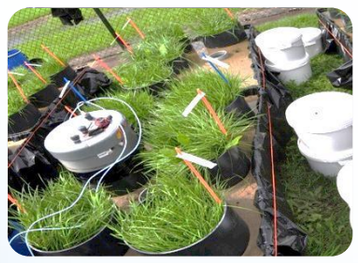

Soil biota and nitrogen cycling in production grasslands with different fertilisation histories Muhammad Imtiaz Rashid 2013

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Soil biota and nitrogen cycling in production grasslands with different fertilisation histories

Muhammad Imtiaz Rashid

Thesis

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This dissertation is dedicated to my
beloved parents and my late brother

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Chapter 1

General Introduction

Muhammad Imtiaz Rashid

1.1 Introduction

In the Netherlands, production grasslands comprise about 1 million hectares. This relatively large area is mainly used for dairy farming. Until recently, these grasslands were intensively managed by the application of high rates of chemical and mineral-rich organic (cattle slurry) fertilisers. This was accompanied with high environmental nitrogen (N) losses (Huijsmans et al. 2003) and loss of soil quality (Ma et al. 1990, De Goede et al. 2003). Legislative restrictions on the intensive use of inorganic fertilisers and mineral-rich animal manures (Vellinga 2006) have increased the importance of organic inputs like solid cattle manure (SCM) as a source of N and other nutrients which are necessary for grass production and improvement of soil quality (Velthof et al. 2000, Van Eekeren et al. 2009). Besides, production of SCM increased in recent years due to growing interest of farmers in straw-based housing systems for reasons of better animal health and welfare (Ellen et al. 2007).

A high short-term N recovery by the grass sward from animal manure is important to reduce environmental N losses (Smith et al. 2002). However, crop N availability from SCM varies widely when applied to soils with contrasting fertilisation histories (Mallory and Griffin 2007, Nett et al. 2010). In recent studies on Dutch grasslands, the variation in N recovery from SCM ranged between 20 and 50% in the year of application (Van Dijk 2004, Schröder et al. 2007, Sonneveld and Lantinga 2011, Shah et al. 2012). Since the major part of the N present in SCM is organically bound, its availability for plant uptake largely depends on the balance between mineralisation and immobilisation. These processes are influenced by environmental conditions (i.e. temperature and moisture) and chemical composition of the applied organic matter as well as soil type and fertilisation history (Verhoef and Brussaard 1990, Wardle 2002, Wang et al. 2009, Nett et al. 2010, Shah et al. 2012), which together affect, and

receive feedback from the diversity and functioning of soil organisms (De Vries et al. 2006, De Vries et al. 2007, Mikola et al. 2009, Van Eekeren 2010). However, it is still unknown to what extent biological activity and specific biological functional traits that are affected by the soil environment and/or manure management practices and what the consequences will be for manure N recovery. This is the subject of this thesis, which will be introduced in more detail below.

1.2 Effects of fertiliser management on soil biological and chemical properties

1.2.1 Fertiliser effects on the soil biota

Chemical fertilisers, cattle slurry manure (CSM) and SCM are the key sources of N fertilisation in production grasslands. The organic fraction in SCM and CSM represents the main food source for the soil biota. However, CSM and SCM differ in chemical and physical composition which affects their decomposition and mineralisation patterns in time (Levi-Minzi et al. 1986). Also, repeated applications of either CSM or SCM to grassland may differentially affect given groups of soil biota, resulting in different community structures. For instance, long term applications of mineral-N rich fertilisers (CSM and chemical fertiliser) have been shown to negatively affect the fungal biomass (Bittman et al. 2005, De Vries et al. 2006), whereas frequent SCM additions have demonstrated positive influences on the abundance of soil biota such as, e.g., epigeic earthworms species (Timmerman et al. 2006, Van Eekeren et al. 2009) and microbial biomass (Hopkins et al. 2011) (Fig. 1.1). The amount of readily available carbon (C) in cattle manure is considered to have a large impact on the activity of soil organisms in arable soils (Fauci and Dick 1994 , Griffiths et al. 1998, Sørensen 1998). Fauci & Dick (1994) observed a positive correlation between the amount of available C inputs and the microbial biomass in a soil with a history of long

term applications of organic amendments. On the other hand, prolonged applications of chemical fertilisers decreased the microbial biomass and soil organic matter content (Hopkins et al. 2011). Furthermore, chemical fertilisers, CSM and urine 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, Ma et al. 1990, De Goede et al. 2003). Similarly, microarthropod abundance and diversity decreased following application of chemical fertilisers to grasslands (Siepel and van de Bund 1988). Therefore, it is to be expected that fertiliser management of grasslands based on long-term amendments of SCM will increase the abundance and biomass of soil organisms compared to management based on the application of CSM with or without chemical fertilisers (i.e. non-SCM inputs).

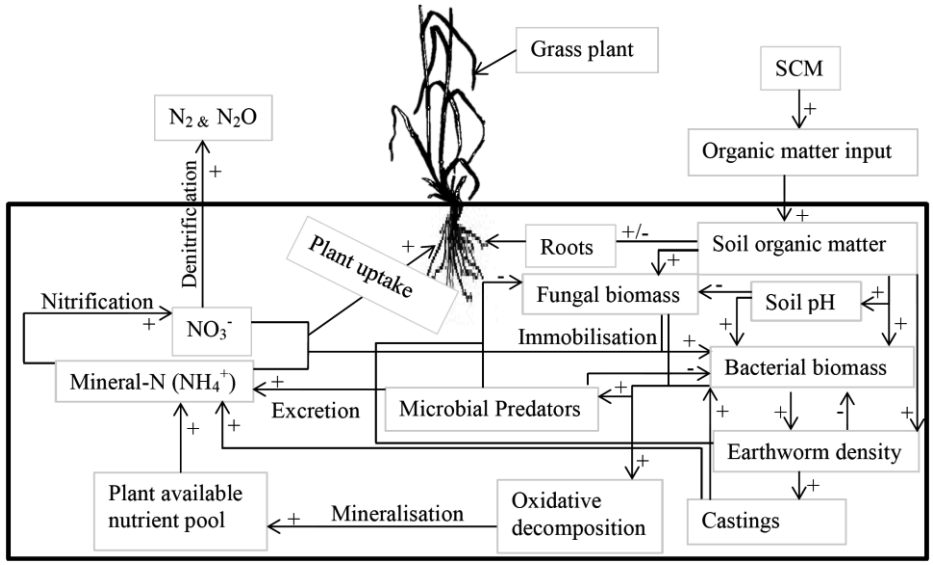

Fig. 1.1 Conceptual diagram of effects of solid cattle manure (SCM) inputs on soil biota and nitrogen (N) cycling. +, – and ± signs show positive, negative effects or no influence of fertiliser input on the given soil parameter, respectively.

1.2.2 Fertiliser effects on soil environmental conditions

Besides being a carbon source, i.e. a food source for the soil biota, applied SCM can also result in changes in soil environmental conditions like an increase in soil pH (Naramabuye and Haynes 2007) (Fig. 1.1) as a result of microbial decarboxylation of the Ca-organic matter complex in SCM and subsequent hydrolysis of Ca^{2+} cations which lead to a release of OH^- anions (Ano and Ubochi 2010). In contrast, ammonical fertilisers release hydrogen cations (H^+) from ammonium during the process of nitrification which results in increased soil acidity (Ma et al. 1990, Guo et al. 2010, Hopkins et al. 2011). The chemical reactions underlying the organic (1) and chemical (2) fertiliser effects on soil pH are given by:

$$\text{SCM} \xrightarrow{\text{Microbial decarboxylation}} Ca^{+2} \xrightarrow{\text{Hydrolysis}} Ca(OH)_2 \qquad (1a)$$

$$2\,Ca(OH)_2 + Al^{+3} \longrightarrow Al(OH)_3 + 2Ca^{+2} + OH^- \qquad (1b)$$

$$NH_4^+\text{-N (chemical fertiliser)} \longrightarrow NO_3^- + 2H^+ \qquad (2)$$

Soil pH-H_2O of 4.0 is a lower limit of tolerance for most species of enchytraeids and earthworms (Standen 1984, Edwards 2004), but earthworms perform best in a neutral-pH of soil (Rengasamy and Olsson 1991). Also, primary consumers such as bacteria and fungi are affected by soil acidification (Rousk et al. 2009). They secrete enzymes into the soil solution so as to execute their external digestion. The performance of these extracellular enzymes, which determines the rate of soil organic matter decomposition, is strongly pH-dependent. At suboptimal soil pH, enzyme morphology is affected and changes in the functional shape disturb the formation of enzyme-substrate complexes (Dancer et al. 1972, Parham and Deng 2000). These changes in soil biota community composition and its functioning in terms of organic matter decomposition and

nutrient mineralisation, may cause long-lasting negative effects on the whole soil ecosystem such as deterioration of soil physico-chemical properties and reduction of agricultural production (Fog 1988, Arnebrant et al. 1990). Since fertiliser management can greatly modify soil pH and thereby the soil biota community composition and activity, it is worthwhile to study the effects of an increased soil pH and earthworm density on SCM decomposition, N mineralisation and crop N recovery in acidic production grasslands.

1.3 Role of soil biota in N cycling

Bacteria, fungi, earthworms, enchytraeids, nematodes, protozoa and microarthropods are representatives of different functional groups in the soil foodweb (Fig. 1.2), acting at different trophic levels (De Ruiter et al. 1994, Bloem et al. 1997, Berg et al. 2001). Bacteria and fungi contribute directly to organic matter decomposition and N mineralisation through excretion of enzymes that break down organically bound N. Microbivores (protozoa and nematodes) mineralise N directly from the microbial biomass, but also exert indirect effects on N mineralisation through stimulating microbial activity (Ingham et al. 1986, Bloem et al. 1997). Holter (1977) calculated that 1 g of earthworms can degrade 0.75 g organic matter (OM) from cattle dung in six weeks. Earthworm casts can be an important source of inorganic N available for plant uptake. Whalen et al. (2000) estimated that the annual N flux from an earthworm population through excretion was 42 kg N ha^{-1} in inorganically fertilised corn agro-ecosystems, which was equivalent to 22% of the crop N uptake. Furthermore, Hameed et al. (1994) showed in a laboratory experiment that 24% of N present in earthworm excreta was taken up by plants during a period of 48 days. Enchytraeids (in particular the species *Cognettia sphagnetorum*) also have direct effects on N cycling and mediating effects on nutrient (also other than N) cycling through modification of microbial performance. Setälä et al. (1991) found that

enchytraeids alone or along with bacterivorous nematodes significantly increased N mineralisation in a microcosm study using forest soil. In another laboratory experiment with mini-ecosystems consisting of coniferous forest floor it was found that these microorganisms can double the N uptake in pine needles compared to non-faunal controls (Setälä 2000). Microarthropods, and amongst them especially springtails, increase the nutrient availability for plant uptake by grazing on microorganisms, thus regulating the size and activity of the microbial community and its functioning (Partsch et al. 2006).

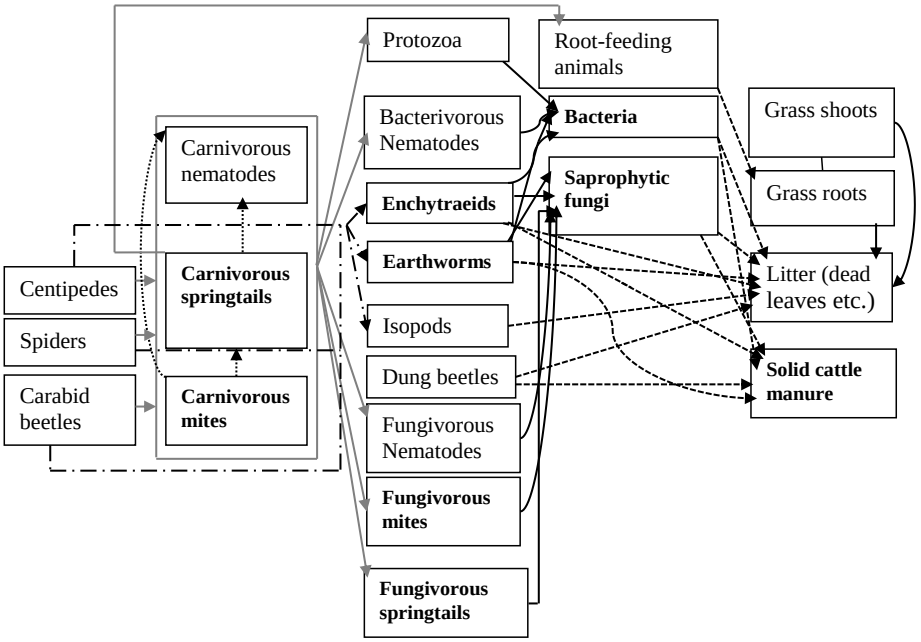

Fig. 1.2 Food web relationships of solid cattle manure-fertilised grassland. This thesis will focus on the role of bacteria, fungi, earthworms, enchytraeids, protozoa and microarthropods in N cycling.

However, higher trophic groups in the food web such as predatory mites or centipedes had no significant effects on primary production and nutrient cycling, despite their often strong influence on the biomass of their prey

(Laakso and Setälä 1999, Laakso et al. 2000, Setälä 2000). Therefore, in this thesis I focus on the role of bacteria, fungi, protozoa, microarthropods, enchytraeids and earthworms in the N cycling of production grasslands (Fig. 1.2).

1.4 Effect of soil properties on soil biota activity

Soil organisms behave differently in soils with contrasting properties such as texture, pH, moisture content, temperature, organic matter content and cation exchange capacity. Organic matter, moisture, temperature and pH affect the activity and distribution of soil organisms (Vonk 1983, Bardgett et al. 1997, Van Vliet et al. 2007). Organic matter-rich soils generally provide more food resources, while acidity and alkalinity of soils affect the biomass of fungi and bacteria. Bardgett et al. (1993) found higher biomass and activity of fungi in acidic conditions whereas biomass of bacteria and earthworms, and density of collembola were lower. Hence, it is worthwhile to take into account the effect of soil physical and chemical properties on mineralisation and availability of N in the soil due to activities of soil organisms in fertilised grasslands with contrasting soil properties.

1.5 Influence of fertiliser management on soil biota, soil organic matter and N dynamics

In general, it is expected that SCM contributes more to a higher soil organic matter content than CSM with or without chemical fertiliser (Fig. 1.1). Hadas et al. (1996), Langmeier et al. (2002) and Nett et al. (2010) found that soils which were fertilised by SCM for a decade or more had higher soil total C and N contents compared to those that received chemical fertilisers only. Stark et al. (2008) found higher microbial activity and N mineralisation from organic matter amendments in soils with a history of organic inputs relative to unfertilised soil. Similarly, Nett et al. (2010) concluded from their own study

along with other published data that net N mineralisation of, and grass N uptake from recently applied SCM was higher in soils with a SCM fertilisation history than in unfertilised soils. However, Mallory and Griffin (2007) found lower N mineralisation rates from recently applied SCM in the soil that had a history of SCM inputs compared to a soil with a history of chemical fertiliser application. They attributed this effect to high C-availability for a more active soil microbial community in the SCM farm soil which immobilised more N from the applied SCM compared to the soil that had a history of chemical fertilisers. Moreover, despite higher levels of microbial biomass in soils that were fertilised by SCM in the past, Hadas et al. (1996) and Langmeier et al. (2002) did not find an increase in net N mineralisation from applied SCM. Therefore, it remains of great interest to better understand how fertilisation history influences N mineralisation and herbage N uptake from SCM applied to production grasslands.

Effects of the soil biota community on decomposition and mineralisation rates of organic matter are addressed in several studies (Setälä et al. 1991, Setälä 2000, Bradford et al. 2002, Wardle 2002). Many researchers have found a strong relation between the chemical composition of applied organic matter and its decomposition rates (Aerts 1997, Strickland et al. 2009b, Wang et al. 2009). However, whether and how continuous inputs of organic matter of a certain chemical composition influence soil biota community composition and its functions is not well understood. It has been observed that plant species composition affects soil ecological functions by influencing the soil biota community (Wardle 2002, Wardle et al. 2004). Strickland et al. (2009a, 2009b) found that through species-specific litter input to soil, plant species can change the soil biota community composition, which affects the local litter decomposition. Several studies indicate that decomposition occurs more

rapidly, when litter is applied in the source habitat (i.e. at home) than it is applied to a habitat with different plant species (i.e. away). This phenomena has been called the home field advantage (HFA) (Gholz et al. 2000, Ayres et al. 2009a, Ayres et al. 2009b, Strickland et al. 2009a, Strickland et al. 2009b, Wang et al. 2009, Milcu and Manning 2011). The HFA is generally stronger in cases where the quality of the input organic matter is lower (higher C:N and/or lignin:N ratio). In contrast, studies that featured similar quality of organic materials to test the HFA hypothesis were generally unsupportive to this concept (Ayres et al. 2006, Gießelmann et al. 2011). It therefore seems that quality of organic matter plays an important role in determining HFAs. Initial differences in chemical composition of litter inputs can determine the soil biota community and their functions. For instance, the lignin content in litter is a principal factor that can structure soil decomposer communities and their capability to decompose litters with different chemical compositions (Freschet et al. 2012). On the other hand, microbial communities rapidly adjust to the quality of a certain type of litter (Gießelmann et al. 2011). This ability could fade away initial differences in decomposition rates of different litters when exposed to one and the same local microbial community, leading to no HFA. Such rapid responses in community structure are not expected for detritivorous soil fauna with relatively long generation times like Collembola and Acarina (Milcu and Manning 2011) and earthworms, many of which selectively feed on litter of a specific chemical composition (C:N ratio) which fulfils their stoichiometric requirements (Bohlen et al. 1997) .

In addition to soil biota community composition, the changes in chemical composition of the litter during various stages of decomposition also influence the size of HFA. According to Ayres *et al* (2009a), relatively large differences in the initial chemical composition of the litter will result in a large HFA in the

early stages of decomposition which would decrease when decomposition of the litter proceeds. In such case, it is to be expected that the chemical compositions of the remaining, more recalcitrant litter will become more similar, eventually resulting in disappearance of HFA (Wallenstein et al. 2013). In contrast, Milcu & Manning (2011) observed a higher HFA of grass litter decomposition in the late-successional stage of natural grassland than in mid- or early-successional stages. Therefore, it remains to be clarified how the change in chemical composition of organic matter during decomposition will affect the size of HFA. Moreover, HFA has only been studied in natural systems. SCM and CSM as used in agricultural systems also differ considerably in composition and quality (lignin:N and C:N ratio) because of differences in bedding materials and manure handling, animal origin, feed ration and processing systems (Tunney 1975, Rotz 2004). As mentioned earlier, long-term application of SCM can influence soil microbes, meso- and macrofauna abundance and activity (Ma et al. 1990, Forge et al. 2005, Timmerman et al. 2006), whereas continuous application of mineral fertilisers to grasslands generally decreases earthworm populations (Ma et al. 1990, De Goede et al. 2003). Hence, also in agro-ecosystems historical factors can shape structural differences in soil biota communities and may be expected to play a role in organic matter decomposition and mineralisation. Therefore, like in natural ecosystems, higher decomposition and N mineralisation of SCM is expected in agro-ecosystems which have a history of SCM inputs compared to those where high quality manure or no SCM has been applied. Hence, it might be incisive to test the HFA concept also for SCM decomposition, N mineralisation and herbage N uptake as well as the contribution of micro-, meso- and macrobiota to a HFA in production grasslands.

1.6 Objectives of this thesis

The main aim of this thesis is to investigate the effect of fertilisation history (SCM and organic-N rich CSM vs. mineral-N rich CSM and chemical fertiliser) on abundance, biomass and activity of the soil biota, and their role in soil N mineralisation as well as SCM decomposition, nitrogen mineralisation and herbage N uptake in production grasslands. More specific objectives of this thesis are to:

1. explore the effects of differences in the prevailing fertiliser management of production grassland, soil type and SCM type (composted vs. stacked) on the DM and N disappearance rate of SCM and herbage N recovery.
2. study whether HFAs of SCM decomposition, N mineralisation and N recovery exist in production grasslands.
3. investigate if manure quality and composition of the soil biota contribute to SCM decomposition and N disappearance HFA
4. study the effects of pH and earthworm density on herbage apparent nitrogen recovery from SCM in acidic peat grassland.

1.7 Experimental approach and thesis outline

My thesis deals with two main topics. The first part of the thesis focuses on effects of fertilisation history on soil biota abundances and their role in soil N supply capacity (Fig. 1.3; Chapter 2) whereas the second part focuses on effects of fertilisation history on decomposition, N mineralisation and herbage N uptake from recently applied SCM (Fig. 1.3; Chapters 3, 4 & 5).

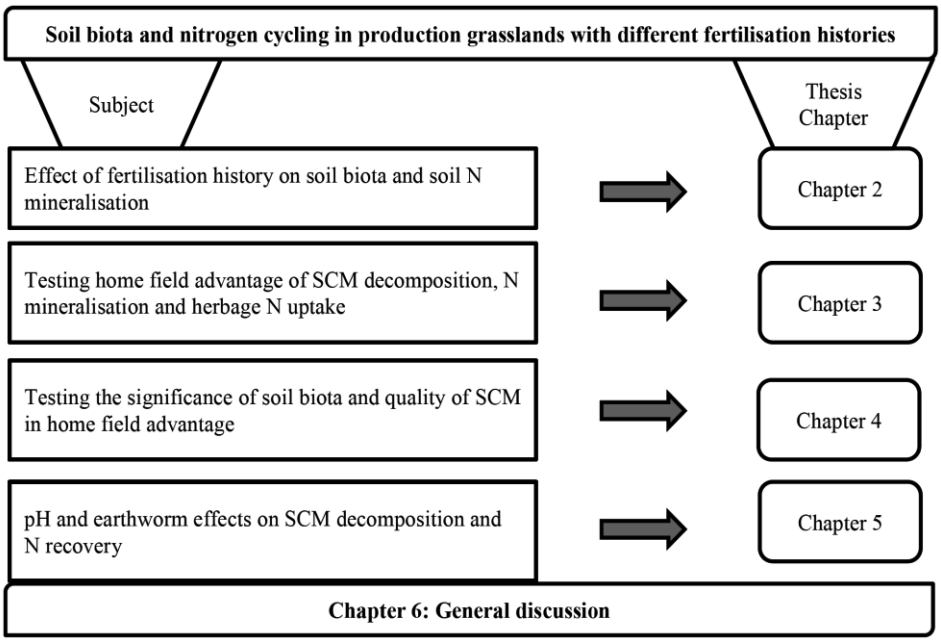

Fig 1.3 Schematic diagram of the thesis content.

Chapter 2 presents the consequences of management history for (i) bacterial and fungal biomass and numbers of earthworms, enchytraeids and micro-arthropods (mites and springtails), and (ii) soil N mineralisation. This chapter also describes an estimation of the N mineralisation by the soil biota obtained through production-ecological modelling and related to *in situ* measurements of herbage N uptake. Moreover, this chapter also compares the different techniques used to measure or predict soil N mineralisation of grasslands.

Chapter 3 presents the results of an experiment to study any home field advantage in terms of decomposition, N release and herbage N recovery of SCMs of different qualities, which were reciprocally exchanged between the grasslands of the farms where the SCMs were produced and also applied on non-SCM farms. The effect of fertiliser management history on manure decomposition and herbage N recovery is also reported.

Chapter 4 presents the contributions of soil micro, meso and macrobiota and manure quality to the home field advantage of SCM decomposition and N release. Also, this chapter compares the contribution of soil biota to decomposition and N release of SCM when applied to grasslands under contrasting fertiliser management histories.

Chapter 5 describes the effects of soil pH and earthworm density on SCM disappearance and herbage N uptake in a mesocosm experiment. It also presents the contribution of earthworms to N_2O emission from unfertilised and manure-amended peat grasslands.

In **Chapter 6**, I synthesise the results of the experimental chapters (2-5). Here, I demonstrate how fertiliser management of grasslands can play a role in affecting the soil biological quality of grasslands and improving herbage N utilisation of SCM.

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Chapter 2

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

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Abstract

We studied two different fertiliser management regimes of grasslands on sand and peat soils: above-ground application of solid cattle and organic nitrogen (N)-rich slurry manure (SCM) versus slit-injected mineral N-rich slurry manure with or without chemical fertiliser (non-SCM). Measurements of field N mineralisation as estimated from herbage N uptake in unfertilised grassland were compared with i) potential N mineralisation as determined from 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 both earthworms and enchytraeids in SCM grasslands were three times higher than in non-SCM grasslands and were positively related to the level of long-term organic N inputs. Fungal biomass and mite density were significantly higher in grasslands on peat than on sand. Mite density was positively related to fungal biomass ($R^2 = 0.51$, $P < 0.001$). On sandy soil, herbage N uptake was 32% higher ($P = 0.001$) in SCM compared to non-SCM grasslands, whereas no differences were found for the peat grasslands. The currently used method in Dutch fertilisation recommendations underestimated actual soil N supply capacity on average by 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 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 grassland.

2.1. Introduction

Accurate assessments of the N delivery capacity of unfertilised 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 nitrogen (N) delivery capacity of unfertilised soil (http://www.bemestingsadvies.nl, section 1.2.2.1) using the method developed by Hassink (1994, 1995). In this method, soil N supply from unfertilised grasslands is calculated by regression models based on the organic N content in the top 0-10 cm soil layer. However, in 17 grassland soils on sand which all had an organic matter content of >32 g kg^{-1} dry soil, this method underestimated the actual soil N supply capacity by on average 31% (42 kg N ha^{-1}) and it was 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 apparent gap 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 (CS) and solid cattle manure (SCM). Repeated applications of CS and SCM have shown to enhance soil organic matter and N content and thereby ecosystem productivity (Shimizu et al. 2009, Müller et al. 2011). However, CS and SCM differ in chemical and physical composition (Levi-Minzi et al. 1986). Thus, repeated applications of

either CS or SCM to grassland may affect a given group of soil biota differently, resulting in diverging community structures. For instance, long term applications of CS have been shown to negatively affect the fungal biomass in the soil due to its high mineral N content (Bittman et al. 2005), whereas frequent SCM additions had positive influences on the abundance of epigeic earthworms (Van Eekeren et al. 2009). These changes in 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 in laboratory incubations (Bloem et al. 1994, Canali and Benedetti 2006). With this method, soil is sieved over 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 mesofaunal 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, e.g., earthworms on N mineralisation are not taken into account.

Trophic interactions in the soil foodweb are important for decomposition and nutrient mineralisation processes (De Ruiter et al. 1994, Bloem et al. 1997, Berg et al. 2001). For a better understanding of the effects of agricultural management on crop nutrient uptake through trophic interactions, models can be useful. 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).

According to De Goede et al. (in review), net soil N mineralisation from grassland soils, calculated as the difference between the main inputs (fertilisation, atmospheric deposition) and outputs of N (crop uptake, leaching) may be up to three times higher than determined with the potential N mineralisation method. They concluded that the level of this mismatch was highly correlated with the *in vivo* density of earthworms which were excluded from the *in vitro* laboratory soil incubation. This is consistent with earlier modelling studies by De Goede et al. (2003) and Van Vliet et al. (2007), who concluded that the gross contribution of earthworms to N mineralisation in grasslands could be as high as 170 kg N ha^{-1} yr^{-1}. However, comparing the results of model calculations with actual crop N uptake, measurements have not been done so far. This might add to a better 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 compare the measurements of actual N mineralisation as estimated from herbage N uptake in unfertilised grassland with i) potential N mineralisation as estimated from laboratory soil incubation, ii) estimated N mineralisation by production-ecological model calculations, and iii) N mineralisation calculated according to the Dutch fertilisation

recommendation for grasslands. 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) herbage N uptake from unfertilised plots in SCM grasslands will be higher than in non-SCM grasslands, (3) the calculated N mineralisation by microbes and mesofauna through production-ecological modelling will approximate the laboratory-determined potential N mineralisation, and (4) any difference between the laboratory-determined potential N mineralisation and herbage N uptake can be explained by the contribution of soil biota not included in the incubations, in particular earthworms, to N mineralisation.

2.2 Material and Methods

2.2.1 Site selection

We selected two dairy farms (A and B) where solid cattle manure (SCM) and organic-N rich cattle slurry (CS) was 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 (CS) was produced. The grasslands on farm C were fertilised with mineral-N rich CS together with chemical fertilisers, and on those of farm D only CS was used. SCM had not been applied for at least 30 years on the grasslands of both farms C and 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 in Tables 2.1 & 2.2, respectively. At each farm, two parallel field experiments (1 and 2) were carried out in grassland fields with a well-documented fertilisation history.

Table 2.1 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	>10	8500	325	75	45	25	0	30
B	Sand	SCM	>10	4960	150	80	70	20	0	5
C	Peat	Non-SCM	>10	4320	275	30	0	30	40	30
D	Sand	Non-SCM	5	3290	145	50	0	50	0	5

Table 2.2 Soil characteristics for the layer 0-10 cm of the four farms (A-D).

