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Belgian Journal of Zoology

AN INTERNATIONAL JOURNAL PUBLISHED BY
THE ROYAL BELGIAN SOCIETY FOR ZOOLOGY

Volume 139 (1) – January 2009



THE BELGIAN JOURNAL OF ZOOLOGY IS COVERED IN CURRENT CONTENTS, SCIENCE CITATION INDEX
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The Belgian Journal of Zoology is published with the financial support of the “Direction générale de l'Enseignement supérieur de la Recherche scientifique” and of the “Universitaire Stichting van België/Fondation Universitaire de Belgique”.

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BJZ

Belgian Journal of Zoology

Published by the
“KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE —
SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE”

Volume 139 (1)
(January, 2009)

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Nematode assemblages in a nature reserve with historical pollution

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ABSTRACT. Nematodes, and especially nematode communities, have significant potential as bio-indicators. The present study aimed to assess the nematode community structure of several sites with different historical pollution. Long-term polluted municipal waste-, tar- and sludge- sites were compared with less disturbed annex sites. At each site heavy metal and PAHs concentrations were measured together with soil texture classes, pH and total organic matter. Identification of three hundred nematodes at each location resulted in the discrimination of 63 genera from 32 different families of which the Cephalobidae, Belonolaimidae, Tylenchidae, Hoplolaimidae, Belonolaimidae and Plectidae were the most abundant families. The sampling sites harbour significantly different nematode communities and significant differences of life-strategy-related parameters (cp-groups, MI indexes) were observed. The significant augmentation of the proportion of the cp 2 nematodes in historically-polluted sites was especially informative. Omitting the cp 1 group from the MI (=MI2-5) better reflects putative historical pollution-induced community changes. However, the current study did not reveal significant relationships between historical pollution and the feeding type composition, or the Shannon-Wiener diversity. The observed results are critically assessed in the light of possible flaws such as sampling and analyzing limitations.

KEY WORDS : bio-indicator, diversity, heavy metals, life strategy, MI, pollution, feeding types

INTRODUCTION

Nematodes, and especially nematode communities, have significant potential as bio-indicators (BONGERS, 1990; RITZ & TRUDGILL, 1999; NEHER, 2001), which makes information on these communities especially useful for soil characterization and assessment of soil conditions. Generally, nematodes are among the most abundant multicellular organisms and often occur in large numbers even at heavily polluted sites. Their life cycle varies from a few days to years making them very sensitive to short term as well as long-term environmental changes. Furthermore, they are represented in nearly all trophic and functional groups in soil food webs (NEHER, 2001). The short and long-term effects of pollution and disturbances in different habitats have been studied on many occasions (WEISS & LARINK, 1991; WASILEWSKA, 1996; HOHBERG, 2003). In all of these studies the nematode communities underwent significant changes in relation to disturbances. In general, species diversity declined due to stress factors while the dominance of r-strategists increased, and the larger and longer living K-strategists were usually eliminated from the soil ecosystem (BONGERS, 1990). Changes in nematode communities, therefore, can be used to assess disturbances of the soil ecosystem including organic pollution and heavy metal pollution.

More specific studies that included long term effects of heavy metals on nematode assemblages were reported by NAGY, 1999; NAGY et al., 2004; GEORGIEVA et al., 2002; YEATES et al., 2003; BAKONYI et al., 2003; in particular, the analysis of the c-p group composition turned out to be a valuable tool to detect heavy metal pollution. Some studies of the effect of low-level pollution on soil inverte-

brates (ERSTFELD & SNOW-ASHBROOK, 1999) and nematode communities (SNOW-ASHBROOK & ERSTFELD, 1998) investigated polycyclic aromatic hydrocarbon contamination (PAH). The effect of PAH contamination on soil food webs and ecological processes was further investigated by BLAKELY et al. (2002).

However, with the exception of some specific pollution elements, the relation of nematode assemblages to a wide range of historical pollutants resulting in chronic low-level contamination over long periods has received relatively little attention. The present study aimed to assess the community structure of several sites with different historical pollution, where the chemical concentrations were expected to exceed background concentrations, but without extreme pollution. More particularly, nematode community characteristics of long-term polluted locations were compared with less disturbed annex sites.

MATERIALS AND METHODS

The study area

The study sites are located in and around the nature reserve Bourgoyen-Ossemeersen near Ghent (Belgium). This reserve is located on an alluvial plain of the river Leie and was used as a dumping site for a variety of waste products up to the 1970s. Three easily accessible areas, characterized by different types of relatively mild industrial and municipal pollution, were sampled in November 2002. In addition to each polluted site, an annex site was sampled. The annex sites are not considered true reference sites since it is simply impossible to find two soils exactly the same with only a difference in degree of pollution.

(1) An old municipal waste site was subdivided into two zones: the municipal bush, a site with a vegetation of trees and bushes (*Fraxinus excelsior*, *Galium aparine*, *Sambucus nigra*, *Tilia cordata*, *Urtica dioica*) and another zone (municipal grass) with a dominance of grasses and weeds (*Arrhenatherum elatius*, *Cirsium arvense*, *Holcus lanatus*, *Medicago lupulina*, *Poa trivialis*). Additionally, a non-polluted site (municipal annex) at 20m separation, was chosen as an annex site with the following vegetation: *Anthistiscus sylvestris*, *Lolium perenne*, *Poa trivialis*, *Rumex acetosella*, *Trifolium pratense*.

(2) The second site investigated was an old tar dumping site (tar) with very dense vegetation. Its non-polluted annex site (tar annex) was 5m away, possessing a similar vegetation (*Alnus glutinosa*, *Arrhenatherum elatius*, *Fraxinus excelsior*, *Rumex acetosa*, *Urtica dioica*) and soil texture.

(3) The third sampled site, currently used as a pasture, is a sludge site (sludge) with sludge originating from the nearby river Leie. The annex site (sludge annex) is situated 20m away and has the same vegetation (*Alopecurus pratensis*, *Dactylis glomerata*, *Lolium perenne*, *Poa trivialis*, *Rumex acetosa*) and soil texture.

The exact composition of the dumped materials, the historical behaviour of the pollutants and possible treatments were unknown.

At each site bulk samples were taken in November 2002; each sample consisted of 15 cores (diameter 5cm, sampling depth 25cm) within a radius of 20cm. Five cores were manually mixed and used for nematode sampling and ten cores for chemical analysis. This procedure was replicated, to a total of three replicates. Total soil Pb, Cd, Cu, Zn, Hg, As, Cr(III) and Cr(VI) were determined after *aqua regia* digestion (VAN RANST et al., 1999). Analyses were subsequently performed, using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Varian Liberty Series II, Varian, Palo Alto, CA). Although reduction of Cr(VI) was as much as possibly avoided by preliminary sample treatment and an appropriate rapid analysis (VITO reference procedure soil inspection, 2006a), Cr(VI) was nevertheless excluded from further analysis. The metal concentrations (mg/kg) for each site are presented in Table 1. The sites were also examined for a variety of polycyclic aromatic hydrocarbons (PAHs) (Table 1). Extractable Organic halogens (EOX) and mineral oils were extracted by a soxhlet extraction (VITO, reference procedure soil inspection, 2006b). EOX was further analyzed by colourimetric titration (EUROGLAS BV, 1998; VITO reference procedure soil inspection, 2006c) and mineral oil was determined by capillary column gas chromatography (Varian CP-3800, Palo Alto, CA; VITO (2006b) reference procedure soil inspection: mineral oil). Accuracy, reproducibility and yield of the applied methods was confirmed with blanks and certified reference material of similar structure to the analysed samples (VITO reference procedure soil inspection, 2006d).

