On-farm impact of cattle slurry manure management on biological soil quality

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Abstract

The effects of dairy cattle slurry management on soil biota, soil respiration and nitrogen (N) mineralization were evaluated in a farm trial across 12 farms and a field experiment on 2 farms located in a dairy farming area in the north of the Netherlands. The slurry management consisted of slit injection or surface application of slurry; the use or no use of additives [Euromestmix® (MX) and Effective Microbes® (EM)] and the type and level of inorganic N fertilization. Slit injection negatively affected epigeic earthworms whereas its effect on anecic and endogeic earthworms was absent or even positive. Enchytraeids were not affected in a consistent way, whereas numbers of nematodes indicative of nutrient-enriched conditions increased. Inorganic N fertilizer had similar effects. Bacterial diversity was not different among the treatments. Nitrifier diversity, however, was high at one of the farms in the field experiment, and was negatively affected by inorganic N fertilizer. The use of MX was usually associated with higher numbers of earthworms. EM affected numbers of earthworms and numbers of bacterial and plant-feeding nematodes, but only in specific combinations of field history, slurry type and slurry application method. We found no effects of EM on the composition of the microbial community. Soil respiration was increased when slurry was surface-applied. The calculated N mineralization by earthworms was in the order of 70–200 kg N ha^{-1} $year^{-1}$. It was highest under farm-characteristic surface application of slurry with MX and lowest under farm-characteristic slit injection of slurry without additives. Compared with the N mineralization by earthworms, that by enchytraeids and nematodes was quantitatively insignificant. Negative treatment effects on earthworms led to corresponding reductions in calculated N mineralization.

Additional keywords: dairy farming, grassland, fertilization, additives, soil fauna, earthworms, enchytraeids, nematodes, microbial diversity

Introduction

Dairy farming in the Netherlands is very intensive. Usually more than 400 kg nitrogen (N) ha^{-1} $year^{-1}$ is applied as inorganic fertilizer. Such high inputs result in N losses to the environment, which are no longer acceptable. So, farmers reduced their inorganic fertilizer application rates and, besides, more and more relied on nutrients mineralized from manure instead of inorganic fertilizers. However, also the application of manure can result in large N losses to the environment, especially to the atmosphere (Neeteson, 2000). Therefore, Dutch farmers are now obliged to inject their cattle slurry manure into the soil to reduce NH_3 emissions to the atmosphere. This in spite of reports that this practice may increase the production of the greenhouse gases N_2O and CH_4 (Ellis *et al.*, 1998; Flessa & Bees, 2000), the latter especially at high groundwater tables (Van Den Pol-Van Dasselaar *et al.*, 1999).

In the northern Frisian Woodlands in the Netherlands farmers add Euromestmix® (MX) to the slurry, and claim that surface application of the slurry does not result in increased N-losses in the form of NH_3, and is less damaging to the soil biota and soil biological processes. MX contains clay minerals that might bind toxic substances and may make N ions in the slurry less reactive. Other farmers in the region apply Effective Microbes® (EM) onto the soil surface. EM (Higa, 1998) is a mix of lactic acid bacteria, photosynthetic bacteria, actinomycetes, yeasts and other micro-organisms, which supposedly lead to enhancement of microbial diversity in soil.

The objective of this study was to evaluate the effects of surface-applied versus slit-injected cattle slurry manure and of slurry manure with versus slurry manure without MX or EM on soil biota, soil respiration and N mineralization. In an across-farm comparison 12 farms were sampled, which included the following three treatments: slit injection of slurry manure with or without surface spreading of EM, and surface application of MX-slurry without surface spreading of EM. We could not find farmers who applied MX in slurry below the soil surface. To make up for this confounding effect we did a field experiment where we compared a MX-slurry with a control-slurry, both applied onto the surface. Absolute reduction of N addition and increasing the share of organically bound N has revived the discussion about the supposedly beneficial effects of cattle slurry manure on soil biota and biological processes. In the experiment we therefore included the treatments 'inorganic N fertilizer only', 'cattle slurry manure only' and 'inorganic fertilizer plus slurry manure'.

Our general hypotheses were:

1. Slit injection reduces the number of soil fauna elements, in particular in the upper 20 cm of the soil. The assumption is that slurry manure has a high osmotic value and electric conductivity, which are considered damaging for most of the soil fauna. Moreover, making injection slits might result in physical damage to the soil fauna, especially earthworms.
2. Inorganic N fertilizer reduces the abundance of soil fauna for the same reason.
3. Slit injection affects the relative abundance of soil organisms in favour of indicators of disturbance and nutrient enrichment.
4. Inorganic N fertilizer has a similar effect.
5. Slit injection negatively affects soil respiration and N mineralization. The assump-

tion here is that this lowers the O_2 concentration, which together with the reduction of the density of soil fauna will inhibit biological soil processes.
6. Euromestmix® reduces the negative effects of slurry on the soil biota and the biological processes on the assumption that it (temporarily) binds NH_4^+. The assumption here is that MX will reduce the reactivity of mineral N compounds in the slurry. More specifically, MX will result in changes in the soil community towards groups that indicate a more gradual mineralization of nutrients, supposedly associated with a higher efficiency of nutrient use.
7. Effective Microbes® will *not* have any effect. The assumption here is that the microorganisms in the suspension will not be able to compete with those in the slurry and the soil.

Materials and methods

Across-farm comparisons

Sixty farms were selected in the northern Frisian Woodlands. Starting in 1995, 20 of them added Euromestmix® (Europlant BV, Appelscha, The Netherlands) to their slurry (2 kg MX m^{-3}), which is applied onto the surface. Another 20 farms, starting in 1998, applied Effective Microbes® (Agriton, Noordwolde Fr, The Netherlands) onto the soil surface (three times during the growing season). EM is applied at a rate of 2 l EM ha^{-1} $year^{-1}$ in the form of a 100× diluted suspension of 1 l EM : 1 l molasses : 8 l tap water, which was incubated for 5 days before slit-injected with the slurry manure. The remaining 20 farms served as a control (CO) group: on these farms no MX or EM was used and slurry manure was slit-injected. Out of each of the three groups four farms were selected that showed as much as possible the same range in other farm(ing) characteristics (Table 1). However, sometimes differences between the groups of selected farms were unavoidable but none of them appeared to be statistically significant ($P \leq 0.05$). The most important differences were as follows:
1. On average the percentage soil organic matter was about 1 percent unit lower on the EM farms (7.9 versus 8.8).
2. Groundwater table class V was not represented among the MX farms (only VI and VII).
3. The soil mineral-N content (0–5 cm) in early spring was approximately 40 kg ha^{-1} lower for MX farms than for EM and CO farms.
4. The potential nitrifying activity (NLV) of the soil showed an average increasing trend from 24 kg N ha^{-1} at EM farms, via 49 kg N ha^{-1} at MX farms to 74 kg N ha^{-1} at CO farms.

On 28 and 29 September 1998 soil samples were taken from one representative field on each of the 12 farms.

Field experiment

The field experiment was laid out in spring 1999 on two farms – Drogeham and

Table 1. Soil characteristics (0–5 cm) and annual N fertilizer rates for the 12 selected dairy farms of the across-farm comparison.

Farm	Treatment[1]	pH-KCl	OM[2]	GWT[3]	Loam class[4]	N_{min}[5]	NLV[6]	Annual N fert. rate[7]	
								Inorganic fertilizer	Slurry manure
			(%)			- - - - - - - - - - (kg N ha^{-1}) - - - - - - - - - -			
Mts. Atsma	MX	5.5	7.6	VI	1	84.8	33.7	230	221
Hansma	MX	5.3	10.3	VI	1	93.0	56.9	304	194
Mts. Hoeksma	MX	5.2	9.5	VII	1	63.0	40.0	193	138
Mts. Postmus	MX	4.2	8.2	VI	0	83.2	65.5	226	219
P. de Jong	C	5.4	7.5	V	1	81.0	42.7	350	115
S. Sikkema	C	5.3	9.6	VII	0	119.4	65.7	348	230
Mts. V/D Veen	C	5.4	8.1	V	0	77.2	53.1	148	317
B. V/D Wal	C	4.8	9.7	V	1	198.3	135.9	256	162
F. Benedictus	EM	6.1	8.1	VI	0	103.2	69.2	0	242
F. Nijboer	EM	6.4	6.7	Vb	1	58.8	15.4	337	270
R. Reinders	EM	4.4	8.4	VI	0	225.9	2.3	385	154
Mts. Veenstra	EM	5.1	8.3	Vb	1	101.3	55.5	280	161
Mean	MX		8.9			81.0	49.0	238	193
	C		8.7			119.0	74.4	276	206
	ME		7.9			122.3	24.4	334[8]	207

[1] MX = Euromestmix® (applied since 1995 except for Mts. Hoeksma, which started application in 1985)
EM = Effective Microbes® (applied since 1998).
C = control (no additives).

[2] OM = soil organic matter.

[3] GWT = groundwater table class.

[4] Loam class: 0 = < 25% loam; 1 = > 25% loam.

[5] N_{min} = $N\text{-}NH_4^+$ + $N\text{-}NO_3^-$.

[6] NLV = potential nitrifying activity.

[7] Fertilizers were applied 3–5 times throughout the growing season.

[8] Excluding the biological farm of F. Benedictus.

