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NITROGEN DYNAMICS IN CONVENTIONAL AND ORGANIC CROPPING SYSTEMS

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List of abbreviations

0N	unfertilized controll
AF	aggregate fraction
Amd	amendment
ANI	added nitrogen interaction
BEDN	bacterial and endogenous debris nitrogen
BIOORG	bio-organic cropping system
C	carbon
Ca	calcium
CONMIN	conventional cropping system
CPMAS-NMR	magic angle spinning nuclear magnetic resonance
CS	cropping system
DM	dry matter
DON	dissolved organic matter
HF	heavy fraction
iPOM	intra-aggregate particulate organic matter
LF	light fraction
MF	mineral-associated organic matter fraction
Mg	magnesium
MineralN	mineral fertilizer ($^{15}NH4^{15}NO_3$)
MIT	mineralization-immobilization turnover
N	nitrogen
^{15}N	stable isotope of nitrogen
N_2	elemental nitrogen
Na	sodium
nd	not determined
NDF	neutral detergent fiber
Ndfle	N derived from the labeled component of the fertilizer/amendment
NEL	net energy for lactation
NH_3	ammonia

NH_4^+	ammonium
NO_3^-	nitrate
NO_2^-	nitrite
ns	not significant
Nsym	symbiotically fixed N
OM	organic matter
P	phosphorus
P	probability level
POM	particulate organic matter
Py-GC/MS	Curie-point pyrolysis-gas chromatography/mass spectrometry
S	sulphur
SDF	size density fraction
SEM	standard error of mean
SlurryF	^{15}N-labeled feces + unlabeled urine
SlurryU	^{15}N-labeled urine + unlabeled feces
SOM	soil organic matter
UDN	undigested dietary nitrogen
WSN	water-soluble nitrogen

Abstract

Intensification of agricultural production has greatly increased fluxes of nitrogen (N) between different compartments of the biosphere and more specifically emission of N compounds from agroecosystems with detrimental effects on the environment. In order to enhance fertilizer N use efficiency and reduce N losses to the environment and to sustain soil N stocks, understanding of N dynamics of animal manure and mineral fertilizer in agricultural cropping systems has to be improved. The impact of a long-term bio-organically (BIOORG) and conventionally (CONMIN) managed cropping system (receiving exclusively mineral fertilizer) and of different N sources (e. g. urine, feces, mineral fertilizer) on N dynamics were investigated in a field experiment. N use efficiency of animal manure and mineral fertilizer was assessed over three vegetation periods (wheat-soybean-maize), for a single application of ^{15}N-labeled slurries (either urine or feces labeled) and ^{15}N-labeled mineral fertilizer to microplots installed in the BIOORG and CONMIN soil. ^{15}N-labeled urine and feces were obtained from a sheep fed on ^{15}N-labeled ryegrass hay. The fate of fertilizer-N not taken up by the crops was investigated by determining ^{15}N recovered in the topsoil (0-18 cm) and by tracking incorporation of fertilizer-^{15}N into different physically separated soil organic matter fractions (SOM). Changes in soil N stock of BIOORG and CONMIN were assessed by calculating N budgets and monitoring soil N content over a 26-years period.

Using ^{15}N-labeled ruminant manure in tracer studies requires uniform labeling of the manure components. Unequal ^{15}N-enrichment of feces fractions indicated that feces were not uniformly labeled. Still, the ^{15}N-enrichment of total feces N could be used to estimate N use efficiency of feces in slurry, because the enrichment of mineralized fecal N was not significantly different from the enrichment of total fecal N. Characterization of feces by Curie-point pyrolysis-gas chromatography/mass spectrometry and solid-state ^{15}N nuclear magnetic resonance spectroscopy revealed that most of the N-containing compounds in feces derived from proteins. In total 50.3%, 40.5% and 14.8 % of ^{15}N applied as mineral fertilizer, urine and feces was recovered by the three crops, with highest recoveries (10-47%) in the year of fertilizer application. In the subsequent years residual fertilizer N effect was low (< 3.5%). Despite higher microbial biomass size and

activity in BIOORG than CONMIN fertilizer N use efficiency by crops was predominantly affected by the form of fertilizer N applied and not by the cropping system. At the end of the third vegetation period 29.4%, 40.1% and 60.7% of mineral fertilizer-, urine- and feces- ^{15}N remained unaccounted, i. e. was not recovered in the three crops nor in the topsoil (0-18 cm). Similar amounts of N were retained in the topsoil independent of the N source applied. Most of fertilizer-derived N was incorporated into the macro-aggregates, which contained 67% of total soil N emphasizing their importance for N storage. The major N sink was the mineral-associated organic matter fraction. After dispersion (i. e. break up of aggregate structure) 37-55% of ^{15}N was lost from the macro-aggregates emphasizing the importance of aggregation for N protection.

Estimated yearly N deficit in the topsoil obtained from the N budget was -77 ±17 kg N ha^{-1} for BIOORG and -89 ±10 kg N ha^{-1} for CONMIN. Measured yearly decrease in topsoil N content was -29 kg N ha^{-1} for BIOORG and -39 kg N ha^{-1} for CONMIN. Hence, decrease in topsoil N stocks was overestimated by the N budget probably because of uncertainties in the budget caused by underlying assumptions, sampling errors and recycling of N. Repeated manure application in BIOORG which was expected to increase SOM and in parallel N content did not cause an increase in soil N stock in BIOORG. Despite inclusion of grass-clover mixtures and green manure crops in BIOORG and CONMIN and application of animal manure in BIOORG, the maintenance of soil N stocks may be difficult due to the high N mineralization potential of the Loess soil at this experimental site. Ongoing decrease of soil N stock may only be countered by reducing soil tillage activities to minimize release of N protected in soil aggregates.

N dynamics was shown to be mainly affected by the form of fertilizer N applied and to a lesser extend by the cropping system. To better synchronize N release with crop demand and thus improving fertilizer N use efficiency a better understanding of N dynamics particularly of recalcitrant organic N compounds contained in manure is still required.