The organic matter content was also determined by loss of mass after ignition. To determine soil pH, 50ml of deionised water were added to 10g of air dried soil and allowed to stand for 24h. Acidity of the supernatant fluid was then measured using a pH electrode (Model 520A, Orion, Boston, MA, USA). Soil structure was analysed with a particle size analyzer (Beckman Coulter LS 100, Fullerton, CA,

USA) (BUCHANAN, 1984) and allocated to a texture class in a texture triangle (GERAKIS & BAER, 1999) (Table 1). Finally, the most abundant vegetation types were identified.

Sampling strategy and nematode analysis

The five "nematode" cores (196cm³) of each bulk sample were fixed with 204ml 4%-formaldehyde (60°C); nematodes were extracted from 100ml of this mixture by the centrifugal-flotation technique (CAVENESE & JENSEN, 1955); the sediment was centrifuged twice with Ludox HS-40 (DuPont Chemicals, Wilmington, USA) and kaolin for 5 minutes at 3500 rotations/minute and the supernatant was rinsed over a 38µm sieve. Nematodes were counted, 100 individuals were picked out randomly using a stereomicroscope (50X magnification). Formaldehyde (4% with 1% glycerol) was heated to 70°C and 4-5ml were quickly added to the specimens to fix and kill the nematodes in one process. The fixed nematodes were processed in anhydrous glycerol following the glycerol-ethanol method (SEINHORST, 1959) and mounted on aluminium slides with double coverslips.

The nematodes were identified to family and genus level and assigned to their feeding type (YEATES et al., 1993a). However, the feeding habits of the so-called "facultative plant parasites" or "plant associates" is unclear (OKADA & HARADA, 2007; BERT et al., 2008). Therefore, a distinction was made between "real" plant-parasitic species (endo- and ectoparasites) dependant on higher plants (=plant-parasites) and those tylenchs that feed on algae, lichens, mosses, epidermal cells or root hairs, apparently without causing damage, of which the exact feeding behaviour is unknown (= "plant associated nematodes", see also YEATES et al., 1993b). Except for the plant-feeding nematodes all individuals were classified according to their colonizer-persister value (cp 1, cp 2, cp 3, cp 4, cp 5 and the combined group cp 3-5). The maturity indices MI and MI2-5 were calculated following BONGERS (1990) and KORTHALS et al. (1996).

Data analysis

Data were analyzed using a combination of multivariate and univariate methods. The correlation structure among the environmental variables was explored by means of Spearman rank correlations. Differences between groups of sites and within a group of sites (*i.e.* all polluted *vs.* all annex sites and the polluted *vs.* the annex site of one site type) of total densities, diversity, maturity indices, the relative abundance of feeding- and cp-groups were analyzed by a one-way ANOVA in SAS v. 9.1.3; this after square root and arcsine transformations for proportional data. Bartlett's and Cochran's tests were used to verify homogeneity of variances prior to the analysis.

Differences in nematode communities among groups of sites were analysed by permutational multivariate analyses of variance (PerMANOVA; ANDERSON, 2001) on square root transformed data, followed by pair-wise comparisons between groups of sites. Site was treated as a fixed factor and the Bray-Curtis dissimilarity measure was applied for all analyses. Each term in the analyses was calculated using 9999 permutations of the appropriate units. Since PerMANOVA is sensitive to differences in multivariate dispersion between groups, possibly increasing the Type I

error, the same model was tested for differences in multivariate dispersion (PERMDISP; ANDERSON, 2004). Furthermore, species indicative for each group of sites were identified by Indicator Species Analysis (INDVAL) (DUFRENE & LEGENDRE, 1997) and their statistical significance was tested by a Monte Carlo Test.

Multivariate patterns were visualised through non-metric Multi-Dimensional Scaling (MDS ordination) applying the Euclidean distance similarity measure (KRUSKAL, 1964) after normalisation of the environmental data and the Bray-Curtis dissimilarity measure on square root transformed nematode data. The correlation between the multivariate nematode and environmental pattern was examined using the BIOSTEP routine (CLARKE & GORLEY, 2001). Finally, in order to allow better visualization between sites and variables, the environmental parameters that best explain the nematode community pattern, indicative genera and certain community characteristics (feeding types groups, cp-groups, diversity, MI) were superimposed on MDS ordination diagrams as circles whose sizes reflect the magnitude of these variables. Mul-

tivariate analyses were performed using the PRIMER v5.0 software package (CLARKE & GORLEY, 2001).

RESULTS

The environmental variables

Detailed results of the analyzed soil parameters for each location are shown in Table 1. Comparison with quality standard values extracted from literature data (after correction for clay and organic matter content) give an indication of the severity of the analysed pollutants (Table 1). The municipal bush and municipal grass, the sludge and the tar were more polluted sites (for organic as well as heavy metal pollution) than their corresponding annex sites. However, as this study is of an observational type, a thorough inspection of the correlation structure among the explanatory variables is a prerequisite for understanding the observed relationships.

TABLE 1

Soil parameters (soiltype, pH, clay percentage, organic matter, total heavy metal concentrations and organic pollutants) in the sampled locations in the Bourgoyen-Ossemeersen. Values expressed as average and standard error.

		Municipal bush	Municipal grass	Municipal annex	Sludge	Sludge annex	Tar	Tar annex
Soil structure		sandy-clay-loam	sandy-clay-loam	clay	clay	clay	sandy-clay-loam	sandy-clay-loam
pH		7.3 (\pm 0.1)	6.9 (\pm 0.2)	6.4 (\pm 0.4)	7.3 (\pm 0.1)	6.4 (\pm 0.1)	7.4 (\pm 0.2)	6.6 (\pm 0.3)
Clay	%	28.2	23.4	64.1	80.5	70.8	26.2	34.8
Organic matter	% C	4.9 (\pm 2)	6.4 (\pm 1.6)	5.9 (\pm 2.3)	17.9 (\pm 1.6)	7.4 (\pm 0.2)	6.6 (\pm 1.2)	6 (\pm 2.3)
As	mg/kg	-	-	-	110^{II} (\pm 25)	-	-	-
Cd	mg/kg	-	1.6 (\pm 0.9)	-	50 (\pm 0.7)	-	2.0 (\pm 1.1)	-
Cr ³⁺	mg/kg	17.6 (\pm 7.1)	-	-	196 (\pm 12)	22 (\pm 8.3)	20.3 (\pm 4.9)	27.6 (\pm 7.7)
Cu	mg/kg	50 (\pm 19)	44 (\pm 18)	-	300 (\pm 24)	57 (\pm 14)	67 (\pm 19)	-
Hg	mg/kg	-	-	-	4.7 (\pm 0.3)	-	-	-
Pb	mg/kg	158 (\pm 67)	203 (\pm 84)	-	759^{III} (\pm 50)	220^I (\pm 74)	346^{II} (\pm 31)	-
Zn	mg/kg	171 (\pm 68)	378 (\pm 121)	138 (\pm 59)	1787^{IV} (\pm 97)	221 (\pm 24)	571 (\pm 126)	115 (\pm 42)
Naftalene	mg/kg	0.5 (\pm 0.2)	-	0.4 (\pm 0.1)	-	-	2.9 (\pm 0.4)	-
Aceanftylene	mg/kg	-	-	-	-	-	-	-
Fluorine	mg/kg	-	-	-	-	-	-	-
Fenantrene	mg/kg	-	-	-	-	-	0.4 (\pm 0.3)	-
Antracene	mg/kg	0.2 (\pm 0.1)	-	-	0.3 (\pm 0)	-	2.1 (\pm 0.1)	0.1 (\pm 0)
Fluorantene	mg/kg	0.1 (\pm 0.1)	-	-	-	-	0.5 (\pm 0.2)	-
Pyrene	mg/kg	-	-	-	1.2 (\pm 0.4)	-	2.0 (\pm 0.1)	-
benzo(a)antracene	mg/kg	-	-	-	1.0 (\pm 0.4)	-	1.2 (\pm 0.1)	-
Chrysene	mg/kg	-	-	-	0.9 (\pm 0.6)	-	0.8 (\pm 0.2)	-
benzo(b)fluorantene	mg/kg	-	-	-	1.3 (\pm 0.9)	-	1.1 (\pm 0.1)	-
benzo(k)fluorantene	mg/kg	-	-	-	2.0 (\pm 0.2)	-	1.5 (\pm 0.1)	-
benzo(a)pyrene	mg/kg	-	-	-	0.5 (\pm 0.2)	-	0.5 (\pm 0.1)	-
indeno(1.2.3-cd)pyrene	mg/kg	-	-	-	0.6 (\pm 0.4)	-	0.6 (\pm 0.1)	-
dibenzo(a,h)antracene	mg/kg	-	-	-	0.8 (\pm 0.2)	-	0.7 (\pm 0)	-
benzo(ghi)perylene	mg/kg	-	-	-	0.3 (\pm 0.4)	-	0.2 (\pm 0.1)	-

