

Soil biological quality of grassland fertilized with adjusted cattle manure slurries in comparison with organic and inorganic fertilizers

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Abstract We studied the effect of five fertilizers (including two adjusted manure slurries) and an untreated control on soil biota and explored the effect on the ecosystem services they provided. Our results suggest that the available N (NO_3^- and NH_4^+) in the soil plays a central role in the effect of fertilizers on nematodes and microorganisms. Microorganisms are affected directly through nutrient availability and indirectly through grass root mass. Nematodes are affected indirectly through microbial biomass and grass root mass. A lower amount of available N in the treatment with inorganic fertilizer was linked to a higher root mass and a higher abundance and proportion of herbivorous nematodes. A higher amount of available N

in the organic fertilizer treatments resulted in a twofold higher bacterial activity (measured as bacterial growth rate, viz. thymidine incorporation), a higher proportion of bacterivorous nematodes, a 30% higher potential N mineralization (aerobic incubation), and 25–50% more potentially mineralizable N (anaerobic incubation). Compared to inorganic fertilizer, organic fertilization increased the C total, the N total, the activity of decomposers, and the supply of nutrients via the soil food web. Within the group of organic fertilizers, there was no significant difference in C total, abundances of soil biota, and the potential N mineralization rate. There were no indications that farmyard manure or the adjusted manure slurries provided the ecosystem service “supply of nutrients” better than normal manure slurry. Normal manure slurry provided the highest bacterial activity and the highest amount of mineralizable N and it was the only fertilizer resulting in a positive trend in grass yield over the years 2000–2005. The number of earthworm burrows was higher in the treatments with organic fertilizers compared to the one with the inorganic fertilizer, which suggests that organic fertilizers stimulate the ecosystem service of water regulation more than inorganic fertilizer. The trend towards higher epigeic earthworm numbers with application of farmyard manure and one of the adjusted manure slurries, combined with the negative relation between epigeic earthworms and bulk density and a significantly lower penetration resistance in the same fertilizer types, is preliminary evidence that these two organic fertilizer types contribute more to the service of soil structure maintenance than inorganic fertilizer.

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Introduction

Soil biota play an important role in ecological processes in the soil and in the provision of various ecosystem services, such as maintenance of soil structure, water regulation, and supply of nutrients (Brussaard et al. 1997; Swift et al. 2004; Mulder 2006; Kibblewhite et al. 2008). In the search for sustainable agricultural grassland systems, these processes and ecosystem services, provided by soil biota, become more important (Brussaard et al. 2007a). Therefore, insight is needed into the mechanisms by which soil biota are influenced by grassland management and what changes in soil biota mean for the functioning of the plant–soil system and the provision of ecosystem services.

Fertilization with inorganic and organic fertilizers is common in grassland management. Especially when initial levels are low, soil organic matter content and soil biological activity can be enhanced through inorganic as well as organic fertilizers. Inorganic fertilizers (containing only mineral N) feed the plant and soil microorganisms directly and the entire soil biota indirectly by increased root biomass and exudates and plant litter. However, inorganic fertilizers involve a high fossil energy consumption and are easily lost from the soil by nitrate leaching and denitrification. Organic fertilizers (20–50% mineral N) on the other hand feed the soil biota directly and the crop both directly (inorganic constituents) and indirectly following mineralization of organic constituents. When more N is organic and mineralized slowly, the risk of leaching is low as long as an actively growing crop is present that can take up the nutrients supplied. In older grasslands with relatively high soil organic matter levels, lower inputs of N may result in crop yields equal to those in grasslands with relatively low soil organic matter levels (Sonneveld and Bouma 2003; Reijs et al. 2007). Many studies, mainly in arable soils, have shown that organic fertilizers can increase organic matter content, soil biological activity, and potential N mineralization (De la Lande Cremer 1976; Mäder et al. 2002; Bittman et al. 2005; Fliessbach et al. 2007; Birkhofer et al. 2008). However, in grassland soils with a crop year-round, dense roots, and no soil tillage, the effects of the crop on the buildup and maintenance of soil organic matter and biological activity may be larger than in arable soils and the added effects of organic fertilizers may be less clear.

Due to legislative restrictions on the use of organic and inorganic fertilizers (Vellinga 2006), animal manure is being revalued, not only as a nutrient source but also as a means to improve soil quality (Velthof et al. 2000). Whereas animal manure was applied as farmyard manure until the 1970s, today it is slit-injected as manure slurry. Some researchers have stated that manure slurry with a low C/N ratio could have adverse effects on soil biological quality through significant amounts of phytotoxic compo-

nents (Van Bruchem et al. 2000). Also, there is a general feeling among many dairy farmers that farmyard manure is the best organic fertilizer to improve soil quality. Although there is a renewed interest in deep-litter stables with a straw bedding, most dairy herds in The Netherlands are kept in a cubicle housing in which animal manure is produced in the form of slurry (feces mixed with urine). To achieve the supposed benefits of farmyard manure, farmers are searching for methods to adjust manure slurry quality to that of farmyard manure by increasing the C/N ratio and achieving a lower N mineral/N total ratio (Verhoeven et al. 2003). This is either done by dietary adjustments (lower crude protein content and/or higher fiber content in the ration) or by mixing and composting manure slurry with other organic materials, like hay. However, little is known about the effects of these adjusted manure slurries on soil quality, especially on soil biological quality. It is also not known whether farmyard manure is really the best organic fertilizer for optimizing soil biological quality in grassland and what differences exist with other organic and inorganic N fertilizers.

Studies on the effect of specific quality aspects of organic fertilizers (higher C/N ratio and lower N mineral/N total ratio) on soil biota are rare. Cotton and Curry (1980) measured little difference in the effect of cattle (relatively high C/N) or pig (relatively low C/N) manure slurry application on earthworms. Griffiths et al. (1998) observed that the number of protozoa responded more quickly to the application of pig manure slurry than cattle manure slurry and explained this by the greater proportion of readily available C in pig slurry compared to cattle slurry. Van Den Pol-van Dasselaar et al. (2006) showed a positive correlation between organic N in cattle manure slurry and bacterial feeding nematodes in the soil and a negative correlation between the mineral N content of manure slurry and predaceous nematodes in the soil. Sørensen (1998) found that the addition of straw to slurry stimulated soil microbial activity (measured as soil respiration). De Vries et al. (2006) measured no difference in bacterial and fungal biomass between manure slurry and farmyard manure applied on grass–clover. De Goede et al. (2003) measured higher numbers of earthworms in slurry amended with the additive Euromestmix[®]. Soil organic matter and organic N accumulation are determined by the input of C rather than the input of N (Ryden 1984; Hassink 1994). Soil organic C increases with the amount of C added to the soil (Van den Pol-van Dasselaar and Lantinga 1995). Fauci and Dick (1994) concluded that long-term organic soil amendments increase biological activity in proportion to the amount of C added. Therefore, we expect that, in the long-term, organic fertilizer with a relatively high C/N ratio (such as farmyard manure) will lead to a higher organic matter content, a higher N mineralization (Reijs et al. 2007), and a higher biomass of soil biota than organic fertilizers with a

relatively low C/N ratio (such as normal manure slurry). In none of the above studies, manure slurries with adjusted qualities were compared with farmyard manure.

