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Simple, inexpensive, and rapid approach to detect changes in the structure of soil free-living nematodes

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Summary

A general limitation of ecological investigations based on nematodes is related to the difficult and time-consuming taxonomic identification of species. Therefore, nematologists are investing many efforts to develop alternative approaches as proxies applicable in biomonitoring assessment. Recently, an alternative method that combines morpho-functional traits was proposed for detecting assemblage changes of marine nematodes. In view of the promising results, it was tested the same approach to document taxonomic structure changes of soil free-living and plant parasitic nematodes. Specifically, this attempt was carried out using three data sets that include studies from various European regions and different types of ecosystems: forests, grasslands and maize crops. Multivariate statistical analysis revealed that the simple combination of the four traits (i.e., buccal cavity cuticularization occurrence, amphideal fovea size and shape, morphology of the cuticle and pharynx) in a single code number perfectly mirrors the taxonomic structure trends of the nematode assemblage at genus level. Therefore, we predict that similar results can be also obtained by directly encoding nematode specimens with the selected traits and we point to new important advances if this procedure can be coupled with advanced machine learning.

Keywords: Nematoda; community structure changes; biomonitoring; morpho-functional traits; future perspective in the ecology assessment

Introduction

Soil plays an important role in providing ecosystem services. Even if invisible to the human eye, soil nematodes are ubiquitous actors in most ecosystem services, acting as biocontrol agents in the transformation of organic matter or regulation of pest organism balance. Nematodes are found in all soil types thanks to their great morphological and functional adaptability. These features, combined with their abundance, found to be 3.2 million/m² (Van Den Hoogen, 2019), omnipresence in all the types of ecosystems (Dionisio *et al.*, 2018) and the introduction of ecological and functional indices (Bongers, 1990; Ferris *et al.*, 2001; Ferris

& Bongers, 2009) have led to the intensive use of nematodes as bioindicators of quality and health of soil over the last 30 years (Du Preez *et al.*, 2022). Bioindicators are organisms or communities of organisms that can be used or observed to evaluate an environmental condition or provide information about an ecosystem. Among the bioindicators, the ecological ones are taxa known to be sensitive to pollution, stresses or environmental disturbances and they are representative of the whole community (Gaugler & Bilgrami, 2004). Indeed, the permeable body wall gives free-living nematodes sensitivity to changes in the surrounding soil environment, making them able to detect possible variations and provide rapid responses (Pires *et al.*, 2022). Furthermore, nematodes'

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Table 1. Geographical location, information on sampling stations and methods used in the three study cases: forest, grassland and maize crop systems.

Charactersits of the study area	Forest	Grassland	Maize crop
Reference	Čerevková <i>et al.</i> , 2021	Čerevková, 2006	Čerevková <i>et al.</i> , 2018
Study Area	Slovakia	Slovakia	Denmark, Spain, Slovakia, and Sweden
Sampling locations and coordinates	Mount Poľana (48°37' N 19°30' E)	Hybe (49°02'N, 19°49'E) Veľký Folkmár (48°51'N, 21°10'E) Ľubietová, Strelníky (48°45'N, 19°22'E) Stropkov (49°12'N, 21°38'E) Telgárt (48°51'N, 20°11'E) Vrbovce (48°47'N, 17°28'E)	Denmark - Slagelse (55°19'N, 11°23'E) Spain – Seseña (40°05'N, 3°40'W) Slovakia – Borovce (48°34'N, 17°43'E) Sweden – Lund (55°45'N 13°2'E)
Environmental factors compared	Managed beech forest (BEE) Managed spruce forest (SPR) Unmanaged mixed forest (UNM)	Permanent meadow (PM) New meadow (NM) Permanent pasture (PP)	Bt maize isogenic hybrids
Sampling date	August 2019	October 2003 – 2004	2013/2014 (during the flowering phase of maize)
No. of plots sampled	15	4	10
No. of replicates for plot	5	5	3
Extraction method	Baermann technique	Cobb's flotation-sieving method	Baermann technique
Total genera found	51	65	45
Type of soil	Cambisol	Hybe: Podzol Veľký Folkmár: Cambisol Ľubietová, Strelníky: Cambisol Stropkov Cambisol Telgárt: Cambisol Vrbovce: Luvisol	Denmark (Slagelse)– Cambisol Spain (Seseña) – Luvisol Slovakia (Bórovce) – Chernozem Sweden (Lund) – Cambisol
Relevant environmental parameters information (pH, temperature etc..)	pH: 4.69 – 4.93; C%: 9.10 – 9.71; N%: 0.76 – 0.82; C/N: 11.66 -12.07	pH: 6.95-7-8.2; Cox 3.42-6.16	pH: 5.97 – 7.55; C%. 1.24 – 1.68; N%: 0.13 – 0.17; C/N: 8.68 – 13.11
Type of vegetation	BEE: <i>Fagus sylvatica</i> L. <i>Acer</i> spp.; SPR: <i>Picea abies</i> (L.) H. Karst.); UNM: <i>F. sylvatica</i> , <i>Abies alba</i> Mill., <i>Acer pseudoplatanus</i> L., <i>Fraxinus excelsior</i> L., <i>P. abies</i> and <i>Ulmus glabra</i> H.	NM: <i>Dactylis glomerata</i> L., <i>Festuca rubra</i> L. and <i>Lolium perene</i> L.; PM: indigenous multispecies vegetation irregularly mown; PP: indigenous vegetation regularly grazed	Bt and isogenic (ISO) hybrids DKC3872YG (Bt maize line MON810) and its near-isogenic line DKC3871 DKC6451YG (Bt maize line MON810) and its near-isogenic line DKC6450

generation time and short lifecycle, easy sampling methods and extraction procedures make them ideal test subjects. The study of the taxonomical and functional nematode structures as a bio-monitoring tool proves to be an effective approach to evaluating the ecological conditions, and as a result, numerous indices have been designed for the assessment of the ecosystem health status (e.g. enrichment, structure, basal and channel indices, see Ferris & Bongers, 2009; Du Preez *et al.*, 2022 for review). However, all of them are inferred through a morphological approach and require at least identification at the genus level. Certainly, as observers, we are used to judging from first sight what we are watching. For this reason, the primary identification and

characterization of nematode communities rely on their morphological and anatomical differences (e.g. Platt, 1981). However, the absence of a simple and fast method based on the morphological approach cannot give the possibility to immediately and easily categorize these tiny animals. On the other hand, environmental DNA is often expected to solve this problem, the approach still has some gaps and cannot be routinely used (Cocozza di Montanara *et al.*, 2022). Thus, it is necessary to conceive alternative methods that leverage easily applicable human abilities. According to Violle *et al.* (2007), functional traits are defined as morpho-physio-phenological traits that impact fitness indirectly via their effects on growth, reproduction and survival. Since some