-: below detection level; **Bold**: exceeding legislated clean-up values, Latin numbers reflect increasing exceeding of norm values (from stringent to less stringent: ^I:nature and forest area; ^{II}: rural area; ^{III}: residential area; ^{IV}: recreation area; ^V: industrial area). Values are corrected for clay and organic matter and compared according to VLAREBO (the Flemisch environmental legislation concerning soil cleanup) as described in VAN GEHUCHTE et al. (1997).

Several environmental data appeared highly correlated. Nearly all metal concentrations of Cd, Pb, Cu, Hg and Zn were significantly positively correlated with each other and with carbon content. Cr(III) and As formed a second group of significantly positively correlated metal concentrations. Evidently, nearly all analyzed PAK's were signif-

icantly positively correlated with each other and total PAK.

Ordination of the environmental variables by an MDS analysis (stress: 0.10) did show a clear separation between replicated annex and polluted sites for Tar and Sludge. The municipal sites did not form a unequivocal pattern (Fig. 1).

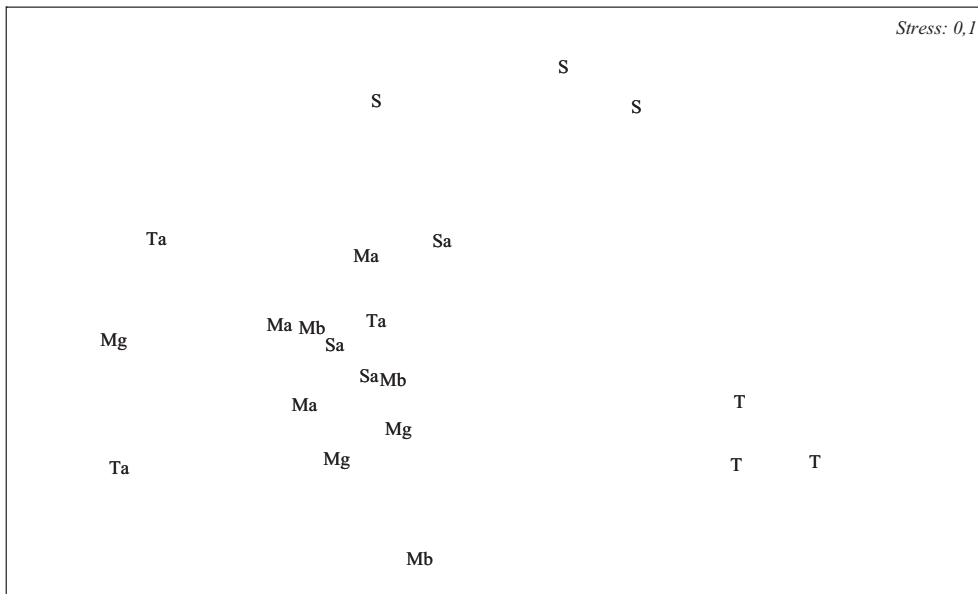


Fig. 1. – Output of non-metric Multi Dimensional Scaling (MDS) on fourth root transformed normalization of the environmental data from seven sampled sites (three replicates) in the Bourgoyen-Ossemeersen (Ghent, Belgium). (Mb): Municipal bush, (Mg): Municipal grass, (Ma): Municipal annex, (S): Sludge, (Sa): Sludge annex, (T): Tar, (Ta): Tar annex.

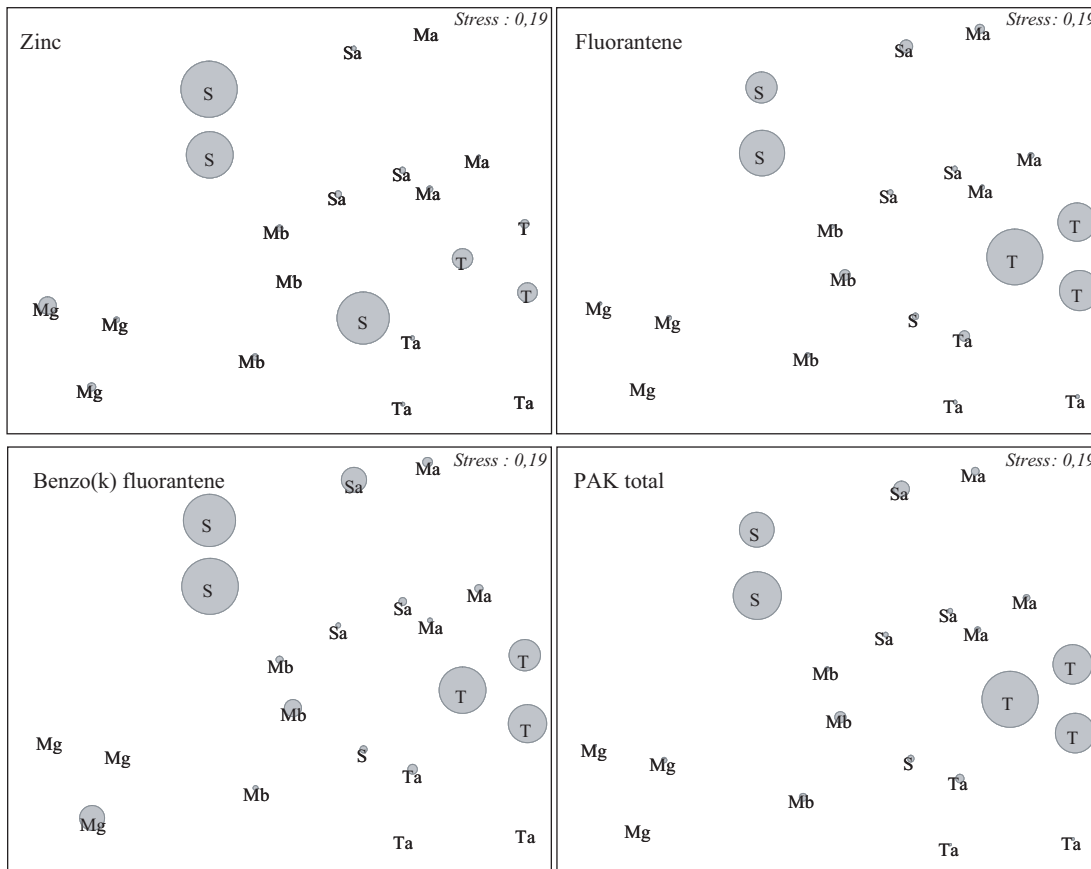


Fig. 2. – Most discriminative environmental parameters superimposed on Non-metric Multi Dimensional Scaling (MDS) biological ordination diagrams. Sizes of shaded circles reflect the relative magnitude of the superimposed variables. MDS output based on square-root-transformed genus abundances from seven sampled sites (three replicates) in the Bourgoyen-Ossemeersen (Ghent, Belgium). (Mb): Municipal bush, (Mg): Municipal grass, (Ma): Municipal annex, (S): Sludge, (Sa): Sludge annex, (T): Tar, (Ta): Tar annex.