Zusammenfassung

Die Zunahme von Stickstoff(N)-Emissionen aus Agrarökosystemen als Folge der Intensivierung der Agrarproduktion wirkt sich schädlich auf die Umwelt aus. Um die N-Ausnutzungseffizienz von Düngern zu erhöhen und die N-Emissionen zu verringern und um den Boden-N-Vorrat zu erhalten, muss die N-Dynamik von Hof- und Mineraldüngern in landwirtschaftlichen Anbausystemen besser verstanden werden. In einem Feld-Versuch wurde der Einfluss eines langzeit organisch-biologisch (BIOORG) und langzeit konventionell (CONMIN) (ausschliesslich Verwendung von Mineraldünger) bewirtschafteten Anbausystems sowie der Einfluss verschiedener N-Quellen (z. Bsp. Harn, Kot, Mineraldünger) auf die N-Dynamik untersucht. Die N-Ausnutzungseffizienz von Hof- und Mineraldünger wurde über drei Vegetationsperioden (Weizen-Soja-Mais) ermittelt. Dazu wurde ^{15}N-markierte Gülle (entweder Harn oder Kot markiert) sowie ^{15}N-markierter Mineraldünger als einmalige Gabe auf Mikroparzellen, welche im BIOORG und CONMIN angelegt wurden, ausgebracht. Der ^{15}N-markierte Harn und Kot stammten von einem mit ^{15}N-markiertem Heu gefütterten Schaf. Um den Verbleib des von den Pflanzen nicht aufgenommenen N zu ermitteln, wurde die Wiederfindung des Dünger-^{15}N im Oberboden (0-18 cm) sowie dessen Inkorporation in verschiedene organische Bodenfraktionen bestimmt. Der Einsatz von ^{15}N-markierter Gülle zur Bestimmung der N-Ausnutzungseffizienz setzt voraus, dass die in der Gülle enthaltenen Komponenten homogen markiert sind. Die unterschiedliche ^{15}N-Anreicherung verschiedener Kotfraktionen wies darauf hin, dass der Kot nicht homogen markiert war. Die Anreicherung des Kots konnte aber dennoch zur Abschätzung der N-Ausnutzungseffizienz verwendet werden, da sich die Anreicherung von mineralisiertem Kot-N nicht signifikant von der Anreicherung im gesamten Kot unterschied. Analysen am Kot mittels Pyrolyse-Gaschromatographie/ Massenspektrometrie und Festphasen-NMR zeigten, dass der grösste Teil der N-haltigen Verbindungen im Kot von Proteinen stammt. Durch die Feldfrüchte wurde 50.3% des Mineraldünger-^{15}N, 40.5% des Harn-^{15}N und 14.8% des Kot-^{15}N aufgenommen, davon der weitaus grösste Anteil (10-47%) im Ausbringungsjahr. Die Dünger-N-Nachwirkung in den nachfolgenden zwei Jahren war mit < 3.5% gering. Trotz der höheren mikrobiellen Aktivität im Boden von BIOORG

als CONMIN wurde die Dünger-N-Ausnutzungseffizienz hauptsächlich durch die Form der applizieren Dünger und weniger durch das Anbausystem beeinflusst. Am Ende der dritten Vegetationsperiode konnte 29.4% des Mineraldünger-^{15}N, 40.1% des Harn-^{15}N und 60.7% des Kot-^{15}N weder in den Feldfrüchten noch im Oberboden wieder gefunden werden. Unabhängig von der applizierten N-Quelle wurde ungefähr dieselbe Menge an Dünger-N im Oberboden zurückgehalten. Die Inkorporation von Dünger-N erfolgte hauptsächlich in die Makroaggregate, welche 67% des gesamten Boden-N enthielten und somit einenwichtigen N-Speicher darstellen. Die wichtigste N-Senke war die Schluf-/Tonfraktion des Bodens. Durch die Dispersion der Aggregate wurde ein beachtlicher Anteil (37-55%) von zuvor vor allem in den Makroaggregaten geschütztem Dünger-^{15}N freigesetzt. Das durch die N-Bilanz geschätzte jährliche N-Defizit belief sich für BIOORG auf -77 ±17 kg N ha^{-1} und für CONMIN auf -89 ±10 kg N ha^{-1}. Die gemessene jährliche Veränderung im Boden-N-Gehalt betrug -29 kg N ha^{-1} für BIOORG und -39 kg N ha^{-1} für CONMIN. Das N-Defizit wurde in der Bilanz überschätz. Annahmen und Schätzungen, die der N-Bilanzierung zugrunde liegen, sowie internes Recycling von N können zu Ungenauigkeiten in der Bilanz führen. Der wiederholte Eintrag von organischem Material durch die Hofdünger in BIOORG führte nicht wie erwartet zu einem Anstieg des Boden-N-Vorrates. Trotz mehrjähriger Kunstwiese und dem Anbau von Zwischenfrüchten in der Fruchtfolge von BIOORG und CONMIN und der Applikation von Hofdüngern in BIOORG könnte es wegen des hohen Mineralisierungspotentials des Loess-Bodens an diesem Standort schwierig werden, den Boden-N-Vorrat zu erhalten. Ein Fortschreiten der Abnahme des Boden-N-Vorrates kann möglicherweise nur dadurch verhindert werden, dass die Intensität der Bodenbearbeitung (v. a. Pflügen) drastisch reduziert wird und somit auch die Freisetzung von in Bodenaggregaten geschütztem N. Die N-Dynamik wurde vornehmlich durch die Art der ausgebrachten Dünger und in geringerem Mass vom Anbausystem beeinflusst. Damit die Freisetzung von Dünger-N besser mit dem N-Bedarf der Pflanzen abgestimmt werden kann, was wiederum zu einer Erhöhung der N-Ausnutzungseffizienz führt, muss vor allem die N-Dynamik von persistenten organischen N-Verbindungen in Hofdüngern besser verstanden werden.

General introduction

Nitrogen dynamics in agroecosystems

Nitrogen (N) plays a key role in agriculture because it is a major yield-limiting nutrient in crop production and because plant N provides the basis of dietary N (protein) of animals and humans (Hauck, 1990; McNeill and Unkovich, 2007). N dynamics in agroecosystems includes different pools and different N forms resulting from different transformation processes (e. g. reduction, oxidation). The most important N fluxes are shown in Figure I1. N enters the soil-plant system trough application of organic fertilizer (e. g. animal manure, composts, crop residues) and/or mineral fertilizer, by atmospheric N deposition and symbiotic N_2-fixation by legumes. Plants take up N primarily from the mineral soil N pool in form of direct available inorganic ammonium (NH_4^+) or nitrate (NO_3^-) ions. Uptake of ammonium and nitrate by the plants depends on the concentration of these ions in the soil solution, on root distribution, on soil water content and on plant growth rate (McNeill and Unkovich, 2007). With a proportion of generally less than 1% of soil N in the soil solution this direct plant-available N pool represents only a small fraction of total soil N (Stockdale et al., 2002) and can limit plant growth. Native soil N as well as applied N inputs contributes to the mineral soil N pool (Cassman et al., 2002). The largest soil N pool is the soil organic matter (SOM) containing most of the soil N reserves (McNeill and Unkovich, 2007; Stockdale et al., 2002). SOM is very heterogeneous and consists of fractions differing in composition, biological function and stability. Amount of organic N in soils can vary between 2-10 T kg N ha^{-1} (Power and Doran, 1984). Most of this organic soil N has first to be transformed into plant-available mineral N forms before it can be taken up by plants. Soil microbial biomass plays a key role in the release of mineral N from freshly applied organic sources and from native SOM (Jarvis, 1996). Within SOM the soil microbial biomass represents a small pool which nevertheless is of prime importance because the living soil microorganisms provide enzymes for the decomposition of organic N and the dead microbial biomass represents a readily available soil N pool (McNeill and Unkovich, 2007). N mineralization by heterotrophic soil microorganisms results in the release of ammonium. Ammonium can further be oxidized via nitrite (NO_2^-) to nitrate by autotrophic or heterotrophic soil microorganisms. Simultaneously N can be immobilized by the soil microbes by

assimilation of mineral N. This process is called mineralization-immobilization turnover (MIT). MIT is influenced by crop management strategy and environmental conditions (Cassman et al., 2002). Immobilization can also occur non-biologically by fixation of NH_4^+ into clay lattices and adsorption to organic matter (OM) (McNeill and Unkovich, 2007). Both biological and non-biological immobilization causes a decrease in the ammonium concentration in the soil solution. Immobilized N can be returned to the available soil N pool by re-mineralization. Elemental atmospheric N_2 can be reduced to NH_3 by specialized microorganisms. As NH_3 is rarely released by healthy N-fixing cells it must pass through an organic form before it enters the N cycle (Schulten and Schnitzer, 1998). N which is not taken up by plants or immobilized in SOM risks being lost from the soil-plant system by leaching of nitrate or by denitrification and volatilization.