BUCCAL MORPHOLOGY

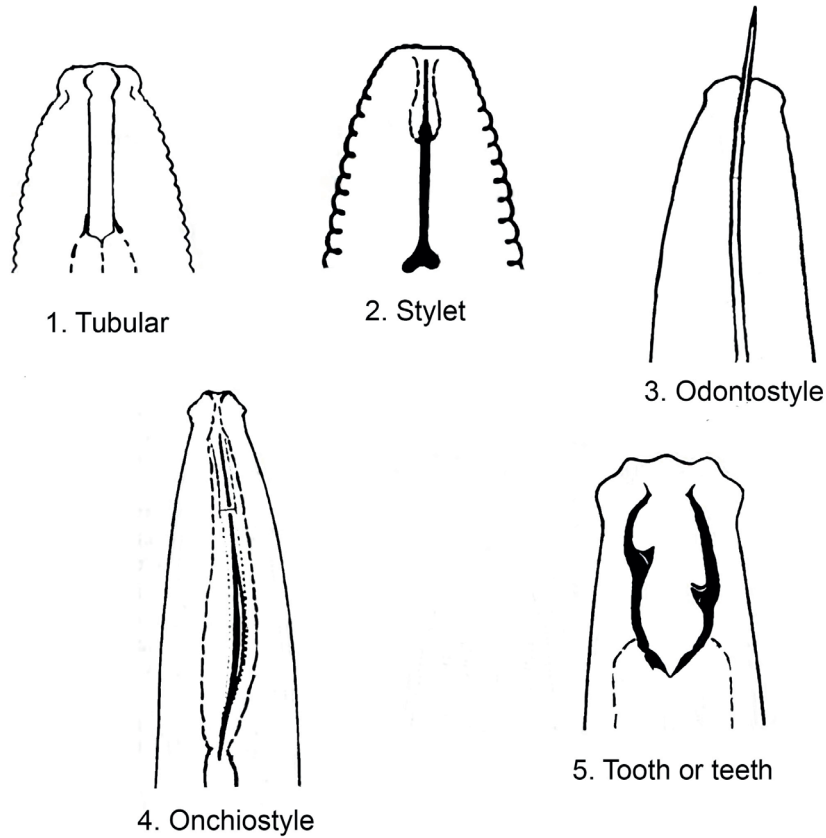


Fig. 1. Occurrence and morphology of possible cuticularizations in the buccal cavity: 1.unarmed buccal cavity; 2. stylet; 3. odontostyle; 4. onchiostyle; 5. tooth or teeth.

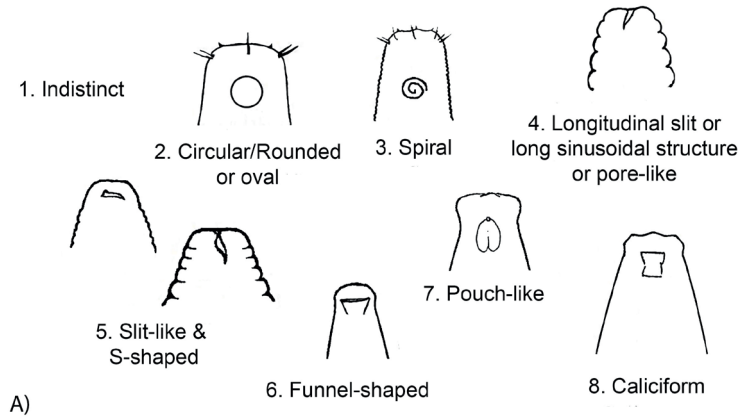
morphological diagnostic characters of nematodes (buccal cavity morphology, amphid, cuticle/external body structure and tails) seem to fit these requirements (Wieser, 1953; Thistle *et al.*, 1995; Thiele *et al.*, 2009; Zullini & Semprucci, 2020). Semprucci *et al.* (2022) explored a cheaper and more user-friendly method based on a combination of morpho-functional traits to assess differences in nematode community structure in Mediterranean marine systems. Particularly, they emphasized the idea that the nematode community structure changes can be revealed by combining a few numbers of morphological diagnostic features in a code as a proxy for taxonomic identification.

Since the simplicity of the method allows us to avoid the time-consuming of activities without renouncing the reliability of results in marine ecosystems (see Semprucci *et al.*, 2022; Justino *et al.*, 2023), we tested the same approach to soil habitats. We considered four morphological characters e.g. buccal morphology, amphideal fovea size and shape, morphology of the cuticle and pharynx. We assume this last trait, as an additional trait since it has a crucial importance in the identification of soil free-living nematodes and its logical relation to feeding habits (Zullini, 2021). On

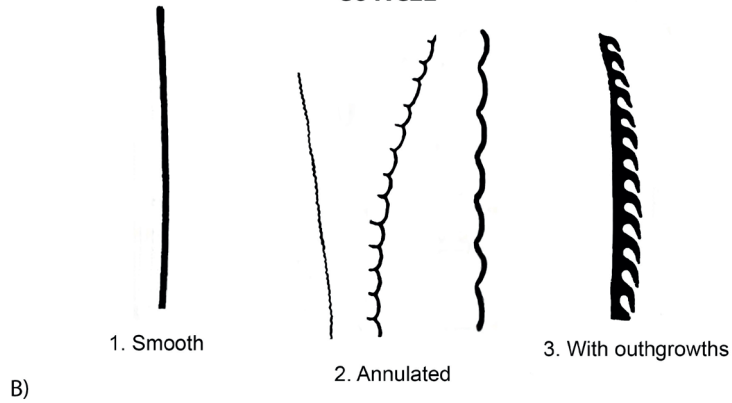
the contrary to the traits selected for the marine nematodes, tail morphology was not considered due to the high variability of this feature, even between males and females of the same soil nematode species.

The aim of the present study is to demonstrate that the combining morpho-functional traits may efficiently mirror the changes in the nematode taxonomic structure, even in the soil system. To deal with this aim, we have considered data coming from different case studies. Since soil conditions affect the boundaries that include plant growth and consequently characteristics of soil biota (Usman & Muhammad, 2016), we decided to examine data from three different soil environments previously studied in three investigations: 1) a natural and temperate forest, 2) a grassland and 3) a field cultivated with maize. Each type of soil ecosystem and its land use can respond differently to potential perturbations, influencing the recovery time of the nematode community structure (Ferris *et al.*, 2001). The diversity of each soil corresponds to a wide differentiation in management practices for maintaining soil quality and soil health. Particularly, food, water and temperature are the three main environmental variables that influence the level of nematode

AMPHIDS



CUTICLE



OESOPHAGEAL TYPES

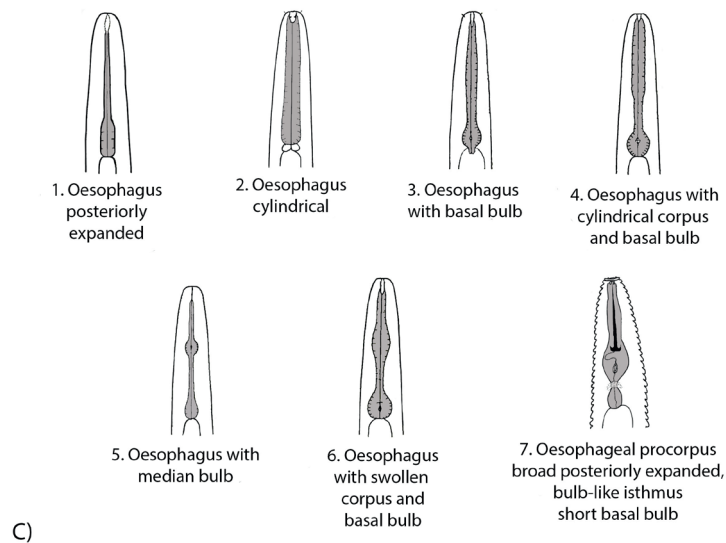


Fig. 2. A) Amphideal fovea: 1. undetected; 2. circular/rounded or oval; 3. spiral; 4. thin, narrow slit or pore-like; 5. large slit-like & S-shape; 6. funnel-shape; 7. pouch-like; 8. caliciform. B) Cuticles: 1. smooth; 2. annulated; 3. with outgrowths; C) Oesophageal shape: 1. oesophagus posteriorly expanded; 2. oesophagus cylindrical; 3. oesophagus with basal bulb; 4. oesophagus with cylindrical corpus and basal bulb; 5. oesophagus with median bulb; 6. oesophagus with swollen corpus and basal bulb; 7. oesophageal procorpus broad posteriorly expanded, bulb-like isthmus short basal bulb.