Nematode communities and relation with the environment

Belonolaimidae, Tylenchidae, Hoplolaimidae, Belonolaimidae and Plectidae were the most abundant families.

Sixty-three genera from 32 different families were identified in this study (Table 2). The Cephalobidae,

TABLE 2

An overview of the identified nematode genera families and genera ordered by feeding type in the sampled locations in the Bourgoyen-Ossemeersen (Ghent, Belgium)

Bacteriovores	Fungal-feeders	Omnivores	Predators	“plant associated nematodes”	Plant-parasites	
<u>Alaimidae</u>	<u>Monhysteridae</u>	<u>Aphelenchoididae</u>	<u>Dorylaimidae</u>	<u>Aporcelaimidae</u>	<u>Tylenchidae</u>	<u>Belonolaimidae</u>
<i>Alaimus</i>	<i>Eumonhystera</i>	<i>Aphelenchoides</i>	<i>Laimydorus</i>	<i>Aporcelaimellus</i>	<i>Aglenchus</i>	<i>Geocenamus</i>
<i>Amphidelus</i>	<i>Monhystera</i>	<u>Aphelenchidae</u>	<u>Nordiidae</u>	<u>Mononchidae</u>	<i>Basiria</i>	<i>Tylenchorhynchus</i>
<u>Bunonematidae</u>	<i>Monhystrella</i>	<i>Aphelenchus</i>	<i>Longidorella</i>	<i>Clarkus</i>	<i>Coslenchus</i>	<u>Criconematidae</u>
<i>Bunonema</i>	<u>Panagrolaimidae</u>	<u>Diphtherophoridae</u>	<i>Thornia</i>	<i>Mononchus</i>	<i>Filenchus</i>	<i>Mesocriconema</i>
<u>Bastianiidae</u>	<i>Panagrolaimus</i>	<i>Diphtherophora</i>	<u>Qudsianematidae</u>	<i>Mylonchulus</i>	<i>Tylenchus</i>	<i>Criconema</i>
<i>Bastiana</i>	<u>Plectidae</u>	<u>Tylencholaimidae</u>	<i>Epidorylaimus</i>	<i>Prionchulus</i>		<i>Crossonema</i>
<u>Cephalobidae</u>	<i>Anaplectus</i>	<i>Tylencholaimellus</i>	<i>Eudorylaimus</i>	<u>Qudsianematidae</u>		<u>Hoplolaimidae</u>
<i>Acrobeles</i>	<i>Plectus</i>		<i>Microdorylaimus</i>	<i>Labronema</i>		<i>Helicotylenchus</i>
<i>Acrobeloides</i>	<i>Wilsonema</i>		<i>Prodorylaimus</i>	<i>Thonus</i>		<i>Rotylenchus</i>
<i>Cephalobus</i>	<u>Prismatolaimidae</u>		<u>Thornenematidae</u>	<u>Nygalaimidae</u>		<u>Nordiidae</u>
<i>Cervidellus</i>	<i>Prismatolaimus</i>		<i>Mesodorylaimus</i>	<i>Nygalaimus</i>		<i>Pungentus</i>
<i>Chiloplacus</i>	<u>Rhabditidae</u>		<i>Opisthodorylaimus</i>	<u>Tripylidae</u>		<u>Pratylenchidae</u>
<i>Eucephalobus</i>	<i>Mesorhabditis</i>			<i>Paratrypila</i>		<i>Pratylenchus</i>
<i>Heterocephalobus</i>	<u>Rhabdolaimidae</u>			<i>Tripyla</i>		<u>Trichodoridae</u>
<u>Desmodoridae</u>	<i>Rhabdolaimus</i>					<i>Trichodorus</i>
<i>Prodesmodora</i>	<u>Teratocephalidae</u>					
<u>Diplopeltidae</u>	<i>Teratocephalus</i>					
<i>Cylindrolaimus</i>	<u>Tylopharyngidae</u>					
	<i>Tylopharynx</i>					

TABLE 3

One-way permutational analysis of variance (Permanova) and one-way permutational test of multivariate dispersion (Permdisp) based on Bray-Curtis square root transformed nematode community data

Source	df	Permanova				Permdisp			
		SS	MS	F	P	SS	MS	F	p
Site	6	16064.0	2677.3	3.16	0.0001	726.2	121.0	1.90	0.1564
Residual	14	11876.3	848.3			891.6	63.7		
Total	20	27940.4				1617.8			

Initial examination by an MDS analysis (stress: 0.19) did show six, not clear cut, groups representing the sampling sites, except for the sludge replicates, which are not grouped (Figs 2-5). However, the MDS plot did not show a clear pattern related to pollution, *i.e.* polluted and non-polluted or annex samples are irregularly grouped on the plot. Nevertheless, a permutational multivariate analyses of variance (PerMANOVA) could demonstrate that the sampling sites harbour significantly different nematode communities. Multivariate dispersions did not differ significantly for the factor Site (Table 3), which indicates that the significant site effect, revealed by the PerMANOVA, is not due to artefacts as a result of variable dispersions. However, *a posteriori* pairwise comparisons showed that the nematode communities from Sludge and Sludge annex were not significantly different from all the other sites. Sludge annex harboured only a significantly different community as

compared to the Tar site, while differences between the communities of the Sludge site and other sampling sites could not be considered significant – most likely because of the considerable community composition differences between the replicates. Indicative species for the seven sampling localities are shown in Table 4 and Fig. 3. Only the species of *Bastiana* and *Ogma* were significantly indicative (Monte Carlo permutation test) for the Municipal bush site and the Tar-annex site respectively. Matching the pattern found in environmental characteristics with that found in nematode communities (BIOSTEP) revealed a relatively small Spearman rank correlation coefficient for any combination of pollutants ($\rho=0.433$). Maximal matching between nematode assemblages and pollutants was explained by Zinc, benzo(k)fluoranteen and PAK total. A combination of Zinc, benzo(k)fluoranteen and fluoranteen resulted in a slightly lower correlation coefficient ($\rho=0.430$).