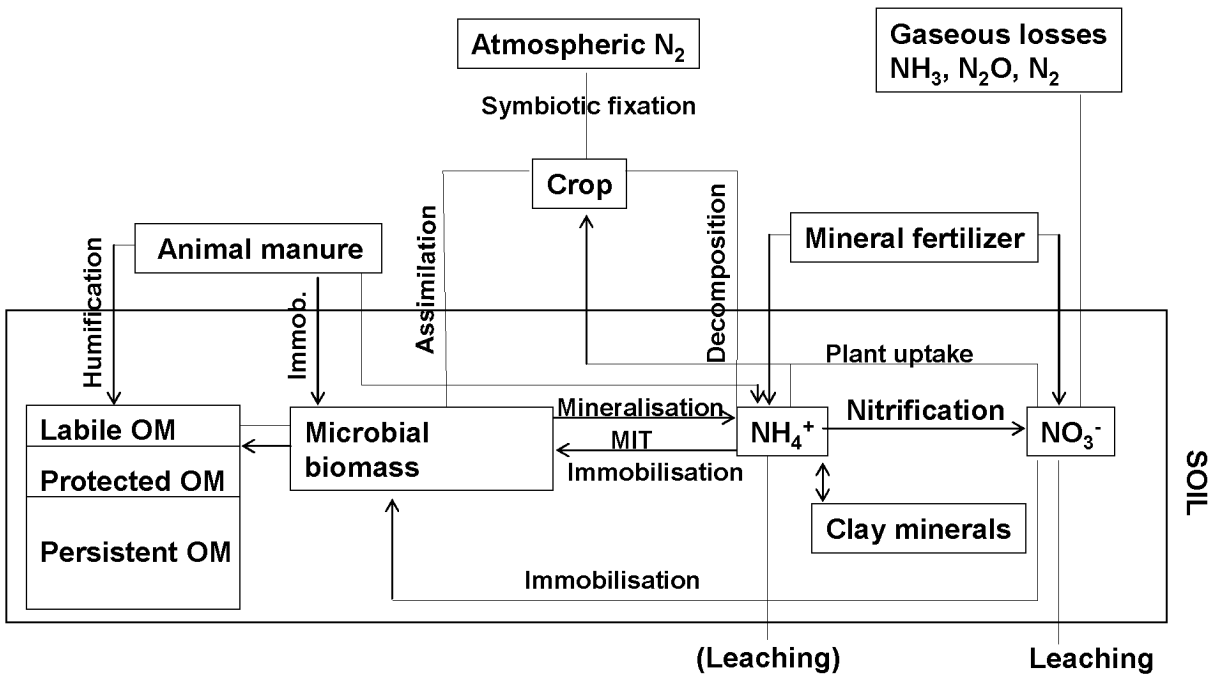

Figure I1: N fluxes and pools of the N cycle.

Motivation for this study

Mineral N fertilization has substantially contributed to increase crop yields by the past 50 years. In parallel, intensive animal production relying on purchased feed resulted in marked increases in milk and meat production (Brown et al., 1998). These practices greatly increased the fluxes of N between the different compartments of the biosphere and more specifically the emission of N compounds from agroecosystems which provoked detrimental effects on the environment (e. g. emission of green house gases, contamination of surface and ground water) (Matson et al., 1997; Tilman et al., 2002). One of the biggest challenges in agriculture is to increase production capacity of agricultural systems and at the same time minimizing N losses to the environment (Hauck, 1990; Tilman et al., 2002). Organic farming is seen as a possible way to reduce N losses from agroecosystems (Dalgaard et al., 1998; Drinkwater et al., 1998; Hansen et al., 2000). In contrast to conventional farming the use of mineral fertilizers is prohibited in organic farming (IFOAM, 2005). Hence, organic cropping systems heavily rely on organic N sources (e.g. animal manure, composts, crop residues, symbiotic N_2-fixation). Consequently, N availability in organic farming is more dependent on microbiologically driven soil processes such as N mineralization from SOM than is the case for conventional farming. Repeated manure application and incorporation of animal and crop residues usually increase organic N content in SOM (Pimentel et al., 2005; Power and Doran, 1984; Tilman, 1998). N release from freshly applied organic N sources or from SOM is hardly predictable and estimation of plant availability of manure N is difficult (Sørensen et al., 1994a). Avoiding N losses from the cropping system and ensuring adequate crop nutrition during the growth season requires optimal use of the manure N. It was shown that around 30% of total N applied with manure and around 50% of total N applied with mineral fertilizer is taken up by crops (Matson et al., 1997; Sørensen and Jensen, 1998; Tilman et al. 2002). N that is not taken up by crops can be incorporated and stabilized to different degree into different SOM fractions. N that is not immobilized in soil or taken up by crops is susceptible to be lost from the system. In order to enhance fertilizer N use efficiency and thus minimizing N losses to the

environment, understanding of N dynamics of animal manure and mineral fertilizer in agricultural cropping systems has to be improved.

Description of the field experiment

The DOC (bio-**d**ynamic, bio-**o**rganic, **c**onventional) long-term field experiment was established in 1978 in Therwil (7°33' E, 47°30' N) near Basel (Switzerland) and is maintained by Acroscope Reckenholz-Tänikon Research Station (ART, Zurich) and the Research Institute of Organic Agriculture (FiBL, Frick). The soil is a loamy silt Typic Hapludalf (USDA, 1999) developed on loess in a temperate climate. The history, conception and experimental design of the field experiment have been described in detail by Mäder et al. (2006). Briefly, two conventional and two organic cropping systems are being compared since 1978. All treatments are cultivated in four replicates in the field with the same seven year crop rotation in a latin square split-split-plot design. The crop rotation which is a compromise between organic and conventional rotations is conducted on three timely postponed rotation units (a, b and c). For this study we included the bio-organic (BIOORG) and a conventional (CONMIN) cropping system. The two cropping systems mainly differ in fertilization strategy and plant protection (Table I1). Crop rotation (including intercrops), crop varieties, residue management and ploughing intensity (frequency, depth) are the same in BIOORG and CONMIN. Mechanical weeding is conducted more frequently in BIOORG than CONMIN. The crops of the four rotation periods are listed in Table I1. In 1977, the year before the field experiment started, the area was cropped with grass-clover. Between 1957 and 1973 the land was under arable crop rotation (including grass-clover ley and manure amendment) and between 1973 and 1976 field vegetables and grain crops were cultivated based on integrated production without manure amendment (Fliessbach et al., 2007). BIOORG is managed according to bio-organic guidelines since 1978 (VSBLO, 2003) and gets exclusively organic fertilizers in form of manure (farmyard manure and slurry) with an average organic carbon (C) input of 2240 kg ha^{-1} yr^{-1} (Fliessbach et al., 2007). Farmyard manure and slurry applied to BIOORG are obtained from an organic farm. CONMIN receives exclusively water-soluble mineral fertilizers since 1985 according to official

