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Production-ecological modelling explains the difference between potential soil N mineralisation and actual herbage N uptake

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ABSTRACT

We studied two different grassland fertiliser management regimes on sand and peat soils: above-ground application of a combination of organic N-rich slurry manure and solid cattle manure (SCM) vs. slit-injected, mineral N-rich slurry manure, whether or not supplemented with chemical fertiliser (non-SCM). Measurements of field N mineralisation as estimated from herbage N uptake in unfertilised plots were compared with (i) potential N mineralisation as determined from a standard laboratory soil incubation, (ii) the contribution of groups of soil organisms to N mineralisation based on production-ecological model calculations, and (iii) N mineralisation calculated according to the Dutch fertilisation recommendation for grasslands. Density and biomass of soil biota (bacteria, fungi, enchytraeids, microarthropods and earthworms) as well as net plant N-uptake were higher in the SCM input grasslands compared to the non-SCM input grasslands. The currently used method in Dutch fertilisation recommendations underestimated actual soil N supply capacity by, on average, 102 kg N ha^{-1} (202 vs. 304 kg ha^{-1} = 34%). The summed production-ecological model estimate for N mineralisation by bacteria, fungi, protozoa, and enchytraeids was 87–120% of the measured potential soil N mineralisation. Adding the modelled N mineralisation by earthworms to potential soil N mineralisation explained 98–107% of the measured herbage N uptake from soil. For all grasslands and soil biota groups together, the model estimated 105% of the measured net herbage N uptake from soil. Soil biota production-ecological modelling is a powerful tool to understand and predict N uptake in grassland, reflecting the effects of previous manure management and soil type. The results show that combining production ecological modelling to predict N supply with existing soil N tests using aerobic incubation methods, can add to a scientifically based improvement of the N fertilisation recommendations for production grasslands.

1. Introduction

Accurate assessments of the natural nitrogen (N) delivery capacity of soils are an imperative component of sustainable, cost-effective and environmentally sound fertilisation management in agro-ecosystems (Velthof et al., 2009). In the Netherlands, fertilisation recommendations for grasslands are based on the N delivery capacity of unfertilised soil (http://www.bemestingsadvies.nl, Section 1.2.2.1) using experimental data collected by Hassink (1994, 1995). In this method, soil N supply from unfertilised grasslands plots is calculated by regression models based on the organic N content in the top 0–10 cm soil layer. However, Van Eekeren et al. (2010) found that with this method in 17 grasslands on sandy soils, which all had an organic matter content of >32 g kg^{-1} dry soil, the actual soil N supply capacity was underestimated by on average 31% (42 kg N ha^{-1}). They therefore concluded that this "legitimises new research to modify the currently used recommendations" (Van Eekeren et al., 2010). The general aim of the present study is to analyse and explain this underestimation and to suggest improvements to the current grassland fertilisation recommendation base.

Fertiliser management practices in temperate production grasslands include applications of chemical fertilisers, cattle slurry and solid cattle manure (SCM). Repeated applications of cattle

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slurry and SCM have been shown to enhance soil organic matter and N content and, thereby, ecosystem productivity (Müller et al., 2011; Shimizu et al., 2009). However, cattle slurry and SCM differ in chemical and physical composition (Levi-Minzi et al., 1986). Thus, applications of either cattle slurry or SCM to grassland may affect a given group of soil biota differently, with repeated applications potentially resulting in diverging community structures. For instance, long term applications of cattle slurry have been shown to negatively affect the fungal biomass in the soil due to its high mineral N content (Bittman et al., 2005). Moreover, long-term chemical fertilizer application to grasslands generally decreases earthworm populations (De Goede et al., 2003; Ma et al., 1990) and microbial biomass (Hopkins et al., 2011). Urine and cattle slurry application to grasslands can be toxic to earthworms and enchytraeids due to high concentrations of ammonia, benzoic acid and sodium sulphide, which also increase soil acidity with negative effects for most soil biota (Curry, 1976; De Goede et al., 2003; Ma et al., 1990). Similarly, microarthropod abundance and diversity decreased following application of chemical fertilisers to grasslands (Siepel and van de Bund, 1988). Conversely, frequent SCM additions have been shown to have positive influences on the abundance of epigeic earthworms (Van Eekeren et al., 2009). These changes in the density and biomass of soil organisms due to historic fertiliser inputs can substantially alter the mineralisation potential of soil organic N (Schon et al., 2012). Therefore, the general hypothesis of the present study is that fertilisation history affects the soil decomposer biota with quantitatively important effects for the soil N delivery capacity.

The capacity of agricultural soils to supply nitrogen for crop uptake is usually estimated as the potential N mineralisation, determined by laboratory incubations (Bloem et al., 1994; Canali and Benedetti, 2006). With this method, soil is sieved to pass a 3–4 mm mesh screen. The sieved soil is adjusted to 60% water holding capacity and incubated at a temperature of 20 °C in the laboratory for six weeks (Bloem et al., 1995). Only microbes and their micro- and meso-faunal predators take account of the decomposition and mineralisation processes, because organisms larger than 3–4 mm in diameter are excluded. Consequently, the possible effects of macrofauna, e.g. earthworms, on N mineralisation, which can be as high as 24 kg N ha^{-1} $month^{-1}$ (De Goede et al., 2003; Van Vliet et al., 2007), are also excluded. Moreover, density and biomass of macrofauna respond differently to different management regimes, especially fertiliser application (Curry, 1976; De Goede et al., 2003; Ma et al., 1990). We therefore hypothesize that current methods underestimate the natural nitrogen supply in grassland soils, which results in higher fertilisation recommendations than needed.

Trophic interactions in the soil foodweb are vital for decomposition and nutrient mineralisation processes (Berg et al., 2001; Bloem et al., 1997; De Ruiter et al., 1994). Models can be useful for aiding our understanding of the effects of agricultural management on crop nutrient uptake through trophic interactions. Models based on the production ecology of soil organisms (Didden et al., 1994) use respiration rates for each group of organisms to calculate energy fluxes, i.e. carbon consumption, assimilation, defecation and production rates. Each taxon of soil organisms, subdivided according to its trophic interactions, is treated as a stand-alone entity with a known body composition and diet (both expressed by C:N ratio), and known assimilation and production efficiencies. Such models assume that soil organisms primarily use N for production (growth and reproduction) and excrete excess mineral N (Persson, 1983). Moreover, these models take into account the fluctuations in the abundance of soil biota throughout the year (Didden et al., 1994). However, comparing the results of model calculations with actual crop N uptake measurements has not been done to date. Such an analysis may improve our understanding of the mechanisms underlying soil organic matter decomposition and crop N uptake and will be of help in the evaluation of the practical use of such models.

The specific aim of the current study is to check whether current methods to estimate background soil N-availability lead to an underestimation because these methods exclude the contribution of larger soil fauna. We hypothesise that (1) the multi-year application of solid cattle manure (SCM) will result in a higher abundance and biomass of all soil biota in the saprotrophic based foodweb, (2) the calculated N mineralisation by microbes and mesofauna through production-ecological modelling will approximate the laboratory-determined potential N mineralisation, and (3) any difference between the laboratory-determined potential N mineralisation and herbage N uptake (corrected for atmospheric N deposition) can be explained by the contribution of soil biota not included in the incubations, in particular earthworms, to N mineralisation.

2. Material and methods

2.1. Site selection

We selected two dairy farms (A and B) where solid cattle manure (SCM) and organic-N rich cattle slurry were produced and applied to the grasslands (SCM grasslands). In addition, two neighbouring farms (C and D) were selected where only mineral-N rich cattle slurry was produced. Differences in the N composition of the cattle slurry were obtained by adaptations in the diet of the cattle (Reijs et al., 2007). The grasslands on farm C were fertilised with slit-injected mineral-N rich cattle slurry together with chemical fertilisers, whereas on those of farm D only cattle slurry was slit-injected. SCM had not been applied for at least 30 years on the grasslands of either farm C or D (non-SCM grasslands). Farms A and C were located in the peat district of the province Utrecht and farms B and D in a sandy soil area near Veenendaal in the province Gelderland, The Netherlands. The distance between farms A and C was about 1 km, whereas farms B and D were situated 15 km apart. Information about grassland management on the four farms and soil characteristics are given

Table 1
Grasslands management information of the four farms (A–D).