TABLE 4

Total nematode abundance, genus abundance, indicative genera, Shannon-Wiener index (H'), Maturity Indices, relative abundance of feeding types and cp-groups in the sampled locations in the Bourgoyen-Ossemeersen (Ghent, Belgium). Results ± standard deviation and with indication of significance differences (one-way ANOVA; *p<0.05; dotted line: significant differences between all polluted and all annex sites; underlined and ↔: significant differences between the polluted and annex site of a single site)

	Municipal bush	Municipal grass	Municipal annex	Sludge	Sludge annex	Tar	Tar annex
Abundance(±SD)							
per 50cm ³ soil	3920 (±53)	3297 (±62)	3980 (±333)	2971 (±241)	3008 (±171)	3289 (±81)	3701 (±268)
# Genera (±SD)	18 (±3)	18 (±2)	21 (±2)	17 (±2)	20 (±5)	23 (±5)	25 (±2)
Diversity (H')	2.63 (±0.16)	2.54 (±0.12)	2.74 (±0.01)	2.51 (±0.17)	2.87 (±0.32)	2.89 (±0.14)	2.98 (±0.13)
Top 3 density	<i>Prismatolaimus</i>	<i>Plectus</i>	<i>Filenchus</i>	<i>Helicotylenchus</i>	<i>Mesodorylaimus</i>	<i>Filenchus</i>	<i>Ogma</i>
	<i>Mesocriconema</i>	<i>Mesocriconema</i>	<i>Helicotylenchus</i>	<i>Eucephalobus</i>	<i>Rotylenchus</i>	<i>Helicotylenchus</i>	<i>Eudorylaimus</i>
	<i>Eumonhystra</i>	<i>Tylenchorhynchus</i>	<i>Mesocriconema</i>	<i>Tylenchorhynchus</i>	<i>Mesocriconema</i>	<i>Alaimus</i>	<i>Eumonhystra</i>
Indicative genera (and indicator value)	<i>Bastiana</i> * (41)	<i>Teratocephalus</i> (78)		<i>Eucephalobus</i> (25)	<i>Mesodorylaimus</i> (33)		<i>Ogma</i> * (66)
	<i>Prismatolaimus</i> (28)	<i>Wilsonema</i> (75)					
MI	2.79 (±0.11)	<u>2.58 (±0.25)*</u> ↔	<u>3.22 (±0.37)*</u>	2.68 (±0.06)	3.24 (±0.46)	2.86 (±0.14)	2.84 (±0.23)
MI (2-5)	2.81 (±0.15)	<u>2.64 (±0.27)*</u> ↔	<u>3.25 (±0.38)*</u>	<u>2.71 (±0.03)*</u> ↔	<u>3.43 (±0.17)*</u>	2.94 (±0.14)	3.02 (±0.14)
Trophic groups %							
Bacteriovores	46.7 (±13.6)	52.8 (±16.8)	20.2 (±7.4)	30.9 (±15.7)	31.7 (±6.0)	36.2 (±4.6)	43.6 (±4.6)
fungal-feeders	1.0 (±1.7)	6.6 (±2.1)	1.9 (±2.5)	0.6 (±1.1)	15.9 (±1.6)	3.7 (±2.5)	1.7 (±2.1)
"plant associates"	4 (±1.7)	0 (±0)	29.5 (±8.3)	9.8 (±6.8)	8.2 (±0.4)	29.2 (±2.7)	11.6 (±2.3)
plant-parasites	35.7 (±16)	25.7 (±12.6)	29 (±4.8)	45.7 (±18.4)	24.5 (±12.1)	21.5 (±6.1)	28.7 (±2.6)
Omnivores	9.6 (±4.5)	6.2 (±1.6)	12.7 (±12.3)	10.3 (±2.8)	20.6 (±11.4)	9.1 (±1.0)	11.2 (±4.1)
Predators	3.0 (±1.0)	8.7 (±6.5)	6.7 (±4.6)	2.7 (±1.6)	9.1 (±8.5)	0.3 (±0.6)	3.3 (±4.1)
Cp-groups %							
cp-1	1.3 (±2.3)	3.2 (±1.8)	1.0 (±1.8)	1.4 (±2.4)	8.4 (±14.7)	4.0 (±0.22)	9.1 (±5.6)
cp-2	<u>44.4 (±8.3)*</u>	<u>56.4 (±9.2)*</u>	27.2 (±11.7)*	<u>60.4 (±5.7)*</u> ↔	<u>25.0 (±4.5)*</u>	<u>42.2 (±6.2)*</u> ↔	<u>29.1 (±4.2)*</u>
cp-3	<u>33.2 (±10.5)</u>	<u>19.4 (±4.8)</u>	<u>29.5 (±2.6)</u>	<u>9.3 (±9.02)</u>	<u>17.8 (±6.02)</u>	<u>22.6 (±2.0)</u>	<u>31.9 (±14.5)</u>
cp-4	16.4 (±4.3)	20.5 (±12.0)	32.9 (±20.5)	26.4 (±1.6)	31.7 (±8.1)	25.8 (±4.8)	28.3 (±5.3)
cp-5	4.7 (±1.9)	0.38 (±0.7)	9.4 (±5.91)	2.5 (±1.16)	17.0 (±7.55)	5.3 (±0.91)	1.5 (±1.3)
cp-(3-5)	<u>54.2 (±8.2)*</u>	<u>40.3 (±7.4)*</u> ↔	<u>71.8 (±13.4)*</u>	<u>38.2 (±6.7)*</u> ↔	<u>66.5 (±10.4)*</u>	<u>53.8 (±7.4)*</u>	<u>61.7 (±13.8)*</u>

Nematode abundance & diversity

The average nematode density ranged from 2971 ind./100cm³ (sludge site) to 3980 ind./100cm³ (municipal annex site), with no significant differences between the sites (Table 4). Disturbed sites were generally less diverse compared to the annex sites, but this difference could not be considered significant (p>0.05). The highest average total number of genera (25) and average value of the Shannon-Wiener diversity index (2.98) was recorded from the tar annex site. Sludge was characterized by the lowest total average number of genera (17) and the lowest (2.51) Shannon-Wiener diversity index. (Table 4; Fig. 4).

Feeding types, "colonizer-persister"-groups and MI

Generally, numbers of bacteriovores and plant-parasites are more than twice the numbers of predators, omnivores, fungal-feeders, and "plant associated nematodes" (=amalgam of tylenchid nematodes that feed on algae, lichens, mosses, epidermal cells or root hairs but of which the exact feeding behaviour is largely unknown). The dis-

turbed sites contained relatively fewer omnivores and predators as compared to the annex sites, however, differences were not significant (Table 4 and Fig. 5). There was also no significant correlation found between any pollutant and the composition of feeding types. Table 4 and Fig. 4 show the relative distribution of the different cp-groups. The disturbed sites contained significantly more colonizers of type cp 2 (p=0.0003) and less persisters (cp 3-5) (p=0.0022) compared to their annex site. Also a direct contrast of individual polluted sites in relation to their respective annex sites with respect to the distribution of the cp groups showed significant differences; sludge (p=0.006) and tar (p=0.049) had significantly more cp 2 colonizers, while municipal grass (0.049) and sludge (0.023) had significantly fewer cp 3-5 persisters. Furthermore, the cp 2/cp 3-5 nematodes were significantly positively/negatively correlated with both groups of intercorrelated metal correlations and PAK's content (r-values and p-values of individual correlations are not given because of the high intercorrelated structure of pollutants related to pollution).