Swiss fertilization guidelines (Walther et al., 2001) and is otherwise managed according to the rules of integrated plant production (KIP, 1999). During the first crop rotation period (1978-1984) CONMIN was used as non-fertilized control. Average total N inputs are slightly lower for BIOORG than CONMIN (1985-2003) while mineral N applied with manure is clearly less in BIOORG than CONMIN where total N equals mineral N (Table I1). Simple average N budgets of both cropping system, calculated as difference between total N input by fertilizers and N removed with harvested products, are negative (Table I1).

Table I1. Management characteristics of the studied cropping systems. Average rate of N fertilization, simple N budgets for the four crop rotation periods (1978-2003), types of fertilizers applied, plant protection strategy and crop rotations (1978-2005).

Cropping system		Bio-organic BIOORG		Conventional CONMIN
	Crop rotation period			
Nutrient management		Total N	N_{min} in manure	Total N
Average N input (kg ha^{-1} yr^{-1})	1978-1984	120	41	0
	1985-1991	100	32	101
	1992-1998	82	25	151
	1999-2003	149	59	136
Simple N budget[a] (kg ha^{-1} yr^{-1})	1978-1984	-68		-172
	1985-1991	-116		-133
	1992-1998	-89		-70
	1999-2003	-99		-88
Type of manure/fertilizer		Slightly aerobically rotted farmyard-manure and slurry from 1.2 (1978-1991) or 1.4 (since 1992) livestock units ha^{-1} yr^{-1}		Unfertilized from 1978 until 1984; since 1985 exclusively water-soluble fertilizers according to official fertilization guidelines
Plant protection:				
Weed control		mechanical		mechanical, herbicides
Disease control		indirect methods		chemical
Insect control		plant extracts, bio-control		chemical
Crop rotation				
	1978-1984	winter barley (*Hordeum vulgare* L.), two years grass-clover, potatoes (*Solanum tuberosum* L.), (intercrop)[b], winter wheat (*Triticum aestivum* L.), (intercrop), white cabbage (*Brassica vulgaris* L.), winter wheat		
	1985-1991	winter barley, two years grass-clover, potatoes (intercrop), winter wheat, (intercrop), beetroots (*Beta vulgaris* L.), winter wheat		
	1992-1998	three years grass-clover, potatoes, winter wheat (intercrop), beetroots, winter wheat		
	1999-2005	winter wheat, two years grass-clover, potatoes, winter wheat, (intercrop), soybean (*Glycine max* L.), (intercrop), maize (*Zea mays* L.)		

[a] Difference between total N input by fertilizers and N output by harvested products.
[b] Green manure rops or fodder intercrop.

Objective of this thesis

The main objective of this study was to investigate the impact of long-term organic and conventional management on N dynamics in different cropping systems. The study was carried out in the DOC long-term field experiment.

Hypotheses and structure of this thesis

In the first chapter results of characterization of the ^{15}N-labeled feces obtained from a sheep fed on ^{15}N-labeled ryegrass hay were shown. Investigating the fate of manure-derived N in the soil-plant system requires uniform ^{15}N-labeling of ruminant excreta. Thus homogeneity of ^{15}N-labeling of feces was assessed by i) comparing the ^{15}N-enrichment of physico-chemically separated feces fractions, ii) determining the proportion of ^{15}N-enriched N-compounds in feces by Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and iii) comparing the enrichment of mineralized fecal N with the enrichment of total feces-N. Additionally homogeneity of ^{15}N-labeling of ryegrass was determined by chemical fractionation and Py-GC/MS. Py-GC/MS furthermore provided information about organic N-compounds contained in feces and ryegrass and about pathways underlying ^{15}N-enrichment of feces. Characterization of feces was completed by solid-state ^{15}N cross polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy.

The second chapter is dedicated to the N use efficiency of animal manure and mineral fertilizer and the fate of fertilizer N not taken up by the crops. Both, sheep slurries where either feces or urine was labeled with ^{15}N and ^{15}N-labeled mineral fertilizer was deployed as a single application to microplots installed in the soil of CONMIN and BIOORG. Over three consecutive years (2003-2005) the recovery of urine-, feces- and mineral fertilizer-^{15}N in the crops (winter wheat - soybean - maize) and the soil was quantified. To study N use efficiency we assessed the proportion of N in the crops derived from the applied fertilizers and calculated the recovery of fertilizer-N in the plant biomass and the soil. Because of a higher microbial activity and biomass in the soil of BIOORG than CONMIN

and the lower amount of mineral N applied with organic fertilizers in BIOORG we hypothesized the N use efficiency of mineral fertilizer and manure and the recovery of fertilizer-N in soils of the two cropping systems to be different.

The third chapter deals with the effect of long-term organic matter (OM) management and different soil biological activity on the incorporation of N added with organic and mineral amendments into soil organic matter (SOM) fractions. After harvest of winter wheat in 2003 soil was sampled from the microplots and physically separated into macro-, micro-aggregates and microstructures. Aggregates were then fractionated into free light fraction (LF), intra-aggregate particulate organic matter (iPOM) and the mineral-associated organic matter fraction (MF). Amendments and soil biological activity is expected to affect the incorporation of OM into the different SOM fractions. Thus we hypothesized that i) the distribution of N among the SOM fractions differs between CONMIN and BIOORG soils due to long-term manure input in BIOORG and ii) the incorporation patterns of freshly applied ^{15}N-labeled animal manure and mineral fertilizer N differ because of different forms of N applied with the amendments and because of OM input with animal manure.

The fourth chapter concerns the impact of long-term organic and conventional N management on soil N stock. The change in soil N stock of the organically and conventionally managed cropping system was estimated by calculating soil-surface N budgets for each of the four crop rotations and a total N budget over a 26-years period (1978-2003). The estimated change in soil N stock obtained from the total N budget was compared with the measured change in soil N stock of the topsoil (0-20 cm) which was assessed by the difference in soil N content at establishment of the field experiment in 1977 and on soil sampled in 2003. Based on soil samples taken in 1977, 1984, 1987, 1991, 1994, 1998 and 2003 the change in N stock of the topsoil of BIOORG and CONMIN was moreover followed. Furthermore change in N stock of the subsoil (30-50 cm) of BIOORG and CONMIN between 1977 and 1998 was investigated. In contrast to CONMIN repeated input of organic N sources over long-term to the soil of BIOORG is expected to maintain or increase soil N stock.