Example of the assignment of the genus *Aglenchus* into the trait categories

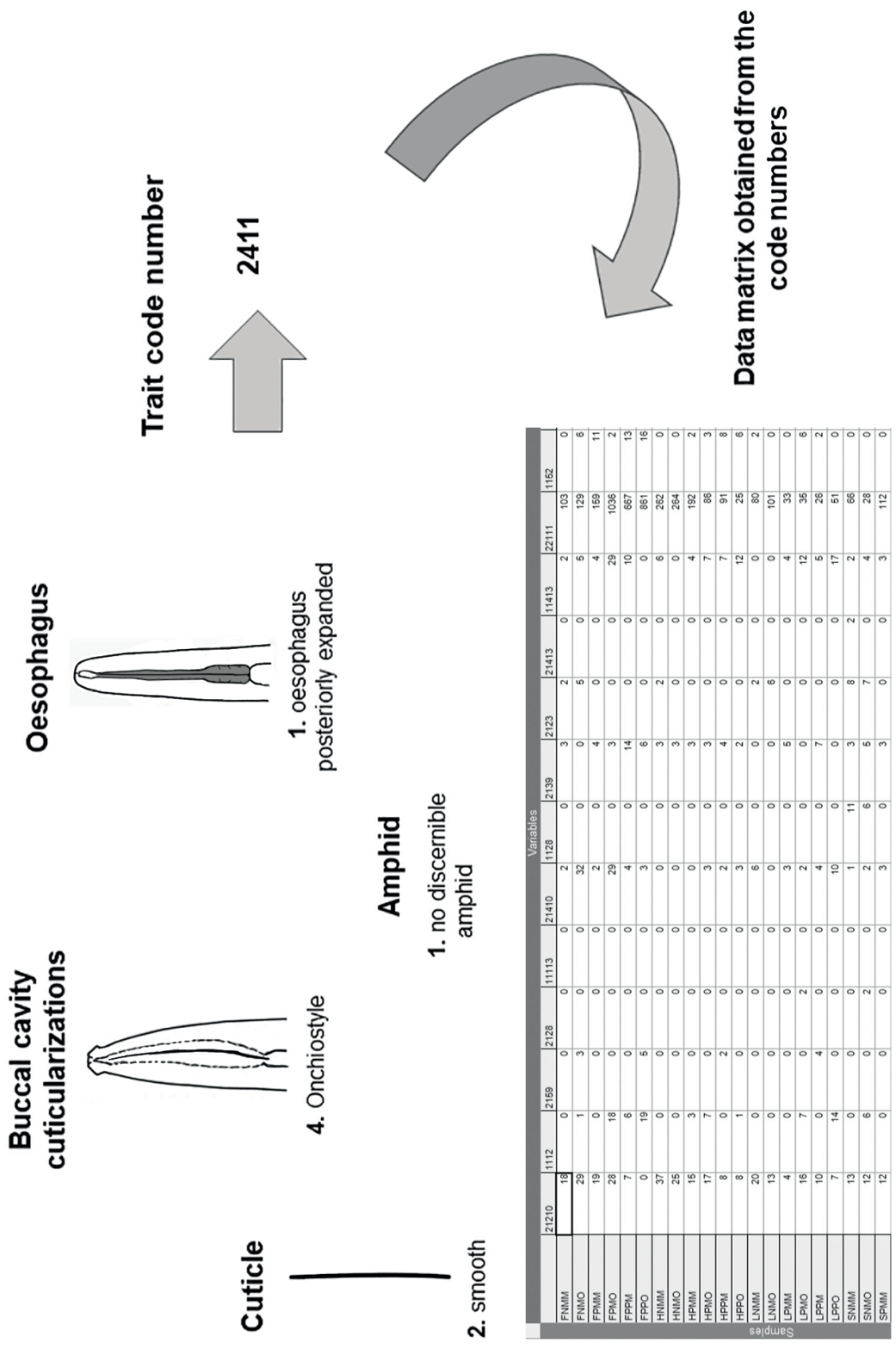


Fig. 3. Assignment of each genus in the four morpho-functional traits and creation of the data matrix based on their combinations.

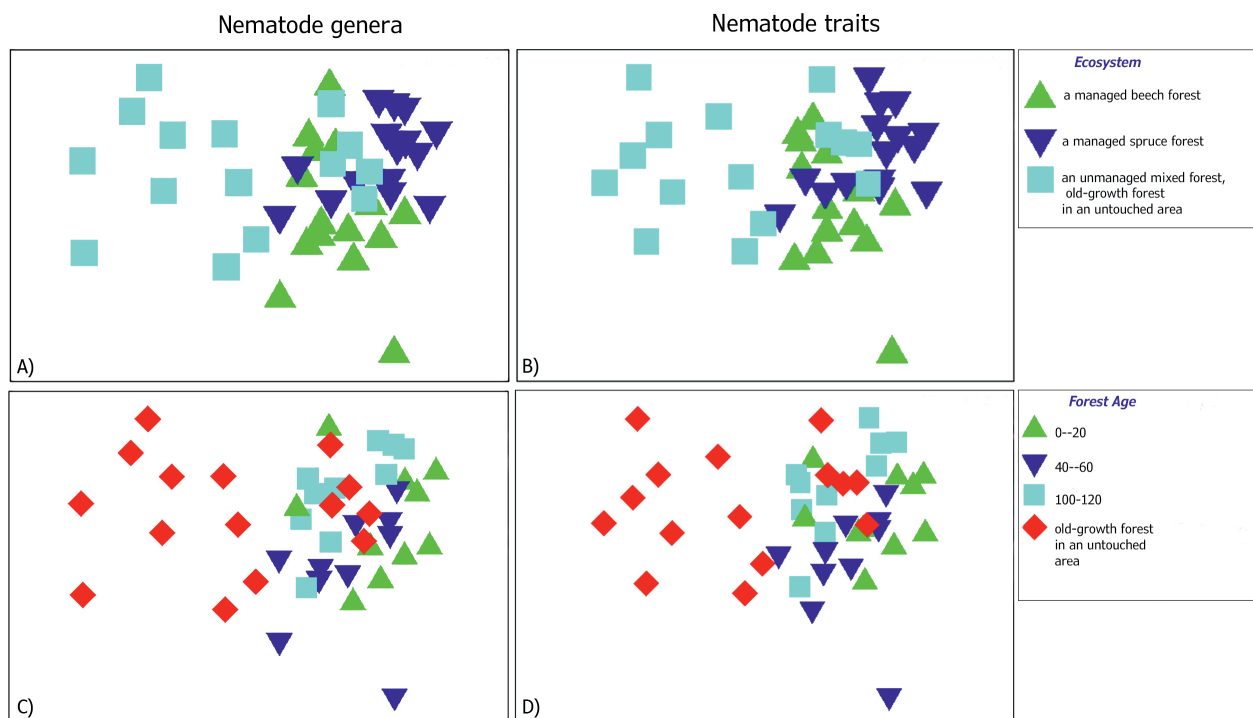


Fig. 4. nMDS carried out on both nematode genera and trait codes (Bray–Curtis similarity, untransformed data) from forest ecosystems in Slovakia. Factors analyzed: A, B) managed beech forest (BEE), managed spruce forest (SPR), and unmanaged mixed forest (UNM). C-D) BEE and SPR plots were stratified by stand age: 0-20, 40-60, and 100-120 age.

diversity and the faunal composition. The soil nematode community structure is mostly driven by the different responses of the various species to the habitat conditions (e.g. food resources, soil physicochemical properties, seasonal variations etc.), contributing to the variability in the ecological processes (Ferris *et al.*, 2001; Gaugler & Bilgrami, 2004).