Farm	Fertilisation History	Soil type	OM	N_{total}	DOC	pH-KCl
			%	g kg^{-1}	mg L^{-1}	
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

2.2.2 Monitoring soil biology (Experiment 1)

At each of the four farms, a grassland field of about 3 ha was selected. Every field was divided into four blocks. In each block, a plot of 15x15 m^2 was selected at random for monitoring soil biological parameters. Soil samples for biological and chemical analyses were collected from each plot on 20 October 2009, and on 12 April and 16 August 2010. In addition, earthworms were also sampled on 20 October 2010. Bacterial and fungal biomass and their activity were analysed from the soil sampled in October 2009 and August 2010.

2.2.2.1 Earthworms

To measure earthworm density and biomass, two soil blocks with a volume of 20×20×20 cm^3 were randomly sampled in each 15x15 m^2 plot. Thus, in total 8 soil blocks were sampled per farm at each sampling time. Earthworms were hand-sorted in the field and taken to the laboratory. Within one day after sampling, they were rinsed in tap water, counted and placed in an incubator at 15°C for two days to empty their gut content. Afterwards, their fresh weight was measured and they were fixed in alcohol prior to species identification. Classification was done according to Bouché (1977) by distinguishing three ecological groups: epigeic, anecic and endogeic species. Numbers and fresh biomass weight of earthworms were expressed per m^2.

2.2.2.2 Enchytraeids

In each 15x15 m^2 plot, two soil cores were taken at random using a cylindrical auger of 15 cm length and a diameter of 5.8 cm. This auger holds 6 PVC rings of 2.5 cm height with which the soil core can be separated into 6 intact soil layers. The soil samples were stored at 4 ^oC 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 m^2.

2.2.2.3 Micro-arthropods

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 (Siepel and

van de Bund 1988, Römbke et al. 2006) 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. Thus, the microarthropods moved downwards to escape from heat, drought and light and collected in vials containing 70% ethanol. The microarthropods were counted, classified as mites or collembola, and their density was expressed per m^2.

2.2.2.4 Soil microbiological parameters, respiration and N mineralisation

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

A sample of 20 g homogenised and sieved 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 (Michel and Bloem 1993, Bloem and Bolhuis 2006).

Homogenised and sieved (4 mm) samples of about 200 g field-moist soil that was 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 (PNM) (Bloem et al. 1994). Increase in mineral N was measured from week 1 to 6. A sub-sample of 20 g soil was taken from each plastic bag and extracted with 50 ml 1M KCl. The extract was centrifuged for 10 minutes 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).

The PNM 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:

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

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 is 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 intrapolation of the data obtained in April, August and October 2010.

2.2.2.5 Abiotic soil parameters

The homogenised and sieved 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 about 20 g field-moist soil after drying at 40°C for 24 hours. 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).

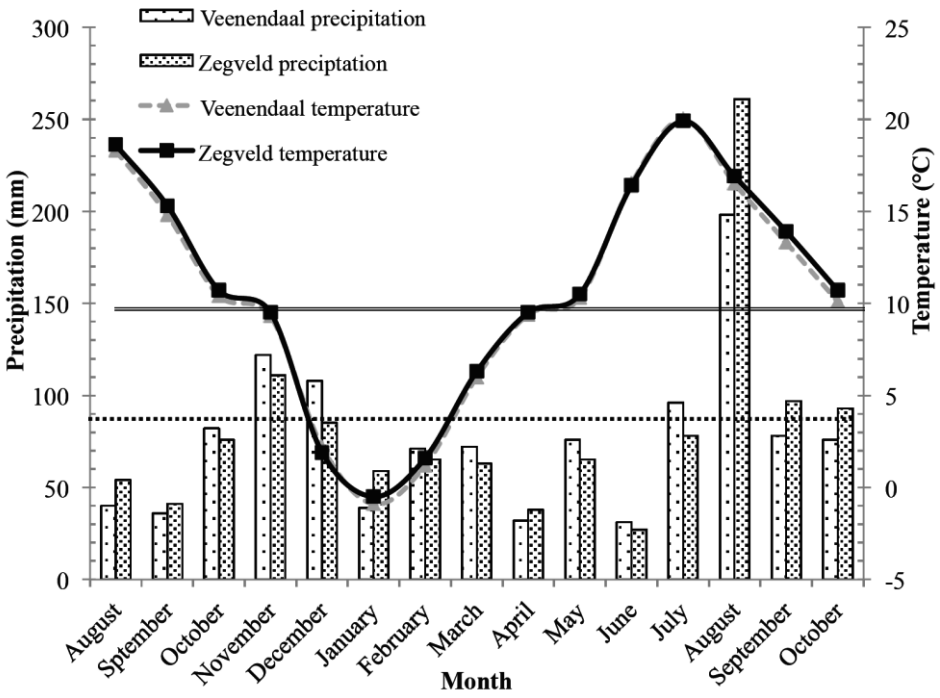

Fig. 2.1 Average monthly temperature and precipitation at Zegveld and Veenendaal (August 2009 – October 2010). Continuous line: mean monthly temperature; dashed line: mean monthly precipitation. Source: KNMI (http://www.knmi.nl).

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. 2.1) for both areas were obtained from two nearby (< 5km) weather stations. Average temperatures in Zegveld (peat) and Veenendaal (sand) for the period October 2009 to October 2010 were 9.8 and 9.6 °C, and total precipitation amounts were 1081 and 1118 mm, respectively.

2.2.3 Dutch N fertilisation recommendation method

Soil N supply capacity, i.e. the non-fertiliser N 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 (Hassink 1994, 1995). In these recommendations, if the soil is sampled to a depth of 10 cm

for total N content from grassland on sand with an age of 4-6 years, the equation to calculate soil N supply is:

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

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:

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

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

The soil N supply capacity for peat grasslands according to Dutch fertilisation recommendations is set 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 farm A and C.

2.2.4 Herbage N uptake (Experiment 2)

In each of the four blocks of the selected fields (see Experiment 1) a grass cage of 4.5×1.25 m^2 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 (unfertilised plot, two manure types, two application rates) have been described in detail in Chapter 3. For the present study, we only used the data from the unfertilised plots regarding herbage dry matter (DM) yield and N uptake. Herbage was harvested 5 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 hours 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 was calculated as:

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

Where NC is herbage N content (g N (100 g DM)$^{-1}$) and DM is herbage DM yield (kg ha^{-1}).

2.2.5 Production-ecological calculations

The potential contribution of soil organisms to N mineralisation during the herbage growing season was estimated following the production-ecological calculation (PEC) method developed by Didden et al. (1994). The model calculations included data on bacteria, fungi, enchytraeids and earthworms as obtained from measurements, 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, De Ruiter et al. 1993b, Van Dijk et al. 2009), but they are very difficult to sample and measure. As they were therefore not sampled in our study sites, we included published amoeba and flagellate biomass data 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). 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 their contribution to soil N mineralisation on a monthly basis, taking into account the changes 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) and are listed in Table 2.3.

Table 2.3 Physiological parameter values for the soil organisms.

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} $ind.^{-1}$ hr^{-1}.

T, temperatures at which a and b were determined.

Source: Persson et al. (1980) and Didden et al. (1994).

The food C:N ratio of earthworms, enchytraeids, bacteria, fungi and protozoa was calculated based on their food preferences and are given in Table 2.4. Based on the epigeic to endogeic ratios of earthworms (Table 2.5) 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 detritivores. Therefore their food C:N ratio was assumed to be equal to the C:N ratio of detritus, which was 14. (De Ruiter et al. 1993a). Protozoa mainly eat

bacteria (Bloem et al. 1997). To account for seasonal fluctuations in density of soil organisms during the whole growing season of 8 months, monthly densities were obtained by intrapolation of the measured densities in April, August and October 2010.

Table 2.4 Food preferences (percentage) for the different taxonomic groups of soil organisms.

Food source	¶Bacteria	¶Fungi	‡Earthworms	‼Enchytraeids	†Protozoa	
					Amoebae	Flagellates
‡Root (C:N= 10)	-	-	20	-	-	-
†Bacteria (C:N= 5)	-	-	10	40	50	100
†Fungi (C:N= 10)	-	-	10	40	0	-
¶Detritus (C:N= 14)	100	100	50	20	-	-
‡Fresh organic matter (C:N= 7)	-	-	10	-	-	-
†Amoebae (C:N= 5)	-	-	-	-	-	-
†Flagellates (C:N= 5)	-	-	-	-	50	-
*Food C:N ratio	14	14	10.3	8.8	5	5

$$*Food\ C{:}N\ ratio = \left(\frac{food\ preference \times C{:}N\ ratio_{food\ source}}{100}\right)$$

† Source: Persson et al. (1980) and Didden et al. (1994)

‡Source: Van Vliet et al. (2007)

‼ Source: De Goede et al. (2003)

‖ Source: Bloem et al. (1997)

¶ adapted from De Ruiter et al. (1993a)

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

$$Q = a \times W^b \qquad (5)$$

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 2.3). The values of parameters a and b in equation 5 presuppose Q as mm^3 O_2 $individual^{-1}$ hr^{-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:

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

Where Q_{FT} is O_2 consumption rate (mm^3 O_2 m^{-2} hr^{-1}) from i^{th} 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 $individual^{-1}$ hr^{-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 (n m^{-2}) in the i^{th} 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:

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

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} hr^{-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:

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

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:

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

$$C_O = \left(\frac{A}{A_e}\right) \quad (10)$$

$$F = C_O \times (1 - A_e) \quad (11)$$

$$P = (A \times P_e) \quad (12)$$

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

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

Where NC represents N consumption (kg N ha^{-1} $month^{-1}$) of a given taxonomic group of soil organisms, C_o 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 1.33x lower C:N ratio in faeces 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:

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

$$NP = \left(\frac{P}{C{:}\,N_{animal\ body}}\right) \quad (15)$$

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

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

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 $C:N_{animal\ body}$ [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.

2.2.6 Statistical Analysis

The effects of treatments 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 results of the soil samples from each plot were averaged resulting in

four pseudoreplicates per grassland. The pseudo replicates within each grassland were considered as block variables in the ANOVA. The main effects of the treatments and their interaction were tested for all sampling dates using time as a covariate.

2.3 Results

2.3.1 Macrofauna

Fertilisation history significantly affected total earthworm numbers and biomass (P< 0.001; Table 2.5), which were on average three times higher in the SCM grasslands than in the non-SCM grasslands (Table 2.5).

Table 2.5 Earthworm parameters in peat (P) and sandy (S) grasslands with different fertilisation history, and corresponding F-values after ANOVA.

Treatments		Earthworms							
Fertilisation history (FH)	Soil type (ST)	Total number	Total biomass	Body weight	Adult	Juvenile	Epigeic	Endogeic	Epigeic/ Endogeic
		n m^{-2}	g m^{-2}	g $worm^{-1}$	n m^{-2}	n m^{-2}	n m^{-2}	n m^{-2}	
SCM†	P	568	174	0.34	184	336	184	347	0.56
	S	554	241	0.46	196	298	221	304	0.82
non-SCM‡	P	268	75	0.28	95	153	59	198	0.32
	S	109	65	0.63	48	53	30	76	0.38
ANOVA table	df	F-values							
FH	1	38.9***	56.9***	4.1	25.6***	24.2***	27.8***	24.9***	6.6*
ST	1	2.1	2.4	68.3***	0.6	2.5	0.01	4.8	1.4
FHxST	1	1.5	4.5	16.5	1.5	0.5	1.2	1.1	0.6
Covariate									
Time	3	4.4*	3.7	10.8	5.5	4.5	1.5	7.4**	1.7
Error (mean squares)	9	14275	1339	0.003	2222	7560	3597	5704	0.068

Values are means with n=16; 4 × 4 [plots × sampling dates (see text)].

df= degrees of freedom

*, ** and *** denote significance level at P<0.05, P<0.01 and P<0.001, respectively.

† Solid cattle manure and organic-N rich slurry manure.

‡ Slurry manure and chemical fertiliser

The abundance of the epigeic and endogeic species in SCM grasslands was 4.6x and 2.4x higher, respectively, than in non-SCM grasslands, also reflected by a higher epigeic to endogeic ratio (Table 2.5).

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, while the endogeic species comprised *Aporrectodea calignosa* 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. calignosa* and *A. chlorotica*. The anecic species *Lumbricus terrestris* L. was found only in the SCM grasslands during August and October 2010, but in low densities (9 m^{-2}). We did not find any anecic species in non-SCM grasslands. Soil type did not affect the earthworm abundance, except for earthworm individual body weight which was significantly higher in the sandy soils than in the peat soils (Table 2.5).

2.3.2 Mesofauna

Enchytraeids, but not microarthropods, were significantly affected by fertiliser history. Enchytraeid abundance and biomass was 2.4x and 3.1x higher in SCM than in non-SCM grasslands, respectively (Table 2.6). Besides, 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. 2.2c). The latter followed a 4-months period of relatively dry weather (Fig. 2.1).

Table 2.6. Mesofauna in peat (P) and sandy (S) grasslands with different fertilisation history, and corresponding F-values after ANOVA

Treatments		Mesofauna			
Fertilisation history (FH)	Soil type (ST)	Enchytraeid density	Enchytraeid biomass	Mite density	Collembolan density
		n m^{-2}	g m^{-2}	n m^{-2}	n m^{-2}
SCM†	P	45947	8.24	19403	10399
	S	27613	4.53	7801	9113
non-SCM‡	P	21870	3.00	10719	3385
	S	8975	1.01	4358	3500
ANOVA table	df	F-values			
FH	1	11.5**	14.4**	3.1	5.1
ST	1	6.1*	2.8*	6.8*	0.0
FHxST	1	0.2	0.6	0.6	0.1
Covariate					
Time	2	6.3*	6.1	15.2**	11.2**
Error (mean squares)	6	1.2E+08	4.0	3.6E+07	23553744

Values are means with n=12; 4 × 3 [plots × sampling dates (see text)].

*, ** and *** denote significance level at P<0.05, P<0.01 and P<0.001, respectively.

df= degrees of freedom

† Solid cattle manure and organic-N rich slurry.

‡ Slurry manure and chemical fertiliser.

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 2.6, Fig. 2.2d).

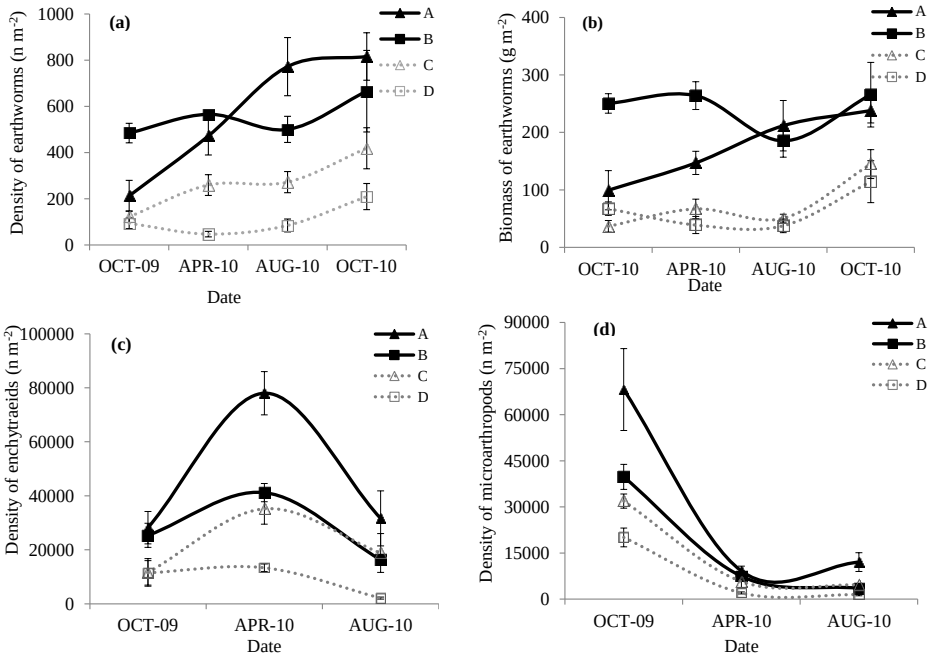

Fig. 2.2 Temporal changes in density (a) and biomass (b) of earthworms, density of enchytraeids (c) and microarthropods (d) in grasslands of farms A (SCM input history on peat), B (SCM input history on sandy), C (non-SCM history input on peat) and D (non-SCM input history on sand).

2.3.3 Microbes

Except for microbial thymidine incorporation, none of the microbial parameters was affected by fertilisation history (Table 2.7). Thymidine incorporation was 2.7x higher in SCM than in non-SCM grasslands. For leucine incorporation, only a trend could be observed in this direction (2x higher). Furthermore, soil type affected only fungal biomass which was 1.6x higher in the peat compared to the sandy soils (Table 2.7). Fungal biomass decreased with increasing soil pH (R^2 = 0.22, P= 0.007).

Table 2.7 Microbial biomass and activity in peat (P) and sandy (S) grasslands with different fertilisation history, and corresponding F-values after ANOVA.

Treatments		Microbial parameters					
Fertilisation history (FH)	Soil type (ST)	Bacterial biomass	Fungal biomass	Fungal activity	Fungi/ bacteria	Thymidine incorporation	Leucine incorporation
		µg C g^{-1} dry soil	µg C g^{-1} dry soil	% of hyphal length	C/C	pmol g^{-1}dry soil ·hr^{-1}	pmol g^{-1}dry soil hr^{-1}
SCM†	P	202.7	82.0	5.8	0.40	90.2	1866.0
	S	80.6	59.2	8.7	0.73	86.3	1203.2
non-SCM‡	P	180.1	71.2	8.7	0.40	35.8	855.9
	S	113.5	34.6	2.5	0.35	28.4	691.2
ANOVA table	df	F-values					
FH	1	0.0	3.6	0.2	1.7	5.4*	2.9
ST	1	6.5	10.0*	0.2	1.7	0.1	0.9
FHxST	1	0.5	0.5	1.2	3.5	0.0	0.3
Covariate							
Time	1	3.3	9.3	3.3	0.2	0.1	4.4
Error (mean squares)	3	277.7	175.8	34.6	0.2	1314	412342

Values are means with n=8; 4×2 [plots × sampling dates (see text)].

*, ** and *** denote significance level at P<0.05, P<0.01 and P<0.001, respectively.

df= degrees of freedom

† Solid cattle manure and organic-N rich slurry.

‡ Slurry manure and chemical fertiliser.

2.3.4 Nitrogen mineralisation

Potential N mineralisation (aerobic incubation; PNM) was significantly higher in peat than in sandy soils (P< 0.05, Table 2.8). However, this was not affected by fertilisation history.

Table 2.8 Potential nitrogen mineralisation (PNM), herbage dry matter yield and N uptake from unfertilised plots in peat (P) and sandy (S) grasslands with different fertilisation history, and corresponding F-values after ANOVA.

Treatments		Microbial parameters		
Fertilisation history (FH)	Soil type (ST)	PNM	Dry matter yield	N uptake
		mg N kg^{-1} dry soil $(5\ weeks)^{-1}$	kg $ha^{-1}yr^{-1}$	
SCM†	P	88.0	14064	352
	S	58.0	11523	305
non-SCM‡	P	87.0	14107	354
	S	46.5	8191	207
ANOVA table	df	F-values		
FH	1	0.4	6.8*	8.0*
ST	1	6.4*	49.9***	33.3***
FHxST	1	0.0	7.6*	8.6*
Covariate				
Time	2	54.3***	-	-
Error (mean squares)	6	2.3	-	-

Values are means with n=12; 4 × 3 [plots × sampling dates (see text)].

*, ** and *** denote significance level at P<0.05, P<0.01 and P<0.001, respectively.

df= degrees of freedom

† Solid cattle manure and organic-N rich slurry.

‡ Slurry manure and chemical fertiliser.

2.3.5 Herbage dry matter yield and N uptake

Herbage DM yield and N uptake were significantly affected by fertilisation history (P< 0.05) and soil type (P< 0.01) (Table 2.8). Pairwise comparison of grasslands based on soil type indicated that herbage DM yield and N uptake were not affected by fertilisation history in case 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).

2.3.6 Contribution of soil organisms to N mineralisation

The production-ecological calculations showed that N consumption by fungi ranged between 107 and 238 kg N ha^{-1} and that they mineralised 62-138 kg N ha^{-1} during the herbage growing season of 8 months for all the grasslands together. 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 only 38-88 and 1-7 kg N ha^{-1}, respectively. It appeared that microbes (bacteria and fungi) accounted for approximately 59% and 58% of the actual soil N mineralisation in the SCM and non-SCM grasslands, respectively (Fig. 2.3). Protozoa and earthworms each contributed 25% to the total soil N mineralisation in the SCM grasslands, whereas in the non-SCM grasslands, their contributions were 29 and 9%, respectively. The estimated contribution of enchytraeids to the total soil N mineralisation was very small in all grasslands (<5%).

2.3.7 Comparison of different approaches to predict soil N mineralisation

The actual and potential N mineralisation (PNM) in the grasslands was measured by herbage N uptake in the field and soil incubation in the laboratory, respectively, whereas it was calculated by PEC and the Dutch fertilisation recommendations (DFR) (http://www.bemestingsadvies.nl, section 1.2.2.1). The results of both the measured and calculated N mineralisation during the growing season of 2010 are given in Fig. 2.3.

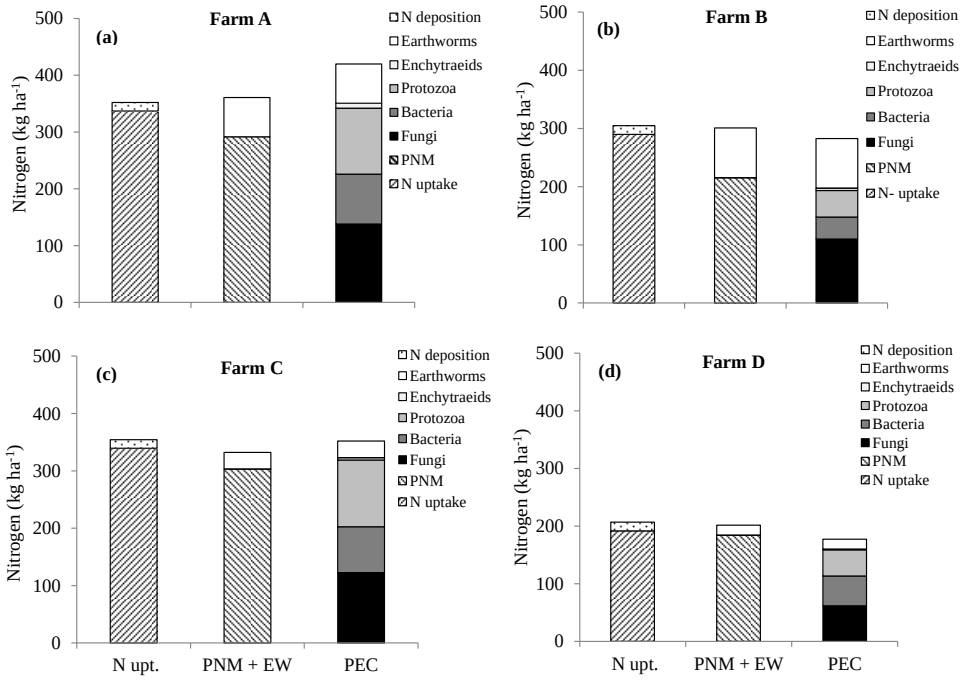

Fig. 2.3 Total herbage N uptake [N upt (non-fertiliser soil N supply + atmospheric N deposition], potential N mineralisation (PNM) + N mineralisation by earthworms calculated by the production ecological model calculations (EW) and simulation of N mineralisation by different groups of soil organisms through production ecological model calculations (PEC) during the growing season of 2010.

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} through regression analysis by Van Eekeren et al. (2010). In both SCM grasslands, net herbage N uptake was significantly higher ($P< 0.05$) than the laboratory-determined PNM. However, no such difference was found for the non-SCM grasslands ($P> 0.05$). The difference between net herbage N uptake and PNM in SCM grasslands disappeared when the earthworm N mineralisation based on PEC was added to the PNM data (Fig. 2.3). When comparing the herbage N uptake from soil with the values obtained by PEC, we found a difference of only 5% for all grasslands together.

However, the DFR method greatly underestimated soil N supply (with 34%, for all grasslands together) with values ranging from 75 to 131 kg N ha^{-1} (Fig. 2.4).

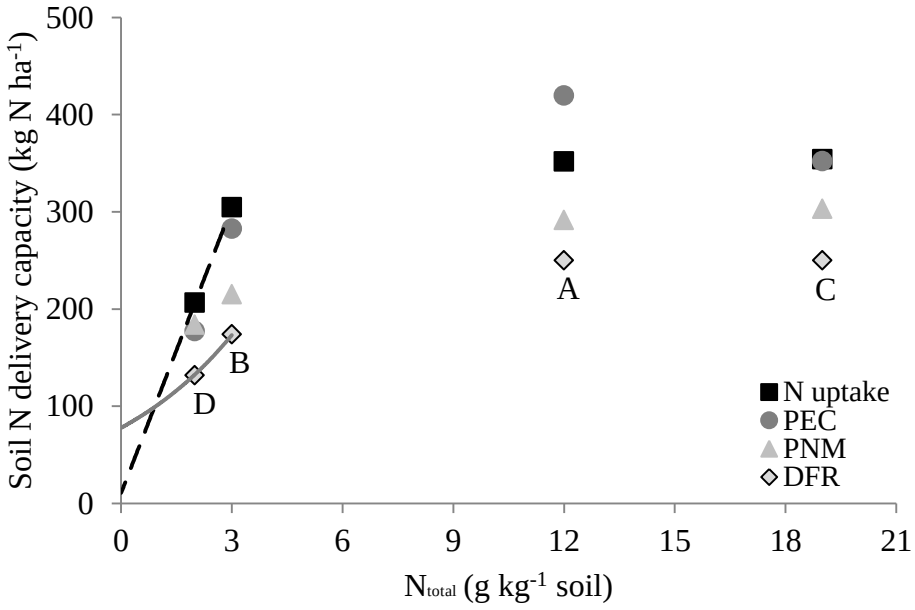

Fig. 2.4 Relationship of soil nitrogen supply (kg N ha^{-1}) estimated as i) herbage N uptake, ii) N mineralisation calculated using Dutch fertilisation recommendation (DFR) for grassland and fodder crops, iii) N mineralisation calculated from the production ecological model (PEC) with soil total N (g kg^{-1} dry soil) in the 0-10 cm soil layer of the grasslands on farms A-D, and iv) potential N mineralisation (PNM). Dotted and solid lines represent linear and curvilinear relations of herbage N uptake and soil N supply (calculated according to DFR), respectively, on the sandy grasslands of farms B and D.

2.4 Discussion

2.4.1 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 with or without chemical fertilisers. In our SCM grasslands the density and biomass of earthworms and enchytraeids (detritivores) were three times higher than in non-SCM grasslands (Tables 2.5 and 2.6). This might be associated with higher fresh food availability (Timmerman et al. 2006, Birkhofer et al. 2008, Van Eekeren et al. 2009), as in the SCM grasslands the organic matter inputs were much higher than in the non-SCM grasslands (Table 2.1). Besides, we found a higher epigeic to endogeic earthworm ratio in the SCM grasslands (Table 2.5). The food preference of epigeic earthworms is plant litter and fresh organic matter inputs (Lavelle 1988), which were higher in the SCM grasslands (Table 2.1). Finally, the rather low abundance of earthworms and enchytraeids on farm C could also be related with the lower soil pH on this farm (Table 2.2), resulting from long-term high inputs of chemical fertiliser N (Standen 1984, Ma et al. 1990, Hopkins et al. 2011).

Bacterial activity measured as bacterial growth rate (thymidine incorporation) was significantly higher in the soil of SCM grasslands. However, there was no effect of fertilisation history on bacterial and fungal biomass (Table 2.7). Probably, these high bacterial growth rates were counteracted in the field by greater grazing activities of microbivores like protozoa, bacterivorous nematodes and microarthropods (Bloem et al. 1997, Laakso et al. 2000). The existence of such a predator-prey relationship was supported by the observed positive correlation between microarthropod density (mites and collembola) and microbial biomass (bacteria and fungi). This can explain why we did not observe an effect of fertilisation history on the fungal and bacterial biomass.

2.4.2 Model prediction of soil N mineralisation

Contrary to our expectations, the measured herbage N uptake and DM yield from the unfertilised plots of SCM and non-SCM grassland on peat were not significantly different. The PEC showed high levels of soil N mineralisation of 420 and 352 kg N ha^{-1} for these grasslands, respectively (Fig. 2.3). Nevertheless, as a mean it was very close (+14%) to the average net herbage N uptake from the two peat grassland soils (338 kg N ha^{-1} excluding N deposition). In line with our expectations, herbage N uptake and DM yield from the unfertilised SCM grassland on sand was higher compared to the non-SCM grassland. PEC estimated soil N mineralisation levels of 283 and 177 kg N ha^{-1}, respectively, which were close to (-5%) net herbage N uptake (Fig. 2.3). For all grasslands together, PEC model prediction was only 5% higher than the obtained net herbage N uptake.