In the general conclusions, the results from the four chapters are synthesized with respect to i) how efficiently different N sources applied to the soil of BIOORG and CONMIN are used by crops, ii) the fate of manure- and mineral fertilizer-N not taken up by the crops and iii) the impact and sustainability of the investigated cropping systems.

Chapter 1: Characterization of ^{15}N-labeled ruminant excreta to be used for nitrogen cycling studies

Abstract

Investigating the fate of manure-derived nitrogen (N) in the soil-plant system requires uniform ^{15}N-labeling of ruminant excreta. We studied homogeneity of ^{15}N-labeling of feces obtained from a sheep fed on ^{15}N-labeled ryegrass hay by i) comparing the ^{15}N-enrichment of physico-chemically separated feces fractions, ii) determining the proportion of ^{15}N-enriched N-compounds in feces by Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and iii) comparing the enrichment of mineralized fecal N with total feces-N. Homogeneity of ^{15}N-labeling of ryegrass was verified by chemical fractionation and Py-GC/MS. Py-GC/MS furthermore provided information about organic N-compounds contained in feces and ryegrass and about pathways underlying ^{15}N-enrichment of feces. Characterization of feces was completed by solid-state ^{15}N cross polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy. Unequal ^{15}N-enrichment of feces fractionations revealed that feces were not uniformly labeled. That also applies to ryegrass. Still, the ^{15}N-abundance of total feces-N could be used to determine the N use efficiency of manure because it was in the range of mineralized fecal N. Most of N-containing pyrolysis products in ryegrass and feces derived from proteins. This was confirmed by high relative intensity of about 70% at -262 ppm in the NMR spectrum assigned to amid functional groups in proteinaceous material. Py-GC/MS suggest that enrichment of feces can be ascribed to excretion of highly resistant peptides contained in ryegrass and of de novo synthesis of proteins.

Introduction

^{15}N-labeling of ruminant excreta represents an appropriate tool to improve the knowledge of manure nitrogen (N) turnover in agricultural cropping systems (Jensen et al., 2000). Using ^{15}N-labeled ruminant manure in tracer studies requires a uniform labeling of the manure components (Powell and Wu, 1999; Sørensen and Jensen, 1998). According to Sørensen and Jensen (1996) urine can be considered as uniformly labeled as only few days after starting ^{15}N-feeding the enrichment of urinary total N and

NH_4-N produced after urine storage was nearly the same. Obtaining feces with homogenous labeled organic N fractions is much more difficult than for urine (Sørensen and Thomsen, 2005a). The homogeneity of ^{15}N-labeling of fecal N depends on the duration of feeding ^{15}N-labeled forage to the animal, on the type of diet and on the metabolism of the individual animal (Powell et al., 2004; Powell and Wu, 1999). Heterogeneity in the labeling of manure components can be caused by i) feeding non-uniform labeled forage resulting from isotopic fractionation during enzymatic incorporation into the plant cell (Macko et al., 1987; Werner and Schmidt, 2002) ii) the different metabolic pathways in the animal where different dietary-N compounds are exposed to (Leng and Nolan, 1984; Nolan and Leng, 1972) and iii) dilution of N by endogenous secretion into the gut e. g. in form of enzymes (Sørensen and Thomsen, 2005a). Thus testing homogeneity of ^{15}N-labeling of feces is important as non-uniform labeling may result in an over- or underestimation of the mineralized amount of fecal N in the soil (Powell et al., 2004; Sørensen et al., 1994b). The homogeneity of ^{15}N-labeling of feces can be determined by either comparing the ^{15}N-abundance of physico-chemically separated feces fractions (Langmeier et al., 2002) and/or the ^{15}N-abundance of mineralized fecal N incubated in quartz sand with the abundance of total feces-N (Sørensen et al., 1994b). Also determining the proportion of ^{15}N-enriched N-containing pyrolysis products in feces identified by Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) can provide information about the homogeneity of ^{15}N-labeling. Pyrolysis degrades macromolecules thermolytically into small volatile fragments by physical cleavage of its chemical bonds (Schulten, 1996; Zang and Hatcher, 2002). The fragments are assumed to be characteristic of the original structure (Galletti and Bocchini, 1995; Schulten, 1996). Pyrolysis is used to study the structure of N in the organic matter of soils and water (Coban-Yildiz et al., 2000; Kögel-Knabner, 2000; Leinweber and Schulten, 1999; Poirier et al. 2005) and in plant material (Galletti et al., 1993; Van Arendonk et al., 1997) and to identify the composition of the macromolecules and analyze their fragments (Galletti and Bocchini, 1995; Knicker, 2004; Veeken et al., 2001). Identification of distinct N-compounds using Py-GC/MS thus can provide information about the composition of forage and feces on a molecular level.

Furthermore results from Py-GC/MS allow the elucidation of pathways of ^{15}N-enrichment during the passage of ^{15}N-labeled forage through the digestive tract of the animal.
Solid-state ^{15}N cross polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy can complete characterization of feces on a structural level. ^{15}N-CPMAS-NMR generally is applied for structural characterization of soil organic matter, plant composts and humic compounds in soils (Knicker et al., 1993; Kögel-Knabner, 1997; Preston, 1996). Solid-state NMR spectroscopy is a non-invasive method which allows examining solid samples in its original state unaffected by chemical or physical pretreatment (Knicker and Nanny, 1997; Kögel-Knabner, 1997). NMR spectroscopy takes advantage of the magnetic properties of NMR-active nuclei and provides chemical structure information (Hatcher et al., 2001; Preston, 1996).

Even though pyrolysis was used for analyzing the composition of ruminant feedstuff (Reeves and Francis, 1997) we could find no study where Py-GC/MS and ^{15}N-CPMAS-NMR were used to characterize ^{15}N-labeled forage fed to an animal and subsequently the feces of the same animal.
Therefore the objectives of this study were to i) test by different approaches the uniformity of ^{15}N-labeling of sheep feces applied in a field study to estimate N use efficiency of ruminant manure, ii) characterize the feces using Py-GC/MS and solid-state ^{15}N-CPMAS-NMR and iii) elucidate pathways of ^{15}N-enrichment of feces.

Materials and Methods

Terminology

Throughout this paper we present absolute ^{15}N abundance in atom% ^{15}N which denotes the proportion of all N atoms of mass 15 on total N multiplied by 100.