Harkema – lying 2.5 km apart. The trial was of a split plot design with two replications per farm and fertilizer-N level (low and high) as the main factor. 'Low' = 76 kg N ha^{-1} $year^{-1}$ (cattle slurry manure, no inorganic fertilizer) and 'high' = 258 kg N ha^{-1} $year^{-1}$ (76 kg N ha^{-1} $year^{-1}$ from slurry manure + 182 kg N ha^{-1} $year^{-1}$ from inorganic fertilizer). At each level of N the following treatments were compared:

– 2 types of slurry manure: slurry with or slurry without MX. [At Drogeham MX had been applied since 1981. Since then soil organic matter content had steadily

Table 2. Soil physical characteristics of the experimental fields at Drogeham and Harkema in 2001.

Location	Soil depth	Bulk density	pH-H_2O	VPW[1] at:		Texture[2]		
				10 kPa	124 kPa	< 2 µm	2–50 µm	≥ 50 µm
	(cm)	(g cm^{-3})		(%)				
Drogeham	0–5	1.02a[3]	5.41ab	46.3d	14.2d	3.6a	15.9b	80.5a
	5–10	1.28c	5.55bc	42.0c	12.7c			
	10–15	1.37d	5.72c	36.8b	10.7b	3.7a	16.0b	80.3a
	15–20	1.41def	5.87c	34.4b	9.3a			
Harkema	0–5	1.13b	5.30a	42.7c	11.3b	3.6a	13.4a	83.1b
	5–10	1.39de	5.26a	36.7b	10.8b			
	10–15	1.46f	5.38ab	30.0a	9.1a	3.6a	13.5a	83.0b
	15–20	1.44ef	5.24b	28.0a	8.6a			

[1] VPW = % water v/v.

[2] The texture data are for 0–10 cm and 10–20 cm soil depth.

[3] The means in a column, followed by the same letter are not statistically different ($P < 0.05$).

increased from about 8% in 1981 to 12.5% 10 years later (Eshuis *et al.,* 2001). At Harkema no MX had been used before the trial started.]

– 2 levels of the additive EM: no application of EM or application of EM three times during the growing season with a total dose of 2 l EM ha^{-1} $year^{-1}$. The treatments that received no EM were sprayed with tap water instead.
– 2 methods of slurry manure application: slit injection (5 cm deep) and surface application.

Furthermore, on each farm there were 'control' treatments that did not receive any organic fertilizer but only 0, 76 or 258 kg inorganic fertilizer-N ha^{-1} $year^{-1}$. The number of replicates per farm for these controls was 4, 2, and 2, respectively, resulting in a total of 80 experimental plots.

Physical soil characteristics are given in Table 2. For chemical soil characteristics and a schematic outline of the field experiment see Schils & Kok (2003).

On 20 June, hereafter referred to as 'summer', and on 31 October and 6 November 2000, hereafter referred to as 'autumn'– after mowing and before fertilization – the plots were sampled for earthworms, enchytraeids, nematodes, bacteria and soil respiration. The samples were transferred to the laboratory and stored at 7 °C until further use. Because of the Foot and Mouth disease crisis in 2001 an envisaged spring sampling in 2001 had to be cancelled. On 20 June, at the end of a dry and warm period, it was exceptionally warm (> 30 °C). This means that this sampling date may be considered representative for the aestivation period of the earthworms, whereas the sampling in autumn may be considered representative for a high-activity period.

Biological analyses

Earthworms

Earthworms were sampled from 6 cores (L × W × D = 20 × 20 × 20 cm) per field in the across-farm comparison and from 1 core per plot in the field experiment. The earthworms were transferred to the laboratory, hand-sorted, counted, weighed, and fixed in formaldehyde 4% until identification (across-farm comparison and autumn samples of the field experiment) of adults. Adults were identified to species. The juveniles were sorted in two groups: tanylobous, including all Lumbricidae, and non-tanylobous (all other specimens). Numbers and biomass were recalculated per m^2. Following Bouché (1977) a distinction was made between (1) epigeic species (pigmented, living superficially in the litter layer, little burrowing activity), (2) endogeic species (living in horizontal burrows at approximately 10–15 cm depth) and (3) anecic species (relatively large worms, living in vertical burrows from which they collect dead organic matter on the surface at night).

Enchytraeids

Enchytraeids were sampled from the top 15 cm (across-farm comparison) or 10 cm (field experiment) in 6 cores per plot, using a cylindrical auger with an inside diameter of 58 mm. For the across-farm comparison each soil core was divided into 6 layers of 2.5 cm from which the enchytraeids were extracted using the O'Connor method (O'Connor, 1955). The extraction proceeded at 20 °C for 30 minutes, after which the temperature was gradually raised to 50 °C over a period of 150 minutes. The 6 cores per plot of the field experiment were mixed and the enchytraeids were extracted from 2 subsamples of 180 g fresh weight as described for the across-farm experiment. The numbers were expressed per m^2.

Nematodes

Nematodes were sampled from the top 10 cm in 50 cores per field in the across-farm comparison and in 20 cores per plot in the field experiment, using a cylindrical auger with an inside diameter of 23 mm. In the laboratory each group of cores was mixed and from a subsample of 100 g the nematodes were extracted using an Oostenbrink elutriator (Oostenbrink, 1960). The nematodes were counted, fixed in hot formaldehyde 4%, and from each sample at least 100–200 randomly selected nematodes were identified to genus and, whenever possible, to species. Nematodes were classified in feeding groups following Yeates *et al.* (1993) and colonizer-persister (CP) groups following Bongers (1990). The Maturity Index was calculated following Bongers (1990) and the Structure, Enrichment and Channel Indices following Ferris *et al.* (2001). In Appendix 1 an explanation of these nematode community indices is given. Numbers were recalculated per 100 g fresh soil. In addition to the 12 farms in the across-farm comparison three more farms were selected (one within each group) where nematodes samples were collected.

Bacterial diversity

Bacterial diversity was investigated in the field experiment in soil samples from the

following treatments: MX, MX + EM, slurry only and slurry + EM – which had received 76 kg N ha^{-1} $year^{-1}$ – and from the control plots receiving 0 and 76 kg N ha^{-1} $year^{-1}$ from inorganic fertlizer only. Only the slit-injection treatments were analysed. The same soil as sampled for nematode analysis in summer was used. DNA was isolated from 0.5 g fresh soil using a DNA kit (Bio 101 Systems: Fast DNA Spin kit; Q-Bio-gene, Omnilabo, Breda, The Netherlands). The V6-V8 variable part of the 16S rDNA was amplified *in vitro* by polymerase chain reaction (PCR), using universal primers to amplify 16S rDNA of all bacteria and nitrifier primers to amplify the 16S rDNA of nitrifying bacteria only. DNA fragments were separated using Denaturing Gradient Gel Electrophoresis (DGGE). The similarity between the DGGE profiles was determined by calculating a band similarity coefficient (SD) (Dice: SD = 2nAB/(nA+nB), where A is the number of DGGE bands in lane 1, B represents the number of DGGE bands in lane 2, and nAB is the number of common DGGE bands (Murray *et al.*, 1996; Gillan *et al.*, 1998; Simpson *et al.*, 1999, 2000). The undiluted EM suspension was likewise analysed.

Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi were investigated on all farms. Because no differences were found between treatments, the results are not reported here.

Soil respiration

The effects on soil respiration were investigated in the field experiment only. From each of the samples used for nematode analysis 35–40 g fresh soil was weighed in an incubation bottle and brought to water holding capacity to ensure that every bottle contained the equivalent of exactly 30 g dry soil. The air-tight bottles were incubated at 25 °C for 14 days, flushed on days 7 and 13, and the CO_2 concentration measured on day 14 using a CO_2 analyser (Innova Multi-gas monitor type 1302, Bruel and Kjaer, Denmark). CO_2 production was recalculated to µl CO_2 per g dry soil per hour.

Statistical analyses

The data from the across-farm experiment were analysed using SPSS version 8.0 for Analysis of Variance. All differences among farms contribute to the variance in the data. In addition, soil biological data usually show a high natural variability, whereas sample size had to be small (only 4 farms per treatment, or 5 for nematodes) because of the laboriousness of the research. Therefore, we used two levels of significance in statistical tests: $P \leq 0.10$ and $P \leq 0.05$.

The field experiment was analysed with GRM and MANOVA using STATISTICA (data analysis software system, version 6, StatSoft, Inc.). The effects of the 4 treatments (type of slurry, slurry application method, EM spreading and inorganic fertilizer rate) were analysed separately for each location and sampling date. Treatments were included as categorical predictors, except for inorganic fertilizer rate, which was included as a continuous predictor. For the categorical predictors all interactions were tested. Significant (interactions between) predictors were selected using a forward

stepwise model that used a $P \leq 0.10$ to enter or remove predictors from the model. In addition, to investigate effects of deviations from the farm-characteristic management, for each location and sampling date a new predictor variable (CHANGE) was calculated based on the four treatments mentioned above. CHANGE was 0 if the combination of treatments in the field experiment was identical to the farm-characteristic management at that specific location, and was 1, 2, 3 or 4 if 1, 2, 3 or all 4 treatments differed from the farm-characteristic management. The predictor variable CHANGE was tested with GRM including location and date of sampling as categorical predictors and CHANGE as continuous predictor, following the procedure as described above. Post-hoc tests (Tukey HSD test) were performed to test for statistically significant differences between individual pairs. In the tables and text only statistically significant ($P \leq 0.05$; if $P \leq 0.10$ this is indicated in the text) main or interaction effects are presented. Consequently, the data presented in the tables and figures often comprise combinations of treatments that by themselves did not result in such statistically significant effects.

Production-ecological calculations

For the field experiment we calculated the potential monthly and annual contribution of the soil fauna groups to N mineralization following Didden *et al.* (1994). The parameters used are listed in Appendix 2.