Farm	Soil type	Fertilisation history	Age of grassland	Manure dry matter applied	Total N applied	N-inputs (%)				Years in management
						Organic-N		Inorganic-N		
			Year	kg ha^{-1} $year^{-1}$		Total from SCM		From slurry manure	From chemical fertilisers	
A	Peat	SCM[a]	>10	8500	325	75	45	25	0	30
B	Sand	SCM[a]	>10	4960	150	80	70	20	0	5
C	Peat	Non-SCM[b]	>10	4324	275	30	0	30	40	30
D	Sand	Non-SCM[b]	5	3290	145	50	0	50	0	5

[a] Solid cattle manure and organic N-rich slurry. [b] Slurry manure whether or not supplemented with chemical fertiliser.

in Tables 1 and 2, respectively. At each farm, two parallel field experiments (1 and 2) were carried out in grassland fields with a well-documented fertilisation history.

2.2. Monitoring soil biology (experiment 1)

At each of the four farms, a grassland field of approximately 3 ha was selected. Every field was divided into four blocks. In each block, a plot of $15 \times 15\ m^2$ was selected at random for monitoring soil biological parameters. Soil samples for biological and chemical analyses were collected from each plot in the autumn of the pre-experimental year on 20 October 2009, and on 12 April and 16 August 2010. In addition, earthworms were also sampled on 20 October 2010.

2.2.1. Earthworms

To measure earthworm density and biomass, two soil blocks with a volume of $20 \times 20 \times 20\ cm^3$ were randomly sampled in each $15 \times 15\ m^2$ plot. Thus, in total 8 soil blocks were sampled per farm at each sampling time. A formaldehyde solution (0.2%) was applied to the pit of each block to extract deeper living anecic earthworm species. Earthworms were hand-sorted in the field and taken to the laboratory. Within one day after sampling, earthworms were rinsed with tap water, counted and placed in an incubator at 15 °C for two days to empty their gut contents. Afterwards, their fresh weight was measured and they were fixed in alcohol prior to species identification. Classification was undertaken according to Bouché (1977) by distinguishing three ecological groups: epigeic, anecic and endogeic species. Numbers and fresh biomass weight of earthworms were expressed per square meter.

2.2.2. Mesofauna

In each $15 \times 15\ m^2$ plot, enchytraeids were sampled in two soil cores at random using a cylindrical auger of 15 cm length and 5.8 cm diameter. This auger holds 6 PVC rings, each of 2.5 cm height, with which the soil core can be separated into six intact soil layers. The soil samples were stored at 4 °C until extraction. Within 4 weeks after sampling, the enchytraeids were extracted from each soil layer separately using a modified wet extraction method (Didden and Römbke, 2001; Römbke et al., 2006). Enchytraeid numbers were counted and their length was measured using a reticle lens mounted on a light microscope. Based on length, fresh weight was calculated according to Abrahamsen (1973) and expressed in g per m^2. The density of enchytraeids was expressed per square meter.

Microarthropods were sampled with the same soil corer as used for the enchytraeids. Two cores were collected from the 0–7.5 cm soil layer of each plot and the microarthropods were extracted by using a Tullgren funnel (Römbke et al., 2006; Siepel and van de Bund, 1988) within 4 weeks after sampling. During extraction, the temperature in the upper compartment of the extractor, holding the soil samples, was gradually increased over one week from room temperature to 30 °C using light bulbs. The temperature in the lower compartment of the extractor was kept at 5 °C. Fauna that escaped from the soil cores was collected in vials containing 70% ethanol. The microarthropods were counted, classified as mites or collembola, and their density was expressed per square meter.

Table 2
Soil characteristics for the 0–10 cm layer of the four farms (A–D). Mean (±1 SE; $n = 3$) soil organic matter (OM), total nitrogen (N_{total}), dissolved organic carbon (DOC) and pH-KCl in the grasslands of solid cattle manure and organic-N rich cattle slurry application histroy (SCM) and slit-injected, mineral N-rich slurry manure, whether or not supplemented with chemical fertiliser history farms (non-SCM).

Farm	Fertilisation history	Soil type	OM %	N_{total} g kg^{-1}	DOC mg L^{-1}	pH-KCl
A	SCM	Peat	44	12	764	5.0
B	SCM	Sand	6	3	253	5.3
C	Non-SCM	Peat	52	19	1066	4.4
D	Non-SCM	Sand	4	2	190	5.6

2.2.3. Soil microbiological parameters, respiration and N mineralisation

In October 2009 and August 2010, 80 core samples were taken in each plot using a grass plot sampler (Eijkelkamp, The Netherlands). This sampler consisted of a tube of 10 cm length and of 2.3 cm diameter, attached to a soil collection beaker. All sample cores were mixed thoroughly to obtain a field-moist composite sample from each plot. These composite samples were sieved to pass a 4 mm screen and used to measure soil microbiological and abiotic parameters.

A sample of 20 g sieved and homogenised field-moist soil taken from each plot was used to measure fungal and bacterial biomass and bacterial growth rate. From this sample, soil smears were prepared to measure microbial parameters as described by Bloem and Vos (2004). The grid intersection method was used to measure fungal hyphae. Confocal laser scanning microscopy and automatic image analysis were used to measure bacterial numbers and cell volumes (Bloem et al., 1995). Bacterial biomass was calculated from the bacterial cell volume. Bacterial growth rate was determined by the incorporation of [^{3}H]thymidine and [^{14}C] leucine into bacterial macromolecules (Bloem and Bolhuis, 2006; Michel and Bloem, 1993).

Sieved (4 mm) and homogenised samples of approximately 200 g field-moist soil, adjusted to 60% water holding capacity, were incubated in plastic bags. The bags were sealed and incubated at 20 °C in darkness for six weeks to measure potential N mineralisation (Bloem et al., 1994). Increase in mineral N was measured from week 1 to week 6. A sub-sample of 20 g soil was taken from each plastic bag and extracted with 50 ml 1 M KCl. The extract was centrifuged for 10 min and 1.5 ml of the supernatant solution was diluted with 4.5 ml 1 M KCl for further analysis. Mineral N content was measured by Skalar Segmented Flow Analysis (Breda, The Netherlands).

Potential N mineralisation was measured from homogenised and sieved field-moist soil sampled in April, August and October 2010 and corrected for field temperature using a Q_{10} value of 3 (Bloem et al., 1994) by:

$$PNM = q^{\frac{(FT - T_0)}{10}} \times N_L \quad (1)$$

Where PNM [mg N (kg soil)$^{-1}$(5 weeks)$^{-1}$] is the potential N mineralisation at field temperature (FT; °C), q is Q_{10} value, N_L [mg N (kg soil) $^{-1}$ (5 weeks)$^{-1}$] is the N mineralisation measured in the laboratory at temperature T_0 (20 °C). PNM was up-scaled to kg ha^{-1} using soil bulk density (kg m^{-3}) and depth of soil sample (m). To account for seasonal fluctuations during the growing season of 8 months, monthly PNM values were obtained by interpolation of the data obtained in April, August and October 2010.

2.2.4. Abiotic soil parameters

The sieved and homogenised composite samples of field-moist soil taken from each plot were also used for chemical analyses. Moisture content was measured by determining weight loss of approximately 20 g field-moist soil after drying at 105 °C for 24 h. Soil pH was measured in a 1 M KCl solution (1:10, w:v ratio). Soil organic matter (SOM) was determined by loss-on-ignition (Ball, 1964). Soil temperatures were measured in the field by using a digital metallic rod thermometer that was inserted to a soil depth of 10 cm. Precipitation and temperature data (Fig. 1) for both areas were obtained from two nearby (< 5 km) weather stations. Average temperatures in Zegveld (peat) and Veenendaal (sand) for the period October 2009–2010 were 9.8 and 9.6 °C, and total precipitation amounts were 1081 and 1118 mm, respectively.