We carried out a 5-year fertilization experiment with four different organic fertilizers, one inorganic N fertilizer, and an untreated control (i.e., no N fertilizer) to assess differences in species richness, abundance, and activity of earthworms, nematodes, bacteria, and fungi. We measured bacteria and fungi as primary consumers in the soil food web. Nematodes were measured as representatives of the secondary consumers in the soil food web, while earthworms were measured as representatives of the ecosystem engineers. Our objectives were (1) to compare the effects of an inorganic N fertilizer and four organic fertilizers on soil biota and (2) to explore the relevance of changes and/or differences in soil biota for the provision of ecosystem services (maintenance of soil structure, water regulation, and supply of nutrients) in the plant–soil system. We hypothesized that, compared to inorganic N fertilizer, organic fertilizers would increase the species richness of earthworms, nematodes, and microorganisms and enhance the microbial activity. Furthermore, we hypothesized that, among the organic fertilizers, farmyard manure would have the most positive effect on the species richness of the soil biota, while normal manure slurry would stimulate the activity of the soil biota. Adjusted manure slurries would take an intermediate place between normal manure slurry and farmyard manure.

Materials and methods

Sampling site and experimental design

The experiment was established on a sandy soil (Gleyic podzol) in the southern part of the Netherlands (51°31' N, 5° 42' E) in a permanent grassland sown in the autumn of 1997 with a seed mixture of *Lolium perenne* cultivars. In the years before the experiment started, the grassland had been fertilized with animal excreta during grazing (approximately

150 kg N total ha⁻¹ year⁻¹), manure slurry (approximately 150 kg N total ha⁻¹ year⁻¹), and inorganic fertilizer (approximately 150 kg N ha⁻¹ year⁻¹). In the spring of 2000, the treatments were installed comprising an untreated control (CON) and five different fertilizer types: inorganic N fertilizer (calcium ammonium nitrate 27%, CAN), “normal” cattle manure slurry (NMS), manure slurry of cattle fed a ration low in protein (LMS), manure slurry mixed and composted with hay from roadsides (CYM), and farmyard manure from a deep-litter stable with dairy cows (FYM). For details, see Table 1. Plot size was 8×2.80 m; the samples were taken from the center, 6×1.5 m of the plots. Two thirds of the total amount of fertilizers was applied before the first harvest of the grass and one third before the second harvest. The applied effective N with the fertilizers in 2004 was calculated according to the Dutch official advice on grassland fertilization as (working coefficient mineral N× N mineral) + (working coefficient organic N × N organic) (<http://www.bemestingsadvies.nl>). The two manure slurries (NMS and LMS) were slit-injected, whereas the solid manures (CYM and FYM) were surface-applied. All treatments were replicated four times in four randomized blocks. The plots were harvested with a mower four to five times a year and not grazed. All plots received a basal fertilizer application of triple super phosphate (19.6% P) and muriate of potash (33.2% K).

Soil sampling, chemical and physical measurements, and grass production

On the 4th of October 2004, five growing seasons after the start of the experiment, soil samples for analysis of chemical, physical, and biological soil quality were taken. Per plot, a bulk sample of 70 cores (0–10 cm, ø 2.3 cm) was collected, sieved through 1 cm mesh, homogenized, and stored at field moisture content at 4°C before analysis. Subsamples were taken for chemical analysis, nematode analysis, and microbiological analysis.

Prior to chemical analysis, subsamples were oven-dried at 40°C. Soil acidity of the oven-dried samples was measured in

Table 1 Average application rate (in kilograms per hectare per year) per treatment and characteristics of the fertilizer types

kg ha ⁻¹	Treatments					
	CON	CAN	NMS	LMS	CYM	FYM
Total organic matter	0	0	3,361	3,718	4,161	6,347
Mineral N	0	150	104	102	13	46
Organic N	0	0	98	104	170	171
C/total N	–	–	7.5	8.1	10.2	13.2

CON unfertilized, CAN fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%), NMS “normal” cattle manure slurry, LMS manure slurry of cattle fed a ration low in protein, CYM manure slurry mixed and composted with hay from roadsides, FYM farmyard manure from a deep-litter stable with dairy cows

1 M KCl (pH-KCl) with a standard electrode. Total C was measured by incineration of dry material at 1,150°C, after which the produced CO₂ was determined by an infrared detector (LECO Corporation, St. Joseph, MI, USA). For determination of total N, evolved gases after incineration were reduced to N₂ and detected with a thermal conductivity detector (LECO Corporation, St. Joseph, MI, USA). The N supply capacity of the soil was calculated from N total in the soil, according to the Dutch official advice on grassland fertilization (<http://www.bemestingsadvies.nl>).

Soil bulk density was measured in the 5- to 10-cm layer, in three undisturbed ring samples containing 100 cm³ soil each (Eijkelkamp, Giesbeek, The Netherlands). Soil penetration resistance was measured with an electronic penetrometer (Eijkelkamp, Giesbeek, The Netherlands) with a cone diameter of 1 cm² and a 60° apex angle. Cone resistance was recorded per centimeter of soil depth and expressed as the average value of ten penetrations per plot in the soil layers of 0–10, 10–20, 20–30, 30–40, and 40–50 cm. Soil structure was determined in one block (20×20×10 cm) per plot. Soil of this block was assessed by visual observation as crumbs, subangular blocky, and angular blocky elements (FAO 2006). Amounts were weighed per type and expressed as a percentage of total fresh soil weight. To determine root biomass, three soil cores (0–10 cm, ø 8.5 cm) per plot were taken. After the soil in the samples was thoroughly washed out with water, root mass was sieved (mesh size 2 mm) and root dry matter was determined after oven-drying at 70°C.

In 2004, grass was cut four times (14th of May, 22nd of June, 3rd of August, and 28th of September), using a “Haldrup” small-plot harvester. The harvested area was 8×1.5 m. Grass was cut at a stubble height of 6 cm, weighed, and sampled for dry matter analysis. Dry matter was determined after drying at 70°C.