Because of the outbreak of Foot and Mouth disease no observations could be made in spring and data are available for summer and autumn only. As a result we estimated the monthly densities of soil fauna groups as follows: January/February (as in summer, hibernation), March–May (half of the densities as measured in autumn), June/July (as in summer, aestivation), August–November (linear increase to densities as measured in autumn), December (as in August). These trends in seasonal densities were based on data presented by Edwards & Bohlen (1996) for *Aporrectodea caliginosa,* which was the most abundant species in the present research, and on earthworm densities in a selection of fields at Drogeham that were sampled in March, April and May 2002. The C/N ratio of the detritus was calculated based on measurements given by Schils & Kok (2003) for soil and slurry manure, assuming a soil organic matter:slurry manure ratio of 1:1 in the soil fauna diet.

Results

Across-farm comparison

Earthworms

Six species of earthworms were found throughout the 12 farms: 1 epigeic species *(Lumbricus rubellus)*, 2 anecic species *(L. terrestris* and *Aporrectodea longa)* and 3 endogeic species *(A. caliginosa, A. rosea* and *Allolobophora chlorotica)*.

The results on *slurry application method* are given in Table 3. Total earthworm numbers were higher with slit injection than with surface-applied slurry. With slit

Table 3. Effect of cattle slurry manure-application method on total numbers (m^{-2}) of earthworms of different ecological groups (adults only).

Ecological group	Slurry-application method		Significance of difference[1]
	Surface	Slit injection	
Epigeic	23	9	*
Endogenic	135	174	ns
Anecic	6	20	*
Total	478	642	*

[1] * = statistically different ($P \leq 0.05$); ns = no statistical difference.

Table 4. Effect of adding Euromestmix® (MX) and Effective Microbes® (EM) to cattle slurry manure on total numbers (m^{-2}) of earthworms of different ecological groups (adults only).

Ecological group	Slurry	Slurry + MX	Slurry + EM
Epigeic	8a[1]	25b	12a
Endogenic	155a	143a	175a
Anecic	18a	6a	18a
Total	672b	478a	572ab

[1] Means in the same row, followed by the same letter are not statistically different ($P \leq 0.10$).

injection higher numbers of adult anecic earthworms and lower numbers of adult epigeic earthworms were found. No differences were found for adult endogeic earthworms, which were the most abundant.

The results on *additives* are given in Table 4. Total numbers were significantly lower with slurry + MX than with slurry without additives. Higher numbers of adult epigeic earthworms and a tendency towards lower numbers of adult anecic earthworms were found with slurry + MX than with slurry without additives. With EM, results were intermediate.

Enchytraeids

As to *method of slurry application*, the numbers of enchytraeids in the top 15 cm were higher for the combination surface-applied slurry + MX than for the control ($0.05 < P < 0.10$). This was largely accounted for by the numbers in the layer 0–2.5 cm ($P = 0.008$). EM did not have a statistically significant effect (Figure 1).

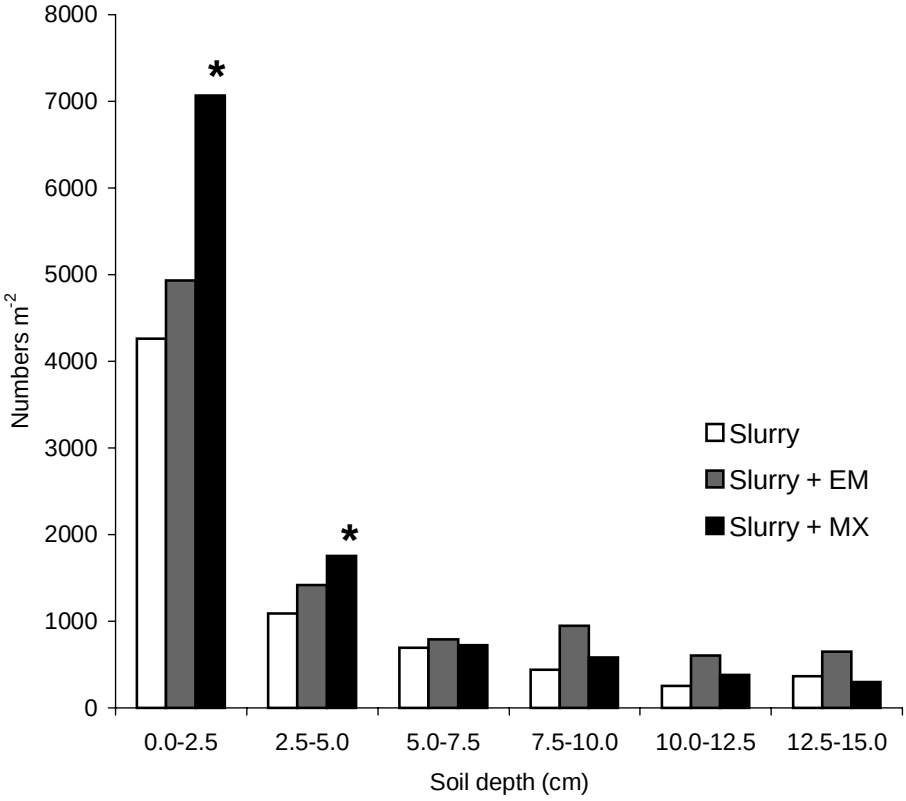

Figure 1. Total numbers of enchytraeid (m^{-2}) per soil layer in fields that were fertilized with cattle slurry manure plus Euromestmix® (MX), slurry manure plus Effective Microbes® (EM), or slurry manure only. * = statistically different ($P \leq 0.05$) from the slurry-only treatment.

Nematodes

Identification to the most detailed level possible yielded 84 taxa of nematodes (species, genera and families). Additives had an effect on the enrichment opportunistic nematodes, i.e., nematodes characteristic for eutrophic, fertilized conditions: their numbers were lower with surface-applied slurry + MX than when slit injection was used without additives ($P < 0.10$). Consequently, for the fields where slurry + MX had been surface-applied the Enrichment Indices (IE) were lower (60–65%) too (Figure 2). Among the fields where no additives had been used there were two exceptions (lowest two closed dots in Figure 2), both of which had received a relatively low level of inorganic fertilizer.

Figure 2 also includes data for the nematode fauna of 36 grasslands on sandy soils located elsewhere throughout the Netherlands (Van Der Waarde, 2002). The five fields with an EI < 50% were unfertilized grasslands that were managed as nature reserves, whereas all other grasslands were part of conventionally fertilized dairy farms.

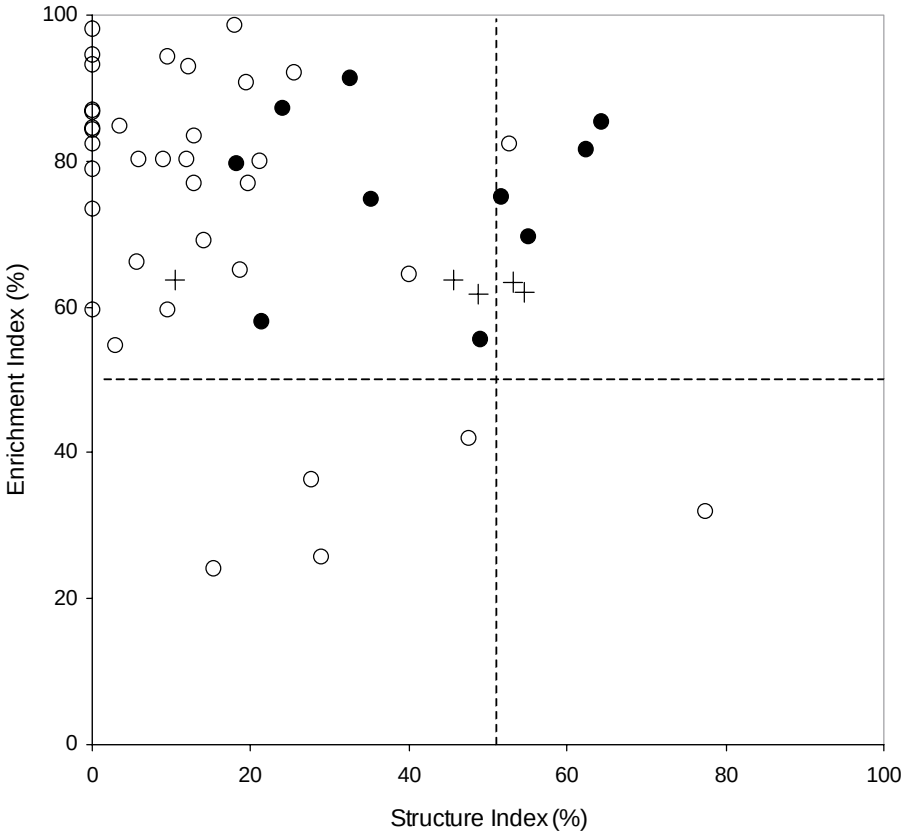

Figure 2. Nematode Structure Index and Enrichment Index for the fields of the across-farm comparison that were fertilized with surface-applied cattle slurry manure plus Euromestmix® (+) or that were slit-injected with slurry manure without Euromestmix® (closed circles). The open circles refer to data from Van Der Waarde *et al.* (2002) for 36 Dutch grasslands on loam-poor sandy soils.

Field experiment on the two farms

Earthworms

In the field experiment at Drogeham and Harkema eight species of earthworms were found, six of which occurred on both farms. The results on earthworms are given in Table 5. At Drogeham total numbers and numbers of adults in each of the ecological groups were higher than at Harkema.