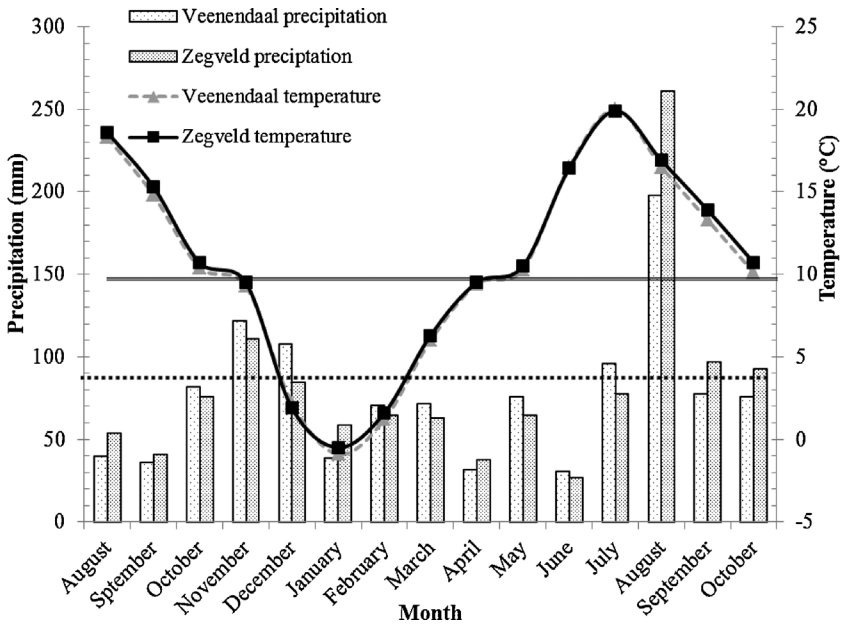

Fig. 1. Average monthly temperature and precipitation at Zegveld and Veenendaal (August 2009–October 2010). Continuous line: mean yearly temperature; dashed line: mean yearly precipitation. Source: Royal Netherlands Meteorological Institute.

2.3. Dutch N fertilisation recommendation method

Soil N supply capacity, i.e. the non-fertiliser N or background supply, was calculated from the total soil N content according to the Dutch fertilisation recommendations for grasslands and fodder crops, which include atmospheric N deposition. If the soil is sampled to a depth of 10 cm, the equation to calculate soil N supply in grassland on sand with an age of 4–6 years is:

$$\text{Soil N supply} = 78 + 28.36 \times [\text{total N}(\text{g.kg}^{-1}\text{soil})]^{1.0046} \quad (2)$$

This equation was used to calculate soil N supply from the grassland of farm D.

For grassland with an age of >9 years, soil N supply is calculated as:

$$\text{Soil N supply} = 78 + 26.57 \times [\text{total N}(\text{g.kg}^{-1}\text{soil})]^{1.0046} \quad (3)$$

This equation was used to calculate soil N supply from the grassland of farm B.

For peat grasslands, i.e. soils with an organic matter content >25%, the Dutch fertilisation recommendations sets the soil N supply capacity in all cases at 250 kg N ha^{-1} $year^{-1}$ (http://www.bemestingsadvies.nl, Section 1.2.2.1). This figure was used as the soil N supply from the grassland of farms A and C.

2.4. Herbage N uptake (experiment 2)

In each of the four blocks of the selected fields (see experiment 1) a wire mesh cage of 4.5 × 1.25 m^2 and 0.5 m height was placed at random (Lantinga et al., 2004). The area inside a cage was divided into five plots of 0.8 × 1.2 m^2 each. The layout and experimental treatments allocated to these plots (i.e. four plots representing all combinations of two manure types and two application rates and one unfertilised plot) have been described in detail by Rashid et al. (2013). For the present study, we used only the herbage dry matter (DM) yield and N uptake data from the unfertilised plots. Herbage was harvested five times during the growing season of 8 months (20 May, 29 June, 9 August, 21 September and 11 November 2010), using a spinach knife and a metallic frame (50 × 50 cm) with pins attached to it to ensure a constant cutting height of 4 cm (Lantinga et al., 2004). Herbage samples were oven-dried at 70 °C for 48 h to calculate dry matter (DM) yield in each plot. After weighing, these samples were ground to pass a 1 mm mesh and analysed for N content by Kjeldahl digestion (MAFF, 1986). The soil herbage N uptake (kg ha^{-1}) was calculated as:

$$\text{N uptake} = \text{N}_{\text{content}} \times \text{DM} \quad (4)$$

where $N_{content}$ is herbage N content (g N (100 g DM)$^{-1}$) and DM is herbage DM yield (kg ha^{-1}). The net herbage N uptake from soil was calculated as the difference between the total measured herbage N uptake and the estimated atmospheric dry and wet N deposition of 15 kg N ha^{-1} as obtained through regression analysis by Van Eekeren et al. (2010).

2.5. Production-ecological calculations

The potential contribution of soil organisms to N mineralisation during the herbage growing season was estimated following the production-ecological calculation method developed by Didden et al. (1994). The model calculations included data on bacteria, fungi, enchytraeids and earthworms as obtained from measurements as discussed previously, and were complemented with literature data for protozoa (amoebae and flagellates). Protozoa are known for their substantial contribution to N mineralisation in agricultural soils (De Ruiter et al., 1993a,b; Van Dijk et al., 2009), but they are very difficult to sample and measure. As such, they were not sampled in our study sites but rather we included amoeba and flagellate biomass data from published literature from grasslands on peat (36.4 and 2.8 kg C ha^{-1}, respectively) (Finlay et al., 2000) and sandy soils (11.3 and 1.9 kg C ha^{-1}, respectively) (Postma-Blaauw et al., 2010). The microarthropod data were not used in the model calculations as microarthropods could not be classified in functional groups. Furthermore, earlier studies have indicated that their direct contribution to soil N mineralisation in agro-ecosystems is small (De Ruiter et al., 1993a,Holtkamp et al., 2011).

In the applied production-ecological approach, assimilation efficiency (A_e), production efficiency (P_e), C:N ratio of body, C:N ratio of food, C consumption and respiration of soil organisms were used to calculate the contribution of each organism group to soil N mineralisation on a monthly basis, taking into account the changes

Table 3
Physiological parameter values for the soil organisms.
Source: (Persson et al. 1980) and (Didden et al. 1994).

Functional group	P_e(C/C)	A_e (C/C)	a	b	T (°C)	Body C:N ratio
Fungi	0.30	1.00	–	–	–	10
Bacteria	0.30	1.00	–	–	–	5
Amoebae	0.40	0.95	13.5	0.8	10	5
Flagellates	0.40	0.95	13.5	0.8	10	5
Earthworms	0.45	0.20	81	0.9	19	5
Enchytraeids	0.40	0.28	33.6	0.67	20	5

P_e, production efficiency, proportion of assimilated energy that is converted into microbial or animal biomass production.
A_e, assimilation efficiency, proportion of ingested food assimilated into blood stream.
a and b, constants for the respiration equation $Q = a\,W^b$ (see text); the constants presuppose Q (oxygen consumption rate) as $O_2\,mm^{-3}\,ind.^{-1}\,h^{-1}$.
T, temperatures at which a and b were determined.

in their density over time. A_e, P_e, and C:N ratio of body of soil organisms were obtained from Didden et al. (1994) who adapted these values from short grass prairie (Hunt et al., 1987) and forest ecosystems (Persson et al., 1980) and are listed in Table 3. The food C:N ratio of earthworms, enchytraeids, bacteria, fungi and protozoa was calculated based on their food preferences and are given in Table 4. Based on the epigeic to endogeic ratios of earthworms (Table 5), and their food preferences, the average C:N ratio of the diet of the earthworm population was calculated according to Van Vliet et al. (2007). The enchytraeid food preferences were taken from De Goede et al. (2003). Bacteria and fungi are mainly decomposers. Therefore, their food C:N ratio was assumed to be equal to the C:N ratio of the detritus and roots (Table 4, De Ruiter et al., 1993a). For protozoa a food C:N ratio of 5 was used as they mainly consume bacteria (Bloem et al., 1997). To account for seasonal fluctuations in the density of soil organisms over the whole growing season of 8 months, monthly densities were obtained by interpolation of the measured densities in April,

Table 4
Food preferences (percentage) for the different taxonomic groups of soil organisms.