Production of ^{15}N-labeled sheep feces and urine

a) Production of ^{15}N-labeled ryegrass hay

Italian ryegrass (*Lolium multiflorum*, Axis) was grown in PVC-boxes (560 x 360 x 129 mm) that were perforated at the bottom and filled with about 30 kg of quartz sand in the greenhouse under controlled conditions (photoperiod 16 hours, 25℃ daytime and 18℃ nighttime temperature). Each box was fertilized with a nutrient solution containing N and all basic macro- and micronutrients. Nitrogen was applied at an amount of 7 g KNO_3 (15.90 atom% ^{15}N) per box and per cut. The nutrient solution was portioned into two doses. For the first cut the first dose was given after germination, the second dose five to six days later. For the following cuts nutrient solution was given by the same way at the same doses; the first dose directly after cutting, the second dose five to six days later. All micronutrients were applied in the first dose after each cut. Nutrition solution not retained by the ryegrass was collected in boxes installed below each box containing ryegrass. These boxes were filled with 10 l of water to avoid crystallizing of the unused nutrition solution and covered with a black foil to avoid growth of algae. The collected nutrition solution was pumped back daily to the ryegrass. The ryegrass was cut six times with the first cut being done 34 days after germination. All following cuts were done in intervals of 21 days. After drying of each ryegrass cut for 40 hours at 45℃ the six cuts were thoroughly mixed. Unlabeled ryegrass hay was simultaneously produced under identical conditions, except that KNO_3 of natural abundance was used.

b) ^{15}N-labeling of sheep feces and urine

The following sequence of a daily amount of 1.4 kg dry matter (DM) ryegrass hay was fed to an adult male sheep (101 kg live weight): unlabeled ryegrass hay (natural abundance of 0.37 atom% ^{15}N) for seven days to adapt the animal to the forage, followed by nine days feeding ^{15}N-labeled ryegrass hay (14.60 atom% ^{15}N) and, in order to evaluate the evolution of ^{15}N-abundance in the excreta after having stopped ^{15}N-feeding, finally offering again unlabeled ryegrass hay for another 3 days as suggested by Sørensen et al. (1994b). From day six onwards, feces and urine were collected

separately once a day for 14 days and immediately frozen at -20℃ until use. For the collection of feces and urine the sheep was put into a cage (170 cm x 70 cm x 185 cm) which was fitted with an automatic drinking bowl and a feeder for the ryegrass hay. The sheep was standing on a grid, so that urine could flow through this grid into a tray attached below the cage. From the tray urine could drain off through a narrow channel into a can that was cooled from the outside with ice to minimize gaseous N losses. Feces were either collected directly from the grid on which the sheep was standing or from another grid that was affixed above the narrow channel in the tray. Because of animal welfare regulations it was not possible to keep the sheep in the cage over the entire 19 day feeding period. Therefore a quantitative sampling of feces and urine was only possible at days six and seven as well as at days 16, 17 and 18 of the experiment when the highest ^{15}N-enrichment in the excreta was expected. This animal experiment was approved by the Cantonal Veterinary Office of Zug, Switzerland.

Sample preparation and analyses of ryegrass hay

Total N, ^{15}N abundance and mineral content

The ryegrass hay was finely ground using a ball mill Retsch® (Retsch GmbH, Germany) prior to total N and ^{15}N analysis. Total N and ^{15}N abundance analyses were carried out on a continuous flow Roboprep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England).

Concentration of phosphorus (P), magnesium (Mg), calcium (Ca) and sodium (Na) was determined on 0.5 g of dried ryegrass that was ashed at 550 ℃ for 8-10 h. The ash was dissolved in 2 ml 20% HCl, the solution was heated shortly, filtered (Whatmann no. 40) with addition of distilled H_2O and made to 50 ml volume prior to measurement by an ICP-OES Liberty 220 (Varian, USA).

Fractionation

Prior to neutral detergent fiber (NDF) determination the ryegrass hay was milled with a 0.75 mm sieve using a Rotor Mil Retsch® ZM1 (Retsch GmbH, Germany). Nitrogen

bound to neutral detergent fiber (NDF-N) was obtained by boiling 5 g of ryegrass hay with a neutral detergent solution (Van Soest et al., 1991) for 1 h in a Fibretec System M (Tecator Ltd., Höganäs, Sweden). The detergent-insoluble fibrous material was washed with boiling distilled water, dried over night at 103℃ and subsequently milled using a ball mill Retsch® (Retsch GmbH, Germany). Total N and ^{15}N abundance analyses of NDF were carried out on the mass spectrometer previously mentioned.

Py-GC/MS

The milled ryegrass hay was pyrolyzed at 500℃ for 9.9 s in a Curie-point-pyrolyzer Fischer 1040 PSC (Bonn, Germany). The pyrolysis products were separated on a gas chromatograph Varian 3800 (Walnut Creek, USA) equipped with a 25 m capillary column BPX 5 (SGE, Ringwood, Australia) that was coated with 0.25 µm film thickness and had an inner diameter of 0.32 mm. Following split injection up to 45 s (splitless) at 300℃, the split ratio was 1:100 from 45 s up to 90 s and 1:5 from 90 s on. The flow rate of the helium carrier gas was adjusted to 2 ml min^{-1}. The starting temperature for the gas chromatographic program was 28℃ (5 min), and heate d at a rate of 5℃ min^{-1} to 280℃ (30 min). The gas chromatograph was connected to a double focusing Finnigan MAT 212 mass spectrometer (Bremen, Germany). Conditions for mass spectrometric detection in the electron impact mode were 3 kV acceleration voltage, 70 eV electron energy, 2.2 kV multiplier, 1.1 s $(mass\ decade)^{-1}$ scan speed, and *m/z* (mass-to-charge ratio) 48 to 450 mass range. Mass spectra corresponding to peaks in the gas chromatograms were identified by comparison with the Wiley mass spectral library, software edition 6.0. The ^{15}N enriched compounds were identified by an increased isotope peak [M+1] of the corresponding mole peak, when compared to the library spectra.

Sample preparation and analyses of ruminant excreta

Total N and ^{15}N abundance of feces and urine

Thawed feces and urine samples collected from day six onwards were acidified with concentrated H_2SO_4 prior to freeze-drying to minimize gaseous N losses. After freeze-drying the samples were finely ground (except urine) using a ball mill Retsch® (Retsch GmbH, Germany). Comparison of total N amount in freeze-dried feces and urine, respectively, with total N amount in thawed samples determined with a Nitrogen Analyzer 1500 (Carlo Erba, Italy) revealed that despite the addition of H_2SO_4 an average of 5.8% of urinary N was lost during the process of freeze-drying while no N was lost from feces. Total N and ^{15}N abundance analyses of feces and urine was carried out on a continuous flow Roboprep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England).

Fractionation of feces

Feces collected at the end of ^{15}N-feeding (9 days after start of feeding ^{15}N-labeled ryegrass hay) was separated by the method described by Mason (1969) and as modified by Kreuzer and Kirchgessner (1985) into the following fractions according to their origin from the sheep metabolism: i) undigested dietary N (UDN) mainly consisting of undigested lignified cell wall components derived from the plant and thus more or less equivalent to undigested NDF-N, ii) bacterial and endogenous debris N (BEDN) which contains mainly bacterial protein from the rumen, the hindgut and endogenous protein as well as some forage protein and iii) water-soluble N (WSN) mainly originating from endogenous digestive secretions derived from the pancreas, bile and intestinal wall but also from soluble cell content of disrupted microbes. The UDN and water-insoluble N were determined in separate subsamples of a homogenate of 18 g fresh feces and distilled water containing 3-6% DM. The UDN and water-insoluble N fractions were exactly obtained as described by Langmeier et al. (2002). The total amount of N and the ^{15}N-abundance of the UDN and water-insoluble fraction can be determined directly. The values for BEDN were calculated from the water-insoluble N fraction which is composed

of UDN and BEDN. Values for WSN were calculated as difference of total feces-N and water-insoluble N. Sediment containing water-insoluble N of feces was freeze-dried and UDN was dried at 103℃. Subsequently the samples we re finely ground using a ball mill Retsch® (Retsch GmbH, Germany) prior to total N and ^{15}N abundance analyses carried out on a continuous flow Roboprep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England).