At Harkema deviation from the farm-characteristic management resulted in a significant increase in earthworm numbers, especially in autumn. Numbers then increased from 463 m^{-2} in treatments with the Harkema farm-characteristic management to 1275 m^{-2} in treatments with surface-applied MX-slurry, use of EM, or no inorganic fertilizer (Figure 3a). Such a clear response of total earthworm numbers was not observed at Drogeham (Figure 3b), although deviation from the farm-characteristic

Table 5. Total numbers (m^{-2}) of earthworms per season and per ecological group (adults only) and frequency of occurrence of species present in autumn in 40 experimental plots at Drogeham and Harkema.

	Drogeham	Harkema	Significance[1] of difference
Numbers m^{-2}			
Total in summer	142	59	**
Total in autumn	926	830	*
Epigeic	14	8	*
Endogeic	161	23	**
Anecic	7	5	ns
Frequency in autumn (%)			
Epigeic:			
Lumbricus castaneus	18	0	
L. rubellus	28	25	
Endogeic:			
Allolobophora chlorotica	8	13	
Aporrectodea caliginosa	95	20	
A. limicola	0	20	
A. rosea	48	5	
Anecic:			
L. terrestris	13	10	
A. longa	15	8	

[1] * = statistically different at $P \leq 0.10$; ** = statistically different at $P \leq 0.05$; ns = no statistical difference.

management resulted in a slight decrease of earthworm numbers in summer (data not shown). At Drogeham the numbers of endogeic adults, however, increased from 75 to 200–222 m^{-2} in treatments with a management that deviated most from the Drogeham farm-characteristic management (Figure 3d). On the other hand, at Harkema the endogeic adults did not show any response (Figure 3c).

In summer, slit injection at Drogeham, where slurry is normally applied to the surface, led to a significant reduction of earthworm numbers (Table 6). At Harkema, where slurry is normally slit-injected, surface application did not lead to an increase in earthworm numbers, which were low in any case. In autumn, however, slit injection resulted in higher numbers of adult endogeic earthworms at both farms (Table 6). For epigeic earthworms, i.e., the non-tanylobous species, no statistically significant effects of slurry application method were found in the experimental fields. But at Drogeham their numbers were highest (81 m^{-2}) across fields in treatments with surface-applied

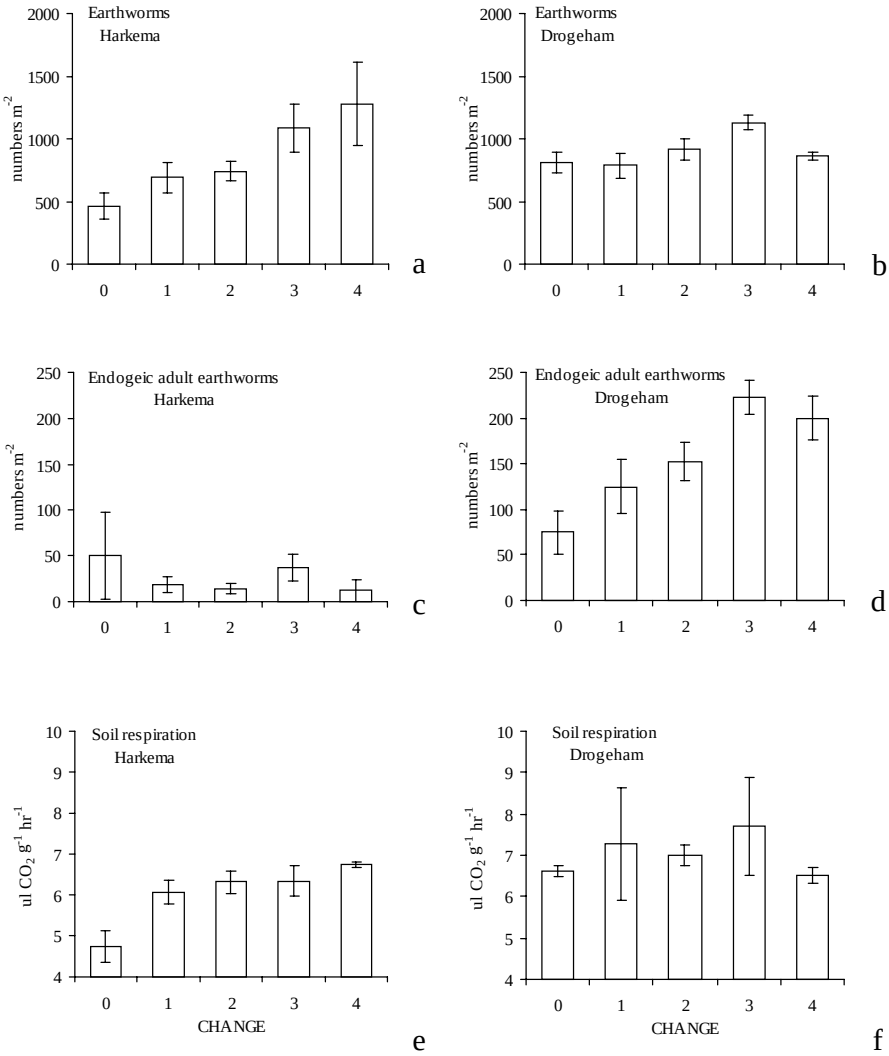

Figure 3. Numbers of earthworms (a–d), soil respiration (e, f), numbers of nematodes (g–j) and Nematode Enrichment Index (k, l) in plots with a farm-characteristic management (CHANGE = 0) and in plots with a management that deviated from this farm-characteristic management in respect of 1, 2, 3 or 4 characteristics (CHANGE = 1, 2, 3 or 4, respectively) in the experimental fields at Harkema and Drogeham in autumn. Bars indicate ± 0.95 × standard error.

MX-slurry whereas they were lowest (28 m^{-2}, $P \leq 0.05$) at Harkema in treatments with slit-injected Harkema slurry. At Harkema, earthworm numbers (916 m^{-2}) were significantly ($P \leq 0.05$) higher with MX-slurry than with farm-characteristic slurry (706 m^{-2}). This positive effect of MX-slurry was not found in combination with EM. Also at Drogeham, MX-slurry when surface-applied resulted in higher earthworm numbers

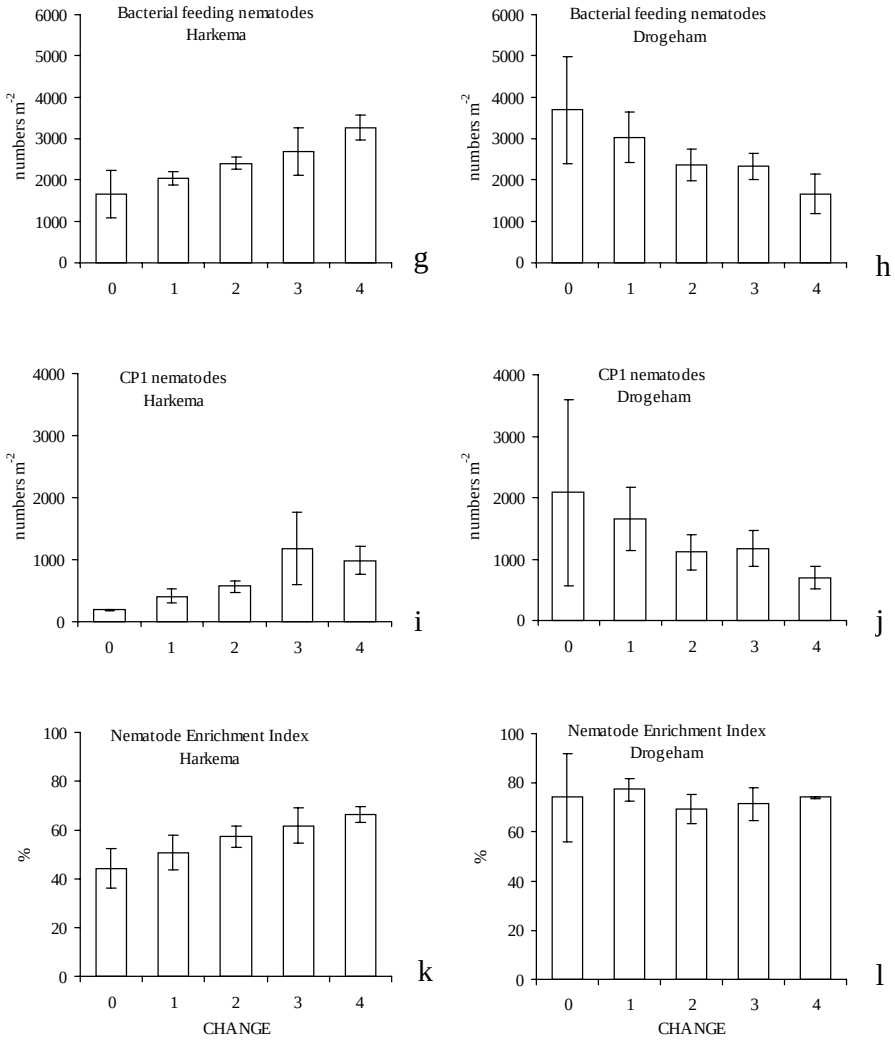

Figure 3. Continued.

than Harkema slurry (Table 7). Moreover, when slurry was surface-applied the use of EM at Drogeham also led to higher earthworm numbers (Table 7). At Drogeham an EM effect was also found in summer, whereas Harkema slurry then resulted in very low earthworm numbers (86 m^{-2}). These numbers increased after application of EM (184 m^{-2}, $P \leq 0.05$). However, in combination with MX-slurry (156 m^{-2}) EM did not have a statistically significant effect.

Application of inorganic fertilizer-N had marked negative effects on the total number of earthworms in autumn at both farms (Table 8).

Table 6. Effect of cattle slurry manure-application method on total numbers (m^{-2}) of earthworms and functional earthworm groups present in summer and autumn in experimental fields at Drogeham and Harkema.