Food source	Bacteria[f]	Fungi[f]	Earthworms[c]	Enchytraeids[d]	Protozoa[e]	
					Amoebae	Flagellates
Root[c] (C:N = 10)	–	–	20	–	–	–
Bacteria[b] (C:N = 5)	–	–	10	40	50	100
Fungi[b] (C:N = 10)	–	–	10	40	0	–
Detritus[g] (C:N = 14)	100	100	50	20	–	–
Fresh organic matter[c] (C:N = 7)	–	–	10	–	–	–
Amoebae[b] (C:N = 5)	–	–	–	–	–	–
Flagellates[b] (C:N = 5)	–	–	–	–	50	–
Food C:N ratio[a]	14	14	10.3	8.8	5	5

[a] FoodC : Nratio $= \left(\frac{(\Sigma \text{foodpreference}) \times \text{C:Nratio}_{\text{foodsource}}}{100}\right)$.
[b] Source: (Persson et al. 1980) and (Didden et al. 1994).
[c] Source: (Van Vliet et al. 2007).
[d] Source: (De Goede et al. 2003).
[e] Source: (Bloem et al. 1997).
[f] Adapted from (De Ruiter et al. 1993a).
[g] Own measurement.

Table 5
Soil biota parameters, herbage dry matter yield and herbage nitrogen (N) uptake in peat (P) and sandy (S) grasslands with different fertilisation history, and corresponding F-values after ANOVA.

Treatments		Soil biota parameters, herbage dry matter yield and N uptake									
Fertilisation history (FH)	Soil type (ST)	Earthworm density	Earthworm biomass	Enchytraeid density	Mite density	Collembolan density	Bacterial biomass	Fungal biomass	PNM[d]	Herbage dry matter yield	Herbage N uptake
		$n\,m^{-2}$	$g\,m^{-2}$	$n\,m^{-2}$	$n\,m^{-2}$	$n\,m^{-2}$	$\mu g\,C\,g^{-1}$ dry soil	$\mu g\,C\,g^{-1}$ dry soil	mg $N\,kg^{-1}$ dry soil $5\,week^{-1}$	$kg\,ha^{-1}$	$kg\,ha^{-1}$
SCM[a]	P	568	174	45947	19403	10399	202.7	82.0	88.0	14064	352
	S	554	241	27613	7801	9113	80.6	59.2	58.0	11523	305
Non-SCM[b]	P	268	75	21870	10719	3385	180.1	71.2	87.0	14107	354
	S	109	65	8975	4358	3500	113.5	34.6	46.5	8191	207
ANOVA table df F-values											
FH	1	38.9***	56.9***	11.5**	3.1	5.1	0.0	3.6	0.4	6.8*	8.0*
ST	1	2.1	2.4	6.1*	6.8*	0.0	6.5	10.0*	6.4*	49.9***	33.3***
Time (covariate)	2,3,4[c]	4.4*	3.7	6.3*	15.2**	11.2**	3.3	9.3	54.3***	–	–
Error (mean squares)	9	14275	1339	1	36700000	23553744	277	175	2	–	–

*, ** and *** denote significance level at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
df: degrees of freedom.
[a] Solid cattle manure and organic-N rich slurry.
[b] Slurry manure whether or not supplemented with chemical fertiliser.
[c] Microbial parameters, mesofauna (collembolan, mites and enchytraeids) and earthworms sampled 2,3 and 4 times, respectively, during the experimental period.
[d] Potential nitrogen mineralisation.

August and October 2010 (De Goede et al., 2003). Interpolated densities of soil biota were used to calculate monthly N mineralisation. These were added to derive N mineralisation over the growing season.

The respiration of earthworms, enchytraeids and protozoa was calculated based on their fresh body weight and oxygen consumption rate according to (Persson et al. 1980) as:

$$Q = a \times W^b \tag{5}$$

where Q is oxygen (O_2) consumption rate per soil organism, W indicates individual fresh weight (g) of the soil organism, and a and b are constants for a specific taxonomic group and obtained at a particular temperature (see Table 3). The values of parameters a and b in Eq. (5) presuppose Q as mm^3 O_2 ind.$^{-1}$ h^{-1}. Since Q is temperature dependent, adjustments for actual field temperature were made using a Q_{10} value of 2 for earthworms, enchytraeids and protozoa (Didden et al., 1994). Field O_2 consumption rate of a given taxonomic group of soil organisms was calculated as:

$$Q_{FT} = Q \times q^{\frac{(FT-T_0)}{10}} \times TN_i \tag{6}$$

where Q_{FT} is O_2 consumption rate (mm^3 O_2 m^{-2} h^{-1}) from ith taxonomic group of soil organisms at field temperature (FT; °C) which was measured at a soil depth of 10 cm. Q is O_2 consumption rate (mm^3 O_2 ind.$^{-1}$ h^{-1}) at temperature T_0 (°C) i.e. the temperature at which a and b constants were obtained, q indicates the Q_{10} value. TN is total number of individuals (nm^{-2}) in the ith taxonomic group of soil organisms.

Field O_2 consumption (Q_{FT}) was converted into C respiration rate of a given taxonomic group according to De Goede et al. (2003) who assumed a respiratory quotient of 0.43 [mg C (mm^3 O_2)$^{-1}$] per individual. The relation is given as:

$$R = 0.43 \times Q_{FT} \times (7.2 \times 10^{-3}) \tag{7}$$

where, R denotes C respiration rate (kg C ha^{-1} $month^{-1}$), and 7.2×10^{-3} is the conversion factor for up-scaling mg C m^{-2} h^{-1} into kg C ha^{-1} $month^{-1}$.

We could not find a and b values in the literature for bacteria and fungi. Therefore, their respiration (kg C ha^{-1} $month^{-1}$) was calculated based on:

$$R_{BF} = q^{\frac{(FT-T_0)}{10}} \times C_R \times B \times 30 \tag{8}$$

where, R_{BF} (kg C ha^{-1} $month^{-1}$) represents respiration rate of bacteria or fungi at field temperature (FT; °C) and q indicates Q_{10} value which is 2.2 for both bacteria and fungi according to Goulden et al. (1996). C_R denotes the respiration rate constant which is 0.27 and 0.29 (kg C respiration per kg C consumed day^{-1} per kg biomass) for bacteria and fungi, respectively (Anderson and Domsch, 1975; Stamatiadis et al., 1990) at T_0 (25 °C). B represents biomass of bacteria or fungi (kg C ha^{-1}) and 30 is the conversion factor for up-scaling day into month.

C respiration was used to calculate C assimilation of a given taxonomic group of soil organisms and then C consumption, defecation and production of that group of soil organisms were calculated according to (Persson et al. 1980) as:

$$A = \left(\frac{R}{1 - P_e}\right) \tag{9}$$

$$C_0 = \left(\frac{A}{A_e}\right) \tag{10}$$

$$F = C_0 \times (1 - A_e) \tag{11}$$

$$P = (A \times P_e) \tag{12}$$

where A, R, C_0, F and P in units of kg C ha^{-1} $month^{-1}$ denote C assimilation, respiration, consumption, defecation and production