Py-GC/MS of feces

Py-GC/MS on freeze-dried and milled feces samples collected at the end of ^{15}N-feeding was carried out as described for ryegrass hay.

Solid-state ^{15}N-CPMAS-NMR of feces

Solid-state ^{15}N-CPMAS-NMR was conducted on sheep feces collected at the end of ^{15}N feeding. The ^{15}N solid-state spectrum was obtained on a Bruker DMX 400 at a frequency of 40.55 MHz with magic angle spinning at 5 kHz using freeze-dried, finely ground and not pretreated feces. A commercial double-bearing probe and a phase-stabilized zirconium rotor with an outside diameter of 7 mm was used. The Hartmann-Hahn contact time was 1 ms during which a ramped ^{1}H-pulse starting at 100% power and decreasing until 50% was applied to circumvent Hartmann-Hahn mismatches (Peersen et al., 1993). Approximately 2000 scans were accumulated using a pulse delay of 0.3 s. Line broadening of 50 Hz were applied prior to Fourier transformation. The relative N distribution was determined by integration of the signal intensity in the different chemical shift regions using an adapted integration routine supplied with the instrument software. The spectrum is referenced to external nitromethane (= 0 ppm).

Incubation experiment

To determine the ^{15}N-abundance of mineralized fecal N, fresh sheep feces collected at the end of ^{15}N-feeding was incubated in quartz sand for 112 days. The incubation period and sampling intervals were the same as those applied in a microplot study carried out in a field experiment where the sheep slurries were used to evaluate the N use efficiency of ruminant manure and the fate of fertilizer N not taken up by the plant in different cropping systems (Chapter 2). To simulate decomposition conditions in soil, 50 g of quartz sand that was sterilized at 105°C for 3 days was inoculated with 2.5 g of soil DM from a bio-organic cropping system containing 3.75 mg N before incubation with the feces. 318.5 mg fresh feces containing 10 mg N was mixed with 50 g of inoculated quartz sand in 250 ml bottles (Sørensen et al., 1994b). The water content was adjusted to 55% of the water-holding capacity of the quartz sand (0.2 ml distilled H_2O g^{-1} quartz sand). To reduce losses of water the bottles were covered by perforated lids of aluminum foil and incubated at 20°C (Sørensen et al., 1994b). The moisture content of the quartz sand was controlled weekly by weighing and if necessary readjusted. At each sampling three replicates of the sand-soil-feces-mixture were extracted with 2 *M* KCl as described by Sørensen et al. (1994b) 11, 47, 75, 97 and 112 days after beginning of incubation. Analogous, as a control treatment, soil was incubated in quartz sand and treated the same as the sand-soil-feces mixture to evaluate the amount of N mineralized from the soil. The ^{15}N-abundance of the mineralized fecal N was corrected for the dilution by mineralized soil N obtained from the control treatment which accounted for 0.04-0.09 mg N g^{-1} soil and caused a dilution that ranged between 2.4-4.8%.

Mineral N (NH_4- and NO_3-N) in the sand-soil-feces mixture and the sand-soil mixture from the incubation experiment was extracted with 2 *M* KCl. NH_4-N and NO_3-N in the KCl-extracts was analyzed colorimetrically on a SAN^{plus} Analyzer (Skalar, Netherlands). ^{15}N abundance analyses of NH_4- and NO_3-N in the KCl-extracts of the sand-soil-feces mixture and the sand-soil mixture was carried out on the mass spectrometer previously mentioned after diffusion of ^{15}N from the KCl-extracts to acidified glass filters (Goerges and Ditter, 1998; Sørensen and Jensen, 1991).

Statistical analysis

Analysis of variance was performed by using the statistical analyses package SYSTAT 11 (Systat Software Inc., USA) to compare the ^{15}N-abundance of the neutral detergent fiber fraction with complete ryegrass hay as well as the ^{15}N-enrichment of the feces fractions and to test the effect of the incubation time on the ^{15}N-abundance of mineralized fecal N. In case of significant effects, separation of means was tested using Tukey's HSD (honestly significant difference) test with a significance level of $P \leq 0.05$.

Results and Discussion

Composition of forage

The ^{15}N-enriched ryegrass hay contained 2.4 % total N in DM (weighted mean over six cuts) with an abundance of 14.6 atom% ^{15}N (weighted mean over six cuts) (Table 1.1). Due to the re-circulation of the nutrient solution in forage production and the cultivation of the ryegrass on quartz sand in order to avoid ^{15}N immobilization by soil microorganisms, 54% (mean over 6 cuts) of the originally applied ^{15}N was recovered in ryegrass shoots (data not shown). The ^{15}N recovery was lowest (29.7%) at the first cut as the root system still was not fully established, and highest (64.5%) at the third cut. The ^{15}N-abundance was lowest at the first cut (13.46 atom% ^{15}N) increasing to 14.46 atom% ^{15}N at the second cut and then remaining between 14.71 atom% ^{15}N and 14.85 atom% ^{15}N at the subsequent cuts (data not shown). For a representative decomposition of the animal excreta applied to the soil an adequate digestibility of the forage is necessary. The composition of the ryegrass hay (Table 1.1) fulfilled the standard of forages for small ruminants (RAP, 1999). From the point of view of animal nutrition feeding only ryegrass hay and extra N-free mineral salt and vitamins to an adult male sheep over the period of experimental duration of 19 days represented a conventional feeding standard.

Table 1.1. Composition of unlabeled and ^{15}N-labeled ryegrass hay (NDF = neutral detergent fiber, OM = organic matter, NEL = net energy for lactation).

Ryegrass	total N	atom% ^{15}N	NDF	OM	NEL	P	Mg	Ca	Na
	g kg^{-1} DM	%	g kg^{-1} DM		MJ kg^{-1} DM	……g kg^{-1} DM……..			
unlabeled	24.9	0.37	492	894	5.3	5.9	4.4	3.4	0.5
labeled	24.3	14.6	550	888	5.0	7.0	4.6	3.6	0.5

Results of the abundance of NDF-N in ryegrass showed that the forage was not homogenously labeled. The ^{15}N-abundance of NDF-N was lower than of total N in ryegrass hay, suggesting that the digestible N must have been higher enriched (Table 1.2).