Soil biological parameters

Earthworms

To determine earthworm parameters, one soil block of 20×20×20 cm was sampled per plot. Only one sample per plot could be taken to prevent disruption of the experiment. This limited the statistical analysis of the earthworm data. Immediately after sampling, earthworm burrows with a diameter >2 mm were counted on the horizontal surface (20×20 cm) exposed at 10 cm depth. Hereafter, the blocks were transferred to the laboratory where the earthworms were hand-sorted, counted, weighed, and fixed in 70% ethanol prior to identification. Numbers and biomass were expressed per square meter. Adults were identified to species, with a distinction between (1) epigeic species (pigmented, living superficially in the litter layer, little

burrowing activity), (2) endogeic species (living in predominantly 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 from the surface at night) (Bouché 1977).

Nematodes

Free-living nematodes were extracted from a subsample of about 100 g field-moist soil, using the Oostenbrink elutriator (Oostenbrink 1960). Total nematode numbers were counted and expressed per 100 g fresh soil. Nematodes were fixed in hot formaldehyde (4%), and at least 150 randomly selected nematodes from each sample were identified to genus and, whenever possible, to species. Nematode genera and species were assigned to trophic groups, following Yeates et al. (1993), and allocated to the colonizer-persister groups (cp groups), following Bongers (1990) and Bongers et al. (1995). The nematode channel ratio (NCR) was calculated to express the relative contributions of bacterivorous (B) and fungivorous (F) nematodes to the total nematode abundance (NCR=B/(B+F)) (Yeates 2003). The maturity index was calculated as the weighted mean of the individual cp values, in accordance with Bongers (1990) and Korthals et al. (1996). The maturity index is an ecological measure which indicates the condition of an ecosystem based on nonplant-parasitic nematode life history group composition.

Microbial parameters

A subsample of 200 g field-moist soil was taken from the bulk soil sample, adjusted to 50% water-holding capacity, and preincubated at 12°C for 4 weeks to avoid the effects of temperature and moisture fluctuations in the field and to stabilize soil conditions (Bloem et al. 2006). After preincubation, fungal and bacterial biomass, bacterial growth rate, and community-level physiological profiles (CLPP) were determined. Microscopic soil smears were prepared and measured as described by Bloem and Vos (2004). Fungal hyphae were measured using the grid intersection method. Bacterial numbers and cell volumes were measured by confocal laser scanning microscopy and automatic image analysis (Bloem et al. 1995). Bacterial biomass was calculated from biovolume. Bacterial growth rate was determined as the incorporation of [³H]thymidine and [¹⁴C]leucine into bacterial DNA and proteins, respectively (Bloem and Bolhuis 2006; Michel and Bloem 1993).

The CLPPs of the bacterial communities in the soil extracts were determined with ECO-plates from Biolog (Hayward, CA, USA). These plates contain a triplicate set of 31 different C substrates, a control, a freeze-dried mineral medium, and a tetrazolium redox dye. For each

bacterial extract, a dilution series was made using 10 mM BisTris buffer at pH 7. Each dilution series (3^{-1} down to 3^{-12}) was used to inoculate four ECO-plates with a volume of 100 μ L per well. The color formation in the plate was measured every 8 h for 7 days with a plate reader spectrophotometer at 590 nm. The CLPPs were calculated from the color formation in the wells and corrected for inoculum density using a regression approach applied to the average well color development (AWCD) as described by Rutgers et al. (2006). This produced CLPPs describing the relative abundance from substrate conversion (31 substrates; log-scaled). To survey the bacterial community activity in the ECO-plate, the AWCD was calculated after 7 days of incubation. The CLPP-slope parameter was calculated from the color development in the ECO-plates. This parameter indicates the rate at which the capacity of the soil to degrade a set of carbon and energy substrates disappears upon dilution. A low slope parameter is indicative of a slow disappearance rate and can be considered a measure of high physiological diversity (Gomez et al. 2004; Rutgers et al. 2006).

Soil respiration (potential C mineralization) was measured by gas chromatography weekly as CO_2 evolution after a 6-week period of incubation. Soil was homogenized, sieved (5 mm mesh size), and brought to 50–60% of the water-holding capacity. Subsamples of 200 g soil were incubated in the dark at 20°C in 1.5 L air-tight jars supplied with a gas septum. The gas chromatograph was a Carlo Erba 6000 with a column switching system, equipped with a 4-m Porapak q and a 2 mmol sieve 5A column. The detector (HWD) temperature was 180°C, the column temperature was 50°C, and the injection volume was 1 mL (Bloem et al. 1994). Soil respiration (potential C mineralization) was expressed as milligrams of C respired per kilogram of soil per week. The results of the first week were not used to avoid effects of soil homogenization.

In the same jars in which respiration was measured, the potential N mineralization rate was determined as the increase in mineral N (ammonium plus nitrate) between week 1 and week 6. The first week was not used to avoid the effects of soil homogenization. Subsamples of 80 g soil were extracted with 200 mL M KCl. After 1 h shaking, the extracts were filtered over a paper filter and mineral N contents (ammonium and nitrate) were determined by Skalar Segmented Flow Analysis (Breda, The Netherlands).

In addition to potential N mineralization by aerobic incubation of soil, potentially mineralizable N was measured by anaerobic incubation of a soil sample under water (in slurry) for 1 week at 40°C (Keeny and Nelson 1982; Canali and Benedetti 2006). These warm and anoxic conditions are optimal for a quick mineralization of organic matter by anaerobic bacteria. The lack of oxygen prevents conversion of released NH_4^+ to NO_3^- (nitrification), so N

losses by denitrification cannot occur. The amount of mineral N (NH_4^+-N) released is a measure of the quality (N content and decomposability) of the organic matter, and thus, for biological soil fertility (Sparling and Schipper 2002).

Statistical analyses

The data were analyzed with GENSTAT (8th Edition, VSN International, Hemel Hempstead, UK). All parameters were analyzed using one-way analysis of variance (ANOVA) in randomized blocks with treatments (CON, CAN, NMS, LMS, CYM, and FYM) as factors. Treatment effects were apportioned on the basis of least significant difference. *P* values of the tests are presented in Tables 2, 3, 4, and 5. In case no significant ($P > 0.05$) individual treatment effects were found, an unbalanced ANOVA in randomized blocks was carried out with CON, CAN, and organic fertilizers combined (OFC) as factors. When relations between parameters were expected, a regression analysis was carried out. Data of nematode taxa and CLPPs were square root-transformed and subjected to redundancy analysis (RDA) (CANOCO 4.5, Biometris, Wageningen, The Netherlands). A Monte Carlo permutation test (499 random permutations) was performed to test for statistically significant ($P \leq 0.05$) relationships between community structure and treatment effects following a multivariate analysis of variance design (Van Dobben et al. 1999).