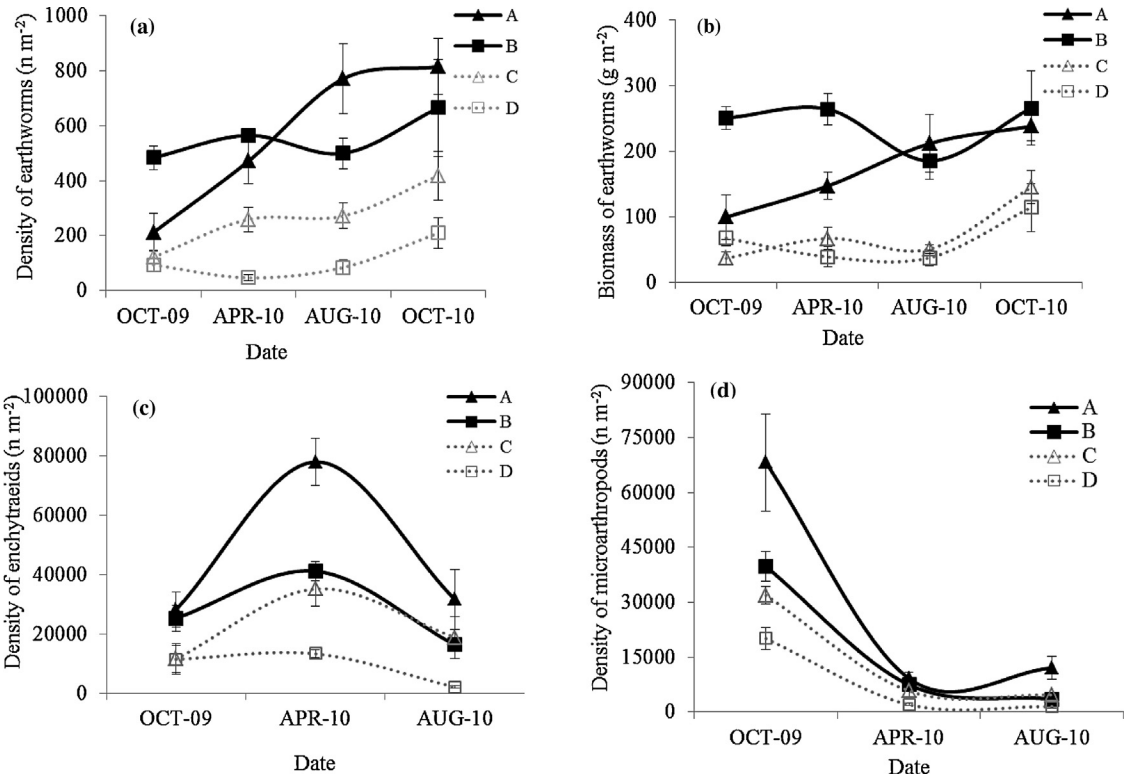

Fig. 2. Temporal changes in density (a) and biomass (b) of earthworms, density of enchytraeids (c) and microarthropods (d) in grasslands of farm A (above-ground application history of a combination of organic N-rich slurry manure and solid cattle manure (SCM) on peat soil), farm B (SCM input history on sandy soil), farm C (slit-injected, mineral N-rich slurry manure, whether or not supplemented by chemical fertiliser (non-SCM) history on peat) and farm D (non-SCM input history on sand).

of a given taxonomic group of soil organisms, respectively. P_e and A_e denote production and assimilation efficiencies of that group of soil organisms, respectively.

N consumption, assimilation, defecation and production of a given taxonomic group of soil organisms were calculated according to (Persson et al. 1983), assuming that C consumption to N consumption ratio and C production to N production ratio of soil organisms were similar to the C:N ratios of food sources and body, respectively. Thus, N consumption was calculated as:

$$NC = \left(\frac{C_0}{C : N_{food}}\right) \quad (13)$$

where NC represents N consumption (kg N ha^{-1} $month^{-1}$) of a given taxonomic group of soil organisms, C_0 indicates the C consumption (kg C ha^{-1} $month^{-1}$) and $C:N_{food}$ [kg C (kg N)$^{-1}$] represents C:N ratio of the food of that taxonomic group of soil organisms.

Persson et al. (1983) assumed that the assimilation efficiency of N in food was higher than the C assimilation efficiency which resulted in a 1.33× lower C:N ratio in feces of the given taxonomic group of soil organisms than that of food consumed. Therefore, N defecation, production, assimilation and mineralisation by that group of soil organisms were calculated as:

$$NF = \left(\frac{F}{1.33 \times C : N_{food}}\right) \quad (14)$$

$$NF = \left(\frac{P}{C : N_{animalbody}}\right) \quad (15)$$

$$NA = (NC - NF) \quad (16)$$

$$N_{min} = (NA - NP) \quad (17)$$

where NF is the N defecation (kg N ha^{-1} $month^{-1}$). F and P, both in units of kg C ha^{-1} $month^{-1}$, represent the C defecation and production, respectively. $C:N_{food}$ and $C:N_{animalbody}$ [kg C (kg N)$^{-1}$] indicate C:N ratios of food and body of a given taxonomic group of soil organisms, respectively. NA, NC, NF and NP, all in units of kg N ha^{-1} $month^{-1}$, denote N assimilation, consumption, defecation and N used in body tissues or cell production, respectively. N_{min} indicates N mineralisation (kg N ha^{-1} $month^{-1}$) by a given taxonomic group of soil organisms.

2.6. Statistical analysis

The effects of treatments on soil biota, potential N mineralisation and herbage N uptake were analysed using analysis of variance (ANOVA) with GENSTAT (13th edition, VSN International, Hemel Hempstead, UK). Treatments were fertilisation history (SCM, non-SCM) and soil type (peat, sand). The interaction between fertilisation history and soil type could not be estimated due to the lack of appropriate replicates. The results of the soil samples from each plot were averaged resulting in four replicates per grassland. In the ANOVA, the replicates within each grassland were nested within farm. The main effects of the treatments were tested for all sampling dates using time as a covariate.

3. Results

3.1. Macrofauna

Total earthworm numbers and biomass were on average three times higher in the SCM grasslands than in the non-SCM grasslands ($P < 0.001$; Table 5). In total, 7 species were observed in SCM grasslands and 3 species in non-SCM grasslands. The dominant epigeic species in the SCM grasslands were *Lumbricus rubellus* Hoffmeister and *Lumbricus castaneus* Savigny. The endogeic species comprised *Aporrectodea caliginosa* Savigny, *Allolobophora chlorotica* Savigny, *Aporrectodea rosea* Savigny and *Aporrectodea limicola* Michaelsen. In contrast, *L. rubellus* was the only epigeic species in non-SCM grasslands, while the endogeic species were dominated by *A. caliginosa* and *A. chlorotica*. The anecic species *Lumbricus terrestris* L. was found only in the SCM grasslands during August and October 2010, but at low densities (9 m^{-2}). We did not find any anecic species in non-SCM grasslands. Earthworm abundance did not differ between sandy and peat soils (Table 5). Earthworm density and biomass progressively increased during the study period (Fig. 2a and b).

3.2. Mesofauna

Enchytraeid abundance and biomass was 2.4 times and 3.1 times higher in SCM than in non-SCM grasslands, respectively (Table 5). Microarthropod abundance and biomass were not significantly different. The density and biomass of enchytraeids in peat soils were about twice as high as in sandy soils. Season significantly affected enchytraeid abundance, which was highest in April 2010 and lowest in August 2010 (Fig. 2c). The latter followed a 4-months period of relatively dry weather (Fig. 1). Enchytraeid abundance was positively correlated with soil moisture content ($R^2 = 0.26$, $P < 0.001$). Abundances of collembola and mites were positively correlated with fungal biomass ($R^2 = 0.51$ and 0.34, respectively; $P < 0.001$) and negatively with soil temperature ($R^2 = 0.25$, $P = 0.001$). Mite, but not collembola abundance, showed a positive correlation with bacterial biomass ($R^2 = 0.20$, $P = 0.012$). The density of microarthropods was much lower during the two sampling periods in 2010 than in October 2009 ($P < 0.01$; Table 5, Fig. 2d).

3.3. Microbes

Fungal biomass was 1.6 times higher ($P \leq 0.05$) in the peat compared to the sandy soils (Table 5). However, fertilisation history did not affect bacterial or fungal biomass (Table 5). Fungal biomass decreased with increasing soil pH ($R^2 = 0.22$, $P = 0.007$).

3.4. Nitrogen mineralisation

Potential N mineralisation (aerobic incubation) was significantly higher in peat than in sandy soils ($P < 0.05$, Table 5). However, no relationship was found with fertilisation history.

3.5. Herbage dry matter yield and N uptake

Pairwise comparison of grasslands based on soil type did not show any difference in herbage DM yield or N uptake between the SCM and non-SCM input farms of the peat soils ($P > 0.05$). However, in the sandy SCM grassland, herbage DM yield and N uptake were higher ($P = 0.001$ and $P = 0.006$, respectively) than in the non-SCM grassland (29 and 32%, respectively).

3.6. Contribution of soil organisms to N mineralisation

The production-ecological calculations showed that fungi mineralised 62–138 kg N ha^{-1} out of the consumed 107–238 kg N ha^{-1} during the herbage growing season of 8 months when comparing the four farms. Earthworms consumed between 77 and 376 kg N ha^{-1} of which 18–86 kg N ha^{-1} was mineralised, whereas the contribution of protozoa to N consumption and mineralisation was 76–200 and 45–116 kg N ha^{-1}, respectively. In addition, bacteria and enchytraeids consumed 238–549 and 3–31 kg N ha^{-1} but they mineralised 38–88 and 1–7 kg N ha^{-1}, respectively. It appears that microbes (bacteria and fungi) accounted for

approximately 60% of the herbage N uptake in both the SCM and non-SCM grasslands (187 and 158 kg N ha^{-1}, respectively) (Fig. 3). Protozoa and earthworms contributed 26% (81 kg N ha^{-1}) and 25% (77 kg N ha^{-1}) to herbage N uptake in the SCM grasslands, respectively, whereas in the non-SCM grasslands, their contributions were 29 (81 kg N ha^{-1}) and 9% (23 kg N ha^{-1}), respectively (Fig. 3). The estimated contribution of enchytraeids to the herbage N uptake was very small (2%) in all grasslands (5 kg N ha^{-1}).

