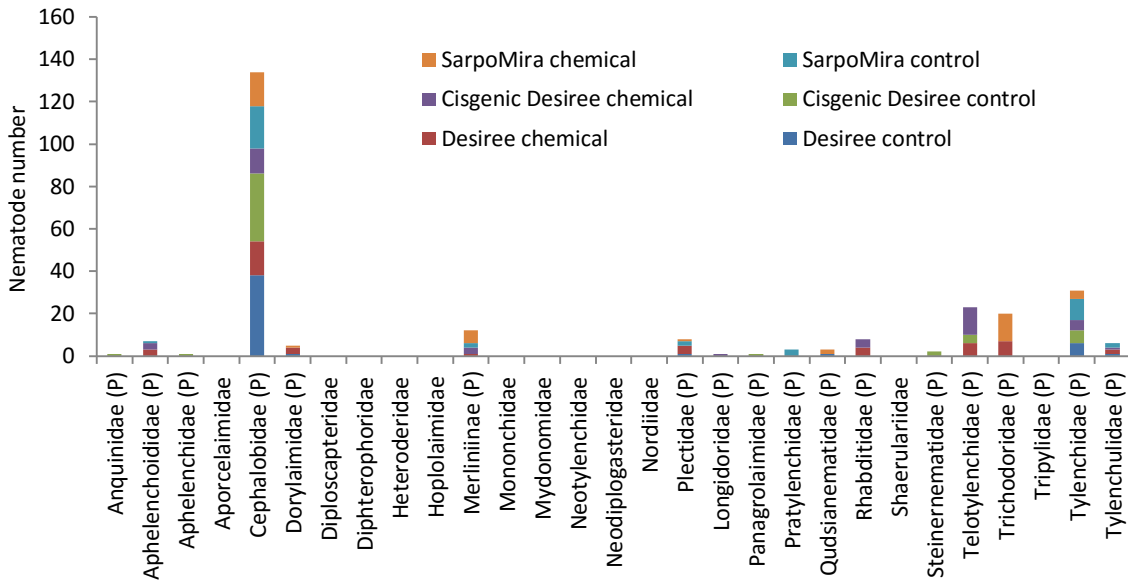
12



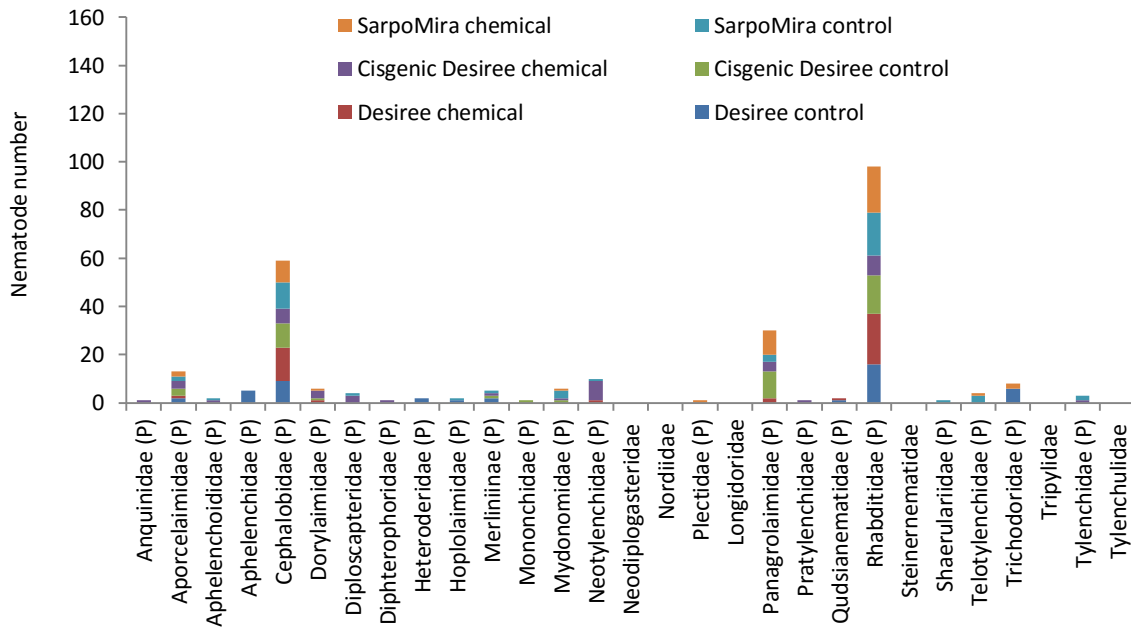


1 **Appendix Figure 1.** Distribution of nematode families relative to each potato genotype x  
 2 management combination for (A) 2013, (B) 2014 and (C) 2015.