Therefore, we compared the results obtained from the different studied areas, considering all the correlated variables to verify whether, by combining the morphological traits, there is a correspondence that mirrors the taxonomic composition results in the soil nematode community identified at the genus level.

Material and Methods

In the present study, data sets, were extracted by three previous surveys carried out in different types of ecosystems: grassland, arable soil and forest (Čerevková, 2006; Čerevková *et al.* 2018; Čerevková *et al.*, 2021) in order to compare the effectiveness of the trait combination of nematodes for the detection of nematode structure changes.

All the information on the characteristic of ecosystems, localities and sampling design and routines is reported in the original papers and summarized in Table 1.

We used the three dataset of identified nematodes on genus level and selected a total of four morphological traits: 1. buccal cavity cuticularization occurrence, 2. amphideal fovea size and shape, 3. morphology of the cuticle and 4. morphology of the pharynx to obtain the trait combinations.

In detail, we identified five main categories in relation to the general morphology of the buccal cavity i.e. presence/absence or the look of the cuticularizations within the buccal cavity: 1. unarmed buccal cavity; 2. stylet; 3. odontostyle; 4. onchiostyle; 5. tooth or teeth (Fig. 1). In relation to amphids, we followed a distinction based on the shape and size of the amphideal fovea that were grouped into eight main categories: 1. amphid punctiform or not discernible; 2. circular/rounded or oval; 3. spiral; 4. thin, narrow slit or pore-like; 5. large slit-like & S-shape; 6. funnel-shape; 7. pouch-like; 8. caliciform (Fig. 2A). The cuticle morphology was recognized on the basis of its morphology and thickness in the following three types: 1. Smooth or nearly so; 2. annulated; 3. with outgrowths (Fig. 2B). Oesophageal shape was subdivided into seven categories: 1. oesophagus posteriorly expanded; 2. oesophagus cylindrical; 3. oesophagus with basal bulb; 4. oesophagus with cylindrical corpus and basal bulb; 5. oesophagus with median bulb; 6. oesophagus with swollen corpus and basal bulb; 7. oesophageal procorpus broad posteriorly expanded, bulb-like isthmus short basal bulb

Table 2. Results of Analysis of Similarities (ANOSIM) carried out to detect the potential occurrence of significant differences among the factors under scrutiny in each of the three study cases (n.s. indicates when significant differences were not found and abbreviations are as follows: BEE: managed beech forest; SPR: managed spruce forest; UNM: unmanaged mixed forest, 0-20, 40-60, and 100-120 stage age; NM: new meadow; PM: permanent meadow; PP: permanent pasture).

Case study	Genus level structure		Trait combination level	
	Global statistic	Pairwise Test	Global statistic	Pairwise Test
Forest ecosystem	R = 0.29; p = 0.001	SPR vs UNM: R = 0.39; p = 0.001 BEE vs SPR: R = 0.27; p = 0.001 BEE vs UNM: R = 0.25; p = 0.002	R = 0.29; p = 0.001	SPR vs UNM: R = 0.40; p = 0.001 BEE vs UNM: R = 0.27; p = 0.002 BEE vs SPR: R = 0.22; p = 0.002
Forest age	R = 0.15; p = 0.001	0-20 vs old forest: R=0.34; p=0.002 40-60 vs old forest: R=0.21; p=0.013 0-20 vs 100-120: R=0.19; p=0.02 100-120 vs old forest: R=0.15; p=0.04 0-20 vs 40-60: R=0.14; p= 0.03 40-60 vs 100-120: R= 0.14; p=0.04	R = 0.16; p = 0.001	0-20 vs old forest: R=0.35; p=0.004 40-60 vs old forest: R=0.23; p=0.006 0-20 vs 100-120: R=0.20; p=0.012 100-120 vs old forest: R=0.15; p=0.027 40-60 vs 100-120: R=0.14; p=0.036
Grassland ecosystem	R=0.12; p=0.005	NM vs PP: R= 0.23; p=0.006 NM vs PM: R= 0.15; p=0.001	R=0.12; p=0.012	NM vs PP: R= 0.26; p=0.005 NM vs PM: R= 0.11; p=0.042
Grassland site	R=0.24; p=0.002	Stropkov vs Telgárt: R=0.67; p=0.002 V.Folkmar vs Stropkov: R=0.58; p=0.001 Hybe vs Stropkov: R=0.50; p=0.004 Lubietová vs Telgárt: R=0.47; p=0.004 V.Folkmar vs Lubietová: R=0.41; p=0.001	R=0.18; p=0.004	Stropkov vs Telgárt: R=0.55; p=0.002 V.Folkmar vs Stropkov: R=0.49; p=0.004 V.Folkmar vs Lubietová: R=0.44; p=0.004 Lubietová vs Telgárt: R=0.44; p=0.002 Hybe vs Stropkov: R=0.33; p=0.032
Grassland date	p=n.s.	p=n.s.	p=n.s.	p=n.s.
Type of maize crop	p=n.s.	p=n.s.	p=n.s.	p=n.s.
Country	R = 0.38; p = 0.001	Sweden vs Slovakia: R=0.53; 0.001 Spain vs Slovakia: R=0.44; p=0.001 Denmark vs Spain: R=0.38; p=0.001 Sweden vs Spain: R=0.38; p=0.001 Denmark vs Slovakia: R=0.33; p=0.001 Denmark vs Sweden: R=0.23; p=0.001	R = 0.28; p<0.001	Sweden vs Spain: R=0.52; p=0.001 Denmark vs Spain: R=0.36; p=0.001 Sweden vs Slovakia: R=0.33; 0.001 Spain vs Slovakia: R=0.31; p=0.001 Denmark vs Slovakia: R=0.24; p=0.001 Denmark vs Sweden: R=0.10; p=0.001
Year	R=0.19; p=0.001		R=0.07; p=0.002	