2.4.3 Role of soil organisms in N mineralisation

In the grasslands we studied, PEC indicated that the N mineralisation through earthworms, 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. This was lower than the range of 85-170 kg N ha^{-1} yr^{-1} found 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 and especially the absence of anecic species in our grasslands. The latter species have a preference of high quality food with a lower C:N ratio than endogeic species eat (Bouché 1977, Lavelle 1988), and therefore they mineralise relatively more N than endogeics (Van Vliet et al. 2007). 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. This contribution could be explained by their relatively higher turnover rates and biomass than those of other groups of soil biota (De Ruiter et al. 1993b, Bloem et al. 1994). Protozoa are important in soil N mineralisation due to their high specific death rate (6 yr^{-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, this was between 16 and 35%.

2.4.4 Comparison of different approaches to estimate soil N mineralisation

We hypothesised that any difference between PNM and herbage N uptake could be explained by the contribution of earthworms to soil N mineralisation (De Goede et al., in review). Indeed, we found that after adding the contribution of earthworms to the N mineralisation, as calculated by the PEC model, to the measured PNM (PNM+EW in Fig. 2.3), no longer a difference with herbage N uptake was found (Fig. 2.3).

Soil N supply calculated according to the Dutch grassland fertilisation recommendations (DFR; 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 of grasslands on sand (Fig. 2.4). 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 DFR. On the latter sites, herbage N uptake was measured from grasslands which were not fertilised for several years, whereas in case of Van Eekeren at al. (2010) the grasslands remained unfertilised only in the year of their experiment. From the herbage N uptake of our grasslands on sand, we estimated an input of 10 kg N ha^{-1} through atmospheric N deposition

(see intercept of dotted line with Y-axis in Fig. 4). Despite that this value is based on only two data points, it does not deviate much from the 15 kg N ha^{-1} established by Van Eekeren et al. (2010). As a consequence, the value of 78 kg N ha^{-1} for atmospheric N deposition used in DFR (see intercept of solid line with Y-axis in Fig. 2.4) cannot be correct. These findings underline the need to modify the currently used fertilisation recommendations for grassland on sand in The Netherlands.

2.5 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 with or withoutchemical 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. 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 from exclusion of earthworms from the incubations and was 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. In so doing, N fertilisation recommendations for production grasslands can be greatly improved.

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Chapter 3

Home field advantage of cattle manure decomposition affects the apparent nitrogen recovery in production grasslands

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Abstract

Based on evidence from forest ecosystems that litter decomposition is highest in its home habitat, the so-called home field advantage (HFA), we tested whether HFA also occurs in production grasslands, to which solid cattle manure (SCM) was applied. Two dairy farms were selected which differed in type of home-produced SCM (stacked or composted) and soil type (sand or peat). Disappearance patterns of manure dry matter (DM) and nitrogen (N) were monitored from litterbags (4 mm mesh size) during the grass growing season. At the same time, apparent herbage N recovery (ANR) of SCM, applied at two rates (200 and 400 kg N ha^{-1} yr^{-1}), was measured. On average, manure DM and N disappearances on the home farms were 20 and 14% greater, respectively, than on away farms. Differences in ANR were also very pronounced (on average 14 and 53% higher at home than away for the two respective application rates). The two SCM types were also studied on two neighbouring dairy farms (one on sand and one on peat soil) where no SCM had been applied for many years. Here, manure DM and N disappearances from the litterbags were much lower ($P < 0.01$). This experiment provides strong evidence for a home field advantage in production grasslands differing in fertilisation history, showing that site-specific manure management affects the soil-plant interactions regulating plant N-availability. These findings have to be taken into account when changing fertilisation regimes in production grasslands. This is the first report to quantify a HFA from an agricultural ecosystem. HFA values we report here have not been established in any ecosystem thus far.

3.1. Introduction

Plant nitrogen (N) availability from solid cattle manure (SCM) when applied to the surface of cultivated grasslands varies widely. In a number of recent Dutch

experiments the apparent herbage N recovery from SCM ranged between 20 and 50% in the first year (Van Dijk 2004, Schröder et al. 2007, Sonneveld and Lantinga 2011, Shah et al. 2012a, Shah et al. 2012b). Since the major part of the N present in SCM is organically bound, its net availability for plant uptake largely depends on the balance between decomposition, mineralisation and immobilisation. These processes are influenced by environmental conditions (i.e. temperature and moisture), abiotic factors like soil pH and clay content, chemical composition of the applied organic matter and the composition of the soil decomposer community (i.e. bacteria, fungi and invertebrates) (Myers et al. 1982, Verhoef and Brussaard 1990, Wardle 2002, Ayres et al. 2009b, Wang et al. 2009).

Globally, the observed variation in decomposition of organic matter in terrestrial ecosystems is explained for about 70% by environmental conditions together with the chemical composition of organic matter (Ayres et al. 2009b). The remaining 30% is influenced by other factors like the soil decomposer community and their interaction with specific characteristics of the added organic matter together with the local soil chemical and physical properties (Tian et al. 1995, Ayres et al. 2009b, Bardgett 2005). However, the strength of these factors and their interactions vary among ecosystems and biomes (Wall et al. 2008). In case of significant interactions, the chemical composition (lignin:N or carbon:N ratio) of the organic inputs was found to determine the composition of the associated decomposer communities and the routes and rates of the decomposition process at local scale (Negrete-Yankelevich et al. 2008, Ayres et al. 2009a). Such an association has been reported to explain why decomposition commonly occurs more rapidly when organic matter is applied to its home habitat than when it is applied elsewhere ("away"), an effect called home field advantage (HFA) (Hunt et al. 1988, Gholz et al. 2000, Vivanco and

Austin 2008, Ayres et al. 2009b, Strickland et al. 2009a, Milcu and Manning 2011).

The HFA is generally stronger in cases where the organic matter quality is lower. However, despite large differences in the chemical composition of the litter from natural grasslands and forests, St. John et al. (2011) did not observe a HFA in a reciprocal grassland-forest litter transplantation experiment. They explained that lack of a HFA was due to a shift from bacterial- to fungal-based decomposition of grass litter when it was incubated in forest, whereas tree litter decomposition was not shifted from fungal- to bacterial-based during incubation in grassland.

SCM varies greatly in its composition and quality (lignin:N and C:N ratio) because of differences in animal origin, feed ration, bedding materials and manure handling as well as processing systems (Tunney 1975, Rotz 2004). Forge et al. (2005) observed that repeated additions of cattle manure to grassland increased the soil faunal biomass as well as its activity and according to Stark et al. (2008) organic matter amendments boosted the activity of enzymes important in the decomposition process. In contrast, long-term application of mineral fertilisers to grasslands generally decreases earthworm populations (Ma et al. 1990, De Goede et al. 2003) and microbial biomass (Hopkins et al. 2011), resulting mainly from a decrease in soil pH. Hence, also in agro-ecosystems historical factors can shape structural differences in soil biota communities and may be expected to affect organic matter decomposition and mineralisation. Some indication for the effect of manure management history on N mineralisation from recently added SCM was obtained in a 10-week pot experiment (Nett et al. 2010). They found that organic fertilisation increases the N mineralisation of and N uptake from recently added SCM but this effect was not always dominant. Therefore, it remains of great interest (i) if HFA exists in

production grasslands, and (ii) what its impact can be on soil N transformations and plant N uptake. Accordingly, the aim of the present study was to investigate the effects of differences in the prevailing fertilisation management of production grassland and type of SCM (composted vs. stacked) on the DM and N disappearance rate of SCM and herbage N recovery. We hypothesized that (i) the DM and N disappearance rate from SCM will be higher in grasslands with a history of SCM application than in grasslands with a history of non-SCM inputs, and (ii) the N mineralisation rate and herbage N uptake from home-produced SCM will be higher on the home farm than when applied on an away farm.

3.2 Materials and methods

3.2.1. Site description

Two dairy farms (A and B) were selected which differed in the type of home-produced and applied SCM. In addition, two neighbouring farms (C and D) were selected where only cattle slurry manure was produced. Farms A and C were located on peat soil in the western peat district of the province of Utrecht, whereas farms B and D were both located on a sandy soil near Veenendaal in the province of Gelderland. The distance between farms A and C was 1 km and farms B and D were located 15 km from each other. Farms A and B used cereal straw as bedding material applied at a rate of approximately 5 kg per livestock unit (= 500 kg live weight) per day in a litter barn. The manure from the litter barn on farm A (AM) was extensively composted by adding regularly organic-N rich slurry and turning the manure heap 2 to 3 times during a storage period of 6 to 8 months. Within this manure handling system, the straw particles were reduced in size to approximately 2 cm length by the turning operations. This

Table 3.1 Mean (±1 SEM; n = 3) dry matter (DM), N_{total}, $N_{mineral}$, $N_{organic}$, C:N ratio and lignin:N ratio of solid cattle manure (SCM) from farms A and B.

Farm	SCM processing method	DM	N_{total}	$N_{mineral}$	$N_{organic}$	C:N ratio	Lignin:N ratio
		(%)	g kg^{-1}DM				
A	Composted	19.0 ± 0.2	25.3 ± 0.3	1.1 ± 0.04	24.2 ± 0.3	20 ± 0.3	13 ± 0.5
B	Stacked	20.1 ± 0.1	21.8 ± 0.1	0.8 ± 0.02	21.0 ± 0.1	23 ± 0.1	8 ± 0.6

resulted in a partly composted SCM (Table 3.1). On farm B, the manure from the litter barn was stacked (BM) during a storage period of several months and contained longer pieces of straw of up to about 10 cm. Farm C was characterized by the application of artificial fertiliser and mineral N-rich slurry manure, and on farm D only slurry manure was used. No SCM had been used on the grasslands of farms C and D for at least 30 years (non-SCM farms). Grassland management information of the four farms is given in Table 3.2.

Table 3.2 Grassland management information of the four farms (A-D).

Farm	Soil type	N-input (%)			
		Organic-N		Inorganic-N	
		from SCM	Total	from slurry manure	from artificial fertilisers
A	Peat	45	75	25	0
B	Sand	70	80	20	0
C	Peat	0	30	30	40
D	Sand	0	50	50	0

3.2.2 Litterbag experiment (experiment 1)

This experiment was conducted to estimate the dry matter disappearance (DMD) and nitrogen disappearance (ND) of the two SCM types from litterbags on the four farms during the growing season of 2010.

On each farm, one permanent grassland field of about 3 ha was selected (Table 3.2). Soil fertility parameters from each grassland field at the start of the experiment are presented in Table 3.3. Each field was divided into four blocks

and in each block a grass cage of 4.5 m × 1.25 m was placed at random (Lantinga et al. 2004). Inside each cage, 18 litterbags (2 manure types, 3 sampling dates and 3 mesh sizes) were randomly placed on the grassland soil surface in the last week of March 2010. The distance between the centres of two adjacent litterbags (100 mm x 100 mm x 15 mm, l x b x h) was 50 cm. For the purpose of this paper, we used only the data obtained with the largest mesh size (4 mm) which allowed the entrance of all size classes of detritivorous soil fauna.

Table 3.3 Soil characteristics for the layer 0-10 cm of the four farms (A-D).

Farm	Soil type	OM	N_{total}	C:N ratio	pH-KCl
		(%)	(g kg^{-1})		
A	Peat	44	12	14	5.0
B	Sand	6	3	10	5.3
C	Peat	52	19	16	4.4
D	Sand	4	2	16	5.6

One day before placement in the field, the litterbags were filled with approximately 150 g fresh manure, corresponding to about 30 g DM for both manures. Before placement of the litterbags, the herbage was mown to a stubble height of 4 cm by using a motorized mower, and a 10 x 10 cm soil surface for each litterbag was gently roughened with a spade to facilitate soil-litterbag contact. In total, 96 litterbags (2 manure types × 4 replicates × 3 sampling dates × 4 farms) were used. The litterbags were collected for analysis on days 60, 120 and 240. In order to monitor material loss from litterbags during transportation, placement on grasslands and retrieval, they were put in traveller bags (Bradford et al. 2002). This material loss appeared to be negligible which

confirmed that only mesh size and soil biota were responsible for the observed DM and N disappearances.

3.2.3 Manure analysis

SCM that remained in the litterbags after removal from the grasslands was oven-dried at 105°C for 24 hours, ground to pass 1 mm sieve and analysed for dry matter, N and ash content. N was measured by Kjeldahl digestion (MAFF 1986). Ash content was determined by loss-on-ignition at 550°C for 4 hours. DMD and ND rates were calculated from the difference between the initial and remaining manure dry weight and N content, after correction for soil contamination according to Potthoff and Lofttfield (1998):

$$SC = \frac{AC_{AR} - AC_{BP}}{AC_S} \qquad (1)$$

SC is the dry weight of soil contamination (g), AC_{AR} is the ash content of SCM in litterbag (mg) after removal, AC_{BP} is the ash content of SCM in litterbag (mg) before its placement, and AC_S is the ash content of the soil (mg g^{-1}).

3.2.4 Manure application experiment (experiment 2)

In a parallel experiment on the same field, again under cages, AM and BM were surface-applied at two application rates (200 and 400 kg N ha^{-1} yr^{-1}) on the grass swards of the four farms. These cages were positioned very close (≤ 1 m) to the cages in which litterbags were placed. The area inside a cage was divided into five plots each with a size of 0.8 m × 1.2 m. Five treatments were allocated at random to these five plots: a control (C_0) receiving no N fertilisation, composted SCM from farm A at rates of 200 (AM_{200}) and 400 kg N ha^{-1} $year^{-1}$ (AM_{400}), and stacked SCM from farm B at rates of 200 (BM_{200}) and 400 kg N ha^{-1} $year^{-1}$ (BM_{400}). The most important characteristics are given in Table 3.1.

3.2.5 Herbage harvests

On all farms, the herbage plots were harvested 5 times during the growing season (20th May, 29th June, 9th August, 21st September and 11th November 2010). This was done with a spinach knife (OTTER-Messer, Solingen, Germany) using a metallic frame (50 cm × 50 cm) with attached pins (Lantinga et al. 2004) to ensure a constant cutting height of 4 cm. Herbage samples were oven-dried at 70°C for 48 hours to calculate the DM yield from each plot. After weighing, these samples were ground to pass a 1 mm sieve and analysed for their N content by means of Kjeldahl digestion (MAFF 1986). The apparent nitrogen recovery (ANR) was calculated as:

$$ANR\ (\%) = \frac{(NC_{MP} \times\ DM_{MP}) - (NC_{CP} \times\ DM_{CP})}{N_{applied}} \times 100 \qquad (2)$$

Where NC_{MP} is the herbage N content (g N (100 g DM)$^{-1}$) and DM_{MP} is the herbage DM yield (kg ha^{-1}) in the fertilized plots, NC_{CP} is the herbage N content (g N (100 g DM)$^{-1}$) and DM_{CP} is the herbage DM yield (kg ha^{-1}) in the unfertilized plots, and $N_{applied}$ is the total manure-N applied (kg ha^{-1}).

3.2.6 Abiotic soil parameters

From each block, 80 cores at a soil depth of 10 cm were sampled using a core sampler and per block they were combined into one bulk sample for chemical analysis. Moisture content was measured by determining weight loss of about 20 g fresh soil after drying at 40°C. Soil pH was measured in 1 M KCl (1:10, w:v ratio). Soil organic matter was determined by loss-on-ignition (Ball 1964).

3.2.7 Statistical analysis

Treatment effects on DMD and ND from the litterbags and total herbage ANR from the manure were analysed using analysis of variance with GENSTAT (13th edition, VSN International, Hemel Hempstead, UK). Treatments were

fertilisation history (SCM and non-SCM), manure type (AM and BM), application rates (200 and 400 kg N $ha^{-1}yr^{-1}$) and soil type (sand and peat). The main effects of the treatments were tested separately for the harvests on days 60, 120 and 240. The interaction between fertilisation history and soil type could not be estimated due to the lack of appropriate replicates. In case of manure type, the main effect and its interaction with fertilisation history and soil type was estimated. The block effect was nested within the individual farms.

The home field advantage (HFA) indices for DMD, ND and herbage ANR from AM and BM on farms A and B were calculated in two steps by relationships adapted from Ayres et al. (2009b):

$$AM_{R\,A} = \frac{AMA}{AMA + BMA} \times 100 \quad (3A)$$

$AM_{R\,A}$ = Relative DM disappearance / N disappearance / herbage ANR from farm A manure on farm A;

AMA = Percentage DM disappearance / N disappearance / herbage ANR from farm A manure on farm A;

BMA = Percentage DM disappearance / N disappearance / herbage ANR from farm B manure on farm A.

Similar equations were used to calculate $AM_{R\,B}$, $BM_{R\,B}$ and $BM_{R\,A}$.

Subsequently, the HFA was calculated as:

$$HFA = \left(\frac{AM_{R\,A} + BM_{R\,B}}{AM_{R\,B} + BM_{R\,A}} \times 100\right) - 100 \quad (3B)$$

Where a positive score for HFA represents a higher DMD, ND, or herbage ANR at the home compared to the away farm.

The relative magnitude of manure DMD and ND as well as herbage ANR on away farms compared to its home application was calculated by:

$$RM = \frac{HMH - HMA}{HMH} \times 100 \quad (4)$$

RM = Relative magnitude of the manure DM disappearance / N disappearance / herbage ANR on the away farm compared to its home application;

HMH = Percentage of home manure DM disappearance / N disappearance / herbage ANR on its home farm;

HMA = Percentage of a manure DM disappearance / N disappearance / herbage ANR on away farm;

3.3 Results

3.3.1 Disappearance of solid cattle manure and N from litterbags

After 240 days of field incubation, 39 to 80% of the DM and 56 to 98% of the N had disappeared from the litterbags (Table 3.4). Fertilisation history and manure type had strong effects ($P \leq 0.001$) on manure DMD. On SCM farms, DMD was on average 37% higher than on non-SCM farms, and the fertilisation history effect was largest (57%) for AM. Overall, BM had a 22% higher DMD rate ($P \leq 0.001$) than AM. This difference was more pronounced on the non-SCM farms and sandy soils where their respective values were 42 and 44%. Also, ND was 45% higher ($P \leq 0.001$) on the SCM farms than on the non-SCM farms, and the effect was largest (65%) for AM. On sandy soils, ND was on average 23% higher than on peat soils and the effect was largest for BM (24%).

A HFA was found both for DMD (20%; $P = 0.002$) and ND (14%; $P = 0.03$) (Figs. 3.1a and b). DMD and ND on the two non-SCM farms (C and D) were in all cases lower compared to their incubations at home (Figs. 3.1a and b; Table 3.4).

Table 3.4 Dry matter disappearance (DMD) and N-disappearance (ND) from litterbags, and herbage apparent N recovery (ANR) 240 days after application of two types of solid cattle manure to grasslands with different fertiliser history (FH) and soil type (ST; peat (P), sand (S)), and corresponding p-values after ANOVA.

Treatment				DMD	ND	ANR	
Manure type (MT)	FH	Farm	ST	(%)		200 kg N ha^{-1}*	400 kg N ha^{-1}*
AM	SCM†	A	P	**80**	**90**	**27.5**	**27.3**
		B	S	60	95	12.5	8.9
	non-SCM‡	C	P	50	56	-0.5	1.4
		D	S	39	56	18.8	15.1
BM	SCM	A	P	74	71	25.5	19.2
		B	S	**80**	**98**	**15.2**	**14.6**
	non-SCM	C	P	63	63	3.4	-2.6
		D	S	63	70	12.2	13.2
ANOVA (p-values)							
Treatment	df						
FH	1			<0.001	<0.001	0.104	0.025
ST	1			0.077	0.021	0.992	0.608
MT	1			<0.001	0.369	0.918	0.595
MTxFH	1			0.007	<0.001	0.984	0.625
MTxST	1			<0.001	0.004	0.772	0.491

Values are means with n = 4; results from home applied manure in bold

† Solid cattle manure and organic-N rich slurry

‡ Slurry manure and artificial fertiliser

* Manure N application rate

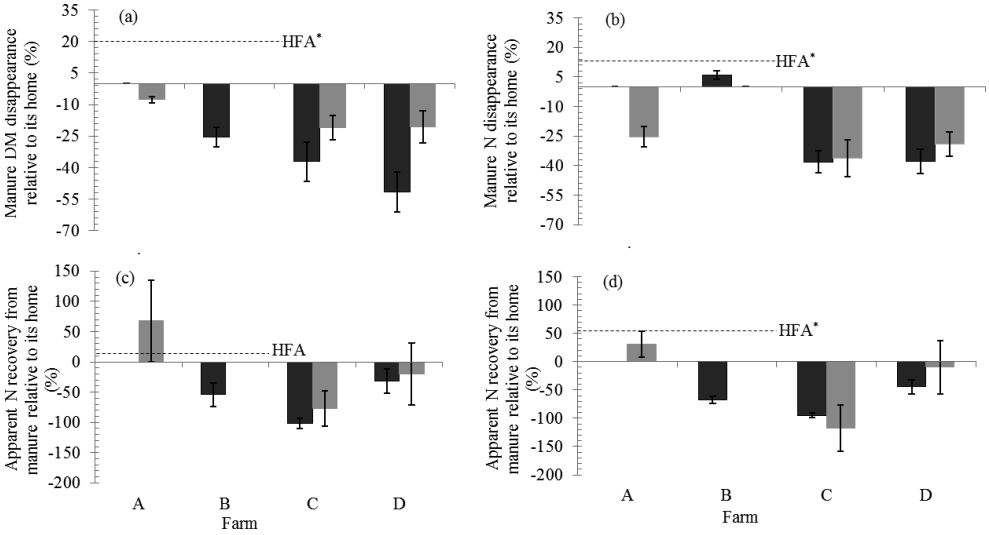

Fig. 3.1 Dry matter (a) and nitrogen disappearance (b) after 240 days from litterbags containing two types of SCM collected from farms A and B (AM (black bars) and BM (grey bars), respectively) and herbage apparent nitrogen recovery (ANR) after surface spreading of AM or BM at rates of 200 (c) and 400 kg N ha^{-1} (d) at away farms relative to their home application. Negative values correspond to lower manure disappearance and herbage ANR at away farms relative to the home application, whereas positive values correspond to higher rates. The dashed line indicates the home field advantage (HFA) for the SCM farms A and B (*: significantly different from zero). Error bars are standard errors of means

3.3.2 Herbage apparent N recovery

Overall, herbage ANR ranged between -2.6 and 27.5% (Table 3.4). On the non-SCM farms, herbage ANR was on average 39% lower than on the SCM farms ($P \leq 0.05$). However, no effect of N application rate on herbage ANR could be established ($P > 0.05$). Reciprocal exchange of SCM between farms A and B resulted in a HFA for herbage ANR that was higher than zero ($P \leq 0.05$) at an application rate of 400 kg N ha^{-1} (Figs. 3.1c and d). At home, herbage ANR was 53% higher than on the away farm for this application rate. For the lower

application rate of 200 kg N ha^{-1}, only a tendency towards a positive herbage ANR could be observed at home (+14%).

3.3.3 Relationships between manure N disappearance and herbage N recovery

Cumulative relationships between ND from litterbags and herbage ANR from SCM throughout the growing season were established using the data collected on days 0, 60, 120 and 240 (Fig. 3.2). Overall, this resulted in S-shaped curves with a small increase in herbage ANR during the first 60 days.

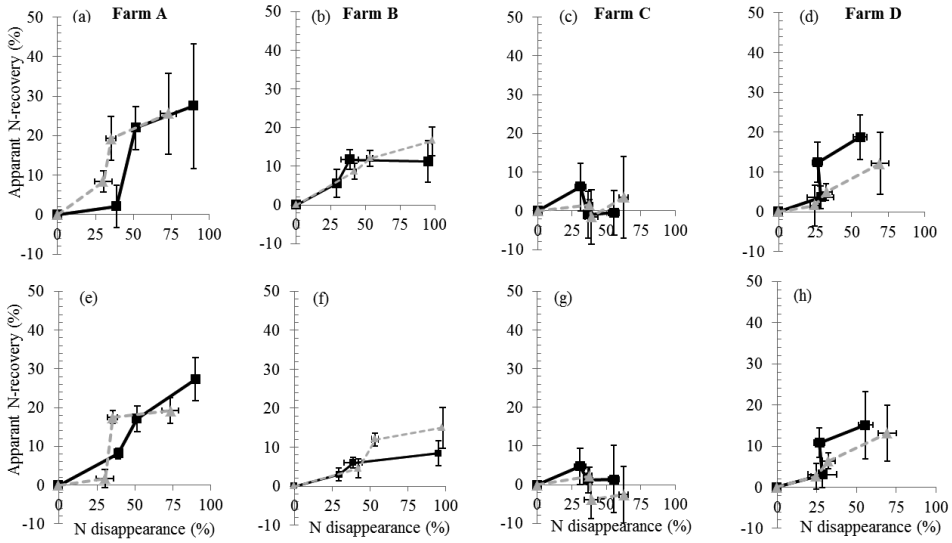

Fig. 3.2 Relationship between progressive disappearance of N (%) from litterbags and herbage apparent N recovery (%) from solid cattle manures AM200 and BM200 (a-d) and AM400 and BM400 (e-h) on the four farms (A-D). AM200 and AM400: manure collected from farm A (black line) and applied at rates of 200 and 400 kg N ha^{-1}, respectively. BM200 and BM400: manure collected from farm B (dashed grey line) and applied at rates of 200 and 400 kg N ha^{-1}, respectively. The four data points indicate day numbers 0, 60, 120 and 240, respectively. Error bars are standard errors of means.

Thereafter, the relationship became steep between days 60 and 120 on farms A, B and D, but not on farm C, and levelled off towards day 240 (Fig. 3.2). On farm C, the change in herbage ANR was negative between days 60 to 120 and neutral between days 120 to 240 (Figs. 3.2 c and g).

3.4 Discussion

We hypothesized that, similar to forest ecosystems, HFA will also occur in grasslands under specific fertiliser application management. In our production grasslands, we found greater HFA effects for dry matter and N disappearances as well as herbage ANR (13 to 53%) compared to those reported by Ayres et al. (2009a) from forest ecosystems (-9 to 29% with a mean of 8%). These effects were more prominent in case of solid cattle manure from farm A (AM) which had a wider lignin:N ratio than the manure from farm B (BM) (13 and 8, respectively). This corroborates results of Ayres et al. (2009a) and Strickland et al. (2009b) who reported for forest ecosystems that the HFA was larger from the litter with a wider lignin:N ratio, i.e. litter of a lower quality. According to Strickland et al. (2009b) this effect could be explained by differences in the species composition of the soil biota community that was characterised by more specialists in soils with low-quality litter input. There is evidence that in this respect microarthropods play a part in our farms. Schouten et al. (2004) found the density and diversity of microarthropods (collembola and mites) to be five times higher on farm A than in conventional peat grassland farms in the same region. Moreover, about half of these microarthropods were fungal grazers that can digest cell walls next to cell contents. These are indications that the mesofauna composition was adjusted to the specific manure quality on this farm.

Overall, higher N disappearance from home-produced SCM in the litterbags on the home farm coincided with high herbage ANR (Figs. 3.2a, b, e and f). The magnitude of HFA for herbage ANR from SCM at an application rate of 400 kg N ha^{-1} was even more pronounced than observed for dry matter and N disappearances (Fig. 3.1). This was mainly due to lower herbage ANR from AM_{400} on away farm B compared with its home application (Fig. 3.1d). Besides, on this farm higher N disappearance from AM in the litterbags was observed compared with its home farm A (Fig. 3.1b). However, on this farm, we found that activity and biomass of bacteria and fungi as well as the density of mesofauna, i.e. enchytraeids and microarthropods, was about half that of farm A (Chapter 2). This could have attributed to lower N mineralisation from lignin-N rich manure and in turn herbage ANR. In addition, the lower N disappearance on farm A from the away manure (BM) coincided with lower herbage ANR from BM_{400} on this farm (Fig. 3.2e). This manure contained long pieces of straw (~10 cm) which did not significantly contribute to its total weight but could have played an important role in manure N immobilisation (Cheshire et al. 1999). Visual observations revealed that on farm A the straw pieces from the away manure of farm B disappeared much slower than at home. This leads us to the inference that the soil fauna community on farm A was less capable to decompose longer pieces of straw compared to those on farm B. Consequently, primary decomposers like bacteria and fungi, which are responsible for straw decomposition after fragmentation by the soil fauna, required more mineral N to degrade C-rich straw pieces. As a consequence, they immobilised more N from this fresh straw-rich manure source (Jensen 1931, Cheshire et al. 1999). Furthermore, our findings resemble those of Groot et al. (2007) and Van der Ploeg et al. (2007). They observed higher herbage ANR from home-produced slurry in a reciprocal transplantation experiment of slurry

manure in combination with its application method (slurry injection or broadcasting). They concluded that the grassland management system on the home farms was "tuned" to stimulate herbage ANR from home slurry when applied by the home fertiliser application method compared to the application of away slurry with its own fertilisation technique. We suggest, based on our findings, that this was also a HFA.