3.7. Comparison of different approaches to predict soil N mineralisation

The results of both the measured and calculated N mineralisation during the growing season of 2010 are given in Fig. 3. In the SCM grasslands, net herbage N uptake was significantly higher ($P < 0.05$) than the laboratory-determined potential N mineralisation. However, no such difference was found for the non-SCM grasslands ($P > 0.05$). The difference between net herbage N uptake and potential N mineralisation in SCM grasslands disappeared when the earthworm N mineralisation based on production ecological calculation was added to the potential N mineralisation data (Fig. 3). When comparing the herbage N uptake from soil with the values obtained by production ecological calculation, we found a difference of only 5% for all grasslands together. In contrast, the Dutch fertilisation recommendation method greatly underestimated soil N supply (by 34%, for all grasslands together) with values ranging from 75 to 131 kg N ha^{-1} (Fig. 3).

4. Discussion

4.1. Effects of fertilisation history on soil biota

We hypothesised that multi-year application of SCM would stimulate the abundance and biomass of soil biota more than cattle slurry whether or not supplemented with chemical fertilisers. In our SCM grasslands the density and biomass of earthworms and enchytraeids (detritivores) were three times higher than in non-SCM grasslands (Table 5). This might be associated with higher fresh food availability (Birkhofer et al., 2008; Timmerman et al., 2006; Van Eekeren et al., 2009), as in the SCM grasslands the organic matter inputs were much higher than in the non-SCM grasslands (Table 1). The rather low abundance of earthworms and enchytraeids on farm C could be related to the lower soil pH on this farm (Table 2), resulting from long-term high inputs of chemical fertiliser N (Hopkins et al., 2011; Ma et al., 1990; Standen, 1984). Fertilisation history did not affect the population density of microarthropods (Table 5). The density of microarthropods was positively correlated with fungal biomass ($R^2 = 0.51$ and 0.34, respectively; $P < 0.001$) suggesting that microarthropods were bottom-up controlled (Sjursen et al., 2005; Booher et al., 2012).

4.2. Model prediction of soil N mineralisation

The production ecological model calculation showed high levels of soil N mineralisation of on average 314 kg N ha^{-1} for the SCM grasslands (Fig. 3). The estimated average N mineralisation was close (+12%, with CV 15% only) to the measured herbage N uptake excluding N deposition (353 kg N ha^{-1} with CV 9% only). For non-SCM grasslands, production ecological calculation estimated an average soil N mineralisation of 244 kg N ha^{-1}, which was very close to (−8% with 29% CV) net herbage N uptake (266 kg N ha^{-1}, with CV 14% only) excluding N deposition; (Fig. 3). For all grasslands together, production ecological model prediction was only 5% (22% CV) higher than the measured net herbage N uptake (290 kg N ha^{-1}, with CV 12%, excluding N deposition). Production ecological calculation gave good estimates of soil N mineralisation.

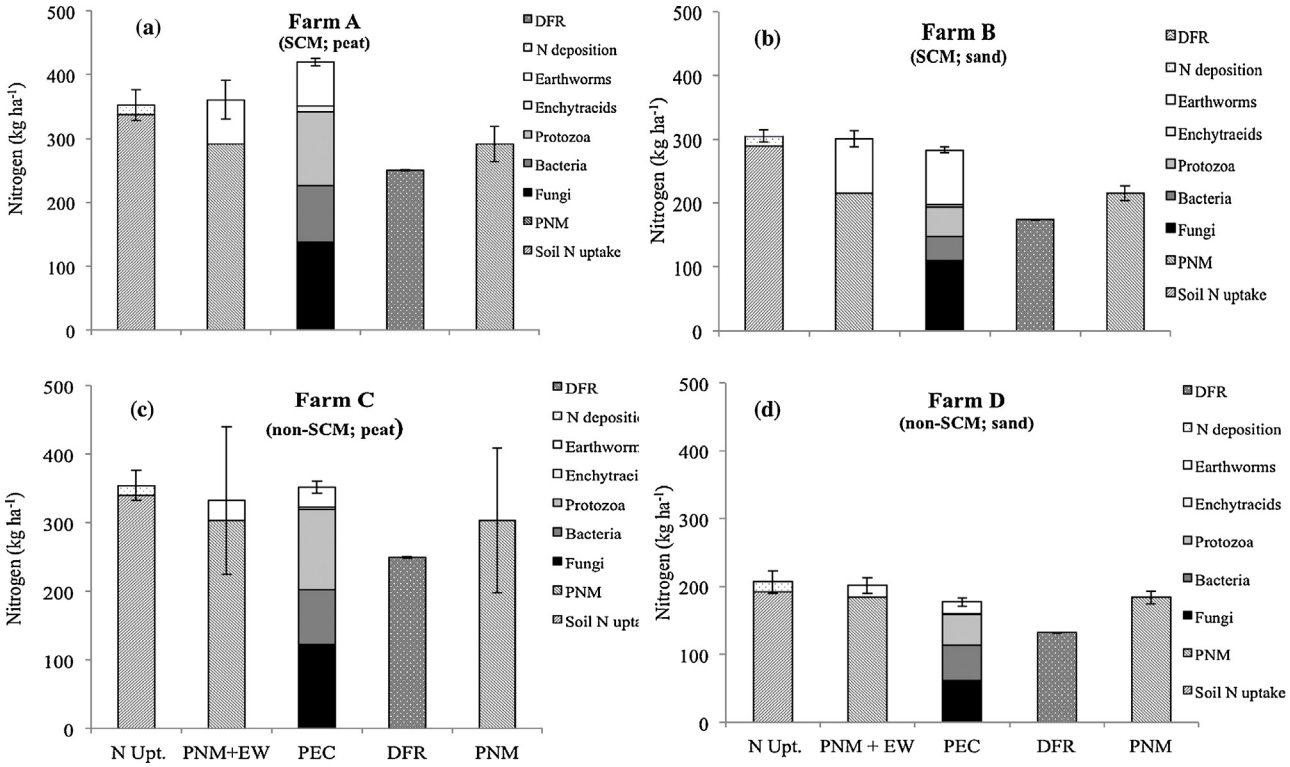

Fig. 3. Total herbage N uptake [N upt (non-fertiliser soil N supply + atmospheric N deposition], potential N mineralisation (PNM), +N mineralisation by earthworms calculated by the production ecological model calculations (EW), simulation of N mineralisation by different groups of soil organisms through production ecological model calculations (PEC), N mineralisation calculated using Dutch fertilisation recommendation (DFR) for grassland and fodder crops and PNM during the growing season of 2010 in SCM (above ground application history of a combination of organic N-rich slurry manure and solid cattle manure) farms A–B and non-SCM (history of slit-injected, mineral N-rich slurry manure, whether or not supplemented with chemical fertiliser) farms C and D.

However, it needed assumptions on certain soil biota parameters as well as on protozoa abundance.

Model predictions mainly depend on the estimated values of physiological parameters of the soil biota that contribute most to N mineralisation (Hunt et al., 1987). In our study, these were fungi, protozoa and earthworms. Sensitivity analysis showed that the prediction of N mineralisation very much depended on parameter values for food C:N ratio, production efficiency and biomass and density of soil biota. Production efficiency values were mainly adapted from short grass prairie (Hunt et al., 1987) and forest ecosystems (Persson et al., 1980). When varying production efficiencies by 25%, fungi showed the largest response (10% difference in N mineralisation), whereas responses related to similar changes in the production efficiency of protozoa and earthworms affected our N mineralisation predictions with only 0.5 and 2%, respectively. Similar changes (25%) in food C:N ratio, resulted in a stronger response of the predicted N mineralisation (46, 27 and 45% for fungi, protozoa and earthworms, respectively). The high responsiveness of fungi probably depends on the fact that fungi have a high body C:N ratio while their food C:N ratio is low (Table 3). The food C:N ratio of earthworms was calculated based on earthworm food preferences i.e. detritus C:N ratio from the selected sites and C:N ratio of the bacteria (Van Vliet et al., 2007). Overall, food C:N ratios had more pronounced effects on the calculated N mineralisation than other parameters. However, as C:N ratios of food sources can relatively accurately be determined, they would have had minor effect on the actual model predictions of N mineralisation.