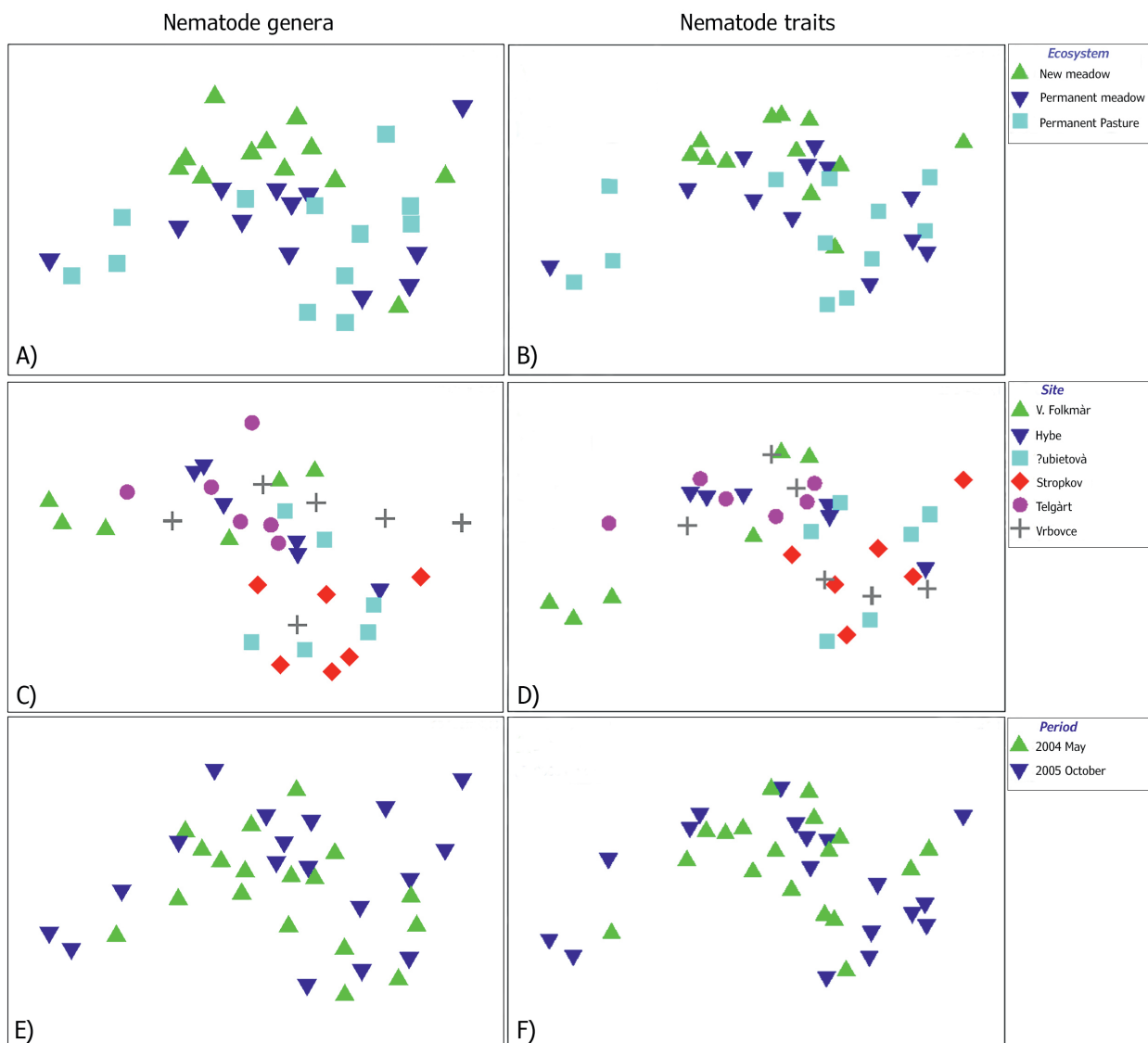


Fig. 5. nMDS carried out on both nematode genera and trait codes (Bray–Curtis similarity, untransformed data) from grassland types of various regions of the Slovak Republic. Factors analyzed: A, B) localities placed around Slovakia (i.e. Ľubietová, Stropkov, Telgárt, Vrbovce and Veľký Folkmár); C, D) type of grassland: NM (established during last 3 years), PM (covered with indigenous multispecies vegetation irregularly mown) and PP (with indigenous vegetation regularly grazed); E, F) period (May 2004 and October 2005).

(Fig. 2C). Categorization of the buccal cavity cuticularizations, amphidial fovea and cuticle type were made according to Andrásy's books (Andrásy, 2005, 2007, 2009), while the oesophageal shape was based on Zullini guide (Zullini, 2021).

After defining of the various categories of each morpho-functional trait (buccal cavity, amphid, cuticle and pharynx), each genus was assigned to the most suitable category based on its morphological appearance and each taxon was given its own number code (Fig. 3).

Data analysis

By previously assigning a number to each morphological trait, we related each taxon with a suite of numbers up to identifying the taxon with a code. Nematodes identified with the same code were arranged together and put in an excel matrix to produce the statistical analysis of the single study cases. Excel matrices were processed through the software package Primer v.6 (Clarke & Gorley, 2006) to perform the multivariate analyses based on the abundance of the customary identification of nematodes at genus level compared to the recently proposed trait combination.