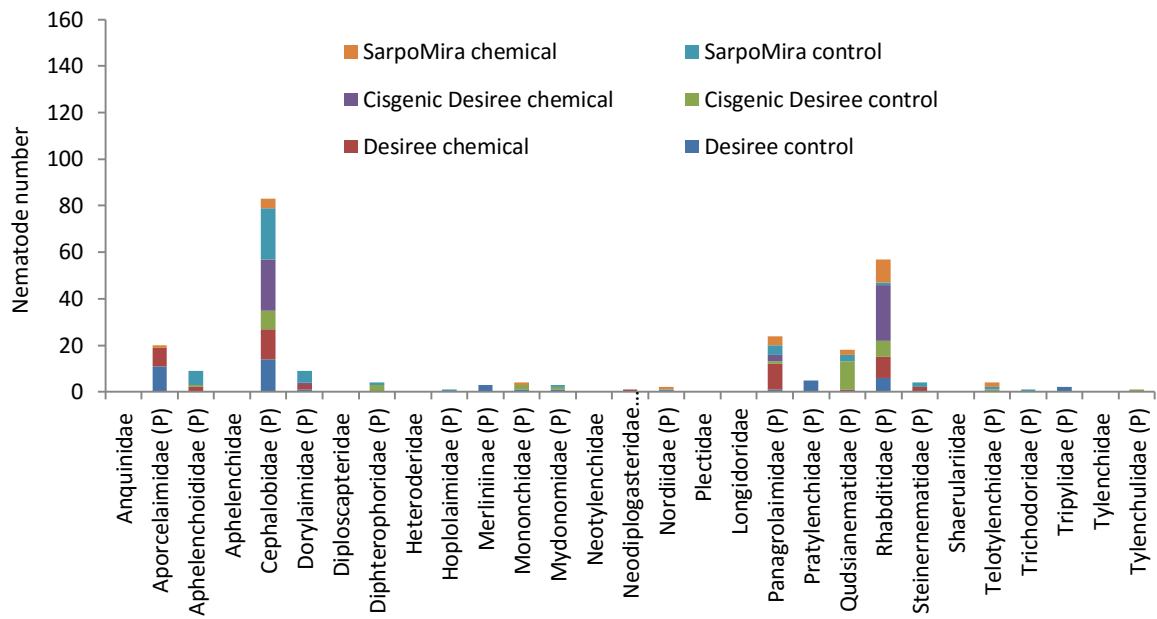
3 (A).



4 (B).



6 (C).



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1 **Appendix Table 3:** Basic data to evaluate the influence of site (location)location (site) (Ctry): SK – Slovakia; DK – Denmark, S – Sweden; variant, ISO – isoline  
 2 and BT – *Bt* maize hybrid and year (2013, 2014) on the abundance of nematodes (Sum) in soil samples and the abundance of nematodes from selected  
 3 genera (for details see material and methods). Each variant (Var.) included 10 repetitions.  
 4