4.3. Role of soil organisms in N mineralisation

In the grasslands we studied, production ecological calculations indicated that the N mineralisation by earthworms, enchytraeids, fungi and protozoa together added up to, approximately, all N mineralisation measured as herbage N uptake. The calculations showed that earthworms mineralised 18–86 kg N ha^{-1} during the herbage growing season of 8 months (Fig. 3). This was lower than the range of 85–170 kg N ha^{-1} $year^{-1}$ reported by De Goede et al. (2003) and Van Vliet et al. (2007) in fertilised Dutch grasslands. This could be ascribed to a mean 33% lower abundance of earthworms in our grasslands. In our grassland fields, the calculated contribution of earthworms to N mineralisation was 9–30% of the net herbage N uptake from soil. The estimated contribution of fungi to the N mineralisation ranged from 62–138 kg N ha^{-1} which accounted for 32–41% of net herbage N uptake from soil (Fig. 3). This contribution could be explained by their relatively high biomass and lower N requirements compared to the other soil biota. Protozoa are important in soil N mineralisation due to their high specific death rate (6 $year^{-1}$) and N rich food (Bloem et al., 1997). De Ruiter et al. (1993a) and Bloem et al. (1997) estimated their contribution to N mineralisation as up to 48% in arable fields. In our grassland, their contribution to N mineralisation was between 16 and 35%. The estimated contribution of enchytraeids to the N mineralisation was lowest (<5%) of all included soil biota groups, probably due to their relatively low biomass (average for all grassland: 4.2 g C m^{-2}).

4.4. Comparison of different approaches to estimate soil N mineralisation

We hypothesised that any difference between potential N mineralisation and herbage N uptake could be explained by the contribution of earthworms to soil N mineralisation as earthworms are excluded from the in vitro laboratory soil incubation to measure potential N mineralisation. The difference between potential N mineralisation and N uptake corresponded to N-mineralization rates by earthworms as calculated by production ecological model estimation (Fig. 3).

Soil N supply calculated according to the Dutch grassland fertilisation recommendations (http://www.bemestingsadvies.nl, Section 1.2.2.1) was about 103 kg N ha^{-1} lower than the average net herbage N uptake (153 vs. 256 kg N ha^{-1} = 40%) from the unfertilised plots in the grasslands on sand. This underestimation was even greater than the 42 kg N ha^{-1} found by Van Eekeren et al. (2010). They explained this effect from the difference in fertilisation history of their grasslands with the experimental sites of Hassink (1994, 1995), which formed the basis of the Dutch fertilisation recommendation. On the sites used by Hassink (1994, 1995), herbage N uptake was measured from grasslands which were not fertilised for several years, whereas in case of Van Eekeren et al. (2010) and our study, the grassland plots remained unfertilised only in the year of their experiment and received fertilisers in the foregoing years. The latter approach will better represent the yearly needs of the grass sward, as long-term cessation of fertilizer application will result in nutrient mining and consequently in an underestimation of the background N supply capacity of fertilised grassland soils. These findings underline the need to modify the currently used fertilisation recommendations for grassland on sand in The Netherlands.

5. Conclusions

Multi-year application of solid cattle manure to grasslands increased the number and biomass of detritivorous soil biota (earthworms and enchytraeids) compared to the application of cattle slurry and/or chemical fertiliser inputs.

Production ecological modelling was found to be a suitable tool to evaluate the contribution of the soil biota to N mineralisation in grasslands under different fertilisation management practices on different soil types. Under the conditions studied, fungi, bacteria, protozoa and earthworms contributed most to N mineralisation, whereas enchytraeids played a minor role.

Laboratory-determined potential N mineralisation was significantly lower than measured net herbage N uptake from grassland soils. The gap could be explained by the exclusion of earthworms from the incubations and it could be bridged by adding the modelled N mineralisation caused by earthworms. Hence, a combination of soil N tests using aerobic incubation methods and production ecological modelling to predict soil N supply is recommended. Our model calculations and field observations suggest that in grassland with high earthworm density (viz. >500 ind. m^{-2}), the potential N mineralisation could be corrected by multiplication with a factor 1.3, whereas at low densities (viz <300 ind. m^{-2}) a factor of 1.1 should be used. Additional research is necessary to refine this recommendation.

Taking account of earthworms can improve the N fertilisation recommendations for production grasslands, which can reduce costs and can contribute to a reduction of environmental losses of N from agricultural grassland.

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References

Abrahamsen, G., 1973. Biomass and body-surface area of populations of Enchytraeidae and Lumbricidae (Oligochaeta) in Norwegian coniferous forest soils. Pedobiologia 13, 28–39.

Anderson, J.P.E., Domsch, K.H., 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Can. J. Microbiol. 21, 314–322.

Ball, D.F., 1964. Loss-on-ignition as an estimate of organic matter and organic carbon in non-calcareous soils. J. Soil Sci. 15, 84–92.

Berg, M., Ruiter d, P., Didden, W., Janssen, M., Ton, S., Verhoef, H., 2001. Community food web, decomposition and nitrogen mineralisation in a stratified scots pine forest soil. Oikos 94, 130–142.

Birkhofer, K., Bezemer, T.M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D., Ekelund, F., Fließbach, A., Gunst, L., Hedlund, K., Mäder, P., Mikola, J., Robin, C., Setälä, H., Tatin-Froux, F., Van der Putten, W.H., Scheu, S., 2008. Long-term organic farming fosters below and aboveground biota: implications for soil quality, biological control and productivity. Soil Biol. Biochem. 40, 2297–2308.

Bittman, S., Forge, T.A., Kowalenko, C.G., 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biol. Biochem. 37, 613–623.

Bloem, J., Bolhuis, P.R., 2006. Thymidine and Leucine Incorporation to Assess Bacterial Growth Rate, Microbiological Methods for Assessing Soil Quality. CABI, Wallingford, UK, pp. 142–149.

Bloem, J., de Ruiter, P.C., Bouwman, L., 1997. Soil Food Webs and Nutrient Cycling in Agroecosystems. Marcel Dekker Inc., New York.

Bloem, J., Lebbink, G., Zwart, K.B., Bouwman, L.A., Burgers, S.L.G.E., de Vos, J.A., de Ruiter, P.C., 1994. Dynamics of microorganisms, microbivores and nitrogen mineralisation in winter wheat fields under conventional and integrated management. Agric. Ecosyst. Environ. 51, 129–143.

Bloem, J., Veninga, M., Shepherd, J., 1995. Fully automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Appl. Environ. Microbiol. 61, 926–936.

Bloem, J., Vos, A., 2004. Fluorescent Staining of Microbes for Total Direct Counts, Molecular Microbial Ecology Manual, 2nd ed. Kluwer, Dordrecht, pp. 861–874.

Booher, E.C.J., Greenwood, C.M., Hattey, J.A., 2012. Effects of soil amendments on soil microarthropods in continuous maize in western Oklahoma. Southwest. Entomol. 37 (1), 23–30.

Bouché, M.B., 1977. Stratégies lombriciennes. Soil organisms as components of ecosystems. Ecol. Bull. 25, 122–132.

Canali, S., Benedetti, A., 2006. Soil nitrogen mineralization. In: Bloem, J., Hopkins, D. W., Benedetti, A. (Eds.), Microbiological Methods for Assessing Soil Quality. CABI, Wallingford, UK.

Curry, J.P., 1976. Some effects of animal manures on earthworms in grassland. Pedobiologia 425–438.

De Goede, R.G.M., Brussaard, L., Akkermans, A.D.L., 2003. On-farm impact of cattle slurry manure management on biological soil quality. NJAS - Wagen. J. Life Sci. 51, 103–133.