We did not find a significant HFA for herbage ANR at an application rate of 200 kg N ha^{-1} yr^{-1}, but only a clear tendency. However, this could be linked with the observed large variation in herbage ANR from this treatment among the experimental units of farm A, resulting from rather large spatial differences in the botanical composition of the grassland on this farm.

Our results support the hypothesis that DM and N disappearances from SCM in litterbags would be higher on SCM farms, compared with their neighbouring non-SCM farms (Table 3.4 and Fig. 3.1). Scheller and Joergensen (2008) found higher wheat straw decomposition rates in soil from a farm that regularly received straw compared to that of a non-straw applying farm. They explained this by induction of a fungal community by regular straw inputs that was better adapted to straw decomposition compared with the fungal community in the non-straw applying farm. Their results are in line with those of Strickland et al. (2009b) who observed in a laboratory microcosm study that the soil microbial community which developed on high-quality litter was not adapted to decompose low-quality litter. However, these studies did not address the soil fauna. Application of organic manure is known to increase the abundance and activity of soil organisms (Forge et al. 2005, Van Eekeren et al. 2009), whereas exposure to high artificial fertiliser inputs has the opposite effect due to increased soil acidity (Ma et al. 1990, De Goede et al. 2003, Hopkins et al. 2011). Therefore, we infer that on our non-SCM farms a lower density of soil

organisms and/or a community composition that is not well adapted to decompose SCM could be the cause of lower dry matter and N disappearances.

The lower herbage ANR from SCM on the non-SCM farms coincided with lower N disappearance from SCM in litterbags (Figs. 3c, d, g and h). On the non-SCM farms, the N disappearance from litterbags, averaged over two manure types was two-thirds of that at the SCM farms (Table 3.4). Our results are in accordance with those of Nett et al. (2010), who concluded from their own study along with other published data that the soil biota in SCM-history soil lead to increased net N mineralisation and, subsequently, plant N uptake from recently applied SCM. The average herbage ANR on the non-SCM farms was lowest (~1%) on farm C. On this farm, 70% of the applied N was mineral (Table 3.2); therefore, herbage growth was much less dependent on organic N. Moreover, Hopkins et al. (2011) found that higher mineral N fertilisation to grassland resulted in increased soil acidity. Indeed, the pH on farm C was 0.9-1.2 units lower than on the other farms, which would further explain the near absence of herbage ANR.

A strong HFA in production grassland implies that the fertilisation history of a given soil will affect the plant-available N from recently applied SCM by influencing the N dynamics of the soil. Long-term organic fertiliser applications generally promote the density, diversity and activity of soil fauna and microflora (Forge et al. 2005). In nearly all of the studies reported by Nett et al. (2010) this was accompanied by an increase in net N mineralisation from recently applied SCM. Therefore, it is strongly recommended that both researchers and farmers take the previous fertilisation history into account when carrying out short-term SCM fertilisation experiments and changes in fertilisation regimes, respectively.

3.5 Conclusions

Our study provides the first quantification of home field advantages in production grasslands, in our case established after the application of solid cattle manure. HFAs were found for dry matter and N disappearance rates and herbage uptake of mineralized N from SCM, especially at higher application rate. Ours are also the first quantifications of HFAs in an agricultural ecosystem and its magnitudes were larger (13 to 53%) than those reported from natural ecosystems (-9 to 29%). Our findings indicate that, like in forest ecosystems, the quality of the applied organic material plays a crucial role in determining the HFA. In our case the quality was reflected in the lignin:N ratio and the presence of long pieces of straw in the solid cattle manure.

The balance between gross N mineralisation from organic manure N and gross N immobilisation plays an important role in herbage N uptake from solid cattle manure. To what extent the density, diversity, size class distribution and activity of soil organisms are drivers for this phenomenon needs to be investigated in future studies.

We observed higher dry matter and N disappearances as well as herbage uptake of N from solid cattle manure on SCM farms than on non-SCM farms. Therefore, it appears that farmers will not achieve higher production in the year of conversion of grassland fertilisation management from artificial fertilisers or cattle manure slurry to solid cattle manure.

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Chapter 4

Soil biota community composition and manure quality are drivers of the home field advantage for solid cattle manure decomposition in production grasslands

Muhammad Imtiaz Rashid, Egbert A. Lantinga, Lijbert Brussaard, and Ron G.M. de Goede, Soil biota composition and manure quality are drivers of the home field advantage for solid cattle manure decomposition in production grasslands.

Abstract

Solid cattle manure (SCM) disappeared faster in grasslands on farms at which it was produced (i.e. at home) than when applied to grasslands on away farms. We investigated which body size-classes of soil biota contributed to this home field advantage (HFA). Two dairy farms were selected which differed in type of home-produced SCM (stacked or composted) and soil type (sand or peat). Manure dry matter (DM) and nitrogen (N) disappearances from litterbags with three different mesh sizes (125 μm, 1.5 mm, and 4 mm) were measured after 120 and 240 days of litterbag placement in grassland. On average, manure DM and N disappearances from macro-mesh size litterbags after 120 days of placement were 50 and 45% greater ($P< 0.001$), respectively, in the home farm than in the away farm. Differences had decreased to 24 and 12% ($P< 0.001$), respectively, during 120-240 days of litterbag incubation. This decrease over time could be related to changes in the chemical composition of the manure that remained in the litterbags. After 120 days, the C:N ratio of home SCM was significantly higher than the C:N ratio of away SCM ($P< 0.01$). However, after 240 days, no difference in C:N ratio was observed anymore. All soil biota size groups contributed to the HFA for SCM decomposition, but their effects were size-group specific and different over time. Initially, the HFA for DM disappearance was stronger for meso- and macrobiota compared to that of microbiota (51, 50, and 30%, respectively). Over time the HFA for DM disappearance decreased, mostly for the macrofauna (from 50 to 24%; $P= 0.013$). On the other hand, all soil biota size groups contributed much more to the N disappearance from home SCM than from away SCM during the initial stage of decomposition (0-120 days). However, over time, there was a sharp decrease in the contribution of meso- and macrofauna to the HFA for N disappearance (from 42 to 15% and from 24 to 12%, respectively; $P< 0.01$), but not so for the microbiota. The two

SCM types were also studied on two neighbouring dairy farms (one on sand and one on peat soil) where no SCM had been applied for many years. Here, manure DM and N disappearances from the litterbags were much lower than on the SCM farms ($P< 0.05$), both after 120 and 240 days of litterbag incubation. The contribution of meso- and macrofauna to DM and N disappearance was about 3x as low as on the SCM farms. However, for the contribution of the microbiota no difference was found. The interaction between fertilisation history, soil biota size classes and chemical composition of SCM explained the HFA for SCM decomposition in production grasslands.

4.1 Introduction

Solid cattle manure (SCM) is a valuable source of nitrogen (N) when applied to production grasslands (Schröder et al. 2007). Biological decomposition of SCM is an important agro-ecosystem function that regulates the net ecosystem productivity through mineralisation of complex organic compounds into plant available nutrients. However, in practice, widespread inconsistency exists in decomposition and N mineralisation patterns of surface-applied SCM, which affects herbage N uptake. In recent studies, herbage N recovery from SCM ranged between 20 and 50% in the year of application when broadcast to temperate production grasslands (Van Dijk 2004, Schröder et al. 2007, Sonneveld and Lantinga 2011, Shah et al. 2012). Decomposer communities which were pre-exposed to SCM of local origin were found to be associated with higher decomposition and mineralisation rates than when they were offered SCM from away sites (Chapter 3). This is known as the home field advantage (HFA) (Gholz *et al.* 2000).

The magnitude of HFA established thus far for dry matter (DM) disappearance was on average 8% in forest ecosystems (Ayres *et al.* 2009a) and 20% in

agricultural grasslands (Chapter 3). However, there was a large variation in the size of the observed HFA (-9 to 29%) (Ayres et al. 2009b, Rashid et al. 2013). According to Ayres *et al* (2009a) and Wallenstein et al. (2013) relatively large differences in the chemical composition of the OM will initially result in large differences in HFA, which will decrease when decomposition of the organic matter (OM) proceeds. This is known as the 'chemical convergence hypothesis', as opposed to the 'initial litter quality hypothesis', which states that differences in initial litter chemistry persist throughout the decay sequence (Wickings *et al.* 2012). As in forest litter, the chemical composition of SCM initially varies greatly due to differences in animal species or breeds which produced it, feed ration, bedding materials and SCM handling and processing (composted or stacked) (Tunney 1975, Rotz 2004). Hence, we expected a pattern of decomposition for different-quality SCM when applied to grasslands and when home and away farms are compared, similar to the above-mentioned case of litter in natural systems.

It has been suggested that in addition to initial differences in OM quality, differences in species composition of the soil biota community play an essential role in determining the size of the HFA during decomposition (Ayres *et al.* 2009a). Soil decomposer communities are assumed to develop traits specialized in degrading certain types of organic material leading to some degree of adaptation (Hunt et al. 1988, Gholz et al. 2000, Vivanco and Austin 2008, Ayres et al. 2009b, Strickland et al. 2009a, Milcu and Manning 2011). In addition, the microbial part of decomposer communities might rapidly respond to changes in the local quality of OM by relative changes in abundances of adapted and less-well adapted species (Gießelmann *et al.* 2011). Such rapid responses in community structure are not expected for detritivorous soil fauna like Collembola and Acarina (Milcu and Manning 2011) and earthworms, many of

which selectively feed on litter of a chemical composition that fulfils their stoichiometric requirements (Bohlen *et al.* 1997). In accordance with such observations, Wickings et al. (2012) proposed the 'decomposer control hypothesis', which states that changes in litter chemistry during decomposition do not simply reflect the effects of time (i.e. the stage of decomposition) and/or initial litter quality, but are also strongly influenced by the functional attributes of distinct decomposer communities.

The use of litterbags of different mesh sizes to allow different body size classes of soil organisms access to decomposing organic matter has revealed that in a successional gradient of natural grasslands all body size classes of soil biota, i.e. micro-, meso- and macrobiota, contributed to a HFA for litter decomposition (Milcu and Manning 2011). However, their contributions varied depending on the successional stage of grassland which usually coincides with a certain quality of OM (Milcu and Manning 2011). Rashid et al. (2013) (Chapter 3) likewise suggested that due to differences in initial quality of SCM (C:N ratio, lignin:N ratio), it is to be expected that the contribution of soil biota to HFA varies when applied to its home compared to an away farm.

The aim of our study was to investigate the contributions of micro-, meso- and macrobiota to a HFA for SCM decomposition and N disappearance in production grassland. We hypothesized that (1) HFA for DM and N disappearance will be larger during the first stage of decomposition than in later stages, (2) all soil biota will contribute to a HFA for DM and N disappearances, but their contributions vary depending on the size class of soil biota and the stage of SCM decomposition, (3) HFA will be larger in high lignin:N SCM than in low lignin:N SCM, and (4) DM and N disappearances from SCM by micro-, meso- and macrobiota will be larger in grasslands with a

history of SCM application than in grasslands with a history of non-SCM inputs.

4.2 Materials and methods

We conducted a field experiment to monitor DM and N disappearances of SCM from litterbags with different mesh sizes that were incubated for 120 and 240 days in four production grasslands with a well-documented fertilisation history in The Netherlands.

4.2.1 Site description

Two dairy farms (A and B) that were selected, differed especially in the type of home-produced and applied solid cattle manure (SCM), henceforth called SCM farms. In addition, two neighbouring farms (C and D) were selected where only cattle slurry was produced. Farms A and C were located on peat soil in the western peat district of the province Utrecht, whereas farms B and D were located on sandy soil near Veenendaal in the province of Gelderland. The distance between farms A and C was 1 km and farms B and D were 15 km apart.

On farm A, SCM was composted during storage (AM), whereas it was stacked on farm B (BM) before it was applied to grassland. AM had a higher lignin:N ratio than BM (Table 4.1).

Table 4.1. Chemical characteristics of solid cattle manure (SCM) from farms A and B used in litterbags.

Farm	SCM storage method	DM	N_{total}	$N_{mineral}$	$N_{organic}$	C:N ratio	Lignin:N ratio
		(%)		g kg^{-1}DM			
A	Composted	19.0 ± 0.2	25.3 ± 0.3	1.1 ± 0.04	24.2 ± 0.3	20 ± 0.3	13.0 ± 0.5
B	Stacked	20.1 ± 0.1	21.8 ± 0.1	0.8 ± 0.02	21.0 ± 0.1	23 ± 0.1	8.0 ± 0.6

In addition to SCM, grasslands of farm A and B also received organic N-rich slurry. In contrast, artificial fertiliser and mineral N-rich slurry were applied on the grasslands of farm C, and only mineral N-rich slurry on the grasslands of farm D. No SCM had been used on the grasslands of farms C and D for decades, which are henceforth called non-SCM farms. Details of manure processing on farms A and B and grassland fertilisation management of all farms under study are given in chapter 3.

4.2.2 Experimental setup

On each farm, one field (≥ 5 years old grassland) of about 3 ha was selected. Soil fertility parameters from each grassland field at the start of the experiment are presented in chapter 3. Each field was divided into four blocks and in each block a grass cage of 4.5 m × 1.25 m was placed at random to prevent grazing by cattle (Lantinga et al. 2004). Inside each cage (replicate), 12 litterbags (100 mm x 100 mm x 15 mm, l x b x h) with the treatments (i) three mesh sizes, (ii) two solid cattle manure types and (iii) two sampling dates were placed randomly on the grassland soil surface in the last week of March 2010. The distance between the centre points of two adjacent litterbags was 50 cm. The mesh size treatment consisted of litterbags with micro mesh (diameter: 125 μm) which allows access to all soil microbiota, meso mesh (diameter: 1.5 mm) allowing access to both micro- and mesobiota, and macro mesh (diameter: 4 mm) which allows the entrance of all size-classes of detritivorous soil biota. One day before placement in the field, the litterbags were filled with 150±0.4 g fresh SCM, corresponding to 30±0.3 g DM for both manure types. Before placement of the litterbags, the herbage was mown to a stubble height of 4 cm by using a motorized mower, and a 10 x 10 cm soil surface for each litterbag was gently roughened with a spade to facilitate optimal soil-litterbag contact. In total, 192 litterbags (2 manure types × 4 replicates × 3 mesh sizes × 2 sampling dates × 4 farms) were

used. Litterbags were put in separate plastic bags to monitor mass loss during handling, transportation and placement on and retrieval from grasslands. The litterbags were collected for analysis after 120 and 240 days of their placement.

4.2.3 Manure analysis

After removal of litterbags from the grasslands, SCM that remained inside the bags was oven-dried at 105°C for 24 hours, ground to pass a 1 mm sieve, and analysed for DM, N and ash contents. Kjeldahl digestion (MAFF 1986) was used to measure N content and ash content was determined by loss-on-ignition at 550°C for 4 hours. DM and N disappearances (DMD and ND, respectively) were calculated from the difference between the initial and remaining manure dry weight and N content, after correction for soil contamination according to Potthoff & Loftfield (1998):

$$C_S = \frac{A_{AR} - A_I}{A_S} \qquad (1)$$

Where C_S denotes the dry weight (g) of soil which contaminated the SCM in litterbags, A_{AR} depicts the ash content (mg) of SCM in litterbags after removal, A_I indicates the initial ash content (mg) of SCM in litterbags and A_S is the ash content of the soil (mg g^{-1}).

Mesh size-specific effects representing the role of soil micro-, meso- and macrobiota to DMD and ND were calculated by IMi-FMi = ΔMi, ΔMe-ΔMi, (with ΔMe = IMe-FMe), and (IMa-FMa)-ΔMe, respectively, for each block. Where, I and F represent initial and final DM or N content of the SCM in the litterbags, respectively. Mi, Me and Ma represent the micro-, meso- and macro-mesh size litterbags, respectively.

4.2.4 Statistical analysis

Analysis of variance in GENSTAT (13th edition, VSN International, Hemel Hempstead, UK) was used to test treatment effects on DMD and ND of SCM from the litterbags. The block effect was nested within the individual farms. Therefore, we could not test for interaction effects between fertilisation history and soil type due to the lack of replicates. The main effects of treatments, i.e. fertilisation history (SCM, non-SCM), manure type (AM, BM), litterbag mesh size (125μm, 1.5, 4 mm) and soil type (sand, peat) and the interaction effects of manure type and mesh size with fertilisation history and soil type, were tested separately for the litterbags removed on days 120 and 240. Significance of HFA was tested by one-sample T-test. One-way ANOVA was used to test the differences in HFA among the three mesh sizes of litterbags as well as between the different stages of SCM decomposition. When main effects of mesh sizes were found to be significant, multiple comparisons among these treatments were performed using Tukey-LSD test at a probability level of 5%.

HFA for DMD and ND of AM and BM from litterbags of each mesh size on farms A and B were calculated in two steps by relationships adapted from Ayres *et al.* (2009b) and used by Rashid *et al*. (2013):

$$RAM_A = \left(\frac{AM_A}{AM_A + BM_A}\right) \times 100 \qquad (2A)$$

Where, RAM_A indicates DMD or ND of farm A manure relative to DMD or ND of farm B manure on farm A, AMA is the DMD or ND of farm A manure on farm A, and BM_A depicts the DMD or ND of farm B manure on farm A.

Similar equations were used to calculate RAM_B, RBM_B and RBM_A.

Subsequently, the HFA was calculated as:

$$HFA = \left(\frac{RAM_A + RBM_B}{RAM_B + RBM_A} \times 100\right) - 100 \qquad (2B)$$

A positive score for HFA denotes higher DMD or ND at home compared to the away farm. When the HFAs for different mesh sizes are >0 but do not statistically differ from each other, and each mesh size contributes to the overall DMD or ND, then soil biota size classes contribute equally to the HFA effect. However, when each mesh size contributes to the overall DMD or ND while the HFA effect of a larger soil biota size class is reduced or increased relative to the preceding soil biota size class, then the relative contribution of these larger soil biota to the HFA is also reduced or increased, respectively. In case that a soil biota size class does not contribute to the overall DMD or ND its HFA will consequently be absent, but then the HFA measured for the corresponding mesh size will represent the HFA for the preceding soil biota size class.

4.3 Results

The contribution of the whole soil biota community to DMD and ND was estimated from the macro mesh size litterbags.

Table 4.2. ANOVA results for the effect of fertilisation history, soil type, manure type (composted and stacked solid cattle manure) on dry matter disappearance (DMD) and nitrogen disappearance (ND) from macro mesh litterbags after 120 and 240 days of placement on grasslands of all farms (A-D).

Treatments	120 days					240 days			
	df	F-value		P-value		F-value		P-value	
		DMD	ND	DMD	ND	DMD	ND	DMD	ND
Fertilisation history (FH)	1	14.2	8.7	**<.001**	**0.007**	34.3	62.5	**<.001**	<.001
Soil Type (ST)	1	10.1	0.3	**0.004**	0.574	3.7	6.9	0.077	0.021
Manure type (MT)	1	8.9	0.0	**0.006**	0.870	48.1	0.9	**<.001**	0.369
FH×MT	1	0.1	0.2	0.709	0.677	10.0	21.1	**0.007**	**<.001**
ST×MT	1	0.1	1.8	0.718	0.197	26.5	12.3	**<.001**	**0.004**
Residual	13	1461	3182			358	365		

P values < 0.05 in bold

After 120 days of field incubation, 21-49% DM (Fig. 4.1) and 31-53% N (Fig. 4.2) had disappeared from the macro mesh size litterbags, and after 240 days this fraction was 39-80% for DM (Fig. 4.1) and 56-98% for N (Fig. 4.2). Fertilisation history and manure type had strong effects (P< 0.01) on manure DMD (Fig. 4.1, Table 4.2).

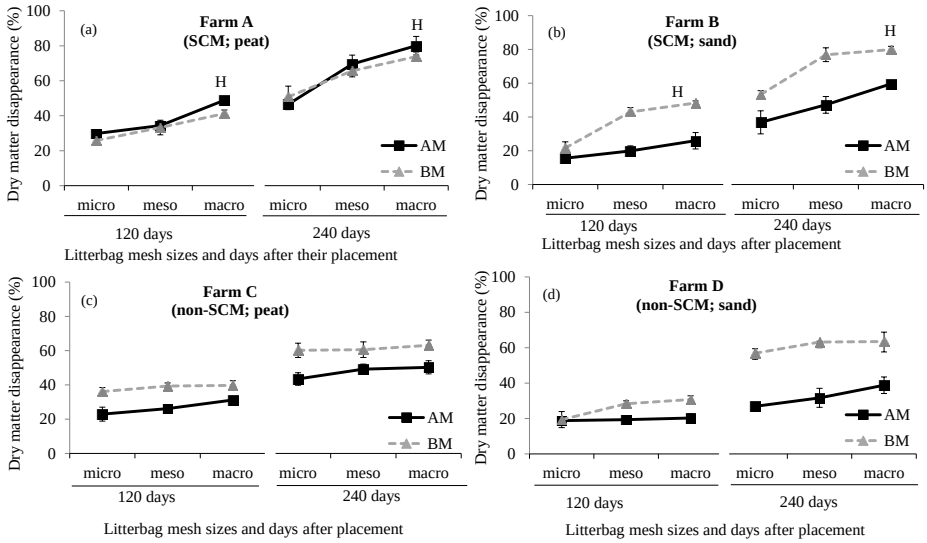

Fig. 4.1 Percentage manure dry matter disappearance from litterbags, as affected by fertilisation history (solid cattle manure and organic N-rich cattle slurry inputs on farms A and B versus mineral N-rich cattle slurry with ad without chemical fertiliser on farms C and D), mesh size, manure type (composted manure from farm A (AM) and stacked manure from farm B (BM)) and their interactions at days 120 and 240. Litterbag treatments are micro (125 μm), meso (1.5 mm), and macro (4 mm) mesh sizes which allow the access to soil microbiota, micro- + mesobiota and micro- + meso- + macrobiota, respectively. Letter H refers to the manure disappearing at its home farm. Bars denote standard error (±1) of means.

After 120 days, DMD from macro mesh size litterbags was on average much higher on SCM farms than on non-SCM farms (41 vs. 30%) (Figs. 4.1a & b vs. Figs. 4.1c & d). The effect of manure type was demonstrated by a much higher

DMD for BM than for AM (45 vs. 35%; Figs. 4.1a & b vs. Figs. 1c & d). Also ND was higher (45 vs. 35%, P< 0.01; Table 2) on the SCM farms than on the non-SCM farms (Figs. 4.2a & b vs. Figs. 4.2c & d).

After 240 days of field incubation, the difference in DMD on SCM farms versus non-SCM farms was almost similar to that obtained after 120 days (37 and 35%, respectively). At this stage, the fertilisation history effect was largest (70 vs. 44%= 57%) for AM (Figs. 4.2a & b vs. Figs. 4.2c & d). BM had a much higher DMD than AM (70 vs. 57%, P< 0.01) and this difference was more pronounced on the non-SCM farms and sandy soils (63 vs. 45%; 42% and 72 vs. 50%= 44%, respectively; Figs. 4.1c & d vs. Figs. 4.1a & b and Figs. 4.1b & d vs. Figs. 4.1a & c, respectively).

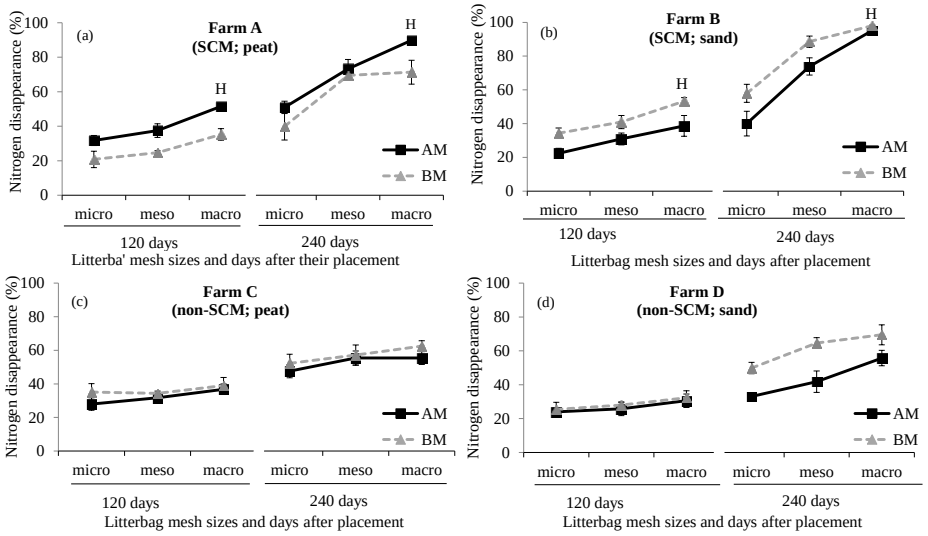

Fig. 4.2 Percentage manure nitrogen disappearance from litterbags. Legend as in Fig. 1

The difference in ND from macro mesh size litterbags between SCM farms and non-SCM farms was larger (Table 4.2) after 240 than after 120 days of field incubation (44% and 29%, respectively; Figs. 4.2a & b vs. Figs. 4.2c & d). After 240 days of incubation, this fertilisation history effect was much larger (93 vs.

56%= 65%) for AM than for BM (Figs. 4.2a & b vs. Figs. 4.2c & d). On sandy soils, the ND was on average much higher (80 vs. 70%; P= 0.02; Table 4.2) than on peat soils and the effect was largest for BM (84 vs. 67%= 25%; Fig. 4.2b & d vs. Figs. 4.2a & c). After 120 days of incubation, the contribution of microbiota to DM disappearance was larger than the added contributions of meso- or macrobiota (Table 4.3). This was much higher on peat than on sandy soil (29 vs. 19%; P= 0.026; Table 4.3).

Table 4.3. Mesh size-specific contributions of soil biota to solid cattle manure (SCM) dry matter disappearance (DMD) and nitrogen disappearance (ND) from micro, meso and macro mesh size litterbags after 120 and 240 days in grasslands with different fertilisation history (FH) and soil type (ST; peat (P), sand (S)), and corresponding p-values after ANOVA.

Treatment				120 days						240 days					
				Micro mesh		§Δ meso-micro mesh		‡‡Δ macro-meso mesh		Micro mesh		§Δ meso-micro mesh		‡‡Δ macro-meso mesh	
Farm	ST	FH	Manure type (MT)	DMD	ND	DMD	ND	DMD	ND	DMD	ND	DMD	ND	DMD	ND
				%											
A	P	SCM†	AM‼	**29.8**	**31.8**	**4.6**	**5.7**	**14.4**	**14.0**	**46.5**	**51.0**	**23.0**	**22.3**	**10.4**	**16.5**
			BM††	25.9	20.8	7.4	3.9	8.0	10.5	50.9	39.9	14.8	29.5	8.1	1.9
B	S		AM	15.6	22.4	3.7	9.2	6.6	7.1	36.9	40.0	10.3	33.9	12.5	21.2
			BM	**21.7**	**34.3**	**21.3**	**6.6**	**5.3**	**12.4**	**53.3**	**57.9**	**23.6**	**30.6**	**3.0**	**9.5**
C	P	non-SCM‡	AM	22.9	28.0	3.2	3.7	5.0	5.1	43.5	47.6	5.7	7.8	1.1	0.0
			BM	36.1	35.1	3.2	1.3	0.4	2.6	60.1	52.2	0.4	4.9	2.6	5.3
D	S		AM	18.8	24.0	0.6	1.8	0.8	4.8	26.9	33.1	4.8	8.7	7.1	13.9
			BM	19.4	25.4	9.0	2.6	2.3	4.4	56.8	49.9	6.3	14.8	0.2	4.8
Treatment		df		ANOVA (p-values)											
FH		1		0.181	0.979	0.250	0.434	0.188	0.141	0.991	0.938	**0.014**	**0.011**	0.182	**0.029**
ST		1		**0.026**	0.558	0.326	0.559	0.343	0.635	0.278	0.852	0.925	**0.047**	0.939	**0.028**
MT		1		0.254	0.410	**0.020**	0.117	0.310	0.900	**<.001**	0.069	0.946	0.887	0.092	0.061
MTxFH		1		0.275	0.710	0.428	0.219	0.689	0.495	0.051	0.495	0.657	0.928	0.512	0.150
MTxST		1		0.618	0.412	0.061	0.703	0.124	0.149	0.055	**0.034**	0.166	0.945	0.126	0.447

Values are means with n = 4; results from home-applied manure in bold; p values < 0.05 also in bold

† Solid cattle manure and organic N-rich slurry

‡ Slurry manure and artificial fertiliser

‼ Solid cattle manure collected from farm A

†† Solid cattle manure collected from farm B

§ Difference between the total DM or N disappearance of meso- and micro-mesh litterbag

‡‡ Difference between the total DM or N disappearance of macro- and meso-mesh litterbag

The added contribution of mesobiota to DMD from BM was much higher than AM (10 vs. 3%; P= 0.02; Table 4.3). After 240 days, 27-60% of the DM was disappeared from the micro mesh litterbags; the added effects of meso- and macrobiota to DMD were 0-24 and 0-13%, respectively (Table 4.3). BM had a much higher added DMD than AM from micro mesh size litterbags (55 vs. 39%; P< 0.001; Table 4.3). The added contribution of mesobiota to DMD was much higher in SCM than in non-SCM farms (18 vs. 4%; P= 0.014; Table 4.3). The ND in the micro-mesh litterbags was 33-58%, which again was larger than the added ND from the meso- and macro mesh litterbags (5-34% and 0-21%, respectively) (Table 4.3). BM had a higher ND than AM from micro mesh size litterbags, but only in the grasslands on sand, not on peat (47%, P= 0.034, Table 4.3). On the SCM farms, the added contribution of meso- and macrobiota to ND was much higher than on the non-SCM farms after 240 days of incubation (29 vs. 9% and 12 vs. 6%, respectively; Table 4.3). Also, on sandy soils the added contribution of meso- and macrobiota to ND was much higher than on peat soil (22 vs. 16% and 12 vs. 6%, respectively; Table 4.3). The interaction between mesh size, manure type and SCM farm (A vs. B) was significant (P < 0.05) in case of DMD after 120 days; this effect tended to disappear (P = 0.107) after 240 days (Table 4.4).