Results

Soil chemical, physical parameters, and grass production

The application of organic fertilizers, considered separately, did not result in higher levels of C total or N total than the application of inorganic fertilizer (CAN) and the untreated control (CON) (Table 2). However, when the organic fertilizers were considered as one overall treatment (OFC), this resulted in higher levels of C total ($P = 0.01$) and N total ($P = 0.004$) in the soil treated with organic fertilizers. The pH-KCl of the individual organic fertilizers was significantly higher than CAN and CON (Table 2). The soil bulk density of the CYM treatment tended ($P = 0.081$) to be lower compared to CAN and CON. In the soil layer 0–10 cm, the soil penetration resistance was significantly ($P = 0.019$) lower in CYM and FYM than CAN. The soil penetration resistance in the 0- to 10-cm soil layer was positively related with the soil bulk density in the 5- to 10-cm soil layer ($R^2 = 0.52$, $P < 0.001$). After 5 years, the treatments had no visible effect on soil structure. The root mass in the soil layer of 0–10 cm was lower in the organic fertilizer treatments than in CAN and CON. The root mass,

Table 2 Soil chemical and physical characteristics in grassland (0–10 cm)

Parameter	Units	Treatments						P value
		CON	CAN	NMS	LMS	CYM	FYM	
C total	g C kg dry soil ⁻¹	20.2	19.5	27.9	25.3	25.4	27.4	NS
N total	g N kg dry soil ⁻¹	1.40	1.41	1.97	2.03	2.05	2.05	NS
pH-KCl		5.30b	5.10b	5.90a	5.90a	5.80a	5.88a	<0.001
Bulk density	g cm ⁻³	1.28	1.29	1.24	1.27	1.17	1.22	NS
Penetration resistance								
0–10 cm	mPa	1.22ab	1.29a	1.18ab	1.23ab	1.05c	1.13b	0.019
10–20 cm	mPa	2.11	2.21	2.05	2.03	1.79	2.00	NS
20–30 cm	mPa	2.62	2.54	2.96	2.66	2.36	2.56	NS
30–40 cm	mPa	2.75	2.60	3.36	2.82	2.85	2.77	NS
40–50 cm	mPa	2.39	2.18	3.18	2.14	2.63	2.24	NS
Soil structure								
Crumb	%	46	41	42	49	49	52	NS
Subangular	%	49	47	48	45	43	45	NS
Angular	%	5	13	10	7	9	3	NS
Root biomass	g DM m ⁻²	1056a	1035a	707b	696b	804b	813b	<0.001
Grass aboveground biomass								
1st harvest	g DM m ⁻²	190d	437ab	533a	421b	323c	431b	<0.001
2nd harvest	g DM m ⁻²	200d	339a	273b	214cd	251bc	226bcd	<0.001
3rd harvest	g DM m ⁻²	72c	133ab	173a	137ab	126b	165ab	<0.001
4th harvest	g DM m ⁻²	99	132	132	105	114	134	NS
Total	g DM m ⁻²	560c	1041a	1111a	878b	813b	956ab	<0.001

Values indicated by the same letter within a row are not statistically different at the 5% error level for the main treatment effect

CON unfertilized, CAN fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%), NMS “normal” cattle manure slurry, LMS manure slurry of cattle fed a ration low in protein, CYM manure slurry mixed and composted with hay from roadsides, FYM farmyard manure from a deep-litter stable with dairy cows

measured in October, was negatively related with the dry matter production of the grass in the first ($P=0.007$), third ($P<0.001$), and fourth ($P=0.047$) harvest, but most of the variance was explained by the data from the third harvest,

in August ($R^2=0.43$, $P<0.001$; Fig. 1). Root mass was also negatively related with the potentially mineralizable N (anaerobic incubation) ($R^2=0.22$, $P=0.015$). The total grass yield was highest for NMS and CAN, while CON had the

Table 3 Earthworm numbers, biomass, species, functional groups, and earthworm burrows in grassland (0–20 cm)

Parameter	Units	Treatments						P value
		CON	CAN	NMS	LMS	CYM	FYM	
Total number	n m ⁻²	144	125	231	175	144	269	NS
Body biomass	g worm ⁻¹	0.43	0.18	0.24	0.34	0.35	0.48	NS
Total biomass	g m ⁻²	58	24	63	67	49	82	NS
Ratio adult/juvenile		1.4	0.7	0.8	1.2	1.6	0.9	NS
Epigeic adults	%	40	43	38	43	70	72	NS
Endogeic adults	%	60	57	62	57	30	27	NS
Earthworm burrows								
At 10 cm depth	n m ⁻²	6	13	25	56	38	63	NS

CON unfertilized, CAN fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%), NMS “normal” cattle manure slurry, LMS manure slurry of cattle fed a ration low in protein, CYM manure slurry mixed and composted with hay from roadsides, FYM farmyard manure from a deep-litter stable with dairy cows

Table 4 Nematodes abundance, trophic groups, life history groups, and community structure indices in grassland (0–10 cm)

Parameter	Units	Treatments						P value
		CON	CAN	NMS	LMS	CYM	FYM	
Total number	$n \times 10^3 \text{ m}^{-2}$	5,381	4,932	4,788	4,804	4,146	5,045	NS
Bacterivorous	$n \times 10^3 \text{ m}^{-2}$	2,082	1,678	2,642	2,289	1,965	2,469	NS
Herbivorous	$n \times 10^3 \text{ m}^{-2}$	2,739ab	2,920a	1,772c	1,999c	1,863c	2,039bc	0.017
Fungivorous	$n \times 10^3 \text{ m}^{-2}$	227	138	98	123	139	96	NS
Omnivorous	$n \times 10^3 \text{ m}^{-2}$	286	220	250	314	150	386	NS
Carnivorous	$n \times 10^3 \text{ m}^{-2}$	0	21	11	66	28	54	NS
Bacterivorous	%	34c	39bc	57a	47ab	47ab	48a	<0.001
Herbivorous	%	56a	54ab	35d	42cd	45bc	41cd	0.002
Fungivorous	%	5	3	2	3	3	2	NS
Omnivorous	%	6	4	5	6	4	8	NS
Carnivorous	%	0	0	0	2	1	1	NS
cp-1	%	31	26	29	22	27	30	NS
cp-2	%	50bc	61a	57ab	57ab	51bc	48c	0.019
cp-3	%	6b	4b	5b	7b	13a	6b	0.031
cp-4	%	5b	2b	7ab	13a	6b	13a	0.007
cp-5	%	9	8	2	1	2	3	NS
Number of taxa		22	23	23	25	24	23	NS
Maturity index (cp1–5)		2.12	2.04	1.97	2.12	2.10	2.10	NS
NCR		0.89b	0.94a	0.96a	0.95a	0.94a	0.96a	0.002

Values indicated by the same letter within a row are not statistically different at the 5% error level for the main treatment effect

CON unfertilized, CAN fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%), NMS “normal” cattle manure slurry, LMS manure slurry of cattle fed a ration low in protein, CYM manure slurry mixed and composted with hay from roadsides, FYM farmyard manure from a deep-litter stable with dairy cows, cp colonizer-persister groups

lowest yield. Values for FYM, LMS, and CYM were intermediate. Total grass yield was positively related with N total in the soil ($R^2=0.14$, $P=0.043$), bacterial growth rate (thymidine incorporation) ($R^2=0.16$, $P=0.030$), the proportion of bacterivorous nematodes ($R^2=0.17$, $P=0.027$), and the effective N from fertilizers ($R^2=0.54$, $P<0.001$). Variance of total grass yield was best explained with a regression model of the sum of the effective N from fertilizers and the N supply capacity of the soil ($R^2=0.63$, $P<0.001$; Fig. 2). The relationship between total grass yield and potentially mineralizable N (anaerobic incubation) was significant ($R^2=0.24$, $P=0.037$) when the CAN treatment was left out of the analysis.