De Ruiter, P.C., Moore, J.C., Zwart, K.B., Bouwman, L.A., Hassink, J., Bloem, J., Vos, J.A. D., Marinissen, J.C.Y., Didden, W.A.M., Lebrink, G., Brussaard, L., 1993a. Simulation of nitrogen mineralization in the below-ground food webs of two winter wheat fields. J. Appl. Ecol. 30, 95–106.

De Ruiter, P.C., Veen, J.A., Moore, J.C., Brussaard, L., Hunt, H.W., 1993b. Calculation of nitrogen mineralization in soil food webs. Plant Soil 157, 263–273.

De Ruiter, P.C., Neutel, A.M., Moore, J.C., 1994. Modelling food webs and nutrient cycling in agro-ecosystems. Trends Ecol. Evol. 9, 378–383.

Didden, W., Römbke, J., 2001. Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. Ecotoxicol. Environ. Saf. 50, 25–43.

Didden, W.A.M., Marinissen, J.C.Y., Vreeken-Buijs, M.J., Burgers, S.L.G.E., de Fluiter, R., Geurs, M., Brussaard, L., 1994. Soil meso- and macro-fauna in two agricultural systems: factors affecting population dynamics and evaluation of their role in carbon and nitrogen dynamics. Agric. Ecosyst. Environ. 51, 171–186.

Finlay, B.J., Black, H.I.J., Brown, S., Clarke, K.J., Esteban, G.F., Hindle, R.M., Olmo, J.L., Rollett, A., Vickerman, K., 2000. Estimating the growth potential of the soil protozoan community. Protist 151, 69–80.

Goulden, M.L., Munger, J.W., Fan, S.-M., Daube, B.C., Wofsy, S.C., 1996. Measurements of carbon sequestration by long-term eddy covariance: methods and a critical evaluation of accuracy. Global Change Biol. 2, 169–182.

Hassink, J., 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem. 26, 1221–1231.

Hassink, J., 1995. Prediction of the non-fertilizer N supply of mineral grassland soils. Plant Soil 176, 71–79.

Holtkamp, R., van der Wal, A., Kardol, P., van der Putten, W.H., de Ruiter, P.C., Dekker, S.C., 2011. Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. Soil Biol. Biochem. 43, 251–260.

Hopkins, D.W., Waite, I.S., O'Donnell, A.G., 2011. Microbial biomass, organic matter mineralization and nitrogen in soils from long-term experimental grassland plots (Palace Leas meadow hay plots, UK). Eur. J. Soil Sci. 62, 95–104.

Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., Rose, S. L., Reid, C.P.P., Morley, C.R., 1987. The detrital food web in a shortgrass prairie. Biol. Fertil. Soils 3, 57–68.

Lantinga, E.A., Neuteboom, J.H., Meijs, J.A.C., 2004. In: Penning, P.D. (Ed.), Sward Methods. British Grassland Society.

Levi-Minzi, R., Riffaldi, R., Saviozzi, A., 1986. Organic matter and nutrients in fresh and mature farmyard manure. Agric. Wastes 16, 225–236.

Ma, W.C., Brussaard, L., de Ridder, J.A., 1990. Long-term effects of nitrogenous fertilizers on grassland earthworms (Oligochaeta: Lumbricidae): their relation to soil acidification. Agric. Ecosyst. Environ. 30, 71–80.

MAFF, 1986. The Analysis of Agricultural Materials, 3rd ed. HMSO, London, United Kingdom.

Michel, P.H., Bloem, J., 1993. Conversion factors for estimation of cell production rates of soil bacteria from [3H]thymidine and [3H]leucine incorporation. Soil Biol. Biochem. 25, 943–950.

Müller, C., Laughlin, R.J., Christie, P., Watson, C.J., 2011. Effects of repeated fertilizer and cattle slurry applications over 38 years on N dynamics in a temperate grassland soil. Soil Biol. Biochem. 43, 1362–1371.

Persson, H., 1983. Influence of soil animals on nitrogen mineralization in a northern Scots pine forest. In: Lebrun, P., Andre, H.M., de Medts, C., Gregoire-Wibo, Wanthy, G (Eds.), New Trends in Biology. Dieu-Brichart, Louvain-la-Neuve, pp. 117–126.

Persson, T., Bååth, E., Clarholm, M., Lundkvist, H., Söderström, B.E., Sohlenius, B., 1980. Trophic structure, biomass dynamics and carbon metabolism of soil organisms in a Scots Pine Forest. Ecol. Bull. 419–459.

Postma-Blaauw, M.B., de Goede, R.G.M., Bloem, J., Faber, J.H., Brussaard, L., 2010. Soil biota community structure and abundance under agricultural intensification and extensification. Ecology 91, 460–473.

Rashid, M.I., de Goede, R.G.M., Brussaard, L., Lantinga, E.A., 2013. Home field advantage of cattle manure decomposition affects the apparent nitrogen recovery in production grasslands. Soil Biol. Biochem. 57, 320–326.

Reijs, J.W., Sonneveld, Sørensen, P., Schils, R.L.M., Groot, J.C.J., EA, 2007. Effects of different diets on utilization of nitrogen from cattle slurry applied to grassland on a sandy soil in the Netherlands. Agric. Ecosyst. Environ. 118, 65–79.

Römbke, J., Sousa, J.P., Schouten, T., Riepert, F., 2006. Monitoring of soil organisms: a set of standardized field methods proposed by ISO. Eur. J. Soil Biol. 42, S61–S64.

Schon, N., Mackay, A., Hedley, M., Minor, M., 2012. The soil invertebrate contribution to nitrogen mineralisation differs between soils under organic and conventional dairy management. Biol. Fertil. Soils 48, 31–42.

Shimizu, M., Marutani, S., Desyatkin, A.R., Jin, T., Hata, H., Hatano, R., 2009. The effect of manure application on carbon dynamics and budgets in a managed grassland of Southern Hokkaido, Japan. Agric. Ecosyst. Environ. 130, 31–40.

Siepel, H., van de Bund, C.F., 1988. The influence of management practises on the microarthropod community of grassland. Pedobiologia 31, 339.

Sjursen, H., Michelsen, A., Jonasson, S., 2005. Effects of long-term soil warming and fertilisation on microarthropod abundances in three sub-arctic ecosystems. Appl. Soil Ecol. 30, 148–161.

Stamatiadis, S., Doran, J.W., Ingham, E.R., 1990. Use of staining and inhibitors to separate fungal and bacterial activity in soil. Soil Biol. Biochem. 22, 81–88.

Standen, V., 1984. Production and diversity of enchytraeids, earthworms and plants in fertilized hay meadow plots. J. Appl. Ecol. 21, 293–312.

Timmerman, A., Bos, D., Ouwehand, J., de Goede, R.G.M., 2006. Long-term effects of fertilisation regime on earthworm abundance in a semi-natural grassland area. Pedobiologia 50, 427–432.

Van Dijk, J., Didden, W.A.M., Kuenen, F., van Bodegom, P.M., Verhoef, H.A., Aerts, R., 2009. Can differences in soil community composition after peat meadow restoration lead to different decomposition and mineralization rates? Soil Biol. Biochem. 41, 1717–1725.

Van Eekeren, N., de Boer, H., Bloem, J., Schouten, T., Rutgers, M., de Goede, R., Brussaard, L., 2009. Soil biological quality of grassland fertilized with adjusted cattle manure slurries in comparison with organic and inorganic fertilizers. Biol. Fertil. Soils 45, 595–608.

Van Eekeren, N., de Boer, H., Hanegraaf, M., Bokhorst, J., Nierop, D., Bloem, J., Schouten, T., de Goede, R., Brussaard, L., 2010. Ecosystem services in grassland associated with biotic and abiotic soil parameters. Soil Biol. Biochem. 42, 1491–1504.

Van Vliet, P.C.J., van der Stelt, B., Rietberg, P.I., de Goede, R.G.M., 2007. Effects of organic matter content on earthworms and nitrogen mineralization in grassland soils. Eur. J. Soil Biol. 43, S222–S229.

Velthof, G.L., Oudendag, D., Witzke, H.P., Asman, W.A.H., Klimont, Z., Oenema, O., 2009. Integrated assessment of nitrogen losses from agriculture in EU-27 using MITERRA-EUROPE. J. Environ. Qual. 38, 402–417.