Table 4.4. The effects of mesh size, manure type (composted and stacked solid cattle manure (SCM)) and SCM-farm (A or B) on dry matter disappearance (DMD) and nitrogen disappearance (ND) after 120 and 240 days of litterbag placement on grasslands. Effects were tested by analysis of variance (ANOVA).

Treatments	120 days					240 days			
	df	F-value		P-value		F-value		P-value	
		DMD	ND	DMD	ND	DMD	ND	DMD	ND
Farm (F)	1	16.6	3.4	**<0.001**	0.074	7.1	14.1	**0.012**	**<0.001**
Manure type (MT)	1	16.6	0.1	**<0.001**	0.726	24.4	0.1	**<0.001**	0.738
Mesh size (MS)	2	42.2	32.9	**<0.001**	**<.001**	58.4	82.2	**<0.001**	**<0.001**
F×MT	1	45.4	55.5	**<0.001**	**<.001**	34.7	15.6	**<0.001**	**<0.001**
F×MS	2	1.7	1.3	0.189	0.292	0.3	1.3	0.783	0.292
MT×MS	2	3.3	0.9	**0.048**	0.397	0.7	2.5	0.505	0.100
F×MT×MS	2	3.5	1.0	**0.042**	0.367	2.4	0.14	0.107	0.869
Residual	33	997	1161			1649	3025		

P values < 0.05 in bold

In contrast, for ND this interaction was not significant (P> 0.1) after 120 or 240 days (Table 4.3). Manure DMD and ND from each mesh size litterbag was higher when it was incubated at home compared to away after 120 and between 120-240 days (HFA: Figs. 4.1, 4.2 and 4.3), as was indicated by a significant interaction for both DMD and ND between farm and manure type (Table 4.4, P< 0.01).

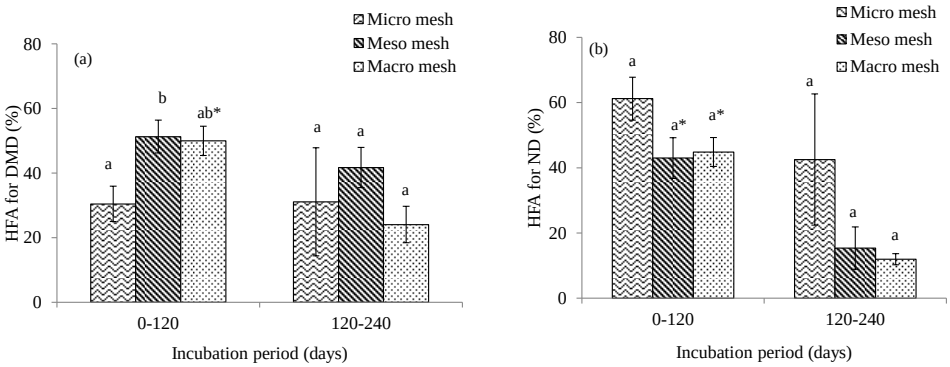

Fig. 4.3 Home Field Advantage (HFA) based on absolute percentage dry matter disappearance (DMD; a) and nitrogen disappearance (ND; b) from micro (125 μm), meso (1.5 mm), and macro (4 mm) mesh litterbags in the early (0-120 days) and late (120-240 days) phases of decomposition. Different small letters indicate significant differences among mesh size treatments within each stage of solid cattle manure decomposition (P< 0.05). Asterisks indicate significant differences in HFA between earlier (0-120 days) and later stage (120-240 days) of manure decomposition (P≤ 0.01).

After 120 days of incubation, this HFA was 50% (P< 0.001) for manure DMD and 45% (P < 0.001) for manure ND from the macro mesh litterbags. However, during the second phase of decomposition (120-240 days of litterbag incubation), the HFA decreased to 24% for DMD and 12% for ND (P = 0.013 and P< 0.001, respectively; Fig. 4.3). The HFA for manure DMD from micromesh litterbags was significantly lower than from the meso mesh litterbags during

the first stage (120 days) of decomposition (30 vs. 51%; P= 0.024; Fig. 4.3a). However, there was no difference in HFA among different mesh sizes (P> 0.05) during the following 120 days (Fig. 4.3a). In case of ND, all body size classes of soil biota positively contributed to the HFA. No differences between litterbag mesh size classes were observed (Fig. 4.3b). DMD and ND of both manure types from litterbags placed in the grasslands of the two non-SCM farms (C and D) were lower (P< 0.05) compared to the DM and N losses when placed at their home farms (Table 4.3 and Figs. 4.1a & b vs. Figs. 4.1c & d and Figs. 4.2a & b vs. 4.2c & d).

4.4 Discussion

In agreement with our expectation (hypothesis 1), HFAs for manure DM and N disappearance were higher during the first phase of SCM decomposition (0-120 days) than in the subsequent stage (120-240 days) (Fig. 4.3). Wang, Zhong & He (2012) also observed larger HFAs in DM and N disappearances in earlier than in later stages of litter decomposition. Ayres *et al.* (2009a) found no or even sometimes negative HFAs in later stages of decomposition. Wallenstein *et al.* (2013) found experimental evidence that changes in the chemical composition of home litter during decomposition were more evident than in away litter and, as a result, the initial differences in the chemical composition between both litters diminished over time. In our study, we found that the C:N ratio of the manure that remained in the macro mesh litterbags after 120 days was significantly different between the two manure types at each of the two farms (P< 0.05 at farm A; P< 0.01 at farm B): the C:N ratio of the home manure became (farm A) or remained higher (farm B) than the C:N ratio of the away manure (Fig 4.4). However, this difference was disappeared after 240 days. So, diminishing site-dependent differences in the chemical composition of SCM as decomposition proceeded explain the higher HFA in the first and the lower HFA in the second

phase of decomposition. This appears to be in accordance with the convergence hypothesis of Wickings et al. (2012). Our study may not have lasted long enough to determine whether the differences in HFA would eventually disappear.

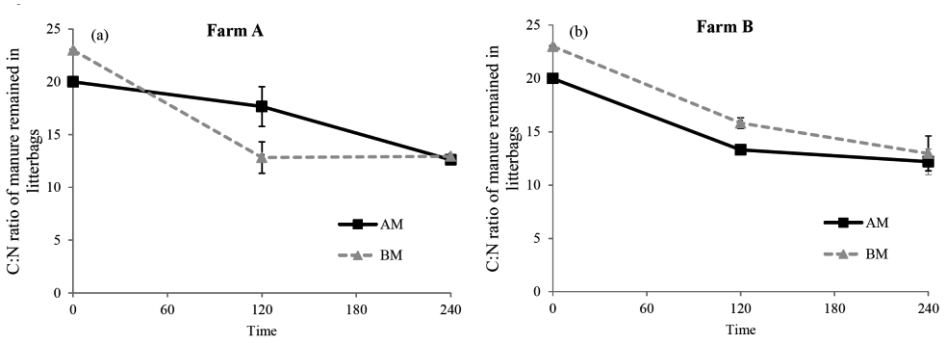

Fig. 4.4 C:N ratio of the manure that remained in the macro-mesh litterbags after 120 and 240 days of placement in the field on SCM farms A (a) and B (b).

In line with our second hypothesis, all body size classes of soil biota, i.e. micro-, meso- and macrobiota, contributed to the HFAs in DM and N disappearance. Their contributions depended on the soil biota size-class and the stage of SCM decomposition. The contribution of soil biota to the HFA for DM and N disappearance was similar in both phases of decomposition (0-120 and 120-240 days) except for microbiota which contributed less to a DM disappearance HFA than mesobiota in the early phase of decomposition (0-120 days; Fig. 4.3). Our findings support observations by Milcu & Manning (2011), who found that all body size classes of soil biota in natural grassland contributed to a litter-decomposition HFA in the late-successional stage, whereas in the mid-successional stage only one group (mesobiota) dominated the HFA. In our study, the HFA for DM disappearance due to mesobiota (Fig. 4.3a) was mainly caused by the low DM disappearance of AM in the meso mesh litterbags at away farm B (Fig. 4.1b). Compared to home-incubated BM, the DM

disappearance from meso mesh litterbags of away AM at farm B was on average 34% lower (59 vs. 39%; Fig. 4.1b) during both decomposition periods. In contrast, the DM disappearance rate of BM from meso mesh litterbags at farm A was only 6% lower than that of AM (49 vs. 52%; Fig. 4.1a). Thus, the mesofauna of farm A could decompose both AM and BM well, but the mesofauna of farm B could decompose BM much better than AM (Fig. 4.1a vs. 4.1b). This clearly indicates that specific adaptations of soil biota are one of the drivers of the HFA.

We hypothesised that the size of the HFA would be affected by the quality of resources. The lignin:N ratio of the SCM produced at farm A was higher than that of farm B (Table 1). Therefore, it was assumed that the decomposition of AM required a more specialized soil biota community than BM. Indeed, our results showed that the decomposition of the low-quality AM was lower at farm B than at its home farm A (Table 4.3, Figs. 4.1a & 4.2a vs. 4.1b & 4.2b). The decomposition of the high-quality BM, however, did not differ much when this manure was incubated at home (farm B) or away (farm A). Moreover, also at the non-SCM farms C and D the decomposition of the low-quality AM was lower than the decomposition of the high-quality BM (Table 4.2, Fig. 4.1 & 4.2). This is in line with the substrate-quality matrix index hypothesis that was proposed by Freschet et al. (2012), which implies that low-quality litter will decompose faster in a poor-quality matrix environment and slower in a matrix of labile compounds (high quality). In accordance with this hypothesis, we suggest that the ability to decompose lignin in AM is a specialized trait of the soil biota, whereas the ability to decompose the relatively labile manure BM is a general trait. Lignin content is one of the principal components of litter that can structure soil decomposer communities and their capability to decompose litters with different chemical compositions (Freschet et al. 2012). Only a few

groups of fungi and bacteria are capable of degrading lignin (Hammel 1997, Paul 2007), and fungi are often more abundant in soils that had a lignocellulose or lignin input history (Ingham et al. 1989). This corroborates our findings (Chapter 2) of 2x higher fungal biomass ($P< 0.05$) and microarthropod densities (springtails and mites, $P = 0.008$) in grasslands of farm A compared to farm B. According to Schouten et al. (2004), about half of these microarthropods were fungivores that can digest cell walls next to cell contents. Therefore, it is likely that soil organisms at farm A are specialized to degrade lignin found in their home manure, whereas the lower decomposition of AM at the away farm B and the two non-SCM farms could be due to the presence of a more generalist soil biota community. Interestingly, N disappearance from both manures was higher when they were applied at home compared to away, irrespective of the initial differences in lignin:N ratio (Table 4.1 and Fig. 4.2). This was mainly caused by microbiota at their home farms (Figs. 4.2a & b and Fig. 4.3). It was visually observed that the long straw pieces from BM disappeared much slower at farm A than at farm B. However, the C:N ratio of this BM manure that remained in the macro mesh litterbags after 120 days of incubation, was lower compared to that of AM at farm A (Fig. 4.4a). This suggests that primary decomposers like bacteria and fungi, required more mineral N to degrade the C-rich straw pieces at the away farm. Consequently, they immobilized more N from this fresh straw-rich manure source (Jensen 1931, Cheshire et al. 1999). Therefore, N disappearance of BM at the away farm A was low (Fig. 4.2a). This led us to conclude that in case of SCM not only the lignin:N ratio but also physical characteristics like the nature of straw particles can be considered as important quality parameters for manure decomposition and N mineralisation.

On SCM farms DM and N disappearance from SCM by meso- and macrofauna was almost 3 times higher compared to non-SCM farms after 240 days,

although this was not statistically significant in 1 of the 4 cases (the added contribution of macrobiota to N disappearance) (Table 4.3). In the non-SCM farms, which had not received SCM for about 30 years, this coincided with significantly lower densities of enchytraeids, microarthropods and earthworms (Chapter 2). This result supports our hypothesis that differences in the abundance of soil biota and/or species composition of soil communities, could be the cause of lower DM and N disappearance rates in non-SCM grasslands (Chapter 3). Contrary to our expectation, the contribution of microbes to DM and N disappearance of SCM was not significantly different between SCM and non-SCM grasslands (Table 4.2). Also, the microbial biomass in the non-SCM grasslands was not significantly different from that in the SCM grasslands (Chapter 2). The absence of a difference in microbial effect on DM and N disappearance between SCM and non-SCM farms is remarkable, because we found that these organisms contributed to the manure decomposition HFA on the SCM farms (Fig. 4.3). This was despite the fact that the contribution of microbiota to the DM disappearance HFA during later stages (120-240 days) of decomposition was not significantly different from zero (Fig. 4.3).

Ayres et al. (2009a), Strickland et al. (2009a) and Strickland et al. (2009b) have shown the existence of a HFA for litter decomposition in natural systems. According to Milcu & Manning (2011) all body size classes of soil biota contribute to such HFA in natural grasslands. We showed for the first time that strong HFAs also exist for SCM disappearance from each of three litterbag mesh sizes. This implies that the fertilisation history of production grasslands likewise affects the functioning of soil biota throughout the whole soil food web. Apparently, long term SCM inputs can alter the soil environmental conditions and thus promote functionally distinct soil biota communities related to the quality of applied OM. Such soil biota will differ in their ability to

assimilate/decompose OM with contrasting chemical compositions, resulting in corresponding differences in HFA. These results support the decomposer control hypothesis of Wickings et al. (2012), but their implication that distinct decomposer communities create functionally distinct decomposer 'funnels' (and hence diverse litter chemistries) that persist throughout decomposition was not confirmed in that the C:N ratio of the different SCMs had converged to the same value after 240 days (Fig. 4.4).

4.5 Conclusions

Our study provides evidence from production grasslands that the soil biota community, represented by various body size classes, explains the sizes of home field advantages for dry matter and N disappearances. The magnitude of the HFA was higher for low-quality than for high-quality solid cattle manure and the HFA decreased as decomposition proceeded. This could be related to changes in the chemical composition (C:N ratio) of the SCM during decomposition. The contributions of meso- and macrobiota to manure dry matter and N disappearance on SCM farms was higher than on non-SCM farms.

Our findings imply that site-specific fertilisation management of agro-ecosystems influences the functioning of soil biota communities that affect C and N release processes. This offers possibilities to develop more sustainable and cost-effective nutrient management strategies in agro-ecosystems.

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Chapter 5

Soil pH and earthworms affect herbage nitrogen recovery from solid cattle manure in production grasslands

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Abstract

We assessed the effects of earthworm density (400 or 700 m^{-2}) at two levels of soil pH (ambient and increased), with or without application of solid cattle manure (SCM), on herbage nitrogen (N) uptake, and CO_2 and gaseous N emissions over a period of 134 days using undisturbed soil cores from an acid peat grassland in a mesocosm experiment. Liming proved to be beneficial for earthworm performance and grassland productivity. A higher soil pH and earthworm density resulted in a higher soil biological activity measured as soil respiration. The combined application of lime and earthworms increased herbage apparent N recovery from SCM with 83% compared to only SCM. In the manured treatments, herbage N uptake was positively correlated with earthworm density (R^2= 0.92). N_2O emissions increased by 37% when SCM was applied compared to the unfertilised control. Following SCM application, the cumulative increase in herbage N uptake was almost ten times greater than the measured total gaseous N losses (N_2O). No relationship was observed between earthworm density and level of N_2O emission. We conclude that N mineralisation and herbage N uptake from SCM in acidic peat grasslands were greatly stimulated by the combined increase of soil pH and earthworm density. This stimulated the activity of soil biota, resulting in an increased herbage N recovery from the applied SCM.

5.1 Introduction

Agricultural intensification of grassland use over the last 50 years included increasing application rates of chemical fertilisers. This has led to soil acidification, because of the release of protons (H^+) from ammonium-based fertilisers during nitrification (Bolan et al. 1991). Application of high rates of chemical fertiliser to grasslands affects the density of the detritivorous soil

fauna, such as earthworms (Standen 1984, Ma et al. 1990, De Goede et al. 2003). Most species of earthworms, which play an important role in maintaining soil fertility and soil structure, thrive best at pH (H_2O) 6-7 (Laverack 1961, Springett and Syers 1984, Edwards 2004). However, some species can be considered as acid-tolerant, e.g. *Aporrectodea caliginosa* (Savigny) and *Lumbricus rubellus* (Hoffmeister), which are amongst the most abundant species in NW-European grasslands. The lower limit of acid tolerance for these two species is approximately pH (H_2O) 3.7 and 4.5, respectively (Laverack 1961, Springett and Syers 1984, Edwards 2004).

In agricultural grasslands, liming is generally used to overcome the problem of acidification (Cole et al. 2006). As a result, the abundance and diversity of detrivorous soil fauna like some species of earthworms are increased (Bishop 2003). This will contribute to an improved organic matter decomposition and nutrient mineralisation (Bradford et al. 2002). However, lime application is costly and also helps to increase greenhouse gas emissions, especially CO_2 (West and McBride 2005, IPPC 2006). Furthermore, it is an end-of-pipe solution. An alternative measure is the use of organic fertilisers, e.g., solid cattle manure (SCM). Acidification is naturally counterbalanced by the release of hydroxyl ions (OH^-) during the decomposition of applied organic residues (Bolan et al. 1991). Earthworm density, microbial activity, soil pH and organic matter were found to be higher on farms with long-term organic fertilisation compared to those using only chemical fertilisers (Bohlen and Edwards 1995, Mäder et al. 2002, Ros et al. 2003).

In a recent study, a wide variation (0.5-27%) in the herbage apparent nitrogen recovery (ANR) from applied solid cattle manure on two peat grasslands in the Netherlands with contrasting N fertilisation history was observed (Chapter 3). The peat grassland with long-term inputs of solid cattle manure and organic-N

rich cattle slurry had higher soil pH and earthworm density than the grassland where artificial N and mineral-N rich cattle slurry were used as fertilisers during the last decades. Hence, it was suggested that differences in soil chemical and biological characteristics due to the prevailing fertilisation history were the major causes of this variation (Chapter 3).

Further to these results, the purpose of the present research was to study the effect of soil pH and earthworm density on herbage N uptake and recovery of N from SCM in an acidic peat grassland. We hypothesised that application of SCM will increase earthworm abundance and microbial activity in limed compared to non-limed treatments (i), which will result in higher greenhouse gas emissions (ii). A higher earthworm abundance will decrease microbial biomass through foraging (iii). The combination of higher earthworm density and liming will increase herbage N recovery from applied SCM (iv).

5.2 Materials and Methods

5.2.1 Study site

The experiment was carried out with soil cores obtained from one of the two peat dairy farms used in Chapter 3. On this farm, cattle slurry and chemical fertilisers have been applied to grasslands for at least 30 years. The farm is located in the central peat district of province Utrecht, The Netherlands (52.140°N; 4.838°E) and the same grassland was used as in Chapter 3. The soil is a "Terric Histosol" according to FAO classification and "Koop peat" according to Dutch soil classification (Langeveld et al. 1997).

5.2.2 Experimental setup

An experiment was carried out in 24 mesocosms using open top and bottom PVC pipes with height 40 cm and diameter 30 cm, filled with undisturbed grassland cores to measure herbage N uptake and apparent N recovery from

surface applied SCM as affected by soil pH and earthworm density (henceforth called mesocosm experiment). In addition, a litterbag experiment was established in 6 mesocosms to measure dry matter (DM) and N disappearances from SCM (henceforth called litterbag experiment).

5.2.2.1 Mesocosm construction and placement

On 23 June 2011, soil cores were randomly collected from an area of about 200 m^2 in the field without disturbing the soil structure by inserting the PVC pipes into the soil to 30 cm depth, followed by digging them out. In this way, we obtained mesocosms containing soil cores that had a native grassland sward and a natural earthworm population. In order to quantify earthworm density and biomass, six soil blocks each with a volume of 20 × 20 × 20 cm were randomly sampled within the same 200 m^2 area. We restricted our earthworm sampling to the 0-20 cm soil depth, because on all previous sampling occasions in this grassland in 2010 no earthworms were found in deeper soil layers using formaldehyde extraction (Chapter 2). Therefore, we assumed that the 0-20 cm soil depth measurements adequately reflect the total earthworm density in the collected soil columns. The earthworms were hand-sorted in the field and taken to the laboratory where they were rinsed with tap water. Subsequently, they were kept at 15 °C in an incubator during two days on moistened paper to empty their guts before they were counted, weighed and classified into ecological groups. Additionally, 30 core samples (0-10 cm) were collected using a grass plot sampler (Eijkelkamp, The Netherlands). All core samples were mixed thoroughly to get a field-moist composite sample. This was used to analyse initial soil pH with 1 M KCl (1:10, w:v ratio).

The mesocosms were brought to Wageningen University on the day of excavation. Of these mesocosms, 6 were placed in two plastic containers (1.2 m length × 0.8 m width × 0.3 m height) each holding three mesocosms (litterbag

experiment) and 24 in six plastic containers each holding four mesocosms (mesocosm experiment). The open spaces between the mesocosms were filled with white sand in order to reduce temperature fluctuations. In the centre of each container, a perforated pipe (10 cm diameter) was placed to visually observe the level of the water table. This was done to guide watering to keep the soil water status at approximately 60% water holding capacity. For this purpose, we applied water regularly using a hand sprinkler while monitoring moisture content by a low-cost moisture meter (FY-901, Hangzhou FCJ I&E Co., Ltd, China). In order to avoid water stagnation in the top 10 cm of the mesocosms, the level of white sand in the containers between the mesocosms was kept 10 cm below the soil level inside each mesocosm. A 3 cm wide Velcro® ribbon was glued inside the top rim of each mesocosm to prevent earthworms from escaping (Lubbers and van Groenigen 2013). The open headspace volume was measured from each mesocosm at the beginning and termination of the experiment. This was used in the calculation of gaseous emissions. A schematic overview of the experimental setup of mesocosm experiment is given in Fig. 5.1.

5.2.3 Treatments

The main treatments included in the experiment were (1) ambient and increased earthworm density, (2) ambient and increased soil pH, and (3) unfertilised and SCM application. Earthworms to be used in the increased density treatments were collected in the same grassland from where the soil cores were taken using the vibration technique and hand sorting (Mitra et al. 2009). They were kept on filter paper in the laboratory at 15°C for two days and were treated as previously explained before introducing them into the mesocosms.

Since SCM was not available from the same farm where the soil cores were extracted, it was collected from a neighbouring farm located about 1 km away.

Its DM content was 190±2 g kg^{-1} of fresh matter, and organic and mineral N contents were 24.2±0.3 and 1.1±0.4 g kg^{-1} DM, respectively. C:N and lignin:N ratios were 20±0.3 and 13±0.5, respectively. The SCM was kept at 4°C before its application to the mesocosms.

At the start of the experiment, the herbage was cut at a height of 3 cm using a spinach knife (OTTER-Messer, Solingen, Germany). To increase the soil pH (KCl) from 4.6 to about 6, lime ($CaCO_3$) was evenly applied to the soil surface of half of the mesocosms at a rate of 1.4 kg m^{-2} two days before SCM application. Subsequently, all the mesocosms were profusely watered so that lime could infiltrate into the soil. One day before SCM application, earthworms were introduced into half of the mesocosms (Table 5.1) with the same epigeic to endogeic ratio (0.39) as observed in the field. Finally, SCM was evenly spread on the sward surface of half of the mesocosms at a rate of 200 Kg N ha^{-1} (Table 5.1), and the first set of gaseous emission measurements was carried out.

5.2.3.1 Litterbag experiment

In this experiment, two treatments were used in three replicates: (1) SCM application without liming at ambient earthworm density (M), and (2) SCM application with liming (1.4 kg m^{-2}) and artificially raised earthworm density (+300 m^{-2}; MLE). The setup comprised a split plot design in which the earthworm density and SCM treatment were nested in the lime application treatment. These treatments, which represented the two extreme cases, were mainly used to make sure that conclusions on N uptake and ANR can be ascribed to N disappearance from the applied manure. Litterbags (10 cm × 10 cm × 1.5 cm) of 4 mm mesh size allowing the entrance of all soil biota were filled with 300±3 g fresh SCM corresponding to 58±1 g dry weight. The litterbags were placed firmly on the vegetation and fixed to their position on soil using metallic pins. After 134 days, the SCM that remained in the litterbags

was oven-dried at 105 °C during 48 hours. Once weighed, the samples were ground to pass a 1 mm sieve and analysed for N content by Kjeldahl digestion (MAFF 1986).

5.2.3.2 Mesocosm experiment

The experimental setup was a split-plot nested design, in which earthworms, SCM and unfertilised control treatments were being nested separately within the lime and non-limed treatments to avoid pH related interferences between them.

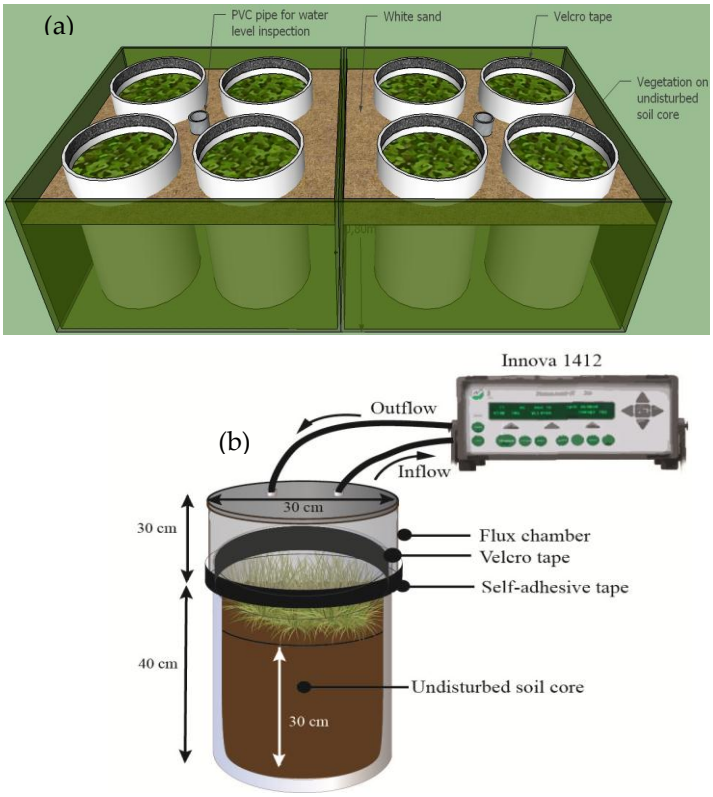

Fig. 5.1 Schematic view of one block comprising two containers, each holding four mesocosms with undisturbed soil columns (a), and of a mesocosm with the static flux chamber on top, connected to a mobile gas monitor (b)

Therefore, one block consisted of two separate containers each with a different level of soil pH and holding four mesocosms (Fig. 5.1a).

In each container, the treatments (earthworm abundance and SCM application) were randomised. In total, there were three blocks and each block consisted of eight treatments. The treatments and their abbreviations are listed in Table 5.1.

Table 5.1 Experimental methods.