Soil biological parameters

Earthworms

Total earthworm biomass was not significantly different between the treatments since variance was high and only one sample per plot could be taken (Table 3). The total earthworm biomass was positively related with the proportion of crumbs in the 0- to 10-cm soil layer ($R^2=0.26$, $P=$

0.007). The earthworm species richness in this experiment was limited; only *Lumbricus rubellus* (epigeic adults) and *Aporrectodea caliginosa* (endogeic adults) were found. No anecic species were found, not even after mustard extraction in a selection of the plots. *L. rubellus* dominated the earthworm adult population after 5 years of FYM and CYM application, while *A. caliginosa* was more dominant in the other treatments. The proportion of epigeic adults was negatively related with the soil bulk density in the 5- to 10-cm soil layer ($R^2=0.41$, $P<0.001$). The number of earthworm burrows was significantly higher in the organic fertilizer treatments ($P=0.041$) when compared as one group (OFC) with CAN and CON.

Nematodes

Nematode abundance was not affected by 5 years of fertilization with different fertilizer types (Table 4). However, CAN and CON were dominated by herbivorous nematodes ($P<0.001$), whereas the treatments with organic fertilizer were dominated by bacterivorous nematodes ($P=0.002$). No N fertilization (CON) for 5 years resulted in the highest proportion of herbivorous nematodes and the lowest

Table 5 Microbial biomass, activity, CLPP, N mineralization, and respiration in grassland (0–10 cm)

Parameter	Units	Treatments						<i>P</i> value
		CON	CAN	NMS	LMS	CYM	FYM	
Bacterial biomass	µg C g dry soil ⁻¹	51	41	56	57	53	52	NS
Fungal Biomass	µg C g dry soil ⁻¹	15	14	15	16	14	17	NS
Thymidine incorporation	pmol g dry soil ⁻¹ h ⁻¹	43c	41c	101a	65bc	74b	53bc	<0.001
Leucine incorporation	pmol g dry soil ⁻¹ h ⁻¹	583	591	901	652	720	638	NS
Fungal activity	% of hyphal length	5	5	1	6	10	1	NS
CLPP								
Slope		0.40	0.39	0.40	0.44	0.38	0.41	NS
ES50	µg dry soil	827	877	941	872	806	928	NS
Potential N mineralization	mg N kg dry soil ⁻¹ wk ⁻¹	8.97a	7.22b	9.57a	9.45a	10.10a	9.65a	0.024
Potentially mineralizable N	mg N kg dry soil ⁻¹	65.1c	58.8c	88.9a	72.8bc	82.6ab	80.1ab	0.004
Potential C mineralization	mg C kg dry soil ⁻¹ wk ⁻¹	88.8	81.4	83.4	94.9	117.8	109.3	NS
Microbial metabolic quotient (<i>q</i> CO ₂)		1.57	1.59	1.23	1.29	1.83	1.66	NS

Values indicated by the same letter within a row are not statistically different at the 5% error level for the main treatment effect

ES50 effective weight of soil needed for 50% substrate utilization, CON unfertilized, CAN fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%), NMS “normal” cattle manure slurry, LMS manure slurry of cattle fed a ration low in protein, CYM manure slurry mixed and composted with hay from roadsides, FYM farmyard manure from a deep-litter stable with dairy cows

proportion of bacterivorous nematodes, whereas applying NMS for 5 years resulted in the highest proportion of bacterivorous and the lowest proportion of herbivorous nematodes. The abundance of herbivorous nematodes was positively related with a higher root mass in the 0- to 10-cm layer ($R^2=0.15$, $P=0.037$). The proportion of bacterivorous nematodes increased with a higher bacterial growth rate (viz. thymidine incorporation or DNA synthesis) ($R^2=0.27$,

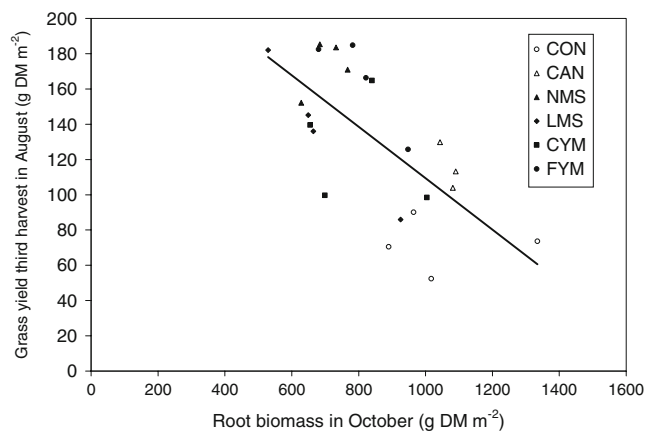


Fig. 1 Relationship between root biomass (g DM m⁻²) in the 0- to 10-cm soil layer and the dry matter yield of grass (kg DM ha⁻¹) in the third harvest ($R^2=0.43$, $P<0.001$). Treatments: unfertilized (CON) or fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%, CAN), “normal” cattle manure slurry (NMS), manure slurry of cattle fed a ration low in protein (LMS), manure slurry mixed and composted with hay from roadsides (CYM), and farmyard manure from a deep-litter stable with dairy cows (FYM)

$P=0.006$). The relatively low proportion of bacterivorous nematodes in CON resulted in a significantly ($P=0.002$) lower NCR in CON compared to all individual fertilizer treatments. The life history group distribution in CAN showed a high percentage of nematodes in the cp-2 group where a high percentage of enrichment opportunists (cp-1) was expected. Differences between treatments in percentages of nematodes in the cp-2 to cp-5 groups did not result in differences in the MI (cp1–5) and MI (cp2–5) (not shown). In a multivariate analysis (RDA) of all nematode taxa, no statistically significant effects of the different treatments were found.

Microbial parameters

Bacterial and fungal biomass and the fungal/bacterial biomass ratio were not affected by 5 years of fertilization with different fertilizer types (Table 5). However, the bacterial growth rate (thymidine incorporation or DNA synthesis) was more than twofold higher in NMS than in CAN and CON ($P<0.001$). CYM, LMS, and FYM were intermediate. A higher thymidine incorporation was negatively related with root mass ($R^2=0.46$, $P<0.001$). The fungal activity did not show significant differences between the treatments.