Season	Location	Functional groups	Slurry-application method		Significance[1] of difference
			Surface	Slit injection	
Summer[2]	Drogeham	Total	142	106	*
	Harkema	Total	66	64	ns
Autumn	Drogeham	Total	818	939	ns
		Tanylobous spp.	55	55	ns
		Non Tanylobous spp.	746	869	ns
		Endogeic adults	128	198	*
	Harkema	Total	839	717	ns
		Tanylobous spp.	59	38	ns
		Non Tanylobous spp.	755	658	ns
		Endogeic adults	14	34	*

[1] * = statistically different at $P \leq 0.05$; ns = no statistical difference.

[2] No functional group data available for the summer sampling.

Table 7. Effect of cattle slurry manure-application method and type of slurry or Effective Microbes® (EM) on total numbers (m^{-2}) of earthworms present in autumn in the experimental field at Drogeham.

Treatment	Slurry-application method	
	Surface	Slit injection
Harkema slurry	705a[1]	1003b
Drogeham slurry + MX[2]	1044b	875ab
Without EM	717a	938ab
With EM	1019b	941ab

[1] Means for treatment combinations in the first two rows and for combinations in the second two rows, followed by the same letter are not statistically different ($P \leq 0.05$; Tukey HSD test).

[2] MX = Euromestmix®.

Enchytraeids

For enchytraeids the marginally significant ($P = 0.055$) overall trend was that deviation from the farm-characteristic management resulted in decreased numbers per m^2 (data not shown). In summer and autumn the application of inorganic fertilizer-N resulted

Table 8. Effect of inorganic N fertilizer on total numbers (m^{-2}) of earthworms present in autumn in the experimental fields at Harkema and Drogeham.

Location	Inorganic N fertilizer (kg N ha^{-1} $year^{-1}$)			
	0	76	182	258
Harkema	1014a[1]	863ab	548b	413b
Drogeham	999a	638ab	774b	513b

[1] Means in the same row, followed by the same letter are not statistically different ($P \leq 0.05$; Tukey HSD test).

Table 9. Effect of inorganic N fertilizer and cattle slurry manure-application method on total numbers (m^{-2}) of enchytraeids in summer and autumn in the experimental fields at Harkema and Drogeham.

Season/ location	Inorganic N fertilizer (kg N ha^{-1} $year^{-1}$)				Slurry-application method		Significance[1] of difference
	0	76	182	258	Surface	Slit injection	
Summer							
Harkema	10786a[2]	9780ab	16858b	15043ab	14646	11474	ns
Drogeham	3596a	2392a	11770b	4874ab	7097	6528	ns
Autumn							
Harkema	10135a	16381ab	22303b	34285b	18359	13766	*
Drogeham	16342a	15348ab	28378b	24091ab	19141	25025	*

[1] * = statistically significant ($P \leq 0.05$) effect of slurry-application method; ns = no statistically significant ($P \geq 0.05$) effect of slurry-application methods.

[2] Treatment means of season/location combinations in the same row, followed by the same letter are not statistically different ($P \leq 0.05$; Tukey HSD test).

in higher numbers of enchytraeids at both farms (Table 9). In autumn, slit injection resulted in higher numbers of enchytraeids at Drogeham, but in lower numbers at Harkema (Table 9).

Nematodes

The total number of nematode taxa found in the field experiment was 53. Results of the nematode analyses are summarized in Table 10. Total numbers were not significantly different between summer and autumn. Opposite differences were found between the two farms in nutrient enrichment indicators (CP1, Enrichment Index, Channel Index) in summer and autumn: compared with Harkema the indicators pointed to relatively low and high nutrient enrichment at Drogeham in summer and autumn, respectively.

Table 10. Total numbers (m^{-2}) of nematodes, trophic groups, CP groups and nematode community indices in summer and autumn for the experimental fields of Drogeham and Harkema.

Parameters	Summer				Autumn			
	Drogeham	Harkema	Significance[1] of difference	D/H[2]	Drogeham	Harkema	Significance of difference	D/H
Total numbers	10365	8410	*	1.23	6818	9204	*	0.74
Trophic groups								
Plant feeding	6023	4598	*	1.31	3561	5734	*	0.62
Bacterial feeding	3181	2688	*	1.18	2566	2390	ns	1.07
Fungal feeding	91	107	ns	0.85	125	204	*	0.61
Omnivore	415	376	ns	1.10	250	451	*	0.55
Predator	483	350	*	1.38	274	374	*	0.73
CP groups								
CP1	425	847	*	0.50	1295	682	*	1.90
CP2	2425	1812	*	1.34	1234	1496	*	0.82
CP3	368	112	*	3.29	123	369	*	0.33
CP4	934	853	ns	1.10	555	788	*	0.70
CP5	191	185	ns	1.03	51	136	*	0.38
Community indices								
Trophic Diversity	2.28	2.44	*	0.93	2.24	2.16	ns	1.04
Maturity Index	2.56	2.40	*	1.07	2.13	2.53	*	0.84
Enrichment Index	39.8	61.8	*	0.64	72.4	56.6	*	1.28
Structure Index	70.2	70.9	ns	0.99	68.7	75.4	*	0.91
Channel Index	6.0	3.8	ns	1.58	4.0	12.6	*	0.32

[1] * = statistically different ($P \leq 0.05$); ns = no statistical difference.

[2] D/H = ratio Drogeham/Harkema.

In autumn, deviation from the farm-characteristic management treatments at Drogeham resulted in decreased numbers of bacterivores, whereas an increase was found at Harkema (Figure 3g,h). At Drogeham, numbers decreased from 3692 m^{-2} to only 1669 m^{-2}, i.e., numbers comparable to those found in the Harkema farm-characteristic management treatment (1652 m^{-2}, Figure 3h). On the other hand, at Harkema bacterivores increased to 3267 m^{-2}, i.e., numbers comparable to those found in the Drogeham farm-characteristic management treatment (Figure 3g). It was mainly the enrichment opportunistic bacterivores that were responding (Figure 3i,j). At Harkema the ratio of CP1 to CP2 nematodes increased, resulting in an increasing Enrichment Index (Figure 3k), whereas at Drogeham this ratio, and thus also the Enrichment Index, was not affected (Figure 3k). Compared with surface application, slit injection

Table 11. Effect of cattle slurry manure-application method and soil treatment with Effective Microbes® (EM) on numbers (m^{-2}) of bacterial feeding (BF) and enrichment opportunistic nematodes (CP1), Maturity Index (MI) and Enrichment Index (EI) in summer at Harkema and Drogeham.

Parameter	Harkema		Drogeham		Harkema		Drogeham	
	Surface application	Slit injection	Surface application	Slit injection	– EM	+ EM	– EM	+ EM
BF	2340	2856	2870	3356	2331	2869	2844	3395
CP1	597	1022	273	561	619	989	317	485
MI	2.54	2.28	2.68	2.49	2.52	2.31	2.65	2.55
EI	54.68	68.14	33.15	46.39	56.87	64.86	36.07	42.01

All treatment pairs per location differ statistically ($P \leq 0.05$).

Table 12. Numbers (m^{-2}) of plant-feeding nematodes in summer and autumn at the locations Drogeham and Harkema as affected by method of cattle slurry manure application and type of slurry.

Season	Location	Nematode[1]	Type of slurry	Slit injection	Surface application	Significance[2] of difference
Summer	Drogeham	PF		5664	6564	*
		PF3		962	1352	*
Autumn	Drogeham	PF3	Harkema slurry	328a[3]	185b	
			MX-slurry	240b	199b	
	Harkema	PF5		1954	1685	*

[1] PF = total number of plant-feeding nematodes; PF3 = semi-endoparasites; PF5 = epidermis and root hair feeders.

[2] * = statistically different treatment effects ($P \leq 0.05$).

[3] Means of combinations of type of slurry and method of application, followed by the same letter are not statistically different ($P \leq 0.05$; Tukey HSD test).

in summer resulted at both farms in increased numbers of bacterial feeders and enrichment opportunistic CP1 taxa. This in turn resulted in an increased Channel Index and a decreased Maturity Index (Table 11). Also spreading of EM caused numbers of bacterivores and CP1 nematodes to increase (Table 11).

Slurry type affected hyphal feeders. In June, at Harkema application of MX-slurry resulted in higher numbers of hyphal feeders (MX-slurry: 119 ± 14; Harkema slurry: 83 ± 13; $P \leq 0.05$). In autumn, at Drogeham both slurry types resulted in relatively high numbers of hyphal feeders, but MX-slurry only when the slurry was slit-injected

Table 13. Numbers (m^{-2}) of plant feeding nematodes (PF) in summer and autumn at two locations as affected by type of cattle slurry manure and application of Effective Microbes®.

Season	Location	Nematode[1]	Type of slurry	Effective Microbes®		Significance[2] of difference
				Applied	Not applied	
Summer	Harkema	PF4	Harkema slurry	3018a[3]	2247a	
			MX slurry	2211a	2695a	
Autumn	Harkema	PF2		360	234	*
		PF3	Harkema slurry	1996a	2883a	
			MX slurry	2601a	1771a	
		PF5[4]	Harkema slurry	1653a	1836ab	
			MX slurry	2068b	1754ab	
	Drogeham	PF3		267	167	*

[1] PF2 = migratory endoparasites; PF3 = semi-endoparasites; PF4 = ectoparasites; PF5 = epidermis and root hair feeders;

[2] * = difference in row statistically significant ($P \leq 0.05$).

[3] Means for a combination of type of slurry and Effective Microbes®, followed by the same letter are not statistically different ($P \leq 0.05$; Tukey HSD test). The 2-way interaction is statistically significant ($P \leq 0.05$).