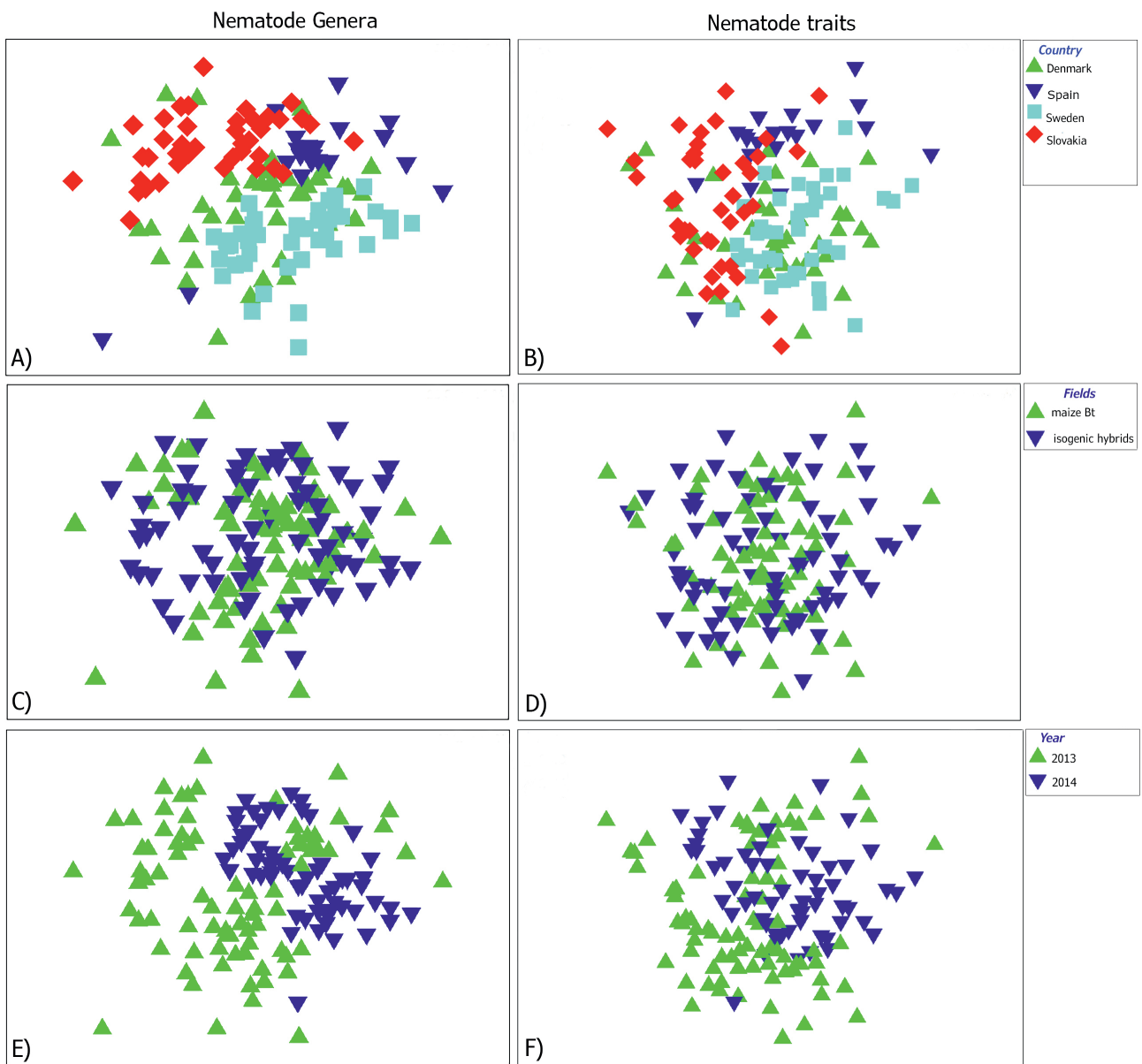


Fig. 6. nMDS carried out on both nematode genera and trait codes (Bray–Curtis similarity, untransformed data) from different maize-growing regions in Europe. Factors analyzed: A, B) country (i.e. Denmark, Slovakia, Sweden and Spain); C, D) maize Bt and isogenic (ISO) hybrids and E, F) period (year 2013, 2014).

To visualize and compare the similarities of the community structure among different factors (e.g. geographical locations, environments or periods), both the genus composition and trait combination were used for the non- Multidimensional scaling (nMDS) analysis after the Bray-Curtis similarity index computation. According to Schratzberger *et al.* (2007) the matrix based on the combination of morpho-functional traits was not transformed and, to make possible comparisons, neither those on the genera. Spatial and temporal trends were further checked by means of the

Analysis of the Similarities (ANOSIM) to assess the presence of significant differences of nematode communities between factors. A visual representation of the biodiversity trends of the free-living nematode communities was provided by a *k*-dominance curve, in which the abundance of each genus and combined trait code were ranked in decreasing order of dominance and cumulatively plotted. The *k*-dominance curve plot can show the richness differences using the two different approaches across the study cases and factors tested.

Results

Overall, 81 genera were assembled in 27 code combinations that were used to understand if this approach can really reflect changes in the taxonomic composition of soil nematodes. In Figures 4 – 6, it is possible to visualize the sample similarity plots of the taxonomic nematode structure and trait combination in light of the several ecological factors analysed. In the first study case from forest ecosystems, the type of forest was the most significant factor influencing both genus (ANOSIM, Global R = 0.29; p = 0.001) and trait code combination (Global R = 0.29; p = 0.001).

Although, there is a certain degree of overlap of the samples in Figure 4A – B, managed spruce forests (SPR) and unmanaged mixed forests (UNM) appeared at the plot extremes highlighting the highest level of dissimilarity. This is confirmed also by the ANOSIM pair-wise test, in which, SPR vs. UNM reported the most marked differences (Table 2). A lesser extent of differences was noticed according to forest age factor (genus structure: Global

R = 0.15; p = 0.001 and trait code structure: Global R = 0.16; p = 0.001, Table 2), but a clear distinction of the old-growth forest in untouched areas was discernible by both nMDS plot and ANOSIM pair-wise results (Table 2). Remarkable, it is that trait combination revealed for forest dataset greater differences than the genus structure in many cases.

In the data set published by Čerevková (2021), the grassland with different ecosystem service (e.g. new and permanent meadows, and permanent pasture), sites, and periods were compared, but significant differences were found only for ecosystem types and localities (Table 2). The values of the global R and probability level for the trait combination were perfectly comparable to the outputs obtained by the genus composition and more marked differences were observed between localities (genus: R=0.24; p=0.002 versus trait combination: R=0.18; p=0.004) than between grasslands types (genus: R=0.12; p=0.005 versus trait combination: R=0.12; p=0.012) (Table 2). In detail, the site with the highest level of dissimilarity was Stropkov (Fig. 5; Table 2), while newly established

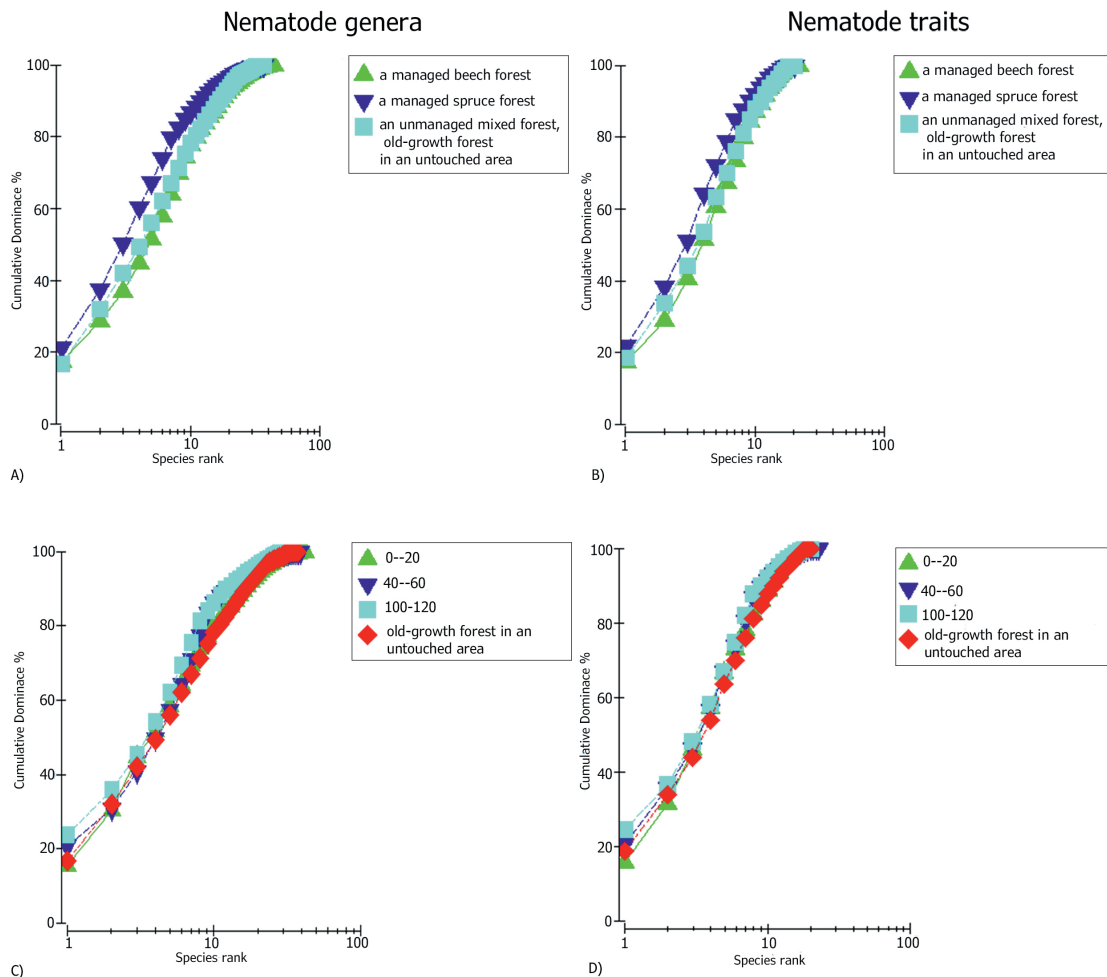


Fig. 7. *k*-dominance curves carried out on both nematode genera and trait codes from forest ecosystems in Slovakia. Factors analyzed: A, B) managed beech forest (BEE), managed spruce forest (SPR), and unmanaged mixed forest (UNM). C-D) BEE and SPR plots were also stratified by stand age: 0-20, 40-60, and 100-120 age.