The CLPP-slope parameter and the amount of extracted soil necessary for conversion of 50% of all substrates in the ECO-plates were not significantly different between the treatments. In a multivariate analysis (RDA) of all CLPPs

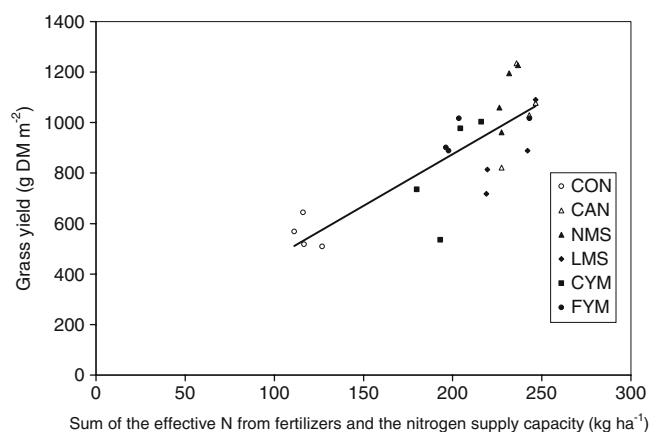


Fig. 2 Relationship between the sum of the effective N from the fertilizers (kg ha^{-1}) and the N supply capacity of the soil with the dry matter yield of grass (g DM m^{-2}) ($R^2=63$, $P<0.001$). Treatments: unfertilized (CON) or fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%, CAN), “normal” cattle manure slurry (NMS), manure slurry of cattle fed a ration low in protein (LMS), manure slurry mixed and composted with hay from roadsides (CYM) and farmyard manure from a deep-litter stable with dairy cows (FYM)

simultaneously (Fig. 3), all canonical axes together were statistically significant ($P=0.012$). A significant relationship was found with FYM ($P=0.006$) and a marginally significance relationship with CYM ($P=0.054$), i.e., both fertilizers with a high C/N ratio (Table 1). Differences in CLPPs originate from differences in the capability of the bacterial communities to degrade a suite of carbon and

energy substrates. In the treatments with FYM and CYM, there was a preference for substrates with carbohydrates (CH; Fig. 3), whereas in all other treatments, substrates like carboxylic acids appeared to be preferred (CA; Fig. 3).

The potential N mineralization (aerobic incubation) was significantly ($P=0.024$) lower for CAN compared to the individual organic fertilizer treatments and CON (Table 5). The potentially mineralizable N (anaerobic incubation) was significantly ($P=0.004$) lower for the CON and CAN treatments. In contrast to the potential N mineralization, the potentially mineralizable N did show differences among the organic fertilizers. NMS had a significantly higher value than LMS. CYM and FYM were intermediate in value. The potential N mineralization (aerobic incubation) increased with a higher pH ($R^2=0.30$, $P=0.003$) and a higher percentage of crumbs ($R^2=0.24$, $P=0.010$). The potentially mineralizable N (anaerobic incubation) increased with a higher C total ($R^2=0.34$, $P=0.002$), a higher N total ($R^2=0.37$, $P<0.001$), a higher soil pH ($R^2=0.32$, $P=0.002$), a higher bacterial growth rate (viz. thymidine incorporation or DNA synthesis) ($R^2=0.43$, $P<0.001$), a higher proportion of bacterivorous nematodes ($R^2=0.25$, $P=0.008$), and a higher earthworm abundance ($R^2=0.22$, $P=0.011$).

Discussion

Effect of inorganic versus organic fertilizers on soil biota

We hypothesized that the abundance of earthworms, nematodes, and microorganisms would be stimulated more by organic than inorganic fertilizers. The results on earthworm biomass, total number of nematodes, and microbial biomass did not confirm our hypothesis, since the differences were not significant. For earthworms, only the number of earthworm burrows was significantly higher in the group of organic fertilizers (OFC) than in CAN, which is an indirect indication that earthworms were stimulated more by organic than inorganic fertilizers. Negative effects of inorganic N fertilizers on earthworms in grassland are mainly related to changes in soil acidity (Ma et al. 1990). Since the pH-KCl was significantly lower in CAN than in the organic fertilizer treatments, this could be the reason for a lower number of earthworm burrows in CAN in our experiment.

For the total number of nematodes, the absence of treatment effects might be the result of differential effects of the inorganic and organic fertilizers on nutrient availability from the fertilizer, mineralization of soil organic matter, and the grass root mass. In turn, this resulted in differential effects on herbivorous and bacterivorous nematodes, respectively. At the end of the growing season, grass root biomass was highest in the CAN treatment which coincided with increased densities (and proportion) of herbivorous

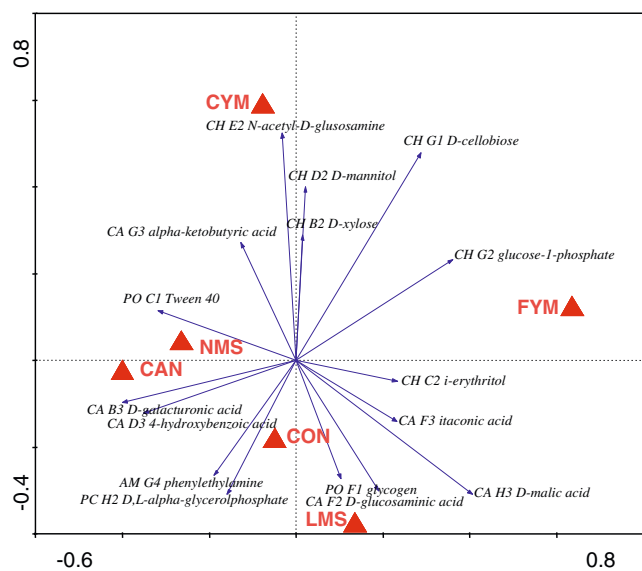


Fig. 3 Ordination diagram of CLPPs of bacterial communities based on RDA. Treatments: unfertilized (CON) or fertilized with inorganic N fertilizer (calcium ammonium nitrate 27%, CAN), “normal” cattle manure slurry (NMS), manure slurry of cattle fed a ration low in protein (LMS), manure slurry mixed and composted with hay from roadsides (CYM) and farmyard manure from a deep-litter stable with dairy cows (FYM). CH carbohydrate, PO polymer, AA amino acid, CA carboxylic acid, AM amine

nematodes. In contrast, organic fertilizer application resulted in an increased proportion of bacterivorous nematodes. Thus, while total abundance of nematodes was the same for the different treatments, the abundance and proportion of herbivorous nematodes was highest in the CAN treatment and the proportion of bacterivorous nematodes was highest in the organic fertilizer treatments. A similar distribution of trophic groups over treatments with inorganic N and organic fertilizers was also found in a microcosm experiment by Van Den Pol-van Dassel et al. (2006). The abundance of herbivorous nematodes was positively related with a higher root mass. Bouwman and Arts (2000) also linked an increase in herbivorous nematodes to root mass, and Todd (1996) reported a significant correlation between the abundance of herbivorous nematodes and the root N content. We conclude that the higher abundance and proportion of herbivorous nematodes in the CAN treatment was probably a fertilizer effect via the increased root biomass. The increased proportion of bacterivorous nematodes in the organic fertilization treatments was probably a fertilizer effect via the increased microbial biomass, consistent with previous laboratory and field studies with grassland soil (Dmowska and Kozłowska 1988; Griffiths et al. 1998; Bardgett et al. 1998; Forge et al. 2005).