Sum	Ctry	Var.	Y	<i>Acrobelloides</i>	<i>Cephalobus</i>	<i>Eucephalobus</i>	<i>Chiloplacus</i>	<i>Rhabditis</i>	<i>Aphelenchoides</i>	<i>Aphelenchus</i>	<i>Eudorylaimus</i>	<i>Filenchus</i>
129	SK	ISO	2013	21	4	5	2	0	22	37	6	0
230	SK	ISO	2013	50	35	7	0	0	24	42	34	24
259	SK	ISO	2013	41	44	0	0	0	29	31	18	43
102	SK	ISO	2013	25	0	0	0	0	44	11	0	5
195	SK	ISO	2013	16	0	9	28	0	17	71	21	10
112	SK	ISO	2013	35	0	2	4	0	21	26	9	3
103	SK	ISO	2013	15	0	7	21	0	23	15	7	7
247	SK	ISO	2013	44	3	2	40	0	25	26	28	67
105	SK	ISO	2013	17	0	1	18	0	27	29	4	4
237	SK	ISO	2013	68	0	1	37	0	24	42	6	30
139	SK	BT	2013	17	16	5	12	0	12	35	11	16
102	SK	BT	2013	22	0	0	25	0	8	0	17	28
303	SK	BT	2013	113	0	2	6	0	12	136	10	16
101	SK	BT	2013	9	0	0	2	5	9	5	4	62
227	SK	BT	2013	92	0	0	20	7	14	21	37	29
130	SK	BT	2013	20	2	1	11	1	9	4	25	56

152	SK	BT	2013	23	0	2	11	4	10	70	14	9
265	SK	BT	2013	8	0	2	4	0	84	99	30	2
148	SK	BT	2013	29	0	11	14	0	0	27	29	18
260	SK	BT	2013	23	8	7	76	0	12	28	25	51
190	SK	ISO	2014	15	60	25	0	19	6	16	10	29
206	SK	ISO	2014	40	39	30	7	23	6	6	6	26
437	SK	ISO	2014	88	60	53	0	63	30	36	12	75
353	SK	ISO	2014	110	55	33	0	45	5	11	5	53
176	SK	ISO	2014	28	61	11	0	18	6	17	5	18
268	SK	ISO	2014	55	18	17	32	37	7	41	19	16
261	SK	ISO	2014	21	0	19	34	76	0	20	0	59
255	SK	ISO	2014	78	6	6	31	23	4	2	6	72
344	SK	ISO	2014	72	25	8	4	140	1	6	7	13
190	SK	ISO	2014	25	6	1	0	30	10	0	2	26
406	SK	BT	2014	125	45	14	0	90	9	35	0	33
124	SK	BT	2014	27	21	4	0	23	2	9	2	11
279	SK	BT	2014	78	44	25	0	22	6	31	8	40
173	SK	BT	2014	33	18	5	5	36		23	5	28
251	SK	BT	2014	36	57	21	0	29	3	15	15	52
245	SK	BT	2014	48	30	22	0	39	38	18	3	33
148	SK	BT	2014	32	8	22	0	12	12	30	5	6

257	SK	BT	2014	30	49	28	7	31	12	45	11	13
188	SK	BT	2014	46	4	1	0	51	10	13	2	20
214	SK	BT	2014	51	15	18	0	47	13	11	0	33
150	DK	ISO	2013	10	13	7	6	0	8	0	25	33
146	DK	ISO	2013	43	0	0	5	0	22	0	23	0
133	DK	ISO	2013	14	8	0	20	0	3	3	12	15
284	DK	ISO	2013	14	2	0	4	0	25	25	22	54
174	DK	ISO	2013	13	0	1	25	0	26	6	0	26
173	DK	ISO	2013	48	0	5	5	2	14	9	4	29
237	DK	ISO	2013	18	0	0	17	13	12	12	3	45
240	DK	ISO	2013	39	0	0	15	27	15	12	0	30
439	DK	ISO	2013	55	0	5	0	15	26	53	0	55
112	DK	ISO	2013	13	0	0	6	4	21	5	0	11
131	DK	BT	2013	4	0	0	2	12	0	2	3	25
129	DK	BT	2013	17	0	0	15	30	30	2	1	11
126	DK	BT	2013	17	0	0	14	44	16	1	0	15
117	DK	BT	2013	13	0	0	11	41	5	5	0	6
185	DK	BT	2013	43	0	0	10	45	3	6	0	33
117	DK	BT	2013	14	0	0	14	10	13	13	0	0
269	DK	BT	2013	15	0	0	9	43	58	19	0	0
168	DK	BT	2013	15	0	0	12	37	10	15	0	0