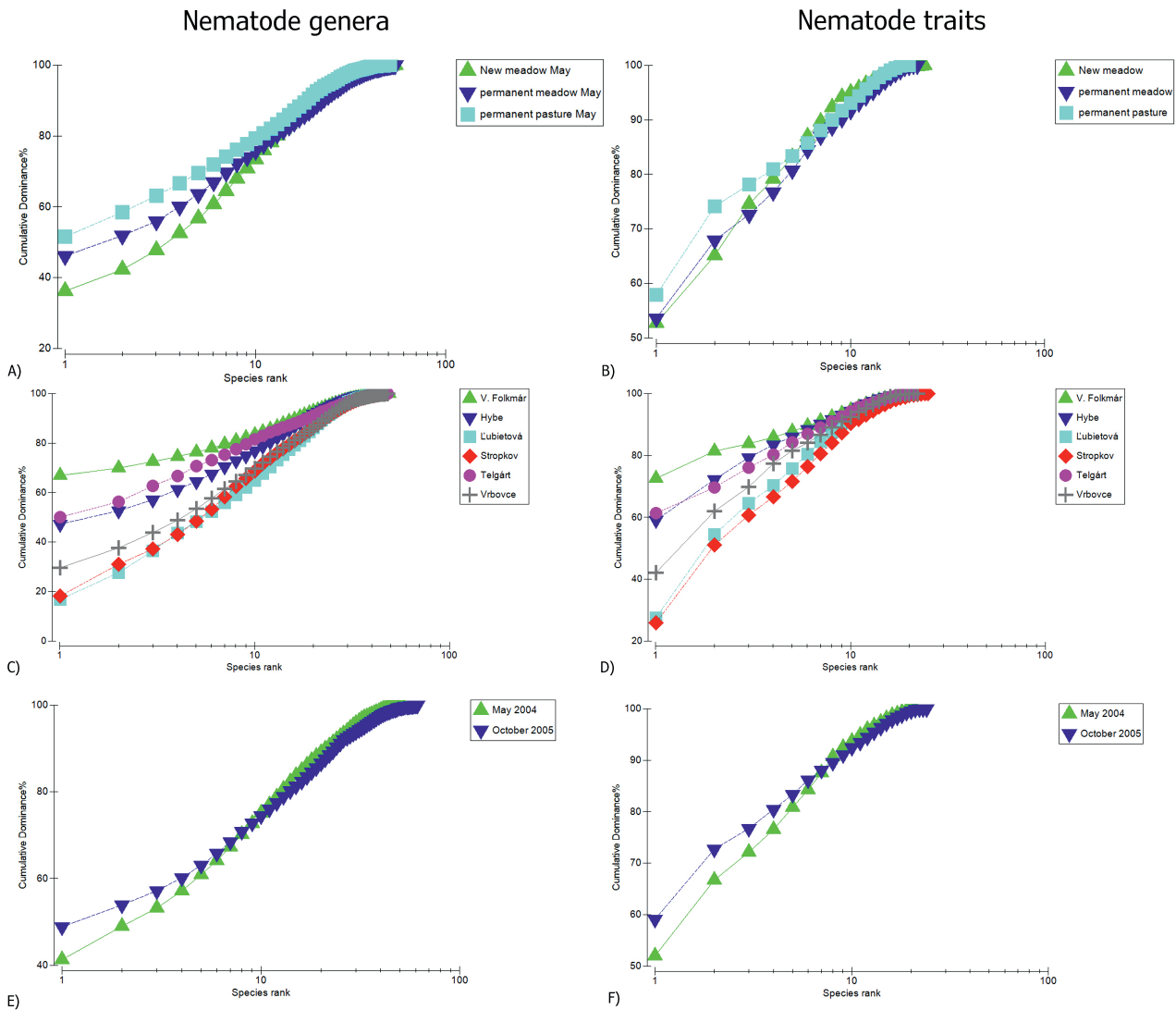


Fig. 8. *k*-dominance curves carried out on both nematode genera and trait codes from grassland types of various regions of the Slovak Republic. Factors analyzed: A, B) type of grassland: NM (established during last 3 years), PM (covered with indigenous multispecies vegetation irregularly mown) and PP (with indigenous vegetation regularly grazed); C, D) localities placed around Slovakia (i.e. Lubietová, Stropkov, Telgárt, Vrbovce and Veľký Folkmár); E, F) period (May 2004 and October 2005).

meadows showed significant differences from both permanent meadows and permanent pastures. Instead, no significant differences were observed between these two types of grasslands (i.e. PM and PP) (Fig. 5 and Table 2).

The study case carried out to evaluate the possible disturbance effects of genetically modified maize did not reveal significant differences between maize Bt and isogenic (ISO) hybrids (Fig. 6; Table 2). Instead, marked differences were observed between countries (genus structure: $R=0.38$; $p=0.001$; trait combination: $R=0.28$; $p=0.001$) with the lowest significant differences noticed between Denmark, Slovakia and Sweden both by genera and

traits (Table 2). Although, the temporal factor resulted significantly different also using traits, Global R resulted very low compared to genus structure results (genus structure: $R=0.19$; $p=0.001$; trait combination structure: $R=0.07$; $p=0.002$).

The above insights are confirmed also by the trends observable from the *k*-dominance curves, where nematode diversity is consistent among genus and code trait combination (Figs. 7 – 9). The only exception as remarked by the multivariate analyses were the comparisons among countries in the study on modified maize (Fig. 9).