The reason for no differences between the treatments for bacterial biomass was less clear than for the nematode abundance. As hypothesized, the bacterial activity, measured as bacterial growth rate (thymidine incorporation or DNA synthesis), was higher for the organic fertilizers compared to the CAN treatment. However, this higher bacterial growth rate (cell production) did not result in a larger bacterial biomass. Possibly, an increase in bacterial biomass was prevented by microbivores like protozoa and bacterivorous nematodes (Bloem et al. 1994). This was supported by the positive relationship between bacterial growth rate and the proportion of bacterivorous nematodes. However, in a Canadian experiment, an increased microbivore population in the organic fertilizer treatment was accompanied by a higher bacterial biomass (Forge et al. 2005; Bittman et al. 2005). A difference between the two experiments was that the fertilizer application and soil sampling in the Canadian experiment was more frequent over the season, whereas in our experiment, the fertilizer treatments were applied before the first and second harvest and sampling took place in October. As a result of this gap between fertilizer application and soil sampling, the direct nutrient availability for the microbial biomass via fertilizer application could have faded away, while the indirect nutrient availability via the plant effects (e.g., root biomass) of the fertilizer treatments could have brought the microbial biomass to the same level. Microorganisms reach higher abundances in the rhizosphere than in bulk soil (Alphei et al. 1996) and thus a higher rhizosphere/bulk soil ratio goes

with an increase in the microbial biomass in the soil. Similar results were found by Bardgett et al. (1999) who measured strong positive relationships between root mass of grasses and total phospholipid-derived fatty acids (PLFA), bacterial PLFA, and fungal PLFA.

Interesting in the whole discussion is the role of the grass root mass in the effect of the different fertilizer treatments on soil biota. The CAN treatment had a higher root mass than the organic fertilizers. In the first instance, this was not expected, since Ennik et al. (1980) found a negative relationship between N supply and grass root mass. However, Ennik et al. (1980) also observed that even a short period of suboptimal N supply could result in a marked increase in root growth. In our experiment, grass root mass in CAN was similar to that in CON. Apparently, the N supply before the date of sampling was low in the CAN treatment. Although the available N was not measured over the year, the dry matter production of the grass could be used as an indirect estimation. There was a significant negative linear relationship between the grass DM production in the third harvest (August) and the root mass determined in October (Fig. 1). Furthermore, the lower available N in the CAN treatment and its effect on root biomass could be seen from the potentially mineralizable N (Table 5) and its significantly negative relationship with root mass. A lower available N in CAN, from the third harvest onwards, could be explained by the application of the fertilizers only before the first and second grass harvest combined with the high direct availability of N from CAN immediately after application. Furthermore, the lower N total in the soil of CAN reduced the mineralization of soil N. The outcomes suggest that the available N (NO_3^- and NH_4^+) in the soil plays a central role in the effect of fertilizers on nematodes and microorganisms. Microorganisms are affected directly through nutrient availability and indirectly through grass root mass. Nematodes are affected indirectly through microbial biomass and grass root mass.

In terms of species richness of soil biota, we hypothesized a higher number of species in the treatments with organic fertilizer compared to CAN. For earthworms and nematodes, no differences in species number were found. For nematodes, only a shift in trophic groups was measured. The CLPP-slope parameter did not indicate a difference in physiological diversity of the bacterial community. Our data suggest that the inorganic fertilizer maintains a similar species richness or community structure of soil biota as the group of organic fertilizers.

Effects of different organic fertilizers on soil biota

We hypothesized that from the four organic fertilizers tested, FYM would stimulate the abundance of the soil biota most. After 5 years, earthworm abundance was not

significantly different between the organic fertilizers tested. Timmermans et al. (2006) measured higher earthworm abundance with farmyard manure compared to manure slurry, although the number of earthworms after application of manure slurry was already very high. Since in grassland large amounts of organic matter are present in the form of root litter, addition of extra organic matter with the different organic fertilizer was not expected to have a considerable effect on earthworm abundance and biomass (Cotton and Curry 1980; Edwards and Lofty 1982). Little or no effect of different organic fertilizers was found on the abundance of nematodes and microorganisms. De Vries et al. (2006) also found no difference in bacterial and fungal biomass after 3 years of application of farmyard or manure slurry on a grass–clover mixture. However, for nematodes, Van Den Pol-van Dasselaar et al. (2006) found a positive relationship between the abundance of bacterivorous nematodes and the organic N content of eight different cattle manure slurries. Although Forge et al. (2005) have shown that the residual effect of organic fertilizer on bacterivorous nematodes can last for at least 1 year, in our experiment, only small differences in trophic group distribution were found. Altogether, the data suggest that, in explaining the effects of organic fertilizers on the abundance of nematodes, the interaction and leveling out of differential effects on various trophic groups via the microbial biomass and the grass root mass may have also played a role. As discussed in the previous paragraph, we suggest that these effects are governed by the available N (NO_3^- and NH_4^+) in the soil and its effect on the microbial biomass and the grass root mass.

Since NMS had a higher labile organic matter content and a higher available N than FYM, we hypothesized that the bacterial activity would be highest in the treatment with NMS and lowest in FYM. The twofold higher bacterial activity, measured as bacterial growth rate (thymidine incorporation), in NMS compared to FYM confirmed this hypothesis.

The bacterial community and the species composition of earthworms was affected by the use of FYM and CYM. The effect of organic fertilizers on the bacterial community was demonstrated by a shift in the substrate preferences in the Biolog plates (Fig. 3). For soils that had received organic fertilizer with a high C/N ratio (FYM and CYM), a preference for a suite of different carbohydrates was observed. We suppose that the bacterial community is more adapted to decomposition of carbohydrate-rich plant residues in the yard manures, compared to the manure slurry which is richer in proteins. Here, it has to be mentioned that the Biolog approach has the advantage of combining both functional diversity and degradation rates, but it is culture-dependent. Still, the culture-dependent part of the microbial community can be effectively studied when replicates contain a similar inoculation density (Nannipieri et al.