140	DK	BT	2013	23	0	0	0	11	2	6	0	24
159	DK	BT	2013	7	0	0	3	27	2	12	3	18
292	DK	ISO	2014	13	7	3	1	111	27	16	3	0
371	DK	ISO	2014	40	3	4	1	51	40	12	10	33
287	DK	ISO	2014	80	5	11	0	29	9	25	1	40
275	DK	ISO	2014	15	0	0	0	165	7	6	0	44
408	DK	ISO	2014	124	3	0	0	112	19	55	3	38
249	DK	ISO	2014	20	0	15	7	30	25	34	9	40
205	DK	ISO	2014	24	6	10	1	63	1	4	7	4
382	DK	ISO	2014	18	2	9	2	145	13	28	2	33
208	DK	ISO	2014	12	4	7	0	92	12	11	9	28
297	DK	ISO	2014	43	6	5	0	138	0	11	0	9
413	DK	BT	2014	43	7	6	0	54	17	11	10	75
285	DK	BT	2014	47	4	2	0	31	16	21	2	62
405	DK	BT	2014	90	30	25	0	47	17	40	2	76
182	DK	BT	2014	41	1	3	0	16	7	51	0	17
209	DK	BT	2014	45	0	6	0	46	4	18	0	37
222	DK	BT	2014	27	1	2	0	29	0	3	3	46
286	DK	BT	2014	53	4	5	0	75	14	9	1	37
262	DK	BT	2014	44	3	4	0	30	8	61	0	20
212	DK	BT	2014	35	2	2	0	37	12	44	0	17

269	DK	BT	2014	15	5	5	0	120	6	33	8	10
305	S	ISO	2013	33	8	4	0	36	27	4	0	0
228	S	ISO	2013	20	11	10	10	29	26	12	7	1
284	S	ISO	2013	43	21	12	2	46	21	0	8	2
204	S	ISO	2013	51	10	12	19	27	22	3		17
367	S	ISO	2013	120	21	20	17	33	19	2	9	17
219	S	ISO	2013	64	3	4	11	25	43	7	1	6
345	S	ISO	2013	58	2	7	3	83	55	2	20	2
209	S	ISO	2013	65	9	3	7	12	29	24	0	3
265	S	ISO	2013	70	6	0	14	30	60	5	0	4
282	S	ISO	2013	85	38	10	19	39	44	0	5	6
301	S	BT	2013	11	20	11	15	29	33	6	10	0
299	S	BT	2013	12	7	0	0	32	8	6	5	3
286	S	BT	2013	114	0	0	0	27	39	10	0	12
157	S	BT	2013	29	7	4	7	8	14	1	2	5
145	S	BT	2013	26	0	0	6	12	28	0	0	19
174	S	BT	2013	37	10	0	8	16	16	10	6	4
223	S	BT	2013	68	1	12	19	28	15	1	9	0
181	S	BT	2013	77	12	3	4	15	19	2	1	4
257	S	BT	2013	125	15	0	4	16	15	5	2	8
153	S	BT	2013	48	5	5	1	24	39	0	0	4

322	S	ISO	2014	16	5	9	0	190	14	3	0	0
375	S	ISO	2014	15	3	5	0	266	8	3	0	5
118	S	ISO	2014	16	3	0	0	43	3	0	2	0
138	S	ISO	2014	10	3	8	0	26	14	5	1	6
210	S	ISO	2014	20	3	8	0	92	12	2	1	7
302	S	ISO	2014	19	2	11	0	190	9	2	0	2
109	S	ISO	2014	18	0	8	2	0	10	4	0	3
343	S	ISO	2014	42	4	12	6	61	15	6	1	22
135	S	ISO	2014	11	0	25	2	28	15	0	0	9
233	S	ISO	2014	30	1	23	3	66	10	0	3	9
151	S	BT	2014	40	0	11	1	30	7	1	3	2
153	S	BT	2014	37	2	8	0	55	1	7	2	0
145	S	BT	2014	24	0	13	0	33	15	2	0	6
339	S	BT	2014	24	2	17	2	184	5	1	2	25
136	S	BT	2014	19	0	5	0	80	1	1	0	2
191	S	BT	2014	27	0	0	0	40	24	4	2	12
148	S	BT	2014	18	0	4	0	62	24	0	0	10
150	S	BT	2014	15	6	10	0	27	13	0	0	17
185	S	BT	2014	19	3	11	0	76	11	1	0	5
280	S	BT	2014	39	0	30	1	93	8	0	7	19