[4] Statistically different at $P = 0.081$.

Table 14. Numbers (m^{-2}) of nematode CP groups[1] and Structure Index (SI) in summer and autumn at two locations as affected by inorganic N fertilizer.

Season	Location	Variable	Inorganic N fertilizer (kg N ha^{-1})			
			0	76	182	258
Summer	Drogeham	CP4	1084a[2]	1304ab	823b	712ab
	Harkema	CP3	160a	176ab	47b	0b
		SI	74.5a	75.5a	68.2a	62.6a
Autumn	Drogeham	CP3	51a	22a	222b	434b
		CP5	76a	153a	25b	21ab

1 See Appendix 1.

[2] Means per row, followed by the same letter are not statistically different ($P \leq 0.05$; Tukey HSD test). The effect of N fertilizer was statistically significant at $P \leq 0.05$ for all variables except CP4 where the effect was statistically significant at $P = 0.06$.

(Harkema slurry: 136 ± 18; MX-slurry slit injected: 149 ± 21; MX-slurry surface-applied: 84 ± 21; $P \leq 0.05$).

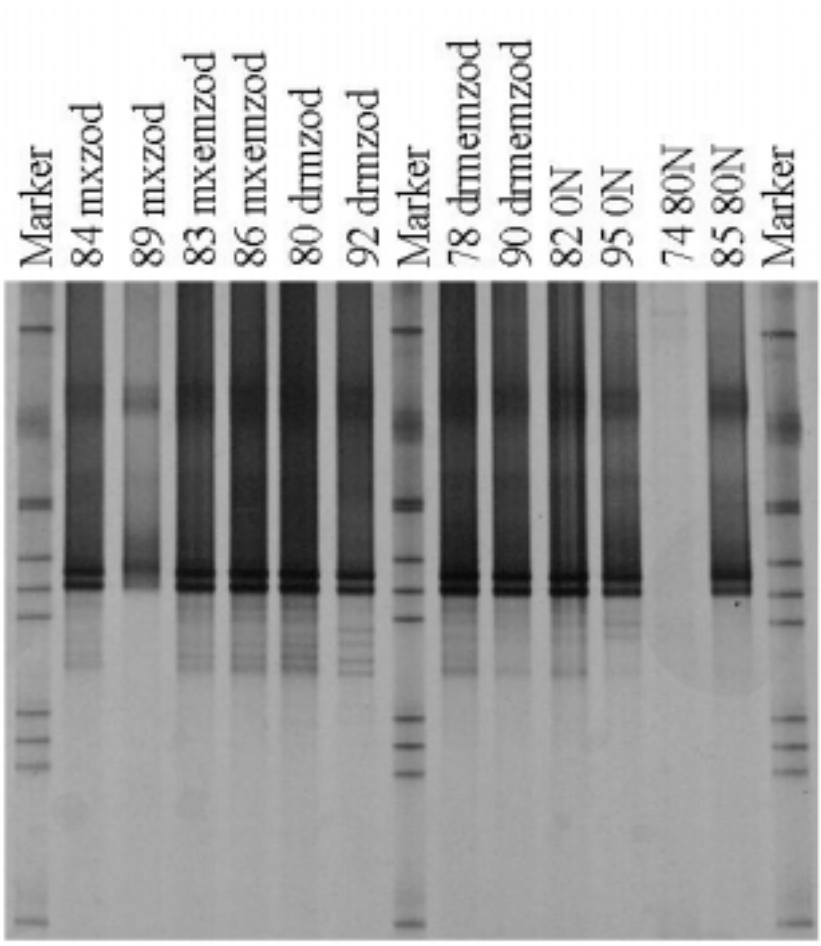

Figure 4. DGGE gel of 16S rDNA fragments for nitrifying bacteria in fields at Harkema that were fertilized with cattle slurry manure (mxzod, mxemzod, drmzod, drmemzod) or inorganic fertilizer (80N; 76 kg N ha^{-1} $year^{-1}$) or that were not fertilized (0N). dr = slurry manure; mx = slurry manure + Euromest-mix®; zod = slit injection; em = Effective Microbes®. The numbers refer to individual fields. (DGGE = Denaturing Gradient Gel Electrophoresis)

Especially plant parasitic nematodes were responsive. However, effects were often complicated interactions depending on location, season, slurry application method, slurry type and the application of EM. In summer, slit injection resulted in lower numbers of plant parasitic nematodes, in particular of the semi-endoparasites (PF_3, Table 12). However, in autumn the numbers of the semi-endoparasites and the epidermis- and root hair feeders were higher with slit injection, both at Drogeham and Harkema, though at Drogeham depending on slurry type (Table 12). In general, application of EM tended to result in lower numbers of plant feeders (PF_2, PF_3, PF_4 and PF_5), but the effects depended on slurry type (with contrasting effects for MX-slurry

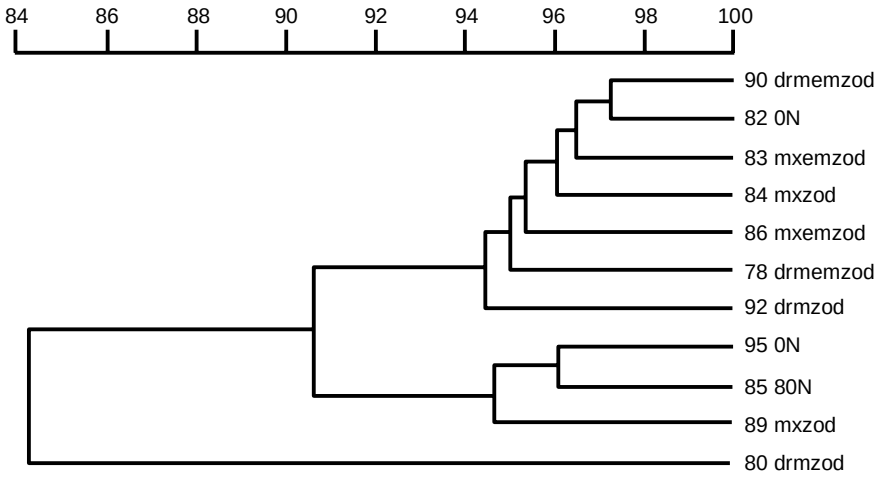

Figure 5. UPGMA cluster diagram (% similarity) of the results of the DGGE analysis for nitrifying bacteria at Harkema. See Figure 4 for explanation. (UPGMA = Unweighted-Pair Groups Method Analysis; DGGE = Denaturing Gradient Gel Electrophoresis)

and Harkema slurry) and were statistically significant only in a specific season and/or location (Table 13).

Application of inorganic fertilizer had a negative effect on the numbers of CP3 and CP4 nematodes in summer and of CP5 nematodes in autumn, which resulted in a lower Structure Index (Table 14). However, the total number of CP3 nematodes in autumn at Drogeham increased with increasing rates of inorganic fertilizer (Table 14).

Bacteria

The universal primers indicated that there were only minor differences in bacterial community structure between Drogeham and Harkema. Also the differences between plots were small. At Harkema, diversity with using nitrifier primers was very small too (Figures 4 and 5) but at Drogeham it was high, both within and among plots (Figures 6 and 7).

Effects of method of slurry application have not been investigated. Using a nitrifier primer revealed that nitrifiers were not present in the EM suspension.

The level of fertilization affected the bacterial community structure most, both for bacteria in general (data not shown) and for nitrifiers (Figure 6). There was no effect of additives on bacterial diversity. Analysis of the EM suspension resulted in 6 bands only (Figure 8).

Soil respiration

Soil respiration in the farm-characteristic management treatment at Harkema was lower (summer 4.4; autumn 4.7 µl CO_2 g^{-1} h^{-1}) than in the farm-characteristic management treatment at Drogeham (summer 5.7; autumn 6.6 µl CO_2 g^{-1} h^{-1}) (Figure 3e,f). At Harkema, deviation from the farm-characteristic management resulted in increased

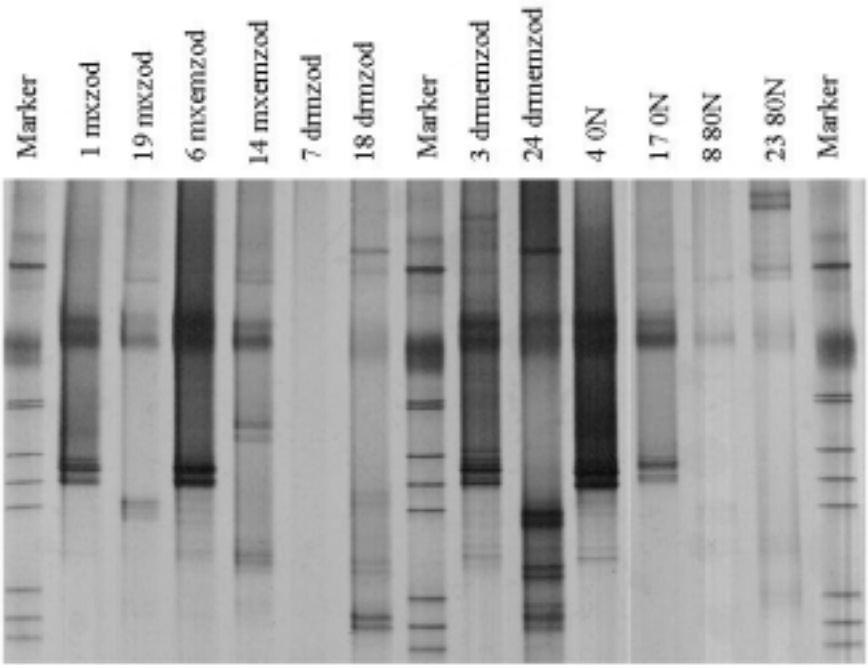

Figure 6. DGGE gel of 16S rDNA fragments for nitrifying bacteria in fields at Drogeham that were fertilized with cattle slurry manure (mxzod, mxemzod, drmzod, drmemzod) or inorganic fertilizer (80N; 76 kg N ha^{-1} $year^{-1}$) or that were not fertilized (0N). See Figure 4 for explanation.
(DGGE = Denaturing Gradient Gel Electrophoresis)