Treatment abbreviation*	Earthworm addition (ind. m^{-2})	Lime application (kg m^{-2})	Manure application (kg N ha^{-1})
C	0	0	0
L	0	1.4	0
E	300	0	0
LE	300	1.4	0
M	0	0	200
ML	0	1.4	200
ME	300	0	200
MLE	300	1.4	200

*C= control with ambient earthworm density; L= lime; E= earthworms; M= solid cattle manure

5.2.4 Measurements

5.2.4.1 Gaseous emissions

Measurements of CO_2, NH_3 and N_2O emissions were carried out daily during the first three days, once a week during weeks 2-10, and once every two weeks from weeks 11 to 19. A cylindrical static PVC flux chamber was fixed onto the mesocosm using self-adhesive tape to make it airtight. Gaseous measurements were recorded by connecting the flux chamber to a mobile infrared photo-acoustic multi-gas monitor using PVC tubes (Innova, model 1412; Air Tech instruments, Ballerup, Denmark) (Fig. 5.1b). The lower detection limits of this

instrument are: 5.1 ppm CO_2, 0.03 ppm N_2O and 0.2 ppm NH_3. Emissions were assumed to be zero if their concentrations were below the detection limit. The instantaneous emission rates were calculated by the fitted initial linear slope between gaseous concentration (mg m^{-3}) and time (h):

$$R = 60 \times B \times \frac{V_T}{A} \qquad (1)$$

Where R is emission rate (mg m^{-2} h^{-1}), 60 is the conversion factor for scaling up a minute to an hour, B is the fitted linear slope of the data between CO_2, N_2O or NH_3 concentrations and time (mg m^{-3} min^{-1}), V_T is total air volume of the mesocosm headspace, flux chamber, PVC tubes and gas monitor together (on average 0.027 m^3); A is the surface area covered by the chamber (m^2).

Cumulative emissions were calculated by assuming linear changes in time between consecutive measurements.

5.2.4.2. Herbage N uptake

Herbage was harvested three times during the experiment (day numbers 36, 85 and 134). Shoots were cut at a height of 3 cm using a spinach knife (OTTER-Messer, Solingen, Germany) and oven-dried at 70 °C during 48 hours. Once weighed to obtain DM yield, the samples were ground to pass a 1 mm sieve and analysed for N by Kjeldahl digestion (MAFF 1986). Herbage N uptake (kg N ha^{-1}) was calculated as:

$$Nuptake = DMY \times N_{cont} \qquad (2)$$

Where DMY and N_{cont} are herbage DM yield (kg DM ha^{-1}) and its N content (g N (100 g DM)$^{-1}$), respectively. Subsequently, herbage apparent N recovery (%) from the applied SCM was calculated as:

$$ANR = \frac{(Nuptake_M - Nuptake_0)}{(Napplied)} \times 100 \qquad (3)$$

Where, $Nuptake_M$ represents the measured N yield in the manured treatments (kg ha^{-1}). $Nuptake_0$ denotes N yield of the unfertilised control (kg ha^{-1}) and $N_{applied}$ is the total amount of applied N (kg ha^{-1}).

5.2.4.3 Final harvest and soil fauna sampling

After 134 days, once the last gaseous measurements and herbage harvests were done, the mesocosms were destructively sampled for soil biological and chemical analysis. From each mesocosm, the soil was removed and earthworms were hand-sorted. Collected earthworms were rinsed in tap water and kept in moistened paper at 15°C during two days to void their guts. Afterwards they were weighed and fixed in ethanol (70%) prior to counting and species identification.

Additionally, one soil sample from the top 15 cm of each mesocosm was taken, sieved through a 4 mm mesh screen, and analysed for pH and microbial biomass-N. Soil pH was measured in a 1:10 soil/0.01 M KCl extract with a pH meter (inoLab pH meter level 1, WTW GmbH & Co. KG, Germany). Microbial biomass N was determined by the chloroform fumigation method, followed by Kjeldahl digestion (MAFF 1986).

5.2.5 Statistical analysis

The results were analysed using a split-plot design with the earthworm and SCM treatments nested within the limed and non-limed treatments (main plots). Treatment effects and interactions were analysed using analysis of variance (ANOVA) with the statistical package Genstat (13th edition, VSN International, Hemel Hempstead, UK). All treatment effects were considered to be significant at 5% probability level. When main factors or interactions were

found to be significant, the comparison among treatments was performed using the protected Fisher-LSD test.

As effects of SCM application on most of the evaluated parameters were supposed to be very strong, hence possibly concealing the effects of earthworms and soil pH, a two-way ANOVA with earthworm density and lime application as factors was performed separately for the treatments either with or without SCM application in which earthworm density was again nested within lime application.

Additionally, a paired two-tailed t-test was performed to compare initial versus final density and biomass of earthworms. SCM disappearance from the litterbags was analysed through analysis of variance (ANOVA) at 5% probability level.

5.3 Results

5.3.1 Effectiveness of the treatments

At the end of the experiment, soil pH (KCl) in the limed treatments was 6.2±0.1 while it was 4.6±0.1 in the non-limed treatments (L, LE, ML & MLE vs. C, M, E & M; $P = 0.004$).

In the treatments with ambient earthworm populations, earthworm density was increased by on average 114% ($P < 0.05$; C, L, M & ML vs. A; Fig. 5.2). In the treatments with increased earthworm abundance, no difference between initial and final density was observed except for MLE (I vs. E, LE & ME; Fig. 5.2). In treatment MLE, a strong increase (1767 vs. 680 n m^{-2}= 160%, $P = 0.013$) in earthworm abundance was observed. Here, more than 50% of the earthworm fauna was comprised of juveniles with a body length of less than 1 cm, which were predominantly located in the top two cm of soil. Adults to juveniles ratio

of earthworms was significantly higher in SCM compared to non-SCM treatments (Fig. 5.2; P= 0.027). Liming significantly increased earthworm abundance compared to non-limed treatments (Fig. 5.2; P= 0.02).

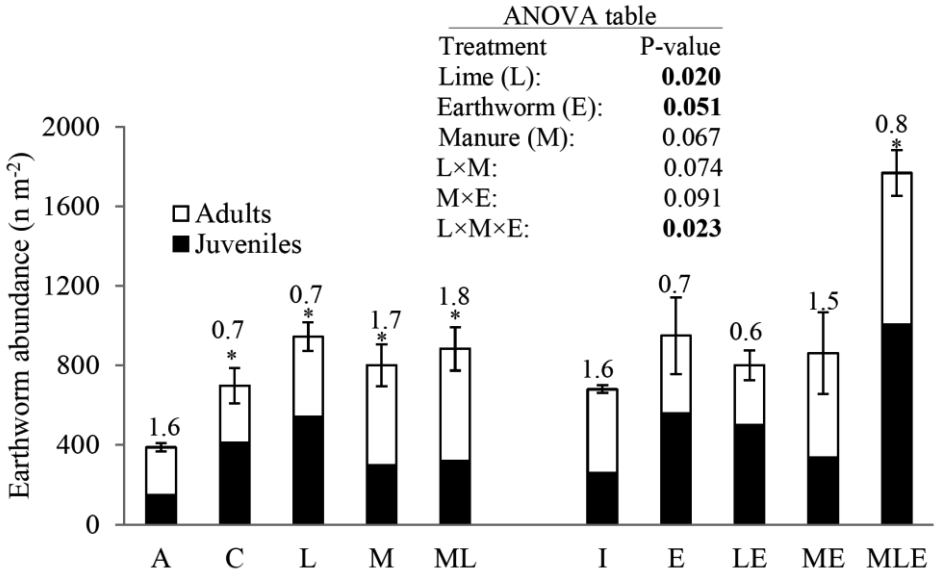

Fig. 5.2 Earthworm initial (A = ambient, I = inoculated) and final abundance (black = juveniles, white = adults) as affected by the following treatments: C = control with ambient earthworm abundance, L = lime, M = solid cattle manure, E = increased earthworm abundance. Error bars represent standard error (±1) of mean (n=3). Asterisks denote significant differences (P<0.05) of A compared with each of the other treatments at ambient earthworm abundance or I compared with each of the other treatments with increased earthworm abundance. The ANOVA table presents the results of the experimental treatments after 134 days. The values above bars represent adult: juvenile ratio of earthworms.

A statistically significant interaction among liming, manure application and inoculation of earthworms was found on earthworm abundance (Fig. 5.2; P= 0.023). Averaged over all treatments, 72% of the earthworm community comprised endogeic species, mainly *Aporrectodea caliginosa* (78%) and *Allolobophora chlorotica* (Savigny) (21%). The remaining 28% were epigeic species

dominated by *Lumbricus rubellus* (99%). Anecic earthworms were not found. The ratio of epigeic to endogeic species did not change throughout the experiment.

5.3.2 Solid cattle manure and nitrogen disappearance from litterbags

At final harvest, almost all the manure DM and N had disappeared from the litterbags. DM disappearances in M and MLE treatments were 99.2±0.2 and 99.8±0.1%, respectively, and the respective N disappearances equalled 99.1±0.3 and 99.7±0.1%.

5.3.3 Soil respiration and microbial biomass

In the SCM treatments, cumulative CO_2 emission was on average 10% higher (P= 0.014) than in the unfertilised treatments (Table 5.2; treatments M, ML, ME & MLE vs. C, E, L & LE). Besides, CO_2 emission increased by 8% (P= 0.018) when earthworm densities were increased in the SCM treatments (Table 5.2; treatments ME & MLE vs. M & ML).

In the treatments with increased earthworm density, microbial biomass N was 8% lower than in those with ambient densities (P= 0.019) (Table 5.2).

Table 5.2 Cumulative gaseous emissions and microbial biomass-N after 134 days

	Treatments	N_2O (kg ha^{-1})	CO_2 (Mg ha^{-1})	Microbial biomass-N (g kg^{-1} soil)
No lime				
	C	13.1 ± 0.2	17.4 ± 1.1	264 ± 16
	E	15.4 ± 0.7	18.1 ± 0.5	235 ± 17
	M	19.0 ± 3.4	17.7 ± 1.6	293 ± 12
	ME	16.9 ± 1.4	19.5 ± 1.6	249 ± 17
Limed				
	L	14.3 ± 0.7	18.7 ± 0.8	302 ± 20
	LE	13.4 ± 0.4	18.6 ± 0.2	293 ± 08
	ML	22.3 ± 5.5	21.0 ± 1.5	306 ± 01
	MLE	18.7 ± 2.1	22.0 ± 1.5	291 ± 15
+ Earthworms (mean of E, ME, LE & MLE)		16.1 ± 1.1	19.6 ± 0.9	267 ± 12
- Earthworms (mean of C, M, L & ML)		17.2 ± 2.4	18.7 ± 1.2	291 ± 14
Split-plot ANOVA: General (P-values)				
	L	0.685 (10.0)	0.364 (6.93)	0.120 (61.3)
	E	0.545 (3.68)	0.215 (1.42)	**0.019 (19.2)***
	M	**0.009 (3.68)****	**0.014 (1.42)***	0.224 (19.2)
	L×E	0.489 (7.66)	0.549 (5.90)	0.173 (47.8)
	L×M	0.415 (7.66)	0.156 (5.90)	0.269 (47.8)
	E×M	0.312 (5.20)	0.427 (2.01)	0.578 (27.3)
	L×E×M	0.811 (8.39)	0.984 (5.90)	0.805 (48.7)
ANOVA: Unmanured treatments, (P-values)				
	L	0.634 (2.61)	0.072 (1.08)	0.155 (91.5)
	E	0.203 (13.4)	0.563 (1.41)	0.189 (33.1)
	L×E	**0.028 (2.07)***	0.457 (1.41)	0.425 (73.9)
ANOVA: Manured treatments, (P-values)				
	L	0.656 (20.9)	0.436 (12.8)	0.103 (40.8)
	E	0.329 (7.08)	**0.018 (0.99)***	0.124 (41.5)
	L×E	0.772 (17.3)	0.340 (12.5)	0.371 (43.4)

P-values and LSD (between brackets) indicate levels of significance: *<0.05; **<0.01; ***<0.001; for treatment abbreviations see Table 5.1

5.3.4 Gaseous nitrogen losses

Application of SCM increased the total N_2O emission with on average 37% (P= 0.009; treatments M, ML, ME & MLE vs. C, E, L & LE in Table 5.2). Liming did not affect the N_2O emission level, neither in the manured (R^2 = 0.01, P= 0.22), nor

in the unfertilised treatments (R^2= 0.69, *P*= 0.44) (Fig. 5.3). In the fertilised treatments, this was true also for earthworm inoculation.

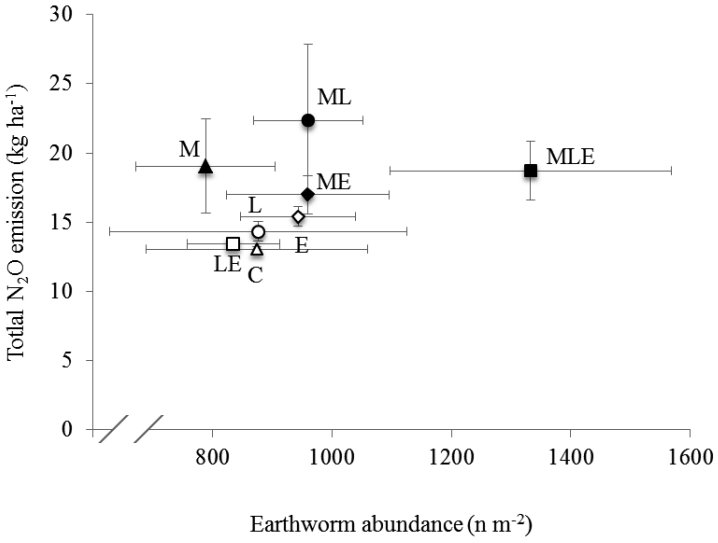

Fig. 5.3 Relationship between total N_2O-N losses and earthworm abundance (mean of initial and final) in manured (solid symbols, R^2 =0.01; P> 0.05) and unmanured treatments (open symbols, R^2= 0.69; P> 0.05). Error bars represent standard error (±1) of mean (n=3). For treatment abbreviations see Fig. 6.2.

However, in the unfertilised treatments earthworms did increase the N_2O emission level, but only in the non-limed treatment (E= 18%; Table 5.2). Detectable emission of NH_3 was only observed during the first day of the experiment and not thereafter due to heavy rainfall (22 mm) in the night after SCM application. Cumulative measured gaseous N_2O losses for the whole experimental period were almost ten times lower than the total herbage N uptake from the applied SCM (Tables 5.2 & 5.3; 5 vs. 48 kg N ha^{-1}).

5.3.5 Herbage N uptake

SCM application increased the cumulative herbage N uptake by 26% compared to unfertilised treatments (P = 0.004; 244 vs. 193 kg N ha^{-1}; treatments M, ME, ML & MLE vs. C, L, E & LE; in Table 5.3). This increase in herbage N uptake was 29, 22 and 21% in harvests 1, 2 and 3, respectively (Table 5.3).

Table 5.3 Herbage N uptake from different treatments after 134 days.

Treatments	N uptake (kg ha^{-1})			
	1st harvest	2nd harvest	3rd harvest	Total
Unmanured				
C	43.2±3.6	66.4±6.3	55.6±2.3	165.2±11.7
L	56.1±3.0	75.9±10.4	69.9±5.2	201.9±10.6
E	62.3±1.7	86.7±10.9	63.3±11.8	212.4±21.2
LE	59.3±3.9	77.1±3.2	68.3±4.9	204.6±7.5
Manured				
M	58.4±4.8	75.1±7.4	70.3±12.2	203.8±20.6
ML	71.2±5.9	101.8±8.5	85.8±11.1	258.8±25.5
ME	72.8±7.1	89.4±7.4	75.8±7.1	237.9±20.7
MLE	82.7±3.6	109.7±11.1	82.4±10.8	274.9±24.6
ANOVA: Unmanured treatments (P-values)				
L	0.064 (5.6)	0.980 (12.3)	0.157 (18.7)	**0.048* (14.1)**
E	**0.035* (9.9)**	0.290 (24.4)	0.738 (23.8)	0.200 (45.1)
L×E	0.090 (9.8)	0.335 (24.1)	0.614 (24.0)	0.243 (44.7)
ANOVA: Manured treatments (P-values)				
L	0.184(24.5)	0.158 (45.8)	0.581 (73.2)	0.281 (135.3)
E	0.057 (13.5)	0.252 (23.1)	0.899 (21.4)	0.279 (55.8)
L×E	0.783 (19.6)	0.720 (36.3)	0.595 (61.3)	0.675 (107.5)
ANOVA: General (P-values)				
L	0.145 (15.0)	0.152 (22.3)	0.418 (44.1)	0.224 (74.7)
E	**<0.001** (5.9)**	0.124 (14.4)	0.735 (12.9)	0.092 (29.7)
M	**<0.001** (5.9)**	**0.021* (14.4)**	**0.033* (12.9)**	**0.004** (29.7)**
L×E	0.113 (11.5)	0.350 (18.8)	0.458 (34.8)	0.273 (57.1)
L×M	0.263 (11.4)	0.099 (18.8)	0.907 (34.8)	0.271 (57.1)
E×M	0.747 (8.4)	0.972 (20.3)	0.867 (18.3)	0.993 (42.0)
L×E×M	0.259 (13.1)	0.636 (26.9)	0.983 (34.4)	0.638 (65.1)

P-values indicate levels of significance: *<0.05; **<0.01; ***<0.001; For treatment abbreviations see Table 5.1

At the first herbage harvest, N uptake was 21% higher in treatments with increased earthworms density than in those with ambient density ($P < 0.001$; treatments E, LE, ME & MLE vs. C, L, M & ML ; 69 vs. 57 kg N ha^{-1}), but this effect decreased in the subsequent harvests (Table 5.3). In total, inoculation of earthworms had increased cumulative herbage N uptake by 15% at the end of the experiment (treatments E, LE, ME & MLE vs. C, L, M & ML; 238 vs. 207 kg N ha^{-1}; Table 5.3). In the SCM treatments, herbage N uptake was highly correlated with earthworm abundance ($R^2 = 0.92$, $P < 0.001$) (Fig. 5.4).

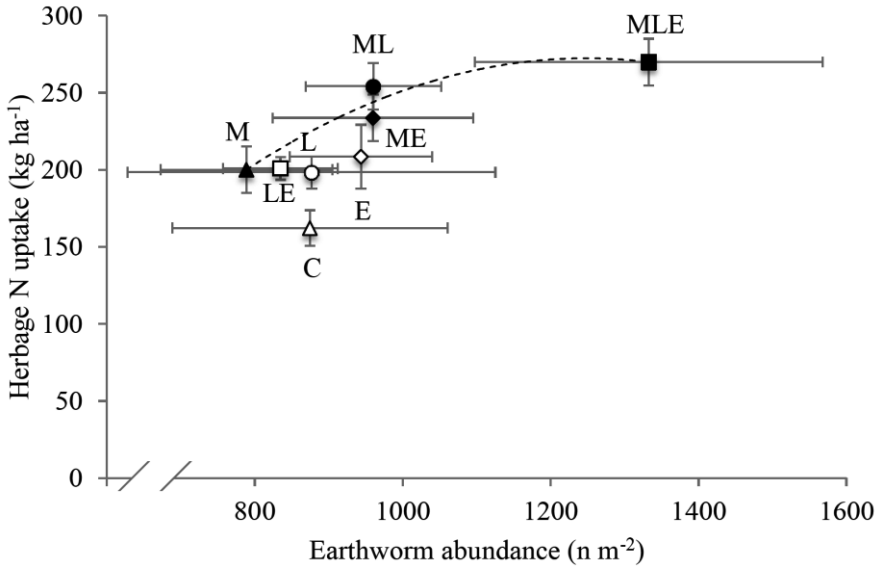

Fig. 5.4 Relationship between total N uptake and earthworm abundance (mean of initial and final) in solid cattle manure (SCM) application (solid symbols, $R^2 = 0.92$; $P < 0.001$) and unfertilised treatments (open symbols, $R^2 = 0.07$; $P > 0.05$). Error bars represent standard error (±1) of mean (n=3). For treatment abbreviations see Fig. 5.2.

Overall, application of lime in non-SCM treatments increased cumulative herbage N uptake with 7% ($P = 0.048$; 203 vs. 189 kg N ha^{-1}; treatments L & LE vs. C & E), whereas it had no effect in SCM treatments ($P > 0.05$; Table 5.3). In

the non-SCM treatments, liming alone increased cumulative herbage N uptake with 22% compared to the non-limed treatment (202 vs. 165 kg N ha^{-1}; L vs. C), whereas increased earthworm density had no effect in case of lime treatment (LE vs. E; Table 5.3).

The average apparent herbage N recovery (ANR) from SCM ranged between 13 and 35% (Fig. 5.5). Although liming did not have a statistically significant effect on total plant N uptake in the SCM treatments, it did increase the herbage ANR from SCM with 98% (treatments ML, MLE vs. M, ME; marginally significant at P= 0.08, Fig. 5.5). An increased initial density of earthworms alone, i.e. without liming, did not affect the herbage ANR from SCM (Fig. 5.5).

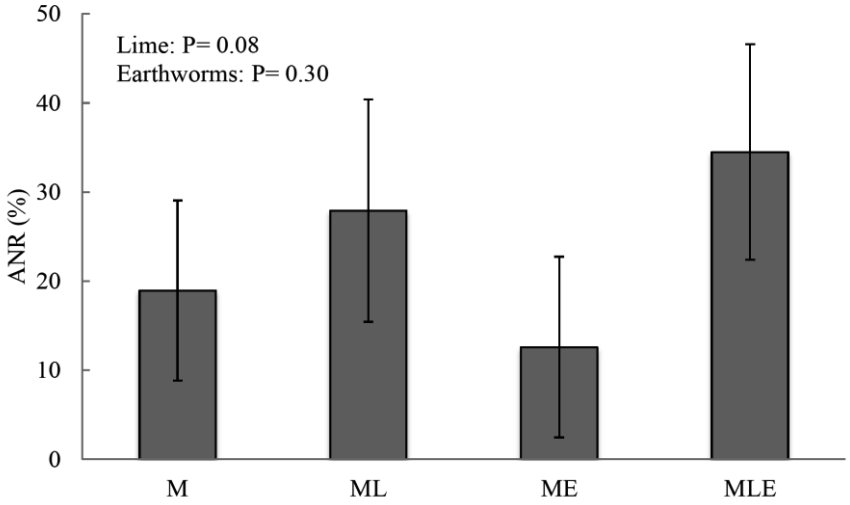

Fig. 5.5 Herbage apparent N recovery (ANR) from solid cattle manure treatments. For treatment abbreviations see Fig. 5.2.

5.4 Discussion

5.4.1 Effectiveness of the treatments

At the end of the experiment, soil pH-KCl was increased from 4.6 to 6.2 in the limed treatments. Thus, the pre-calculated amount of agricultural lime (1.4 kg

m^{-2}) was sufficient to achieve the desired level of soil pH. Earthworm abundance remained higher in earthworm-inoculated treatments compared to the initial field densities (Fig. 5.2; P= 0.05), indicating that inoculation of earthworms was successful. According to our expectation, liming had a positive effect on earthworm numbers, and this effect was strongest in the treatment where earthworms were inoculated as well as manure was applied (MLE; Fig. 5.2). Although the effect of SCM application on earthworm density was only marginally significant, such effect was expected. Moreover, the lack of sound significance in some of the observed effects (i.e. N uptake and herbage ANR) could be associated with the visually observed large variation in botanical composition between the small experimental units which also changed rather quickly throughout the experiment. Some of them became dominated by creeping buttercups (*Ranunculus repens* L) and/or dandelion (*Taraxacum officinale* Weber), which are less productive species than perennial ryegrass (*Lolium perenne* L.).

5.4.2 Herbage N uptake

According to our expectations, herbage ANR from SCM was highest in the treatment with a combined increase of soil pH and earthworm density (Fig. 5.5; MLE vs. M: 35 vs. 19%). We obtained similar results in an earlier study, when we compared the field used for the current mesocosm trial with a nearby grassland on a peat soil with a higher pH-KCl (5.1 vs. 4.4) and earthworm density (568 vs. 268 m^{-2}) (Chapter 3). An explanation could be that soil pH of the latter grassland field was within the optimum range for growth and activity of earthworms (Edwards 2004), as well as for soil microorganisms to secrete extracellular enzymes which are important in the process of organic matter mineralisation (Parham and Deng 2000, Tabatabai et al. 2010). According to Shah et al. (1990), liming increases microbial activity and N mineralisation from

organic matter in grassland soil, while Blair *et al.* (1997) found that earthworms can decrease N immobilisation from the microbial biomass N through their foraging activity on microbes. In line with these findings, we also observed greater microbial biomass (298 vs. 260 g kg^{-1} soil= 14%) and activity (measured as CO_2 emission; 20.1 vs. 18.2 Mg CO_2 ha^{-1}= 10%) after liming (L, LE, ML, MLE vs. C, E, M, ME) and lower microbial biomass N in the increased earthworm density treatments (Table 5.2). Although these differences were not statistically significant, they are consistent with the observed higher ANR from SCM in MLE (Fig. 5.5).

Increased earthworm density alone did not influence herbage ANR (Fig. 5.5; ME vs. M). Parham & Deng (2000) observed that the activity of extracellular enzymes secreted by microbes decreased to zero at a soil pH-$CaCl_2$ of 4.0, which was rather close to the soil pH in the treatments ME and M of our experiment (pH-KCl= 4.6). Moreover, we did not observe differences in CO_2 emission between these treatments (Table 5.2), which is a measure of soil biota activity and organic matter decomposition (Ayres et al. 2006). Thus, at low soil pH increased earthworm populations will not stimulate soil microbial activity and hence SCM decomposition, N mineralisation and herbage N uptake.

CO_2 emission was higher in the treatments with increased earthworm density compared to those with ambient densities (5%; Table 5.2; P= 0.018), indicating greater organic matter decomposition and N mineralisation (Ayres et al. 2006). Concomitantly, microbial biomass N was significantly lower (8%; P= 0.019) in increased earthworm density treatments (Table 5.2). This resulted in less N immobilisation in the microbial biomass, leading to a higher cumulative herbage N uptake (12%; P= 0.092) in treatments with increased earthworm density compared to those with ambient densities (Table 5.3). This effect was only significant at first harvest (17% P< 0.001; Fig. 5.2). However, the created

initial density differences disappeared with time (Fig. 5.2) and this may have diminished the effects of earthworms on herbage N uptake towards the end of experiment.

A non-linear relationship between earthworm density and herbage N uptake was observed in SCM treatments, which levelled off gradually up to a density of approximately 1300 earthworms m^{-2} (Fig. 5.4). This relationship was largely determined by the treatment MLE, which was characterized by a much higher presence of juvenile earthworms (MLE vs. M, ME & ML: 884 vs. 275 n m^{-2}), a much lower adult: juvenile ratio (0.8 vs. 2.1) and a much higher presence of endogeic vs. epigeic earthworms (880 vs. 480 n m^{-2}) than in the other SCM treatments. In MLE, juveniles and endogeics represented 57% and 78% of the total earthworm community, respectively. Schmidt & Ostle (1999) used natural abundance measurements of nitrogen stable isotopic ratios ($^{15}N/^{14}N$, expressed as $\delta^{15}N$ ‰) from cattle slurry in a field experiment and found that the endogeic species *Aporrectodea caliginosa* assimilated more N in its tissue derived from cattle slurry compared to the anecic species *Aporrectodea longa*. Juvenile earthworms have higher consumption and assimilation rates than adults per unit biomass, reflecting their high energy requirements (Curry and Schmidt 2007). They use more N in the production of their body tissues than adults. Therefore, they contribute less to the overall net organic N mineralisation. Accordingly, their increased density (Fig. 5.2) did not further increase the herbage N uptake in our experiment (Table 5.3).

5.4.3 Gaseous N-losses

Application of SCM significantly increased N_2O emission by 37% compared to unfertilised treatments (Table 5.2; ME, ML & MLE vs. C, L, E, & LE). In total, the observed increase in N_2O emission from SCM was 2.5% of the applied N (~5 kg N ha^{-1}). This was almost ten times lower than the increase in herbage N

uptake from SCM (Table 5.2 & 3; 5 vs. 47 kg N ha^{-1}). Thus, gaseous N losses did hardly affect herbage N uptake from SCM.