2003) or when dilution series have been used (this study; Rutgers et al. 2006). The trend towards a higher proportion of epigeic adult earthworms in FYM and CYM could be explained by the application method and/or the food preference of earthworms. Both FYM and CYM were surface-applied, whereas both manure slurries (NMS and LMS) were slit-injected. De Goede et al. (2003) and Van Vliet and de Goede (2006) found a negative effect of slit injection on the abundance of epigeic earthworms compared to surface application, depending on the weather conditions. Since the proportion of epigeic adults in the slit-injected treatments (NMS and LMS) was similar to that in CON and CAN, negative effects of injection did not probably occur. A more likely explanation is the food preference of epigeic earthworms. Edwards and Lofty (1982) and Whalen et al. (1998) showed an increase in the anecic species *L. terrestris* when farmyard manure was applied. In our study, no anecic species were found, but the food preference of both anecic and epigeic earthworms is primarily plant litter and fresh organic residues (Lavelle 1998). Both FYM and CYM have the same consistency, have a higher C/N ratio than manure slurry, and contain fresh organic matter in the form of straw or grass particles.

Relevance of changes in soil biota for ecosystem services

Soil biota are key for a range of ecosystem services, including soil structure maintenance, water retention, and supply of nutrients (Swift et al. 2004; Mulder 2006; Brussaard et al. 2007b; Kibblewhite et al. 2008). Soil structure maintenance is an ecosystem service provided by roots and soil biota like earthworms, bacteria, and fungi. In our experiment, the different fertilizers did not affect visual soil structure. In a field study on arable land, greater aggregate stability was demonstrated in a soil fertilized with farmyard manure compared to inorganic fertilizer only or no fertilization at all (Estevez et al. 1996). Probably, these effects could not be measured in our experiment because the positive effects of grass and its roots on soil structure outweighed the added effects of fertilization on soil structure-forming biota. Williams and Cook (1961) concluded from their study on arable land that a grass ley was much more effective than farmyard manure in improving soil structure. Although in our experiment soil structure was not significantly affected by any treatment, the percentage of soil crumbs significantly increased with a higher earthworm biomass. A similar relationship between earthworm abundance and soil structural stability was found by Estevez et al. (1996). A higher abundance and proportion of epigeic earthworms was in our experiment negatively related with the soil bulk density. Clements et al. (1991) and Hoogerkamp et al. (1983) found a higher bulk density when earthworms were absent. The positive trend towards

more epigeic earthworms in CYM and FYM, the negative relation between epigeic earthworms and bulk density, and the significantly lower penetration resistance in CYM and FYM gave some evidence that both fertilizer types (CYM and FYM) contribute more to the service of soil structure maintenance than CAN, but this needs further study.

Water regulation is an ecosystem service greatly influenced by earthworms in grassland (Clements et al. 1991; Bouché and Al-Addan 1997). Especially earthworm burrows can increase water infiltration (Edwards and Shipitalo 1998). In our experiment, the number of earthworm burrows was significantly higher in the organic fertilizer treatments when compared as one group (OFC) with CAN. Therefore, it is likely that organic fertilization contributes to the ecosystem service of water regulation. Like for soil structure maintenance, this warrants further study.

The ecosystem service “supply of nutrients” is influenced, amongst others, by bacteria, fungi, nematodes, and earthworms. As discussed previously, the abundances of microorganisms and nematodes were not significantly different between CAN and the organic fertilizers, most probably because of an interaction of direct and indirect fertilizer effects. However, after 5 years, the soils with organic fertilizers had, in comparison with CAN, a higher C total and N total (analyzed as one group of organic fertilizers), a twofold higher bacterial activity (measured as bacterial growth rate, i.e., thymidine incorporation), a 30% higher potential N mineralization (aerobic incubation), and 25–50% more potentially mineralizable N (anaerobic incubation). The bacterial activity was positively related with the proportion of bacterivorous nematodes and the potentially mineralizable N. The relation of the potentially mineralizable N with grass yield was significant when the CAN treatment was left out of the analysis. This makes sense because, without chemical fertilizers, crop production is more dependent on mineralizable N. Parfitt et al. (2005) also found, in nine adjacent pastures spanning a wide range of soil fertility, a significant relationship between potential N mineralization and grass yield. In our experiment, with much smaller differences in soil fertility, the variance of total grass yield was best explained by the sum of the effective N provided by the fertilizer (calculated according to the Dutch official advice) and the N supply capacity of the soil (also calculated according to the Dutch official advice). Thus, compared to inorganic fertilizer, organic fertilization increased the C total, the N total, the activity of decomposers, and the supply of nutrients via the soil food web. The processes behind the supply of nutrients (via mineralization of organic matter in soil and manures) translated well into dry matter yield of the grass by the formulas used in the Dutch official advice on grassland fertilization.

The differences between the organic fertilizer treatments for parameters related to the ecosystem service “supply of

nutrients” were small. N inputs were similar, but the FYM treatment received almost twice as much organic matter ($6,300 \text{ kg ha}^{-1} \text{ year}^{-1}$) as the NMS treatment ($3,400 \text{ kg ha}^{-1} \text{ year}^{-1}$). Still, there was no significant (extra) increase in C total and in abundances of soil biota. The potential C mineralization (soil respiration) and metabolic quotient ($q\text{CO}_2$) of FYM were 30% higher than NMS (but not significant), suggesting that part of the (extra) organic matter in FYM is quickly mineralized. There was no net effect on the potential N mineralization rate. There is no indication that NMS has negative effects on biological soil quality, as supposed by Van Bruchem et al. (2000). On the contrary, NMS provided the highest bacterial activity, the highest amount of mineralizable N, and was the only treatment positively associated with an increase in grass yield over the years 2000–2005 (De Boer et al. 2007). Thus, we have no indications that FYM or the other adjusted manure slurries provide the ecosystem service “supply of nutrients” better than NMS, at least not within 5 years.

Conclusions

After 5 years, organic fertilization increased the C total, the N total, the activity of decomposers, and the supply of nutrients via the soil food web in comparison with inorganic fertilization. Within the group of organic fertilizers, however, there was no significant difference in soil C total, in abundances of soil biota, and in the potential N mineralization rate. Thus, we have no indications that farmyard manure or the adjusted manure slurries provided the ecosystem service “supply of nutrients” better than normal manure slurry, at least not within 5 years. The higher number of earthworm burrows in the organic compared to the inorganic fertilizer treatments suggests that organic fertilizers might stimulate the ecosystem service of water regulation more than inorganic fertilizer. The trend towards higher epigeic earthworm numbers with farmyard manure application and one of the adjusted manure slurries, the negative relation between epigeic earthworms and the bulk density, and the significantly lower penetration resistance for these two organic fertilizer types suggest that they contribute more to the service of soil structure maintenance than inorganic fertilizer.

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