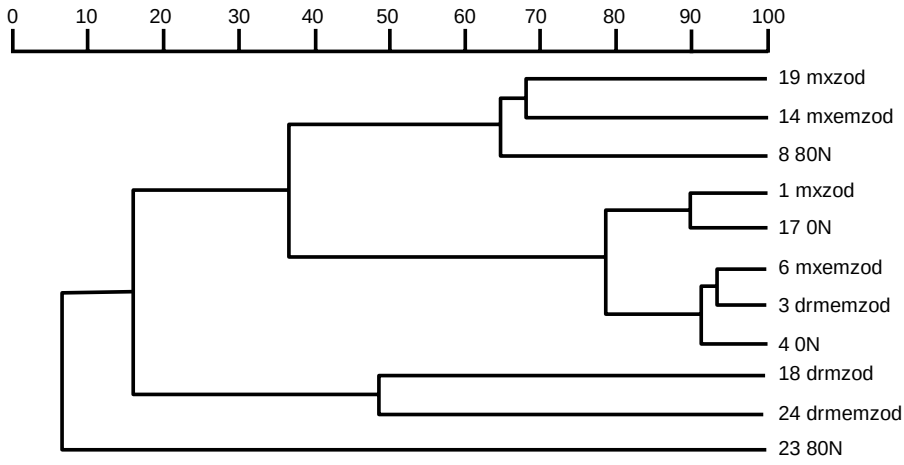

Figure 7. UPGMA cluster diagram (% similarity) of the results of the DGGE analysis for nitrifying bacteria at Drogeham. See Figure 4 for explanation. (UPGMA = Unweighted-Pair Groups Method Analysis; DGGE = Denaturing Gradient Gel Electrophoresis)

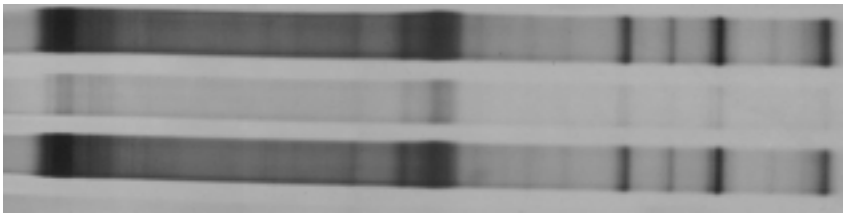

Figure 8. DGGE gel of 16S rDNA fragments for all bacteria in Euromestmix® (middle row). The upper and lower row are markers. (DGGE = Denaturing Gradient Gel Electrophoresis)

soil respiration (summer 5.5; autumn 6.8 µl CO_2 g^{-1} h^{-1}) up to levels comparable with Drogeham, where no significant response was found (Figure 3e,f). The effects at Harkema could be explained by an increased soil respiration when slurry was surface-applied in summer (for both slurry types): slit injection 5.1; surface-application 5.5 µl CO_2 g^{-1} $hour^{-1}$; in autumn (only for Harkema slurry): slit injection 5.8; surface-application 6.4 µl CO_2 g^{-1} $hour^{-1}$). However, this positive effect of surface application in summer was not found when also EM was applied. Moreover, in autumn, also MX-slurry increased soil respiration at Harkema, but only when applied by slit injection (data not shown).

Nitrogen mineralization by the soil biota

Production-ecological calculations were done for those treatments where conclusive effects on the investigated soil fauna had been assessed. Calculated N mineralization was by far the highest for earthworms (Table 15). Enchytraeids did not contribute more than just over 4 kg N ha^{-1} $year^{-1}$, nematodes still less (data not shown). The treatment effects can be summarized as follows (Table 15):

The calculated N mineralization in the farm-characteristic management at Drogeham was 11 kg N ha^{-1} $month^{-1}$ in summer and 21 kg N ha^{-1} $month^{-1}$ in autumn, with a calculated annual N mineralization of 167 kg N ha^{-1}. At Harkema the calculated N mineralization in the farm-characteristic management was 1 kg N ha^{-1} $month^{-1}$ in summer and 12 kg N ha^{-1} $month^{-1}$ in winter. On an annual basis it was 71 kg ha^{-1}, which is 57% less than at Drogeham. At Harkema (but not at Drogeham), deviation from the farm-characteristic management resulted in a significant increase in earthworm numbers. This was accompanied by a 330% increase in the calculated annual N mineralization at Harkema in the treatments that differed most from the farm characteristic management. This strong increase in calculated annual N mineralization at Harkema mainly depended on adaptations in slurry type, slurry application method, and use of inorganic fertilizers that affected earthworm numbers. Application of Drogeham-MX slurry instead of Harkema slurry resulted in a 33% increased calculated annual N mineralization (137 kg N ha^{-1} $year^{-1}$). When surface-applied this slurry even resulted in a 60% increase (165 kg N ha^{-1} $year^{-1}$) compared with slit-injected Harkema slurry (103 kg N ha^{-1} $year^{-1}$). Compared with the treatments that did not receive any inorganic fertilizer-N the calculated annual N mineralization by earthworms in fields with 258 kg inorganic-N ha^{-1} $year^{-1}$ decreased from 172 to 72 kg ha^{-1} $year^{-1}$, which is a

Table 15. Number of earthworms (m^{-2}) and calculated N mineralization by earthworms per month (summer and autumn) and per year (kg N ha^{-1}) at the locations Harkema and Drogeham in relation to type of cattle slurry manure, method of slurry manure application and inorganic fertilizer.

Location	Treatment[1]	Number of earthworms (m^{-2})		N mineralization by earthworms		
		Summer	Autumn	Summer	Autumn	Year
Harkema	typical	13	463	1	12	71
	a-typical	200	1275	10	34	234
Drogeham	typical	225	813	10	21	167
	a-typical	125	863	6	23	151
Harkema	surface + MX slurry	88	991	4	27	165
	surface + Harkema slurry	55	763	3	21	124
	slit inj. + MX slurry	59	841	3	23	137
	slit inj. + Harkema slurry	69	594	3	16	103
Drogeham	surface + MX slurry	163	1044	8	27	186
	surface + Harkema slurry	131	705	6	18	130
	slit inj. + MX slurry	119	875	6	23	145
	slit inj. + Harkema slurry	94	1003	5	26	164
Drogeham	surface – EM	114	717	6	19	128
	surface + EM	197	1019	10	27	190
	slit inj. – EM	100	938	5	25	156
	slit inj. + EM	113	941	6	25	159
Harkema	no inorganic fertilizer	100	1014	5	27	172
	inorganic N fertilizer	50	413	3	11	72
Drogeham	no inorganic fertilizer	115	999	6	26	168
	inorganic N fertilizer	113	513	6	13	99

[1] typical = farm-characteristic fertilization; a-typical = maximum deviation from typical; MX = Euromest-mix®; EM = Effective Microbes®; inorganic N fertilizer = calcium ammonium nitrate at 258 kg N ha^{-1} $year^{-1}$.

42% reduction. At Drogeham, earthworm numbers were affected by slurry type, EM and application rates of inorganic fertilizer. Farm-characteristic surface application of Harkema slurry instead of Drogeham-MX slurry resulted in a 30% lower calculated annual N mineralization (130 kg N ha^{-1} $year^{-1}$). However, spreading of EM in combination with surface application of slurry had a positive effect on earthworm numbers, which resulted in a 48% increase of the calculated annual N mineralization (190 kg N ha^{-1} $year^{-1}$). Also at Drogeham, application of 258 kg inorganic N ha^{-1} $year^{-1}$ resulted in reduced numbers of earthworms and thereby in a 41% reduction of the calculated

annual N mineralization (99 kg N ha^{-1} $year^{-1}$) compared with plots that had not received any inorganic N fertilizer (168 kg N ha^{-1} $year^{-1}$).

Discussion and conclusions

There appears to be no literature on the impact of cattle slurry injection on bacterial diversity and enchytraeids or on the combination of cattle slurry application and the additives Euromestmix® (MX) or Effective Microbes® (EM). We know of only one study on the impact of cattle slurry injection on earthworm populations (Kruk, 1994). Kruk (1994), who compared 15 peat meadows on which slurry was applied by slit injection with 15 meadows where the fertilizer was applied onto the surface, found no statistically significant differences in earthworm biomass during the first three weeks after application. In our across-farm comparison we found lower densities of earthworms with surface application of slurry manure. On the other hand, in the field experiment densities were higher with surface application. However, both studies indicated contrasting effects of slit injection on epigeic and endogeic earthworm species, with the first group being negatively affected and the second being stimulated. The negative effects of slurry injection on earthworms will be partly due to physical damage. Using the line-intersect method of Newman (1966), we estimated that 15% of the epigeic earthworms of 5 cm or longer will be dissected with injection slits 20 cm apart, whereas of those measuring 10 cm or more even 30% will be damaged. Anecic and endogeic species run a much lower risk of being hit, while they probably also escape direct exposure to the injected slurry. This may be the reason why these functional groups are not negatively affected or even enhanced by slurry injection. In literature both positive and negative effects of inorganic fertilizers on earthworms have been reported (Edwards & Bohlen, 1996; Curry, 1998). In our research we found very negative effects. Because it is difficult to believe that these effects will come about through stimulated plant production, it is likely that they result from direct exposure to the manure. Indications for this were the decreasing numbers of nematodes of higher CP-groups, which are indicators of environmental disturbance (Ferris *et al.*, 2001). The suppression of nematode numbers by high rates of inorganic-N fertilizers is well known (Rodríguez-Kabana, 1986; Verschoor, 2001).