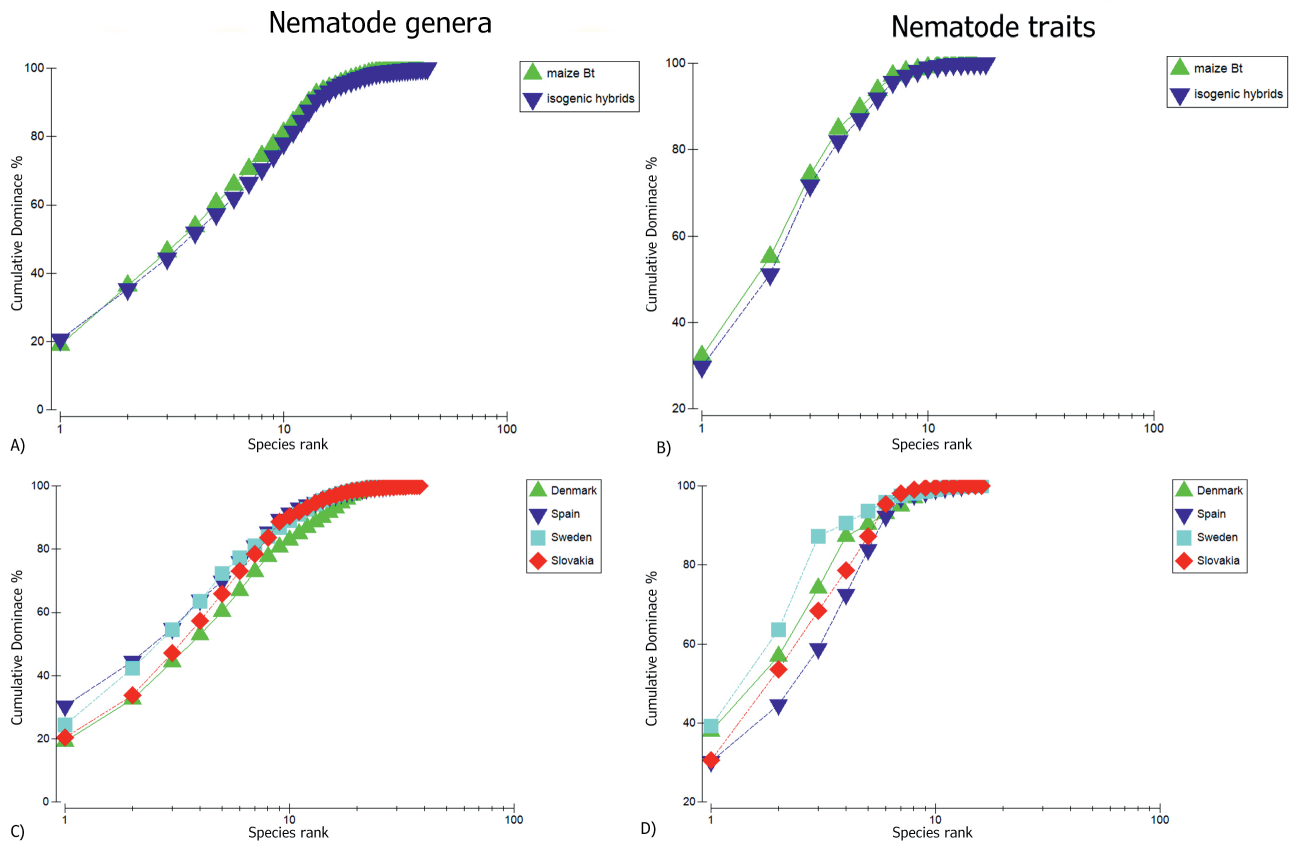


Fig. 9. k -dominance curves carried out on both nematode genera and trait codes from different maize-growing regions in Europe. Factors analyzed: A, B) maize Bt and isogenic (ISO) hybrids and E, F) period (year 2013, 2014); C, D) country (i.e. Denmark, Slovakia, Sweden and Spain).

Discussion

The identification based on morphology is a time-consuming activity especially when reports must return in a short time, and large-scale biomonitoring surveys are required. Therefore, alternative methods must be explored and developed. Environmental DNA (eDNA) is a technique that will increase our ability to quantify ecosystem biodiversity and overcome labour-intensive traditional surveys. However, it cannot be the only forward way due to the existence of some possible problems such as imperfect detection and taxonomic assignment, species quantification, assessing ecological status (Beng & Corlett, 2020), and economic costs not always accessible to each research group or environmental agency. In this scientific context, the trait combination aims to provide a practical and easily accessible approach for reading and quantifying changes in the nematodes' community structure. This approach, coupled with advances in machine learning also referred to as deep learning or artificial intelligence (AI), could open a new avenue for nematode ecological assessment in future (Colin *et al.*, 2017; Bogale *et al.*, 2020). Indeed, the development of machine

learning for the automated detection of a few morphological nematode traits and their immediate combination into a trait code could be even more easily calibrated than in case of genera (Shabrina *et al.*, 2023) and species level (Thevenoux *et al.*, 2021), allowing for the faster deployment of AI systems and the skipping of time-consuming steps in the usual identification process. Promising results have been documented in marine systems using the trait combination (Semprucci *et al.*, 2022). This is the first time that trait combination is tested to detect the community changes in the soil free-living nematodes.

Multivariate analysis performed using the nematodes identified at genus level and compared with those obtained by trait combination revealed consistent results in all the three study cases analysed. In detail, in the forest ecosystem, both approaches showed that the type of forest management was the most relevant factor influencing the nematode structure, followed by the forest age. This supports the idea that forest management and the stand age play key roles in determining the soil nematode composition (Čerevková *et al.*, 2021). Managed spruce forest (SPR) and the unmanaged mixed forest (UNM) showed the most significant

differences that resulted associated also to a marked presence of tolerant nematode taxa in the coniferous SPR forest (Čerevková *et al.*, 2021). Additionally, the combination of traits was more sensitive than taxonomic composition in detecting the differential successional stage of the forest vegetation. The age of the forests showed the highest level of dissimilarity likely due to the highest values of nematode richness, total abundance, biomass, and diversity were found in the youngest forest stands, regardless of forest type (Čerevková *et al.*, 2021).

In the grassland system, a primary effect of the ecosystem's type was discovered in the succession from newly established meadows to permanent pastures and permanent meadows, while no significant differences were observed between permanent meadows and pastures (Čerevková, 2006). It is further noteworthy that the level of significance revealed by ANOSIM among ecosystems was perfectly the same (see Global R level). Furthermore, both approaches highlighted a greater geographic effect, with the Stropkov site revealed the most noticeable differences.

The statistical analysis performed on the response of soil nematode communities to *Bt* maize cultivation was the only case in which the R values of ANOSIM resulted visibly lower than those obtained by taxonomic identification. However, again, both the approaches revealed similar trends: the effect of the crop location and sampling periods on the soil nematode communities was more pronounced than that of the *Bt* genetic modification. Moreover, trait combination supports the idea that, in contrast with data collected by Neher *et al.* (2014) and Manachini and Lozzia (2002), *Bt* maize had no effect on soil nematode communities (Čerevková *et al.*, 2018). Observing the *k*-dominance curves, trait combination perfectly mirrored the genus diversity trends with the only exception being the countries in the *Bt* maize cultivation study. That makes it possible to support the trait combination as indirect measure of biodiversity loss.

As demonstrated, the present approach can certainly allow a survey of environmental disturbance effects on the soil nematodes community structure. Furthermore, even if, at the moment, it is not possible to assign an ecological quality status to an environment with this method, it could be in the future. Indeed, after the analysis of a wide data set of samples in well-discernible environmental disturbance gradient, it could be possible to find recurring combinations of morpho-functional traits that, therefore, could be regarded as indicators of good ecological quality or sentinels of pollution giving a further significant contribution to the biomonitoring assessment with soil nematodes.

Conclusion

Ecological and, above all, biomonitoring surveys require smart and rapid approaches to detect possible variations of the faunal communities after a perturbation. Recently, a combination of morpho-functional traits was proposed as an alternative method to document changes in the marine nematode community struc-

ture. By assigning together each trait with a single number, the authors have created a data matrix based on a series of codes that was able to perfectly mirror the taxonomic structure of the marine nematode community at the genus level. Therefore, the same approach has been used to understand if it is applicable to soil nematodes, but considering an adapted version of morphological traits such as: buccal cavity morphology, amphideal fovea size and shape, morphology of the cuticle, and pharynx. We demonstrated that the matrices based on genus level and trait combination gave the same results in all three study cases considered. Therefore, this approach makes it possible to suggest that its implementation, associated with advances in machine learning, could transform nematode ecological surveys.

Conflicts of Interest

The authors declare no conflict of interest.

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