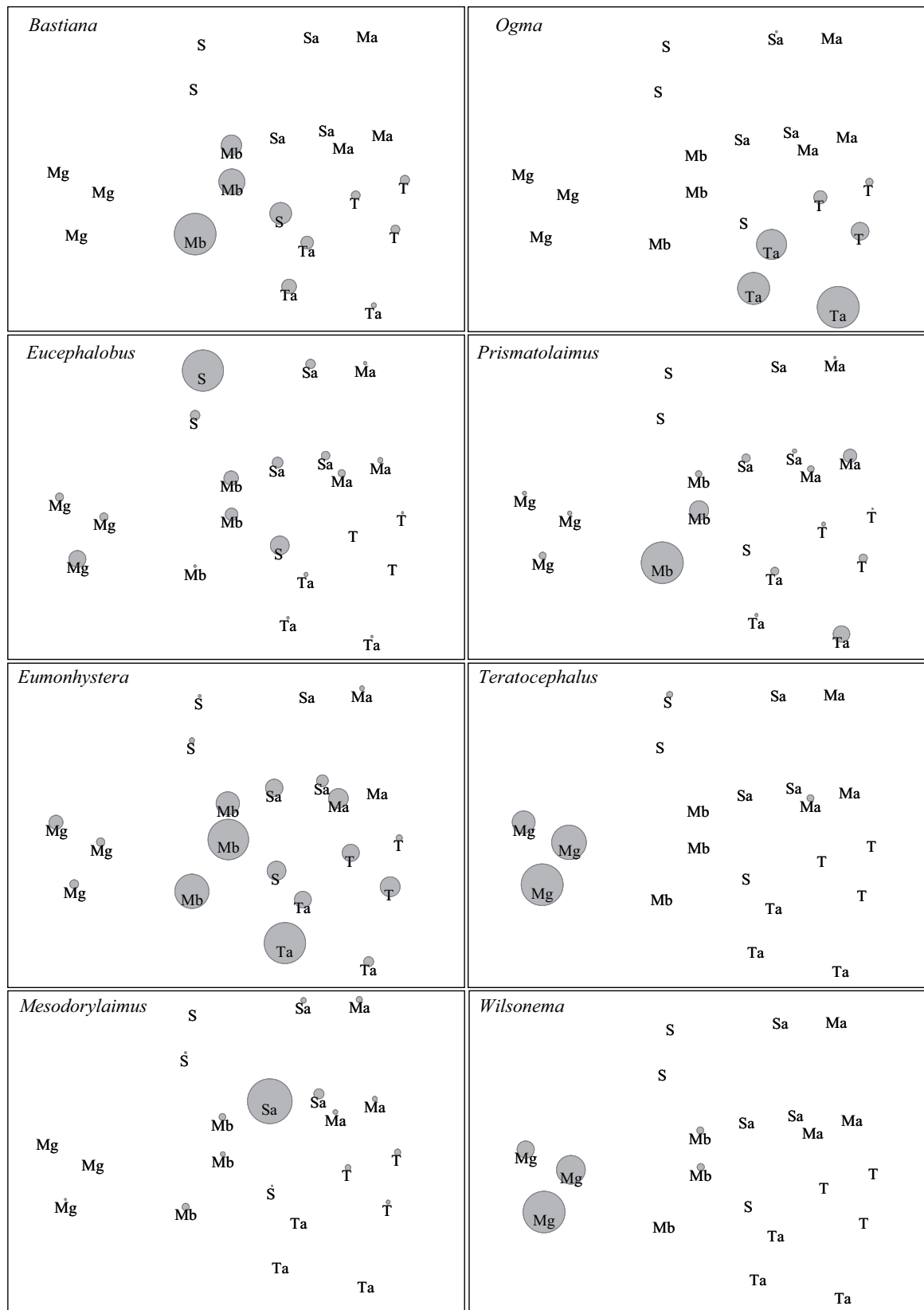


Fig. 3. – Indicative species for the seven sampling localities superimposed on Non-metric Multi Dimensional Scaling (MDS) ordination diagrams. Sizes of shaded circles reflect the relative magnitude of the superimposed variables. MDS output based on square-root-transformed genus abundances from seven sampled sites (three replicates) in the Bourgoyen-Ossemeersen (Ghent, Belgium). (Mb): Municipal bush, (Mg): Municipal grass, (Ma): Municipal annex, (S): Sludge, (Sa): Sludge annex, (T): Tar, (Ta): Tar annex.

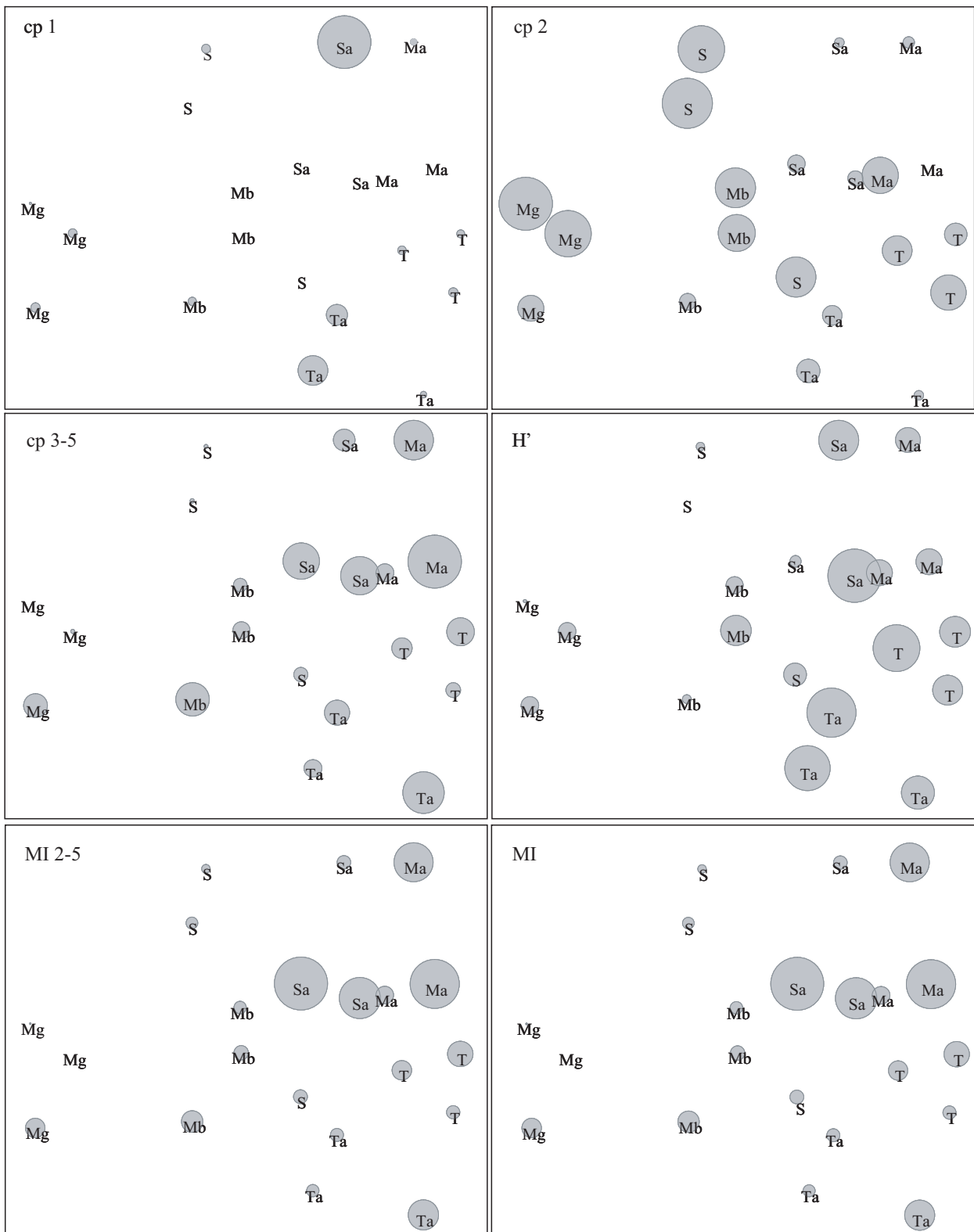


Fig. 4. – Nematode community characteristics (“colonizer-persister”-groups: cp 1, cp 2 and cp3-5; Shannon-Wiener diversity: h' ; Maturity Indexes: MI, MI2-5) superimposed on Non-metric Multi Dimensional Scaling (MDS) ordination diagrams. Sizes of shaded circles reflect the relative magnitude of the superimposed variables. MDS output based on square-root-transformed genus abundances from seven sampled sites (three replicates) in the Bourgoyen-Ossemeersen (Ghent, Belgium). (Mb): Municipal bush, (Mg): Municipal grass, (Ma): Municipal annex, (S): Sludge, (Sa): Sludge annex, (T): Tar, (Ta): Tar annex.