The CHANGE analyses showed that deviation from the farm-characteristic management affected each of the investigated soil fauna groups and soil respiration. In general, earthworms, bacterial feeding nematodes and soil respiration increased when the management that was part of the Drogeham farm replaced the farm-characteristic management of Harkema. In contrast, adjustment of the Drogeham management to the Harkema management resulted in opposite effects for nematodes, but not for earthworms and soil respiration. Enchytraeids responded with decreased numbers to a change in management at both locations. These responses suggest a larger role of soil biological processes for the nutrient dynamics in fields with a management that comprises surface application of MX-slurry.

The calculated N mineralization by earthworms indicates that earthworms accounted for a gross mineralization of 70–230 kg N ha^{-1} $year^{-1}$. Surface application of slurry

with MX resulted in a 33–43% increase in calculated N mineralization compared with surface-applied slurry without MX. Compared with surface application, slit injection resulted for 3 out of the 4 location x slurry type combinations in a 17–22% decrease in the calculated N mineralization. At Drogeham, slit injection of slurry without MX resulted in a 26% increase of the N mineralization. The farm-characteristic management at Harkema (slit injection of slurry without additives) resulted in a calculated annual N mineralization of only 71 kg N ha^{-1} $year^{-1}$, which is even 57% less than for the farm-characteristic management at Drogeham (surface applied MX-slurry; 167 kg N ha^{-1} $year^{-1}$). The correctness of these figures depends on the reliability of the parameter values used and the extrapolations over the season. However, the values could be confirmed by extrapolating the data from Whalen *et al.* (2000) on N mineralization by individual earthworms in the laboratory to the data from our field experiment. It remains to be assessed to what extent negative effects of slurry injection on earthworms might influence grass production and quality.

Contrary to our hypotheses, EM affected earthworms and specific groups of nematodes, i.e., plant feeders and the enrichment opportunistic bacterial feeders, but these effects were found in the field experiment only. For earthworms, application of EM had different effects at the MX-farm Drogeham where numbers were increased, whereas at the conventional farm Harkema no effects were found. Moreover, these effects depended on slurry type and slurry application method. It is not clear how these effects can be explained. EM application did not have any statistically significant effects on the composition of the bacterial community as described by DGGE profiles of all bacteria and nitrifiers. There are, however, indirect indications that EM did affect soil microbial activity. In the field experiment EM application was associated with higher numbers of enrichment opportunistic bacterial feeding nematodes. Such changes in the nematode community structure are indicative of enhanced bacterial biomass (Bongers, 1990; Ettema & Bongers, 1993; Griffiths *et al.*, 1994; 1998). But we did not observe effects of EM on *in vitro* soil respiration.

The analyses of the nematode enrichment indices furthermore suggest that there were large differences in the temporal dynamics of the microbial biomass that can be related to differences in fertilizer management. In summer, the enrichment indicators were higher at Harkema than at Drogeham. Moreover, at Harkema they decreased with increasing deviation from the farm-characteristic management (i.e., slit injection of slurry without additives). This effect was largely explained by a positive relationship between slit injection and the abundance of nutrient enrichment indicators. In autumn, however, nutrient enrichment indicators where higher at Drogeham than at Harkema. This time, deviation from the farm-characteristic management at Harkema resulted in an increase in nutrient enrichment indicators, whereas the opposite was found at Drogeham (farm-characteristic management: surface-applied MX-slurry). At Drogeham the *in vitro* soil respiration was higher in the treatments with surface-applied slurry. Thus, fertilizer management seems to affect the temporal dynamics of the microbial activity, which in turn could indicate effects of fertilizer management on temporal variation in the availability of plant nutrients. Also, the across-farm comparison fields that were fertilized with surface-applied MX-slurry differed in enrichment indicator value from the fields that were fertilized using slit injection. At the time of

sampling, i.e., the end of September, the fields with surface-applied MX-slurry had nutrient enrichment indicator values (Enrichment Index: 60–65%) at the lower end of the range of Dutch agricultural grasslands (Enrichment Index: 55–100%, Figure 2). The only grasslands with an Enrichment Index < 50% were unfertilized grasslands that were managed as nature reserves.

In terms of the hypotheses stated in the introduction, we conclude that:

1. Slit injection reduced the number op epigeic earthworms. Anecic and endogeic earthworms were not reduced or even increased. This may be due to less direct contact with the injection device and/or the slurry. Slit injection did not show a consistent effect on enchytraeids. This may be due to the fact that they are usually more abundant where earthworms are absent. So a more favourable environment for the enchytraeids may compensate the negative effects of slit injection on both earthworms and enchytraeids when earthworms are reduced in number.
2. MX was usually associated with higher numbers of earthworms.
3. Inorganic N fertilizer reduced the number of earthworms, but did not negatively affect enchytraeids, possibly for the same reason as mentioned sub 1.
4. Because slit injection and inorganic N fertilizer negatively affected earthworm numbers, this also reduced the calculated N mineralization by earthworms. Application of MX-slurry had the opposite effect.
5. Production-ecological calculations of gross N mineralization indicated a large contribution from earthworms (70–200 kg N ha^{-1} $year^{-1}$), but minor contributions from enchytraeids and nematodes.
6. As expected, nematodes indicative of nutrient-enriched conditions (CP1) thrived under slit injection, but only in summer. Nematodes of the higher CP groups (CP3–CP5), which are indicators of environmental disturbance, were negatively affected by increased rates of inorganic fertilizers.
7. Soil respiration was positively affected by surface application of slurry.
8. Effects of EM were apparent for earthworms and nematodes under some specific conditions, but not for the composition of the microbial community, which was affected by increased rates of inorganic fertilizer only and not by the other treatments.

We currently are investigating the possible mechanisms of MX and ME in the laboratory.

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Appendix 1

Definitions of the nematode community indices

Colonizer-persister (CP) scale

Assignment of nematode taxa to a 1–5 linear scale according to their r and K characteristics. This scaling comprises only non-plant feeding nematodes and forms the basis for the calculation of several nematode-based soil quality indicators.

CP1: Short generation time, small eggs, high fecundity, mainly bacterivores, feed continuously in enriched media, form dauerlarvae as microbial blooms subside.

CP2: Longer generation time and lower fecundity than the CP1 group, very tolerant of adverse conditions and may become cryptobiotic, feed more deliberately and continue feeding as resources decline. Mainly bacterivores and fungivores.

CP3–5: Longer generation time, sensitive to adverse conditions, larger body size, lower fecundity than *CP1* and *CP2* groups. Fungivores, bacterivores, carnivores and omnivores.

Maturity Index

Weighted mean of *CP* value of the individuals in a sample.

Nematode fauna profile

Based on the *CP* scaling, nematodes are classified as being associated with the 'basal', 'enrichment' or 'structure' component of the soil food web. Based upon this, the Enrichment, Structure and Channel Index are calculated, using weightings (NP_w) that differ from those used for the calculation of the Maturity Index.

Basal foodweb: diminished due to stress, including limitation of resources, adverse environmental conditions or recent contamination. Weighted mean of NP_w of the *CP2* individuals (excluding carnivores) in a sample.

Structured foodweb: when resources are more abundant or when recovering from stress. Weighted mean of NP_w of the *CP3, 4, 5* and carnivores *CP2* individuals in a sample.

Enriched foodweb: when disturbance occurs and resources become available due to organism mortality, turnover, or favourable shifts in the environment, resulting in a flush of microbial activity. Weighted mean of NP_w of the *CP1* and fungivores *CP2* individuals in a sample.

Enrichtment Index

Proportion (%) of nematodes of the enriched foodweb in the basal + enriched foodweb.

Structure Index

Proportion (%) of nematodes of the structured foodweb in the basal + structured foodweb.

Channel Index

Proportion (%) of fungivores *CP2* nematodes in the basal foodweb.

Largely adapted from Ferris *et al.* (2001).

Appendix 2

Energetic parameters used in the production-ecological calculations on the N mineralization by earthworms, enchytraeids and nematodes.[1]

Parameter	Unit/ item	C/N[2] ratio	Taxon		
			Earthworm	Enchytraeid	Nematode
Individual fwt[3]	g		1.31	1.0032 x 10^{-4}	1 x 10^{-7}
Fwt/dwt[4]			4.6	6.7	4
C content	%dwt		50	50	40
C/N body			5	5	5
a[5]			81	33.6	29.25
b[5]			0.9	0.67	0.72
t[6]	°C		19	20	20
Q10			2	2	3
Assimilation efficiency			0.20	0.28	0.41
Production efficiency			0.09	0.11	0.16
C/N food			10.1	10.1	8.1
Diet	% roots	(7.5)[7]	0	0	0.4
	% bacteria	(8)	0.4	0.4	0.45
	% fungi	(10)	0.4	0.4	0.15
	% detritus	([8])	0.2	0.2	0

[1] Data after Didden *et al.* (1994), unless indicated otherwise.

[2] C/N ratio = ratio of C to N in dry weight.

[3] fwt = fresh weight.

[4] dwt = dry weight.

[5] a en b are parameters for the equation $Q = aW^b$.

[6] t = temperature at which a and b were determined.

[7] After Verschoor (2002).

[8] C/N ratio of detritus at Drogeham and Harkema was 12.5 and 11.7, respectively.