Contrary to our expectation, we did not observe a correlation between earthworm density and N_2O emission (Fig. 5.3). Earthworms increased N_2O emission in the non-limed and non-SCM treatments (Table 5.2; P= 0.028), but this increase in N_2O emission was quantitatively very low (E vs. C: 15.4 vs. 13.2 = 2.2 kg N_2O per ha^{-1}; Table 5.2) compared to the total emission from non-limed treatments (14 kg N_2O ha^{-1}; Table 5.2). Speratti & Whalen (2008) did not find an effect of earthworms on N_2O emission from unfertilised soils. According to Nebert *et al.* (2011), cumulative N_2O emission from maize residues was significantly increased by the epigeic *Lumbricus rubellus*. However, interactions of this species with the endogeic *Aporrectodea caliginosa* did not impact on cumulative N_2O emission. Lubbers *et al.* (2011) observed that introduction of *Lumbricus rubellus* increased N_2O emission by 51% in mesocosms fertilised with a liquid solution of NH_4NO_3, KH_2PO_4 and K_2SO_4 whereas this increase was only 4% with introduction of *Aporrectodea caliginosa*. However, they observed that the co-occurrence of these two species in mesocosms resulted in a 14% *lower* N_2O emission as compared to the sum of their individual effects. In our experiment, earthworm species were dominated by *Aporrectodea caliginosa* which comprised 72% of the total community, whereas this fraction was 27% for *Lumbricus rubellus*. The absence of earthworm effects on N_2O emission in our case fits within the results of the above-mentioned studies.

5.5 Conclusions

Earthworms increased herbage N uptake in the first harvest both in the SCM and non-SCM treatments. However, this increase became smaller and insignificant in subsequent harvests. This could be explained by the initial

difference in earthworm abundance between ambient and increased earthworm density treatments which was however disappeared at the end of experiment.

In the SCM treatments, herbage N uptake was highly correlated with earthworm abundance. In the non-SCM treatments, liming alone increased cumulative herbage N uptake whereas increased earthworm density had no additional effect.

Liming increased herbage ANR from SCM, however, an increased initial density of earthworms alone, i.e. without liming, did not affect this.

We observed no effects of earthworm abundance on N_2O emission from solid cattle manure. This is in accordance with literature that reports that combinations of epigeic and endogeic earthworms do not increase N_2O emissions.

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Chapter 6

General Discussion

Muhammad Imtiaz Rashid

6.1 Introduction

Intensification of livestock systems due to a continued increase in consumption of animal products worldwide has resulted in gradually increasing amounts of produced animal manure. In North Western Europe, most of the manure is being handled as slurry from cubicle barns. However, in the Netherlands, production of solid cattle manure (SCM) has increased in recent years due to renewed interest in straw-based housing systems for reasons of better animal health and welfare (Ellen et al. 2007). In recent studies N recovery from SCM ranged from 20 to 50% during the year of application to Dutch grasslands (Van Dijk 2004, Schröder et al. 2007, Sonneveld and Lantinga 2011, Shah et al. 2012). However, a high short-term nitrogen (N) recovery from SCM is important to increase its fertiliser value and reduce environmental losses. Therefore, the search for factors which are responsible for these differences was the major driving force for the current thesis work. In this chapter, it is my aim to discuss the main findings and to integrate the factors which have been detected as plausible causes for these differences. This all might serve as input to the discussion about how to assist farmers in the process of developing more sustainable fertilisation management strategies for their production grasslands.

6.2 Major findings

In this section, I will give a brief overview of the major findings obtained in this thesis research.

Long-term application of SCM increased the abundance and biomass of earthworms and enchytraeids as well as bacterial growth rates compared to the use of only chemical fertiliser and /or cattle slurry manure (non-SCM). Model calculations indicated that this higher abundance and biomass of soil organisms directly resulted in a higher N mineralisation of soil organic matter in SCM than

in non-SCM grassland on sandy soil. However, in peat grasslands, soil N supply was not affected by SCM or non-SCM inputs history. Our results showed that production ecological-modelling can give a good estimate of the natural soil N supply capacity. The integration of N mineralisation estimates based on model calculations of earthworm N excretion and laboratory-determined potential N mineralisation, explained ≥98% of the established soil N supply based on herbage N uptake measurements. In contrast, the currently used method in Dutch fertilisation recommendations greatly underestimated the soil N supply capacity (about one-third) of our studied grasslands (Chapter 2).

Disappearance rates of SCM significantly differed among SCM and non-SCM grasslands. As expected, dry matter (DM) and N disappearance rates as well as herbage N recovery was higher in SCM grasslands compared to those found in non-SCM grasslands (Chapter 3). These differences in DM and N disappearance rates were mainly caused by the meso- and macrobiota, with three times higher disappearance rates in SCM than in non-SCM grasslands. Microbiota did not contribute differently to manure decomposition between these sites (Chapter 4). This was supported by higher abundances of soil meso- and macrobiota and no differences in microbial biomass between SCM and non-SCM grasslands, respectively (Chapter 2). Manure exchange experiments showed that between the two SCM grasslands, SCM decomposition and N mineralisation was 20 and 14% higher, respectively, when the SCM was applied at home. In other words, a clear home field advantage (HFA). This resulted in a higher herbage N recovery from SCM when applied to the home farm grassland (Chapter 3). In SCM grasslands, all body size classes of soil biota contributed to the higher DM and N disappearance rates from home manure. However, their contributions varied depending upon the stage of SCM decomposition (Chapter 4). During the initial

stage of decomposition, i.e. the first 4 months, HFAs for both DM and N disappearance rates were greater than in the next 4 months (Chapter 4). This decrease was related to consistent changes in the chemical composition of the manure. After 4 months, the C:N ratio of SCM remaining in the litterbags was in both cases (peat and sand) significantly higher at home compared to that of away-applied SCM. However, after 8 months, this difference in C:N ratio was disappeared (Chapter 4).

Liming of the low-pH peat grassland increased the herbage N recovery from SCM from 19 to 28%, whereas in combination with artificially raised earthworm abundance this increase was from 19 to 35%. In the latter case, microbial biomass N was reduced indicating higher N mineralisation. However, the increase of earthworm populations without liming, did not stimulate the herbage N recovery in acidic peat grassland (Chapter 5).

Overall, I conclude that continued SCM inputs increased soil biota abundance (Chapter 2) which increased SCM decomposition and herbage N recovery in SCM grasslands (Chapter 3), whereas prolonged cattle slurry with and without chemical fertiliser application decreased soil biota abundance and soil pH, especially in peat grassland (Chapter 2). In the latter case, liming in combination with artificially raised earthworm density increased the herbage N recovery from SCM (Chapter 5).

Part of my findings has resulted in new insights in the mechanisms behind SCM decomposition and its net N mineralisation, which might direct future research in agro-ecosystems like production grasslands. Others contributed to the confirmation of earlier developed insights and explanations.

6.3 Needs for adjusted fertilisation management to improve the biotic and abiotic environmental conditions in grassland soils

The use of organic (SCM and cattle slurry manure) next to chemical fertilisers is common in production grasslands. Fertiliser inputs greatly influence soil biotic and abiotic environmental conditions (Ma et al. 1990, De Goede et al. 2003, Hopkins et al. 2011). Both organic and chemical fertilisers can positively affect the earthworm and enchytraeid abundance and biomass in grasslands (Curry 1976, Standen 1984, Curry et al. 2008, Van Eekeren et al. 2009), as long as the fertiliser application does not increase soil acidification (Ma et al. 1990, Hopkins et al. 2011). Especially ammonium based fertilisers are known to decrease soil pH due to the release of hydrogen ions (H^+) from ammonium during nitrification (Ma et al. 1990, Guo et al. 2010, Hopkins et al. 2011). Application of SCM, however, increases soil pH and thus improves the living environment for soil organisms especially enchytraeids and earthworms which prefer a soil pH-H_2O >4.0 (Standen 1984, Edwards 2004). Besides, SCM also directly provides a fresh food source for detritivorous soil organisms, in contrast to chemical fertilisers. This explains the increased earthworm and enchytraeid abundance in the SCM grasslands compared to non-SCM grasslands (Chapter 2). Moreover, high inputs of inorganic N to grassland reduced the abundance of enchytraeids and microbial biomass due to increase in soil acidity and high concentrations of ammonia, benzoic acid and sodium sulphide (Standen 1984, Hopkins et al. 2011). The application of SCM positively influenced the fungal to bacterial biomass ratio (Chapter 2) which is considered to be an important indicator for changes in fertilisation management from intensive to low N inputs systems. In contrast, chemical fertilisers or high mineral N inputs in grasslands have been reported to decrease the fungal to bacterial biomass ratio (Bardgett and McAlister 1999, Bloem et al. 2004). Grasslands which had a fungal to bacterial

biomass ratio of 0.4-0.9 were considered as fungal dominated and represent a low N-input system which is mainly reliant on self-regulation through soil biological pathways of nutrient turnover (Bardgett and McAlister 1999, Bloem et al. 2004, De Vries et al. 2006). According to this criterion, our SCM grasslands which had a fungal to bacterial ratio of on average 0.6 can be classified as fungal dominated (Chapter 2). This supports the idea that SCM inputs promote sustainable and self-regulatory ecosystems, and is a plea for adoption of this type of fertilisation management.

6.3.1 Soil biota and soil organic matter decomposition and N mineralisation

The majority of the processes in soil are carried out by soil biota (Ritz et al. 2009), but their relationships with soil processes are not always straightforward Ritz et al., (2005). Van Eekeren et al. (2010) found that enchytraeid density was positively correlated with net herbage N uptake from soil. In line with their findings, I also found such positive correlation with herbage N uptake, but it was not significant. The reason for this was not clear.

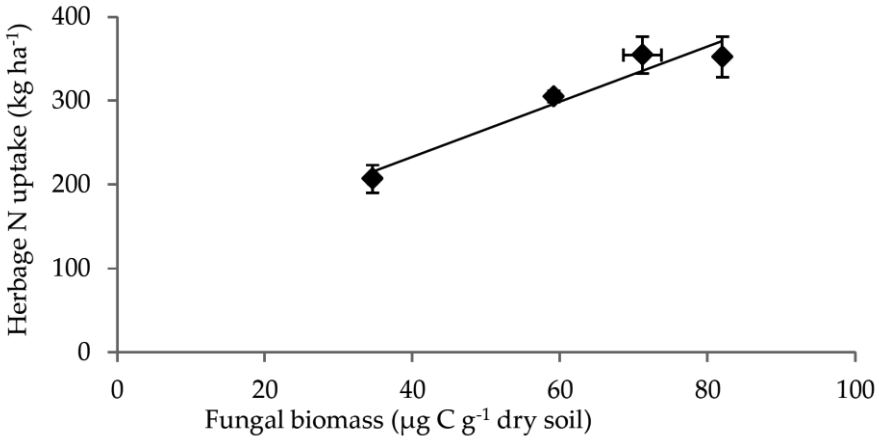

Fig. 6.1 Relationships between herbage N uptake and (a) fungal biomass (R^2 =0.94, P = 0.031) in the grasslands of SCM and non-SCM farms (data from Chapter 2).

Instead, we found a strong linear relationship between net herbage N uptake from soil and fungal biomass (Fig. 6.1).

In spite of lack of experimental evidence on relationships between soil biota and soil N mineralisation, foodweb modelling studies widely acknowledge the contribution of soil biota to N mineralisation in agricultural ecosystems (Hunt et al. 1987, De Ruiter et al. 1993, Holtkamp et al. 2011). Several of these studies showed that microbes contribute the most to N mineralisation, followed by the soil fauna. Other studies, based on foodweb and production ecological modelling, also recognise the importance of the soil fauna (De Goede et al. 2003, Van Vliet and de Goede 2006, Schon et al. 2012). Schon et al. (2012) found a higher contribution of earthworms than microbes to soil N mineralisation in grasslands under both organic and conventional management. De Goede et al. (2003) and Van Vliet et al. (2007) calculated that earthworms even can contribute up to 170 kg N ha^{-1} yr^{-1} to soil gross N mineralisation in production grasslands. In our grasslands, model calculations indicated that the contribution of bacteria and fungi was 37 and 22% of the total soil N mineralisation of 290 Kg N ha^{-1} (mean data from all grasslands), whereas protozoa and earthworms contributed 17% and 27%, respectively. Thus, in addition to a positive correlation with herbage N uptake, production ecological model calculations also confirmed the importance of fungi (Chapter 2).

6.3.2 Need for better methods to predict soil N supply

Accurate assessments of the soil N delivery capacity is an imperative component of sustainable, cost-effective and environmentally sound fertilisation management in agro-ecosystems (Velthof et al. 2009). Several methods to assess the soil N delivery capacity exist in the literature. Among these, biological incubation methods, chemical extraction methods and simulation modelling are commonly used and have their own advantages and

disadvantages (Bloem et al. 1994, Nannipieri and Eldor 2009, Ros et al. 2011). I compared soil N mineralisation measured as herbage N uptake with the laboratory-determined potential N mineralisation (PNM), production ecological calculation (PEC), and N mineralisation calculated according to the Dutch fertilisation recommendation (Chapter 2). The currently used method in the Dutch fertilisation recommendation based on soil N content greatly underestimated (34%) the actual soil N supply capacity from two grassland fields on sandy soil (Chapter 2). The accuracy of this method was already questioned by Van Eekeren et al. (2010) and our findings confirm that this method should be re-evaluated (Chapter 2). The appealing characteristic of the current method is that it is based on one simple soil analysis and linear regression equations between soil N content and soil N mineralisation in the field. However, according to Van Eekeren et al. (2010) and our measurements the slope of these regression lines is in reality much steeper.

Although PECs gave reasonable estimates of soil N mineralisation (on average 105% of the measured N uptake in our four grassland fields), it needed assumptions on certain soil biota parameters as well as on protozoa abundance. Besides, in grasslands with low abundances of soil biota the soil N supply was underestimated. Hence, this method requires further research to prove its validity in a broader range of agro-ecosystems. Nevertheless, the integration of laboratory-determined PNM and PEC estimates of soil N mineralisation related to earthworm abundance proved to be the best among the studied methods (Chapter 2) and this approach could be recommended for practical use. In case there is a preference to continue with the current method, there is a strong need to re-formulate the regression equations.

6.3.3 Need to unravel the differences in herbage N recovery from SCM

It is obvious from the discussion so far and Chapter 2 that fertilisation history can greatly affect a number of important soil biological and chemical parameters in production grasslands. However, the mechanisms behind the effects of fertilisation-induced changes on decomposition and net N mineralisation of applied SCM are not well understood yet. The few studies that were aimed to unravel this yielded contrasting results (Mallory and Griffin 2007, Nett et al. 2010). These studies indicated that continued SCM inputs over time increased microbial activities in the soil, but led to increased N immobilisation in the study of Mallory and Griffin (2007) *vs.* increased N mineralisation in the study of Nett el al. (2010). From natural ecosystems it is known that changes in the chemical as well as in the biotic soil environment directly affect soil functioning in terms of decomposition and nutrient mineralisation from litter (Orwin et al. 2006, Ayres et al. 2009a, Milcu and Manning 2011). Such changes can be induced by the chemical composition of the litter produced in the ecosystem. The home field advantage (HFA) hypothesis, which was originally developed to explain litter decomposition in forest ecosystems, was used to test the significance and nature of effects related to changes in soil biota communities induced by differences in specific local environmental conditions. This hypothesis explains that decomposition occurs more rapidly when locally produced ('home') organic materials are offered than with off-site produced ('away') organic materials. This hypothesis was tested for production grasslands (Chapter 3). My results showed clear HFAs for SCM decomposition, indicating that specific characteristics of the local soil biota communities played a prominent role in the observed differences in DM and N disappearance rates and herbage N recovery from SCM (Chapters 3 & 4). Two mechanisms have been proposed to explain the HFA in natural ecosystems: 1)

differences in abundance and species diversity of soil organisms among home and away sites (Milcu and Manning 2011), and 2) specialisation of soil biota to degrade a particular quality of litter, i.e. the home litter, with which they interact most frequently (Ayres et al. 2009b, Strickland et al. 2009). I found evidence that both mechanisms are responsible for the DM or N disappearance HFA of SCM in production grasslands. As evidence for the first mechanism, we found a significant correlation between DM disappearance of farm A manure (AM) and earthworm density (R^2 = 0.90, P = 0.05), whereas in case of farm B manure (BM) the best correlation (R^2 = 0.97, P = 0.015) was found with earthworm biomass (Figs. 6.2a and b). Similarly, N disappearance of AM showed positive correlations with earthworm density (R^2 = 0.82, P = 0.09) and biomass (R^2 = 0.98, P = 0.01) (Figs. 6.2c and d), whereas there were no clear relationships for BM (Figs. 6.2c and d).

The most striking feature in the latter case was that with both increasing earthworm density and biomass from farm D to A, ND from BM did not increase at all except at its home farm B (Figs. 6.2c & d). This is strong evidence that earthworms on farm B were adapted to decompose and mineralise N from the home manure. Moreover, the lower N disappearance of this manure on away farms could be explained by its physical and chemical composition. This manure contained long pieces of straw (~10 cm) which did not significantly contribute to its total weight but could have played an important role in manure decomposition and N mineralisation (Cheshire et al. 1999).

Visual observations revealed that on the away farms (A, C & D), the straw pieces from manure BM disappeared much slower than at its home (farm B). This leads us to the inference that the soil fauna community on the three away farms was less capable to decompose longer pieces of straw compared to the home community. Thus, the developed specialisation of soil biota in

decomposing the home manure resulted in an increased N mineralisation and led to a higher herbage N recovery at home than on the away farms (Chapter 3).

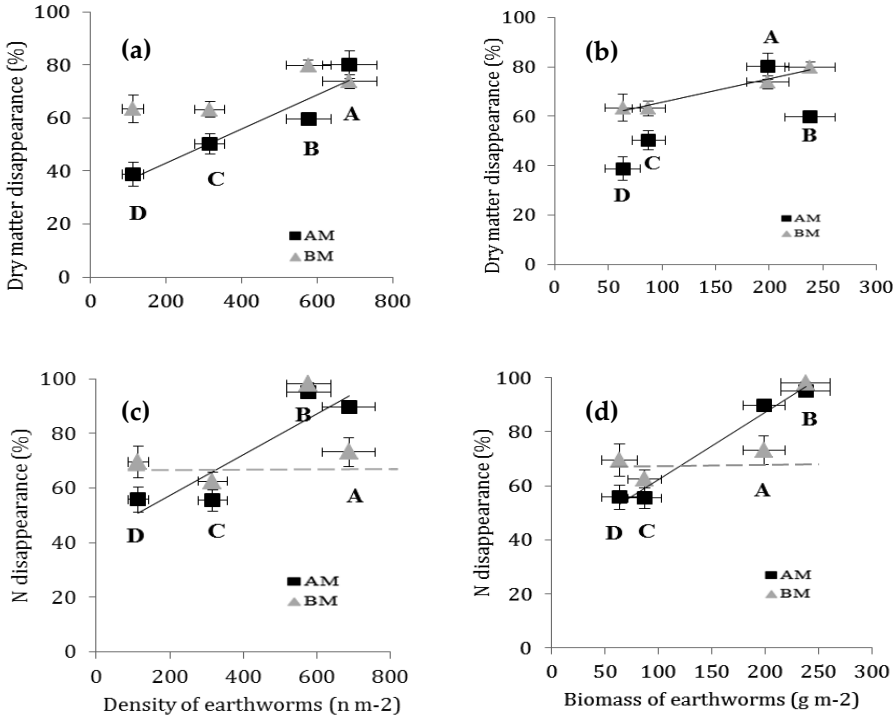

Fig. 6.2 Relationships of earthworm density and biomass with average dry matter disappearance of AM and BM from macro mesh litterbags (a-b) and with average nitrogen (N) disappearance of AM and BM from macro mesh litterbags (c-d) on all four farms. Error bars represent standard error (±1) of mean (n = 4). Solid lines indicate linear relationships between earthworm density and dry matter disappearance of AM (R^2 = 0.90; P = 0.05), between earthworm biomass and dry matter disappearance of BM (R^2 = 0.97; P = 0.015), between earthworms density and N disappearance of AM (R^2 = 0.82; P = 0.09) and between earthworm biomass and N disappearance of AM (R^2 = 0.98; P = 0.01) from litterbags. AM and BM are the manures produced on farm A and B, respectively. A, B, C and D represent the resulting values of dry matter and N disappearance at the end of the growing season on farms A-D respectively. Farm A and B are home sites of AM

and BM, respectively. C and D are non-SCM farms (data from Chapters 2 & 3). The dotted horizontal regression lines for BM (c-d) do not include the data from farm B.

Further exploring the mechanisms of manure decomposition HFA, I found that all body size classes of soil biota contributed to HFAs regarding manure DM and N disappearances and their contributions depended on the stage of decomposition (Chapter 4). The HFAs due to soil biota were higher in the initial than in the later stages of decomposition (Chapter 4). This is in line with Ayres et al. (2009a) who found that HFA of DM loss decreased with time. Wallenstein et al. (2013) observed that changes in home litter during decomposition are more evident than in away litter. Therefore, during the later stages of decomposition the initial differences in the chemical composition of home and away litters diminished and thus HFAs decreased. In line with the above mentioned literature sources I also observed that there was no difference in the C:N ratio of home and away SCM at the final observation after 8 months of SCM decomposition (Chapter 4).

In our production grasslands, we found greater HFAs for DM and N disappearance (Chapters 3 & 4; on average 14-48%) compared to those reported by Ayres et al. (2009b) and Wang et al. (2012) from forest ecosystems (-9-29% with a mean of 8%). These effects were more prominent in case of AM which had a wider lignin:N ratio than BM (13 vs. 8). This corroborates results of Ayres et al. (2009b) and Strickland et al. (2009) who reported for forest ecosystems that the HFA was larger for the litter with a wider lignin:N ratio, i.e. litter of a lower quality. Hence, quality of SCM also plays an important role in determining the extent of the HFA, as in case of forest litter. Therefore, I conclude that both initial quality of organic inputs (here SCM) and soil biota community composition influence the HFA for SCM decomposition and N mineralisation which ultimately affects herbage N uptake from SCM.

A strong HFA in production grasslands implies that the fertilisation history of a given soil will affect the plant-available N from recently applied SCM by influencing the N dynamics of the soil (Chapters 3 & 4). Long-term organic fertiliser applications promote the density, diversity and activity of soil fauna and microflora (Chapter 2). Therefore, it can alter the soil environmental conditions and thus promote functionally distinct soil biota communities. Such soil biota will differ in their ability to assimilate/decompose SCM with contrasting chemical compositions, resulting in higher SCM decomposition and N mineralisation of the home manure and therefore herbage N recovery (Chapters 3 & 4).

Herbage N recovery from SCM was about zero in the non-SCM peat grassland (Chapter 3). Soil pH and earthworm density in this grassland was lower compared to its neighbouring SCM peat farm (Chapter 2) where manure N recovery was on average 25% (Chapter 3). This demonstrates that ammonium based fertiliser input to this grassland had decreased soil pH and the soil biota activity. By testing whether lower density of soil biota or pH was responsible for the difference in N recovery from SCM here (Chapter 3), I concluded in Chapter 5 that liming increased manure N recovery from 19 to 31%. However, an increased initial density of earthworms alone, i.e. without liming, did not affect the herbage ANR from SCM (Chapter 5). This indicates the importance of the soil chemical environment (i.e. soil pH) for the functioning of soil biota in terms of SCM decomposition and N mineralisation. I conclude that it is mainly the fertilisation management which affects both the biological and chemical environment of the soil leading to changes in important soil functions like SCM decomposition, N mineralisation and herbage N recovery.

6.4 Practical implications

Long term application of SCM is a very good option to improve soil biological quality (Chapter 2). In the Dutch soil quality monitoring network reference values for soil biological quality have been determined as criteria for healthy soils (Rutgers et al. 2008, 2009). They have been assessed for soil biota abundance and biomass as well as potential N mineralisation, pH and organic matter etc. In the SCM grasslands, I found higher values for most of these parameters than the reference values given for healthy soil in the Dutch soil quality monitoring network whereas in the non-SCM grasslands the values were lower (Table 6.1).

Table 6.1 Reference values of soil biological parameters from sandy and peat grasslands studied in the Dutch soil quality monitoring network compared with the values obtained from SCM and non-SCM grasslands in this thesis research.

Soil properties	unit	*Non-SCM grasslands		‡Reference value		†SCM grasslands	
		peat	sand	peat	sand	peat	sand
Earthworm density	n m^{-2}	268	109	336	187	568	554
Enchytraeid density	n m^{-2}	21870	8975	31700	24000	45947	27613
Microarthropod density	n m^{-2}	3385	3500	70735	43500	10399	9113
Bacterial biomass	µg C g^{-1} dry soil	180	113	215	146	203	81
Fungal biomass	µg C g^{-1} dry soil	71	35	38	22	82	59
Organic matter	%	52	4	30	6	44	6
pH-KCl		4.4	5.6	4.5	5.2	5.0	5.3
Potential-N mineralisation	mg N kg^{-1} wk^{-1}	17	9	28.2	12	18	12

* Grasslands with application history of cattle slurry and chemical fertiliser or cattle slurry only

‡ Source: Rutgers et al. (2008, 2009)

† Grasslands with application history of solid cattle manure + organic-N rich cattle slurry

Therefore, in non-SCM grasslands, there is great potential for adoption of SCM fertilisation by the farmers to reach the criteria for healthy soils i.e. to improve soil biological and chemical quality.

Assessing the potential of soils to supply N is essential to optimise N fertiliser use efficiency and to minimise environmental N losses. However, to date, methods used to predict soil N mineralisation are very complex, expensive and labour-intensive (Bloem et al. 1994, Nannipieri and Eldor 2009). In Chapter 2, I compared different methods to predict soil N supply and showed that a combination of potential N mineralisation using soil aerobic incubation and production-ecological model estimates is a good method in terms of accuracy and simplicity. Therefore, this method can be seen as a very promising practical option for farmers to predict soil N supply instead of the current procedure included in the Dutch grassland fertilisation recommendation.

Herbage N recovery from SCM in non-SCM grasslands was lower than in SCM grasslands (Chapter 3). Moreover, in the non-SCM grasslands, soil biota abundance and activity, organic matter content (sandy soil) and pH (peat soil) were also lower (Chapter 2). This was because, long-term application of SCM had increased soil pH (Naramabuye and Haynes 2007) and soil biota abundance and activity (Chapter 2). Therefore, for the fertilisation management of such grasslands, especially under organic management, SCM would be the best option to improve all soil biota parameters as well as herbage N recovery from the applied SCM in production grasslands (Chapters 2 & 3).

Lower herbage N recovery in non-SCM grasslands also suggests that farmers will not achieve a higher grassland production already in the year of conversion of their fertilisation management from cattle manure slurry with and without artificial fertilisers to a management based on the application of SCM.

6.5 Conclusions and research perspectives

Based on the results of this thesis study, I conclude that fertilisation management affects the soil biological and chemical (soil pH) environment and thus influences soil functions in terms of decomposition and N mineralisation of organic matter (e.g. soil organic matter and applied solid cattle manure) and herbage N recovery from applied solid cattle manure. I demonstrated that variation in herbage N recovery from SCM is mainly caused by differences in fertilisation management of the grasslands. However, further research is needed for better understanding of fertilisation-induced changes in soil physico-chemical and biological environment (other parameters than mentioned in this thesis), and how these changes influence the nutrient cycling in agro-ecosystems. The main recommendations as a follow up of this thesis are:

- There is a need to investigate how fertilisation management affects the decomposition and mineralisation of other major nutrients, i.e. phosphorous and potassium, from the applied organic fertilisers.
- There is a need to investigate how long-term fertilisation management affects the soil physico-chemical properties (texture, bulk density, cation exchange capacity etc.) and how these changes affect the soil functions in terms of decomposition, nutrient mineralisation and immobilisation etc. and thus overall ecosystem services in agro-ecosystems.
- Although production-ecological calculations (PEC) provided good estimates for N uptake in grasslands with high soil biota abundance, in cases where these abundances were lower PEC underestimated herbage N uptake. Therefore, the model needs to be validated for a range of agro-ecosystems with low to high densities of soil biota.

- In the studied grasslands, earthworms appear to be one of the key biota groups whose communities were highly dominated by a few species i.e., *L. rubellus* and *A. caliginosa*. Therefore, it needs to be investigated whether these species show intra-specific adaptations to changes in local conditions due to fertilisation management.

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Summary

Background and objectives

Cattle contribute to about 60% of the global livestock manure production. In the Netherlands, most of this is collected as cattle slurry manure (CSM) in cubicle barns. However, the production of solid cattle manure (SCM) has increased in recent years due to a growing number of farmers interested in switching back to straw-based housing systems for reasons of better animal health and welfare. Fertilisation management of grasslands in the Netherlands includes the application of chemical fertilisers, CSM and/or SCM. Long-term application of various combinations of these fertilisers can differentially influence the soil physical, chemical and biological environment, thereby affecting the decomposition and nitrogen (N) mineralisation of organic matter from added manure. This could be one of the reasons of the observed large variations in N recovery from SCM (20-50%) during the year of application. A high short-term N recovery is important to maximise the fertiliser value and reduce environmental N losses during the growing season. Therefore, the challenge is to unravel if long-term fertilisation management of grasslands influences soil chemical and biological properties that are important for decomposition and nitrogen mineralisation and, as a consequence, might affect herbage N recovery from SCM **(Chapter 1)**. Thus, the main objective of this PhD thesis is to investigate the effect of fertilisation history (SCM and organic-N rich CSM vs. mineral-N rich CSM with or without chemical fertiliser-N) on abundance, biomass, and activity of the soil biota and their role in soil N mineralisation as well as SCM decomposition, nitrogen mineralisation and herbage N uptake in production grasslands.