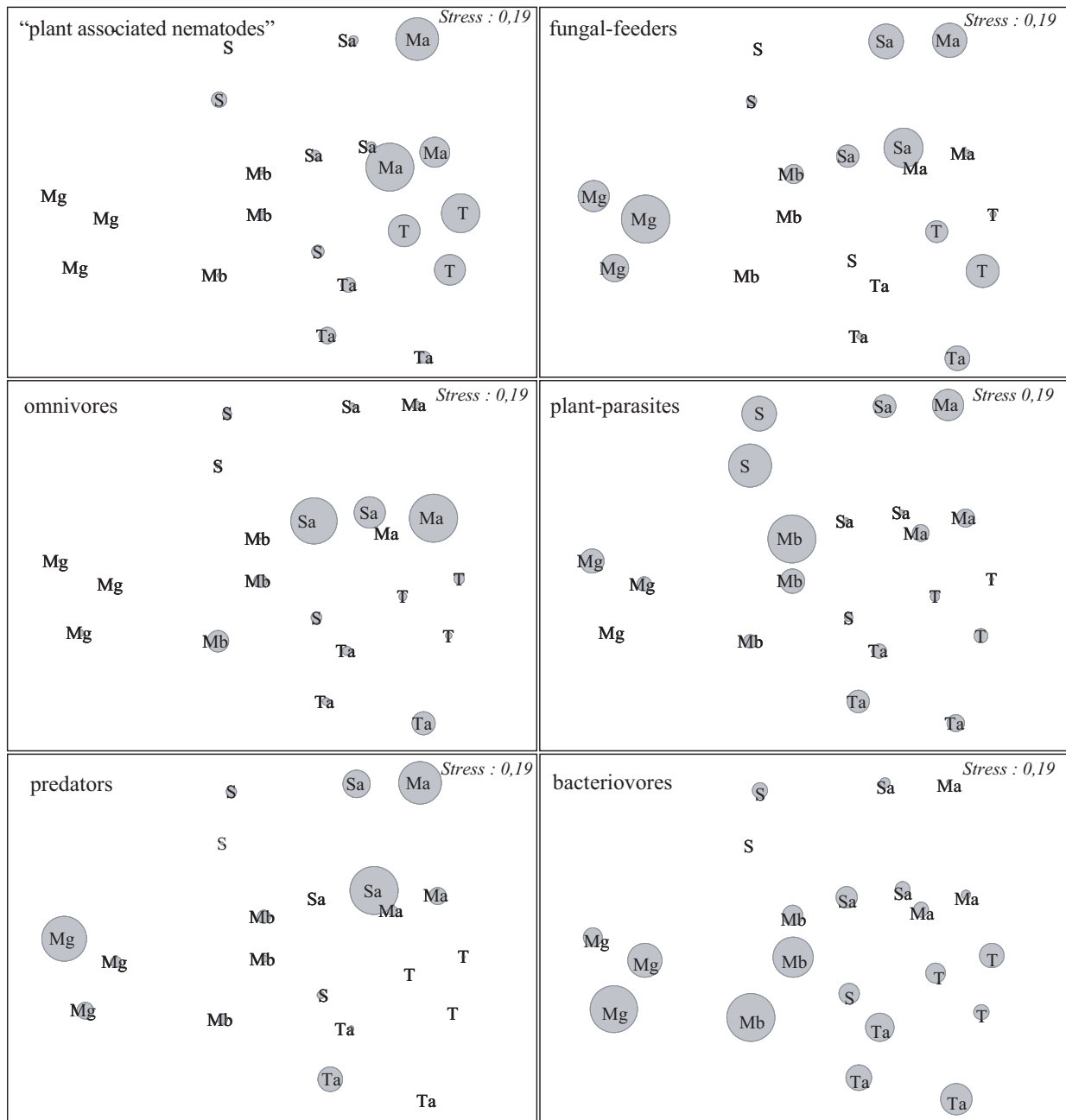


Fig. 5. – Feeding types (bacteriovores, plant-parasites, predators, omnivores, fungal-feeders, and “plant associated nematodes”) superimposed on Non-metric Multi Dimensional Scaling (MDS) ordination diagrams. Sizes of shaded circles reflect the relative magnitude of the superimposed variables. MDS output based on square-root-transformed genus abundances from seven sampled sites (three replicates) in the Bourgoyen-Ossemeersen (Ghent, Belgium). (Mb): Municipal bush, (Mg): Municipal grass, (Ma): Municipal annex, (S): Sludge, (Sa): Sludge annex, (T): Tar, (Ta): Tar annex.

The average maturity indices (MI/MI2-5) respectively ranged from 2.79/2.81 (Municipal bush) to 3.24/3.43 (Sludge annex) (Table 4; Fig. 4). The total average MI/MI2-5 were higher for the annex sites (3.10/3.24) compared with the polluted sites (2.74/2.79). However, only the MI/MI2-5 of the municipal grass were significantly higher compared to their respective annex site ($p=0.003/$

0.004); while for the sludge site differences in life strategy could only be significantly ($p=0.0007$) appointed with the MI2-5 index. Thus, differences related to historical pollution were slightly more pronounced for the MI2-5 values compared to the original MI (absolute differences as well as significance levels).

DISCUSSION

Genera: analyses and diversity

The total number of genera (63) found in this study is high for the latitude 50°-60° where the mean number of species is only 58 (BOAG & YEATES, 1998). In comparison, only 141 genera were recorded from 200 different locations in the Netherlands that were selected to cover the maximal habitat diversity (MULDER et al., 2005). Furthermore, in another period of the year, Manhout et al. (unpublished results) recovered a further 50 genera in our study area that were not recovered in this study. This further points to the remarkably high nematode diversity in the examined alluvial plain. In disparity to the known adverse effect of several heavy metals on nematode diversity (e.g. GEORGIEVA et al., 2002; YEATES et al., 2003), no relationship between pollution and decreasing nematode diversity could be discerned. Possibly, the historical nature of the pollution and time elapsed could have allowed a build-up of the nematofauna to a high diversity. On the other hand, several studies have shown that other, more recent, disturbances did not have a significant effect on nematode diversity (e.g.: urbanization: PAVAO-ZUCKERMAN & COLEMAN, 2007; agricultural management practices: PORAZINSKA et al., 1999). Furthermore, the underlying assumption that larger, more diverse assemblages reflect "more healthy" soils and are thus "desirable" is still under debate (YEATES, 2003). Especially there is no unambiguous evidence to support the view that diversity or complexity predictably affect the stability of ecosystem properties or processes (CRAGG & BARDGETT, 2001).

"Colonizer-persister"-groups and MI

Like other organisms, soil nematodes can be ranked along a gradient referring to their reproductive strategies, from larger (persistent) K-strategists adapted to stable environments because of their long life cycles, towards colonizing *r*-strategists that respond quickly to favourable conditions (BONGERS, 1990; BONGERS & FERRIS, 1999; FERRIS et al., 2001).

This study did not show any relation of enrichment opportunists (cp 1) and historical pollution. The cp 1 group consists of bacteriovores that grow rapidly upon an increase in microbial activity caused by an organic input either biological or anthropogenic (WASILEWSKA & BIENKOWSKI, 1985; FERRIS et al., 1996; YEATES et al., 1997). The cp 1 group can thus show very different sensitivities and reactions to disturbances and therefore are of limited use as bioindicators (GEORGIEVA et al., 2002).

Conversely, the contribution of colonizers of type cp 2 and persisters (cp 3-5) was significantly different between the polluted sites and their annex site. Regarding comparisons of individual sites, the significant augmentation of cp 2 nematodes in the tar samples is especially informative. This because explorative analyses of the environmental variables (irregular scattering of environmental variables of the municipal sites) and the communities (sludge community not significantly different from other sites) reveals that the insight in the relation of the nematode community and pollution within a single site can be best explored for the tar samples. A higher abundance of

general opportunists (cp 2) is an indication of pollution-induced stress (KORTHALS, 1997; BONGERS & FERRIS, 1999). Especially the Cephalobidae are known to increase in proportion as a result of resource limitation such as heavy metal addition (YEATES, 2003). Conversely, omnivores and predators (mostly K-strategist mononchs and dorylaids from the cp 3-5 groups) are well known to be negatively related to pollution or disturbance. Their abundance is negatively affected by heavy metals, especially Pb, Zn and Cu (BARDGETT et al., 1994; YEATES et al., 1994; BAKONYI et al., 2003; GEORGIEVA et al., 2002; NAGY et al., 2004) or by a mixture of pollutants (WRIGHT & COLEMAN, 1988; RUESS et al., 1993; WASILEWSKA, 1996; GEORGIEVA et al., 2002). Thus taxa with higher cp values (3-5) indicate a more stable undisturbed ecosystem (RUESS et al., 1993; KORTHALS et al., 1996). Indirect evidence of a relation between historical pollution and a shift in the relative contribution of the cp 2/cp 3-5 nematodes is corroborated with significant positive/negative correlations with both groups of intercorrelated metal correlations and PAK's content.

Thus, this study showed significant relations between the relative contribution of colonizer/persister and historical pollution, though only for the relative contribution of cp 2 and cp 3-5 nematodes and not for cp 1 nematodes. Logically, omitting the cp 1 group from the MI (=MI2-5) (KORTHALS, 1997) should better reflect (historical) pollution-induced community changes. The average MI and MI2-5 are clearly lower for the polluted sites compared to the annex sites (2.74/2.79 vs. 3.10/3.23) but significance levels are not met because of the relatively high variation within a limited number of replicates. Differences between the minimum and maximum MI/MI2-5 (2.42/2.48 in historically disturbed site vs. 3.63/3.63 in annex site) is also not pronounced as compared to several other MI studies. For example, according to BONGERS & FERRIS (1999) the value of the MI varies from below 2 (for nutrient enriched sites) to about 4 in undisturbed sites. Since the disturbed sites contained significantly more colonizers of type cp 2 and less persisters (cp 3-5) the difference between MI2-5 values is on average more pronounced than the MI range, despite the fact that the latter is composed of a wider array of cp-groups. Furthermore, some differences in life strategy could only be significantly appointed with the MI2-5 index and not with the "overall" MI (see e.g. the sludge analyses).

Critical evaluation of the obtained nematode community characteristics

The current study did not show any significant relation between historical pollution and feeding type composition and the Shannon-Wiener diversity. Only limited, but significant effects were observed on life-strategy-related parameters (cp-groups, MI indexes). Although nematodes are known to reflect long term effects (NAGY, 1999; NAGY et al., 2004; GEORGIEVA et al., 2002; YEATES et al., 2003; BAKONYI et al., 2003), it is experimentally indicated that the nematode assemblages as represented in c-p groups can partly recover after a few years (BAKONYI et al., 2003). Nevertheless, despite nematodes in the current study having had possibly more than thirty years to adapt physiologically and genetically to the contaminants, puta-

tive remaining effects on the community structure were still observed. The supposed relations are here merely based on assessing nematode assemblages as a whole based on genus or family level. But, it has been suggested that several ecological insights should be investigated by addressing diversity within individual functional groups, and species-level identification can provide pivotal information (YEATES, 2003). Furthermore, despite the fact that the nematode community structure is generally acknowledged as an excellent bio-indicator (BONGERS & FERRIS, 1999), future research challenges should include other biological data in order to fully understand the effects of historical pollution.

In the current study there were considerable differences between the replicates, which definitely reduced the statistical power and complicated interpretation of the results. Albeit the analyses were based on bulk samples, compounded of a mixture of 15 cores, several replicates of a single sampling spot were remarkably different. The observed large dissimilarity suggests high spatial differences that are possibly related to high micro-habitat variability. The latter presumably partly explains the observed remarkably high generic variability in a geographically small sampling area. However, patchiness and micro-habitat variability have rarely been considered in ecotoxicological investigations although such variability is certainly not a negligible factor in the interpretation of the results. Insufficient insight into the micro-habitat variability in the current study could possibly have led to the flawed community structure pattern within the sludge samples, and the environmental variables structure within the municipal samples. In order to effectively cope with a high species and habitat diversity, the use of comprehensive mathematical models to obtain the appropriate sample size is possibly the way forward. Such approaches are already common practice in agro-nematology (for an overview, see BEEN & SCHOMAKER, 2006). Thus, future sampling could include systematically more and smaller cores to obtain a bulk sample of a certain optimal size (see BEEN & SCHOMAKER, 2006).

Finally, the possibility of differences in bio-availability and measured pollution content (KORTHALS et al., 1996) dictates caution in interpretations, especially for the current study, which deals with substances that were deposited a long time ago. Biological effects are due to the activity of heavy metals in solution; any bound, inert inactive metal components will not have a direct effect on soil processes. Furthermore, buffered conditions in soil differ markedly from *in vitro* conditions. Thus, although some significant relations between the nematode community and historical pollution were observed in the current study, any assessment of the direct impact of heavy metals, and extension to all pollutants, should be based on their activity rather than total concentration (e.g. YEATES et al., 2003). Use of the cotton strip decomposition method (YEATES et al., 1994; LATTER et al., 1998) is proposed as an example of such an effect-based measurement.

Thus, studies such as the present investigation do not permit elucidation of causal relationships between nematodes and environmental factors. The observed significant relationships between historical pollution and nematode

life strategy characteristics remain to be further analysed with more experimental approaches, though, the obvious constraints associated with studies that cover such a long time-span remain a restricting factor.

ACKNOWLEDGMENTS

The research was conducted on behalf of the Belgian State, Services of the Prime Minister, Federal Office for Scientific, Technical and Cultural Affairs, for the project Nema-biosensor n° MO/36/007. We would like to thank Rita Vandriessche for extracting and mounting all the nematodes and Carl Vangestel for statistical advice. Two anonymous reviewers are acknowledged for their comprehensive remarks.

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Received: October 26, 2005

Accepted: October 9, 2008