The specific objectives of this thesis are:

1. To study the effects of fertilisation history on soil biota abundance, and soil organic matter N mineralisation (**Chapter 2**).
2. To relate the actual soil natural N mineralisation as estimated from herbage N uptake in unfertilised grassland to estimations of the yearly potential N mineralisation as determined by i) laboratory soil incubation studies, ii) production-ecological model calculations, and iii) calculations according to the Dutch fertilisation recommendation for grasslands **(Chapter 2).**

3. To investigate whether differences in long-term fertilisation management between production grasslands result in farm-specific manure decomposition, N release and N uptake **(Chapter 3),** and, whether this can be related to farm-specific adaptation of the soil biota community (**Chapter 4).**
4. To study the effects of liming and earthworm density on herbage N recovery from SCM in an acidic peat grassland **(Chapter 5).**

To pursue the objectives, a number of field experiments on grasslands with contrasting fertilisation management histories **(Chapters 2, 3 and 4)** and a mesocosm experiment with undisturbed soil cores from an acidic peat grassland **(Chapter 5)** were conducted. The field experiments examined effects of prevailing fertilisation management (SCM and organic-N rich CSM vs. mineral-N rich CSM with or without chemical fertiliser) on bacterial and fungal biomass as well as on earthworm, enchytraeid and microarthropod abundance and soil pH in production grasslands on sand and peat soils. Manure exchange experiments were imposed to investigate adaptation of the grassland soil biota to long-term farm-specific fertilisation management, and its consequences for decomposition, N release and herbage N recovery. Two types of manure, i.e. a composted SCM and a stacked SCM, which differed in chemical quality, were exchanged between the farms. The effects of specific soil biota body-size groups on SCM dry matter (DM) and N disappearances were investigated by using litterbags with different mesh sizes. In the mesocosm experiment with undisturbed soil cores, soil acidity and earthworm density were manipulated to study their effects on SCM decomposition, N release and manure N recovery in acidic peat grassland.

Major findings

Fertilisation management history of grasslands significantly affected abundance and biomass of earthworms and enchytraeids. The abundance and biomass of these organisms was higher in SCM grasslands compared to grasslands with a history of cattle slurry application with or without chemical fertiliser (non-SCM). Continued application of SCM also increased the natural soil fertility, i.e. soil N mineralisation (measured as herbage N uptake), compared to non-SCM grassland on sandy soil. However, in peat

grasslands with a SCM or non-SCM input history soil N supply did not differ. The results of production-ecological model calculations (comprising data of bacteria, fungi, protozoa, enchytraeids, and earthworms) for the natural N mineralisation potential of the soil were very close to the actual field measurements. On average, the model estimated 105% of the measured net herbage N uptake from soil. Therefore, our results showed that production ecological-modelling can give a reliable estimate of the natural soil N supply capacity. Furthermore, combining the estimated N mineralisation by only earthworms based on production-ecological model calculations with the potential soil N mineralisation as measured in laboratory incubation, explained 98-107% of the measured herbage N uptake from soil. In contrast, the currently used method in the Dutch fertilisation recommendations underestimated soil N supply capacity of our grasslands with, on average, about one-third **(Chapter 2)**.

DM and N disappearances and herbage N recovery from SCM were higher in SCM grasslands than in non-SCM grasslands **(Chapter 3)**. This difference was strongly affected by 3 times higher SCM disappearance rates due to meso- and macrobiota **(Chapter 4),** and supported by higher abundances of these organisms in the SCM grasslands (**Chapter 2)**. However, microbiota did not contribute to the differences in manure decomposition between SCM and non-SCM grasslands **(Chapter 4)**, nor did microbial biomass differ **(Chapter 2)**. Manure exchange experiments showed that for the SCM grasslands, the SCM decomposition and N mineralisation was 20 and 14% higher, respectively, when the SCM was applied at the home farm compared to the away farm, indicating a home field advantage (HFA). As a result, a higher herbage N recovery from SCM was found when applied to the home farm grassland **(Chapter 3)**. In SCM grasslands, all body size classes of soil biota contributed to higher DM and N disappearance rates from the home SCM. However, their contributions varied depending upon the stage of decomposition **(Chapter 4)**. During the first four months, HFAs for both DM and N disappearance were greater than in the subsequent stages of decomposition **(Chapter 4).** The decrease in HFA was related to consistent changes in the chemical composition of the manure. After 120 days, the C:N ratio of SCM remaining in the litterbags that were applied at home was significantly higher compared to the C:N

ratio of SCM applied away. However, after 240 days this difference in C:N ratio was disappeared **(Chapter 4).**

Herbage N uptake from SCM was positively correlated with earthworm density (R^2= 0.92). A higher earthworm abundance did not increase N_2O emission in the manured grassland swards, probably due to a higher proportion of endogeic than epigeic species. Liming of low-pH grassland increased the herbage N recovery from SCM from 19 to 28% and coincided with a higher soil biota activity measured as CO_2 emission and a lower microbial biomass N, which will have contributed to a lower nitrogen immobilisation. However, the increase in earthworm populations in the absence of liming did not stimulate the herbage N recovery **(Chapter 5).**

Our results indicate that long-term application of SCM increases soil biota abundance **(Chapter 2)** which stimulates SCM decomposition and nutrient release, and thereby stimulating N uptake and N recovery in SCM grasslands **(Chapter 3)**. In contrast, long-term application of cattle slurry with or without chemical fertiliser application decreases soil biota abundance and, in peat grassland, soil pH **(Chapter 2).** In the latter case, both liming and artificially increased earthworm population densities contributed to an increase of the herbage N uptake and N recovery in the year of manure application **(Chapter 5).**

Overall conclusions

This PhD research resulted in the following general conclusions.

- Multi-year application of solid cattle manure to grasslands increase the number and biomass of detritivorous soil biota (earthworms and enchytraeids) compared to grasslands with a history of cattle slurry with or without chemical fertiliser inputs.
- Herbage N uptake from SCM is highly correlated with earthworm abundance.
- A combination of soil N prediction tools, i.e. the combined use of aerobic soil incubation methods and production-ecological model calculations using earthworm abundance data, can be useful to better predict natural soil N supply.

- Home field advantages (HFAs) are found for DM and N disappearance rates and herbage uptake of mineralised N from SCM and their magnitudes (13 to 53%) are larger than those reported from natural ecosystems (-9 to 29%).
- The magnitude of the HFA is larger during the early stages of SCM decomposition and decreases as decomposition proceeds. This can be related to changes in the chemical composition (C:N ratio) of the SCM during the decomposition process.
- Soil biota and the chemical composition of SCM are amongst the main drivers of HFA. Fertilisation history directly impacts on soil biota community composition and, thereby, indirectly controls the decomposition of, and N mineralisation from applied SCM in agro-ecosystems.
- In acidic grassland, liming increases herbage N recovery from SCM through direct and indirect effects i.e., liming and SCM application stimulate earthworm abundance, thereby affecting SCM decomposition and N mineralisation.
- Site-specific fertilisation management of agro-ecosystems influences the composition and functioning of the soil biota communities which affect C and N release.

Implications for grassland management

The results obtained from the experiments during this PhD research have various implications for the management of production grasslands.

- Long-term application of SCM is a promising management strategy to improve soil biota abundance, and will contribute to an increased net N mineralisation rate from organic inputs.
- The combination of soil N tests using aerobic incubation methods and production-ecological modelling to calculate N excretion from earthworms is an attractive method in terms of simplicity and labour intensity to accurately predict soil N supply in production grasslands.
- Use of liming is recommended to improve the nitrogen recovery of SCM/ organic fertilisers in acidic grasslands under organic management.

- DM and N disappearances as well as herbage uptake of N from SCM were higher on SCM farms than on non-SCM farms. This suggests that farmers will not achieve a higher grassland production already in the year of conversion of their fertilisation management from cattle manure slurry with and without artificial fertilisers to a management based on the application of SCM.

Overall, the results of this thesis research contribute to the development of innovative fertilisation strategies in production grassland ecosystems.

Samenvatting

Achtergrond en doelstellingen

Rundvee draagt wereldwijd voor ongeveer 60% bij aan de productie van dierlijke mest. In Nederland wordt rundveemest grotendeels geproduceerd als drijfmest (RDM) in ligboxenstallen. De productie van vaste rundveemest (VRM) is de afgelopen jaren echter toegenomen. De reden hiervan is de groeiende interesse van boeren in de herintroductie van strostallen vanwege de betere leefomstandigheden voor het vee.

Nederlandse graslanden worden doorgaans bemest met kunstmest, RDM en/of VRM. De toediening van combinaties van deze mestsoorten leidt tot uiteenlopende effecten op de fysische, chemische en biologische eigenschappen van de bodem. Dit heeft consequenties voor de netto mineralisatie van stikstof uit de toegediende rundveemest. Tevens kan dit verklaren waarom er grote variaties zijn gevonden in stikstofbenutting van VRM op grasland tijdens het jaar van toediening in diverse proeven (20 tot 50%).

Een hoge stikstofbenutting op de korte termijn is belangrijk om de stikstofverliezen naar het milieu tijdens en na het groeiseizoen te reduceren. Daarom is het van groot belang om de invloed van verschillende bemestingsstrategieën in kaart te brengen. Hierbij zal specifiek gekeken moeten worden naar chemische en biologische bodemeigenschappen die van belang zijn voor de decompositie en stikstofmineralisatie van VRM en de stikstofbenutting door de plant beïnvloeden (Hoofdstuk 1).

Het hoofddoel van deze dissertatie is te onderzoeken wat de effecten zijn van de bemestingsgeschiedenis (toepassing van VRM en RDM die rijk is aan organische stikstof vs. toepassing van RDM met een hoog mineraal stikstofgehalte en kunstmeststikstof) op de diversiteit, biomassa en activiteit van het bodemleven en hun bijdrage aan bodemstikstofmineralisatie,

decompositie en stikstofmineralisatie van VRM, en stikstofopname door de graslandvegetatie in productiegraslanden.

De specifieke doelen van deze dissertatie zijn:

1. Het bestuderen van de effecten van de bemestingsgeschiedenis van productiegrasland op de diversiteit, biomassa en talrijkheid van het bodemleven en de omvang van de stikstofmineralisatie uit organische stof.
2. Het relateren van de natuurlijke bodemstikstofmineralisatie, gemeten als de stikstofopname van onbemest grasland, aan jaarlijkse schattingen van potentiële stikstofmineralisatie zoals bepaald door:
   (i) Incubatiestudies in het laboratorium;
   (ii) Productie-ecologische modelberekeningen;
   (iii) Berekeningen gebaseerd op de Nederlandse bemestingsaanbevelingen voor graslanden (Hoofdstuk 2).
3. Het onderzoeken of verschillen in lange-termijn bemestingsmanagement van productiegraslanden resulteren in bedrijfsspecifieke afbraak van mest, stikstoflevering en stikstofopname (Hoofdstuk 3), en of dit gerelateerd kan worden aan een bedrijfsspecifieke adaptatie van het bodemleven (Hoofdstuk 4).
4. Het bestuderen van de effecten van bekalking en aantallen regenwormen op de stikstofbenutting door planten uit VRM op veengrasland met een relatief lage pH (Hoofdstuk 5).

Om deze doelstellingen te bereiken werden diverse veldexperimenten uitgevoerd op graslanden met een sterk uiteenlopende bemestingsgeschiedenis (Hoofdstukken 2, 3 en 4). Tevens werd er een experiment uitgevoerd met ongestoorde bodemkolommen van laagveengrasland met een relatief lage pH die naar Wageningen werden getransporteerd voor een bekalkings- en regenwormenexperiment (Hoofdstuk 5). Het effect van de bemestingsgeschiedenis (toepassing van VRM en RDM met een hoog organisch stikstofgehalte vs. toepassing van RDM met een hoog aandeel minerale stikstof en kunstmest) op de biomassa

en aantallen van bacteriën, schimmels, regenwormen, potwormen, mijten en springstaarten is d.m.v. veldexperimenten in productiegraslanden op zand- en veengrond onderzocht. Experimenten met mestuitwisseling tussen rundveebedrijven zijn uitgevoerd om de lange-termijnadaptatie van het bodemleven aan het bedrijfsspecifieke mestmanagement te onderzoeken. Ook de consequenties voor decompositie, stikstofbeschikbaarheid en stikstofbenutting werden onderzocht. Hiertoe werden twee typen mest, namelijk gestapelde VRM en gecomposteerde VRM, welke verschilden in chemische kwaliteit, uitgewisseld tussen de rundveebedrijven. De effecten van het bodemleven op de verdwijning van droge stof en stikstof uit toegediende VRM zijn onderzocht met behulp van nylonzakjes gevuld met mest die op de graslandbodem werden gelegd. Door toepassing van zakjes met verschillende maaswijdtes kon onderscheid gemaakt worden in de bijdrage van diverse grootte-groepen van bodemorganismen (respectievelijk macrofauna, mesofauna en microbiota). In het bodemkolommenexperiment werden de bodemzuurgraad en de regenwormendichtheid gemanipuleerd om het effect op de afbraak en stikstofmineralisatie van VRM en de stikstofbenutting door de grasvegetatie te bestuderen in een relatief zuur laagveengrasland.

Belangrijkste bevindingen van het proefschrift

De bemestingsgeschiedenis van grasland had een significant effect op de abundantie en biomassa van regenwormen en potwormen. De aantallen en biomassa van deze organismen waren hoger in graslanden die gedurende lange tijd waren bemest met vaste rundermest dan in graslanden met een historie van toepassing van runderdrijfmest al dan niet in combinatie met kunstmest.

Productie-ecologische modelberekeningen, die gebaseerd waren op het voorkomen van bacteriën, schimmels, protozoën, potwormen en regenwormen, lieten zien dat de gemodelleerde natuurlijke potentiële stikstofmineralisatie goed overeen kwam met de daadwerkelijk gemeten waarden in het veld. De schattingen van het model bedroegen gemiddeld 105% van de in het veld gemeten netto stikstofopname door de grasvegetatie. Onze resultaten laten daarmee zien dat productie-ecologische modellen een betrouwbare schatting kunnen geven van het natuurlijke stikstofleverende vermogen van agrarisch beheerde graslandbodems. Een veel toegepaste praktische methode om het natuurlijke stikstofleverende vermogen te schatten is gebaseerd op incubatie van landbouwgrond (zonder regenwormen) in een laboratoriumopstelling. Deze methode resulteerde in een onderschatting van de stikstofmineralisatie in de door ons onderzochte graslandbodems. Door echter de resultaten van de incubatieproef te combineren met die van de productie-ecologische modelberekeningen (voor alleen regenwormen) ging de schatting van de stikstofopname door het gewas omhoog tot 98-107%. Dit staat in schril contrast tot schattingen op basis van de Nederlandse bemestingsaanbevelingen voor graslanden die een structurele onderschatting opleverden van ongeveer 30% (Hoofdstuk 2).

De gewichts- en stikstofafname van vaste rundermest en de stikstofopname door de grasvegetatie vanuit de vaste rundermest waren hoger in VRM-graslanden dan in niet-VRM graslanden (Hoofdstuk 3). Het verschil werd sterk beïnvloed door een drie keer hogere gewichts- en stikstofafname uit vaste rundermest in aanwezigheid van meso- en macrofauna (Hoofdstuk 4). Microbiota bleken niet bij te dragen aan de verschillen in mestdecompositie tussen VRM en niet-VRM graslanden (Hoofdstuk 4).

Deze resultaten sluiten aan op de waargenomen verschillen in de abundantie van bodembiota in VRM en niet-VRM graslanden, waarbij hogere dichtheden van meso- en macrofauna maar niet van microbiota werden vast gesteld in de VRM graslanden (Hoofdstuk 2). Uitwisseling van mest tussen VRM-bedrijven liet zien dat de decompositie en stikstofmineralisatie van vaste rundermest respectievelijk 20 en 14% hoger was wanneer de mest werd toegediend op het thuisbedrijf, iets dat een zogenaamd 'thuisvoordeel' indiceert. Als gevolg van het thuisvoordeel was ook de stikstofbenutting vanuit de mest hoger in het grasland van het rundveebedrijf waar de mest werd geproduceerd (Hoofdstuk 3). De bijdrage van de bodembiota aan het thuisvoordeel bleek echter afhankelijk van het decompositiestadium van de mest (Hoofdstuk 4). Gedurende de eerste vier maanden na mesttoediening was het thuisvoordeel voor zowel de gewichtsafname van de mest als het verdwijnen van stikstof uit de mest groter dan in de vier maanden daarna (Hoofdstuk 4). Het kleinere thuisvoordeel later in het seizoen stemde overeen met eenduidige veranderingen in de chemische samenstelling van de vaste rundermest. Na 120 dagen was de C:N verhouding van de in de nylonzakjes achtergebleven vaste rundermest van het thuisbedrijf significant hoger dan de C:N verhouding van de mest afkomstig van het andere bedrijf. Na 240 dagen was dit verschil echter geheel verdwenen (Hoofdstuk 4).

In het experiment met ongestoorde bodemkolommen die afkomstig waren uit een relatief zuur laagveengrasland bleek dat de stikstofopname door het gewas vanuit vaste rundermest positief gecorreleerd was met de experimenteel verhoogde regenwormdichtheid (R^2=0.92) (Hoofdstuk 5). In tegenstelling tot onze verwachting resulteerde de experimentele verhoging van het aantal regenwormen in combinatie met toepassing van vaste

rundermest niet in een verhoogde N_2O emissie. Mogelijk hing dit samen met het relatief hoge aandeel van endogeïsche ten opzichte van epigeïsche regenwormsoorten. Bekalking van het relatief zure veengrasland resulteerde in een toename van de stikstofbenutting door de grasvegetatie vanuit de vaste mest met 19 tot 28%. Ook resulteerde bekalking in een hogere microbiële activiteit en tegelijkertijd in een lagere microbiële biomassa, wat geleid kan hebben tot een lagere stikstofimmobilisatie en daarmee tot een hogere stikstofbenutting door het gewas. Indien er niet werd bekalkt, bleek een experimentele toename van de regenwormpopulatie niet te leiden tot een verhoogde stikstofbenutting door het gewas (Hoofdstuk 5).

Onze resultaten suggereren dat het toepassen van vaste rundermest in productiegrasland op de lange termijn de abundantie van het bodemleven vergroot (Hoofdstuk 2). Dit stimuleert de afbraak van de vaste rundermest en het vrijkomen van nutriënten, waardoor het de N-opname en N-benutting in VRM-graslanden verhoogt (Hoofdstuk 3). Anderzijds lijkt het gebruik van runderdrijfmest, al dan niet in combinatie met kunstmest, juist te resulteren in een afname van de abundantie van het bodemleven. Tevens lijkt dit op veengrond samen te gaan met een daling van de bodem-pH (Hoofdstuk 2). In het relatief zure veengrasland leidde bekalking in combinatie met een kunstmatige verhoging van de regenwormabundantie tot een verhoogde stikstofopname door het grasgewas en een verhoogde stikstofbenutting in het jaar van mesttoediening (Hoofdstuk 5).

Algehele conclusies

Aan dit promotieonderzoek kunnen de volgende conclusies worden ontleend:

- Meerjarige toediening van vaste rundermest aan graslanden leidt in vergelijking tot graslanden met een historie van runderdrijfmest al dan niet met kunstmest tot grotere aantallen en een hogere biomassa van de saprovore bodemfauna (regenwormen en potwormen).
- De stikstofopname door het gewas vanuit vaste rundermest is sterk gecorreleerd met de abundantie van regenwormen.
- Het integreren van indicatoren voor het voorspellen van het stikstofleverend vermogen van graslandbodems kan bijdragen aan een verbeterde voorspelling. Dit geldt in het bijzonder voor het gecombineerde gebruik van schattingen gebaseerd op aërobe grondincubatiemethoden en productie-ecologische modelberekeningen met abundantiegegevens van regenwormen.
- Langdurige toepassing van bedrijfseigen mest kan resulteren in een versnelde incorporatie van mest en stikstof in de bodem en een verhoogde stikstofbenutting door de plant. Dit zogenaamde 'thuisvoordeel' varieerde van 13 tot 53% en was groter dan de tot op heden gepubliceerde cijfers voor natuurlijke ecosystemen (-9 tot 29%).
- Het thuisvoordeel is groter gedurende de eerste stadia van de decompositie van vaste rundermest en neemt af naarmate de decompositie vordert. Deze afname van het thuisvoordeel valt samen met structurele veranderingen in de chemische samenstelling (C:N verhouding) van de vaste rundermest gedurende het afbraakproces.
- Het thuisvoordeel wordt in belangrijke mate gestuurd door de aanwezige bodembiota en de chemische samenstelling van de vaste rundermest. De samenstelling van de bodemlevengemeenschap wordt beïnvloed door het mestmanagement van het agrarische bedrijf. Daarmee heeft het mestmanagement direct en indirect invloed op de afbraak en N-mineralisatie van toegediende vaste rundermest in agro-ecosystemen.
- In verzuurd veengrasland kan bekalking de stikstofbenutting door planten vanuit vaste rundermest direct en indirect beïnvloeden. Het gecombineerd toepassen van kalk en vaste mest stimuleert de abundantie van regenwormen, wat leidt tot een verhoging van de decompositie en stikstofmineralisatie van vaste rundermest.

- Locatie-specifiek mestmanagement in agro-ecosystemen beïnvloedt de samenstelling en het functioneren van de bodemlevensgemeenschap en daarmee de koolstof- en stikstofbeschikbaarheid in de bodem.

Implicaties voor het beheer van productiegrasland

- Langdurige toepassing van vaste rundermest is een veelbelovende strategie om de abundantie van het bodemleven te verhogen. Dit zal bijdragen aan een toename van stikstofmineralisatie uit de toegediende organische meststoffen.
- Het integreren van de standaard grondincubatiemethode en productie-ecologische modelberekeningen aan regenwormen lijkt een aantrekkelijke strategie om relatief eenvoudig, doch accuraat, het stikstofleverend vermogen van een graslandbodem te voorspellen.
- Bekalking van relatief zure biologisch beheerde graslanden is een goede strategie om de stikstofbeschikbaarheid uit vaste rundermest (VRM) en andere organische meststoffen te verhogen.
- Toepassing van vaste rundermest op niet-VRM bedrijven resulteert in het significant langzamer verdwijnen van de mest en de daarin aanwezige stikstof dan op VRM-bedrijven. Ook de stikstofopname door het gewas is lager op niet-VRM bedrijven dan op VRM-bedrijven. In zijn algemeenheid draagt dit promotieonderzoek bij aan de ontwikkeling van innovatieve bemestingsstrategieën in agrarische productiegraslanden.

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Muhammad Imtiaz Rashid

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CURRICULUM VITAE

Muhammad Imtiaz Rashid was born on 1 February 1983 in Chak No. 42/K.B Tehsil Burewala District, Vehari (Pakistan). He completed his primary and higher secondary education at Government M.C. Model High School and Degree College, Burewala. Thereafter, he joined the University of Agriculture at Faisalabad in 2002 and completed his BSc (Hons.) Agriculture with major Soil and Environmental Sciences in 2006. During his B.Sc. he performed an internship at Ayub Agriculture Research Institute, where he worked on effects of wheat genotypes on soil physical and chemical properties. In late 2007, he got a scholarship for MSc leading to PhD from the Higher Education Commission (HEC) of Pakistan and joined the Organic Farming Systems group, which is now known as the Farming Systems Ecology group at Wageningen University, the Netherlands. He started his first year (qualifying year for admission to PhD) by undertaking MSc courses. For his MSc thesis, he worked on the interaction between soil and manure types in affecting manure N mineralisation and plant N recovery. In 2008, he got admission to the PhD defence at Wageningen University under the supervision of Dr. Egbert A. Lantinga, Dr. Ron G.M. de Goede and Prof. dr. Lijbert Brussaard. During his PhD, he worked on soil biota and nitrogen cycling in production grasslands with different fertilisation histories. Meanwhile he was an active member of the PhD Council (PPC) and the Education Committee of the graduate school Production Ecology and Resource Conservation.

PUBLICATIONS

Referred scientific journal:

Shah, G.M., **M.I. Rashid,** G.A. Shah, J.C.J. Groot, and E.A. Lantinga, (2012). Mineralization and herbage recovery of animal manure nitrogen after application to various soil types. Plant and Soil. DOI 10.1007/s11104-012-1347-8

Rashid, M.I., R. G.M. de Goede, L. Brussaard, and E A. Lantinga, (2013). Home field advantage of cattle manure decomposition affects the apparent nitrogen recovery in production grassland. Soil Biology & Biochemistry 57 320-326.

Rashid, M.I., R. G.M. de Goede, L. Brussaard, J. Bloem, E. A. Lantinga, Production-ecological modelling explains difference between potential soil N mineralisation and actual herbage N uptake. *Submitted*

Rashid, M.I., E. A. Lantinga, L. Brussaard, and R. G.M. de Goede, Soil biota composition and manure quality are drivers of the home field advantage for solid cattle manure decomposition in production grasslands. *In preparation*

Rashid, M. I., G. A. C. Nuñez, R.G.M. de Goede, L. Brussaard and Egbert A. Lantinga., Soil pH and earthworms affect herbage nitrogen recovery from solid cattle manure in production grassland. *Submitted*

Conference proceedings:

Rashid, M.I. and E.A. Lantinga. 2010. Impact of fertilization history of grasslands on apparent nitrogen recovery from recently applied solid cattle manure. In: S. Keestra, and G. Mol (Eds.), Proceedings of the Wageningen soil meeting. 18–22 September 2011, Wageningen, the Netherlands, pp. 242.

Shah, G.A., G.M. Shah, **M.I. Rashid,** J.C.J. Groot, and E.A. Lantinga. 2012. Effects of bedding additives on N losses during storage of cattle straw manure and maize N recovery after field application. Published in the proceedings of the international conference on "Global assessment for organic resources and waste management" held in Rennes, France in June 2012.

Shah, G.A., **M.I. Rashid,** J.C.J. Groot, P.W.G. Groot Koerkamp, and E.A. Lantinga. 2012. Bedding additives reduce NH_3 losses during storage and improve N utilization from cattle straw manure applied to grassland. Published in the proceedings of the 17th Nitrogen workshop, held in Wexford, Ireland in June 2012.

Shah, G.A., **Rashid, M.I.,** Groot, J.C.J., Groot Koerkamp, P.W.G. & Lantinga, E.A. (2012). Effects of bedding additives on N losses during storage of cattle straw manure and maize N recovery after field application. In *Proceedings of international symposium on emissions of gas and dust from livestock*. St. Malo, France. June 2012.

PE&RC PhD Training Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (5 ECTS)

- Role of soil biota in the decomposition and N dynamics of different types of dairy manures

Writing of project proposal (4.5 ECTS)

- Biology of decomposition and nitrogen mineralization of solid cattle manure in production grasslands

Post-graduate courses (5 ECTS)

- Multivariate analysis; PE&RC (2009)
- Soil ecology: taking global issues underground; WGS (2010)
- Imaging science; WGS (2012)

Laboratory training and working visits (0.3 ECTS)

- Role of soil biota in the nitrogen recovery of organic manure; Louis Bolk Institute, Driebergen, the Netherlands (2009)

Deficiency, refresh, brush-up courses (3 ECTS)

- Soil quality (2007)
- Crop ecology (2008)
- Nutrient management (2008)
- Analysis and design of organic farming systems (2008)
- Biological interactions in soil (2008)
- Advanced statistics (2008)

Competence strengthening / skills courses (2.4 ECTS)

- Techniques for writing and presenting a scientific paper; WGS (2009)
- Information literacy, including Endnote; WUR Library (2010)
- Scientific publishing; WGS (2009)
- How to write a world class paper; WUR Library (2010)
- Reviewing a scientific paper; PE&RC (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.7 ECTS)

- PE&RC Weekend (start) (2008)
- PE&RC Days (2009-2012)
- PE&RC Weekend (end) (2011)

Discussion groups / local seminars / other scientific meetings (7.2 ECTS)

- Statistics, maths and modelling in Production Ecology and Resource Conservation (2010-2011)
- Plant-Soil Interactions (2010-2012)
- Global Soil Fertility seminar (2011)
- Soil Ecology meeting (2011)
- Biochar: the soil is the limit (2012)

International symposia, workshops and conferences (8.8 ECTS)

- British Soil Science Society: Soils and Ecosystem Services: the challenges for science, management and policy (2011)
- Wageningen meeting: Soil Science in changing world (2011)
- 17th International Nitrogen Workshop (2012)
- 4th International Congress Eurosoil (2012)

Supervision of a MSc student (3 ECTS)

- Soil pH and earthworm abundance increase solid cattle manure decomposition and herbage apparent nitrogen recovery