Table 1.2. Percentage of fraction N on total N and atom% ^{15}N of the ryegrass hay fraction and the different sheep feces fractions collected at the end of the ^{15}N feeding period. Standard deviation is shown in brackets. (n = 3).

	Proportion of fraction N on total N	atom% ^{15}N
	……%……	
Ryegrass hay fraction		
Neutral-detergent fiber bound N (NDF-N)	54.7	13.86 (0.04) b
Total N (TN)	100.0	14.60 (0.50) a
Feces fractions		
Undigested dietary N (UDN)	33.0	10.76 (0.05) b
Bacterial and endogenous debris N (BEDN)	53.1	12.02 (0.16) a
Water-soluble N (WSN)	13.6	9.85 (0.50) c
Total N (TN)	100.0	11.28 (0.03) ab

Means of atom% ^{15}N of ryegrass hay fraction and feces fractions followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test.

Isotopic fractionation during enzymatic reactions in plants has been reported in natural abundance studies (Kolb and Evans, 2003; Yoneyama et al., 1998; Yoneyama and Tanaka, 1999). Indication obtained from fractionation about inhomogeneous labeling of the hay was also expressed in the results from Py-GC/MS. Of N-containing compounds in the forage approximately half were ^{15}N-enriched (Table 1.3).

Table 1.3. N-containing and ^{15}N-enriched pyrolysis products in ryegrass hay and feces tentatively assigned by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), mass weight (MW), elemental composition and possible source of origin.

Pyrolysis products	MW	Elemental composition	Found in	^{15}N-enriched in	Possible source of origin	References
1H-Pyrrole	67	C_4H_5N	RH, F	RH	Pr; AA (hypro/pro)	Kögel-Knabner, 2000; Sorge et al., 1993;
1H-Pyrrole, 1-methyl-	81	C_5H_7N	RH, F	RH, F	Pr; AA (hypro/pro)	Nguyen et al., 2003
1H-Pyrrole, 2-methyl-	81	C_5H_7N	F		Pr; AA (hypro/pro)	Nguyen et al., 2003
1H-Pyrrole, 3-methyl-	81	C_5H_7N	F		Pr; AA (hypro/pro)	Nguyen et al., 2003
1H-Pyrrole, 2,5-dimethyl-	95	C_6H_9N	RH, F	RH, F	Pr; AA (hypro)	Chiavari et al., 1992
Pyrrole, 2,4-dimethyl-	95	C_6H_9N	RH	RH	Pr; AA (hypro)	Chiavari et al., 1992
1H-Pyrrole, 1-ethyl-	95	C_6H_9N	RH, F	RH	Pr; AA (hypro)	Chiavari et al., 1992
Pyrrole, 3,4-diethyl-2-methyl-	137	$C_9H_{15}N$	F		Lig	Van Arendonk et al., 1997; Niemann et al., 1995
1H-Pyrrole, 1-pentyl-	137	$C_9H_{15}N$	F		Lig	Van Arendonk et al., 1997; Niemann et al., 1995
Pyridine	79	C_5H_5N	RH, F	RH	Pr; AA (ala/tyr)	Nguyen et al., 2003; Chiavari et al., 1992
Pyridine, 4-methyl-	93	C_6H_7N	RH, F	F	Pr; AA (ala/cys)	Kögel-Knabner, 2000; Chiavari et al., 1992
Pyridine, 4-methyl-1-oxide-	108	C_6H_7ON	F		Pr; AA (tyr); Lig	Schulten, 1996; Sorge et al., 1993
Pyridine, 2,3-dimethyl-	107	C_7H_9N	RH		Pr; AA (tyr)	Sorge et al., 1993
Pyridine, 2,5-dimethyl-	107	C_7H_9N	RH	RH	Pr; AA (tyr)	Sorge et al., 1993
Pyridine, 5-ethenyl-2-methyl-	119	C_8H_9N	RH		Pr; AA (thr)	Sorge et al., 1993
Pyrimidine, 4-methyl-	94	$C_5H_6N_2$	RH	RH	Pr; AA (hypro/tyr)	Nguyen et al., 2003; Chiavari et al., 1992
1H-Pyrazole, 1-methyl-	82	$C_4H_6N_2$	F		Pr; AA (his)	Sorge et al., 1993
1H-Pyrazole, 3-methyl-	82	$C_4H_6N_2$	RH, F		Pr; AA (his)	Sorge et al., 1993
Pyrazine, methyl-	94	$C_5H_6N_2$	F		Pr; AA (hypro/tyr)	Chiavari et al., 1992
Piperidine, 1-methyl-	99	$C_6H_{13}N$	RH		Pr; peptide	Van Arendonk et al., 1997; Schulten, 1996
1H-Imidazole, 2-ethyl-	96	$C_5H_8N_2$	RH		Pr; AA (lys)	Sorge et al., 1993
Ethanone, 1-(4-methyl-1H-imidazol-2-yl)-	124	$C_6H_8ON_2$	RH		Lig	Poirier et al., 2005; Reeves and Francis, 1997

AA = amino acids, ala = alanine, cys = cysteine, F = feces, his = histidine, hypro = hydroxyproline, Lig = lignin, lys = lysine, Pr = protein, pro = proline, RH = ryegrass hay, thr = threonine, tyr = tyrosine.

Evolution of ^{15}N-abundance in feces and urine

Compared to the ^{15}N-abundance in feces a fast increase in the abundance of urine was observed at the beginning of ^{15}N-feeding. For both, feces and urine, the ^{15}N-abundance increased sharply between day 9 and 10 (Figure 1.1). Two days after start of ^{15}N-feeding the abundance of feces exceeded the one of urine and remained higher during the complete sampling period as shown in other studies with sheep (Sørensen et al., 1994b) or cows (Powell et al., 2004). About 5 days after the start of feeding ^{15}N-labeled forage the further increase in the abundance of urine and feces was only very slight. At the end of ^{15}N-feeding the abundance of urine attained its maximum and decreased immediately when unlabeled forage was fed (Figure 1.1). The abundance of feces, in contrast, continued to increase slightly after terminating ^{15}N-feeding showing that the reaction to ^{15}N-feeding was retarded in feces compared to urine due to the different metabolic pathways (Nolan and Leng, 1972). This was also apparent at the beginning of ^{15}N-feeding. At the end of ^{15}N-feeding the abundance of total urinary N was 60% and that of total fecal N 77% of the abundance of the ryegrass hay (Figure 1.1) most likely due to ^{15}N-dilution by unlabeled endogenous N originating from the animals body. A lower enrichment of feces than forage was also shown by Sørensen et al. (1994b) who fed labeled ryegrass hay during a period of 9 days to an adult male sheep and an abundance lower by 28% and 7% in urine and feces, respectively, than in forage was reported by Sørensen and Thomsen (2005a) who fed a diet containing ^{15}N-labeled barley and peas to growing pigs for 11 days. In order to obtain similar abundances of the excreta (urine and feces) and the forage the ^{15}N feeding period would have had to be much more extended since the entire body protein would have to be exchanged by newly synthesized protein labeled with ^{15}N.

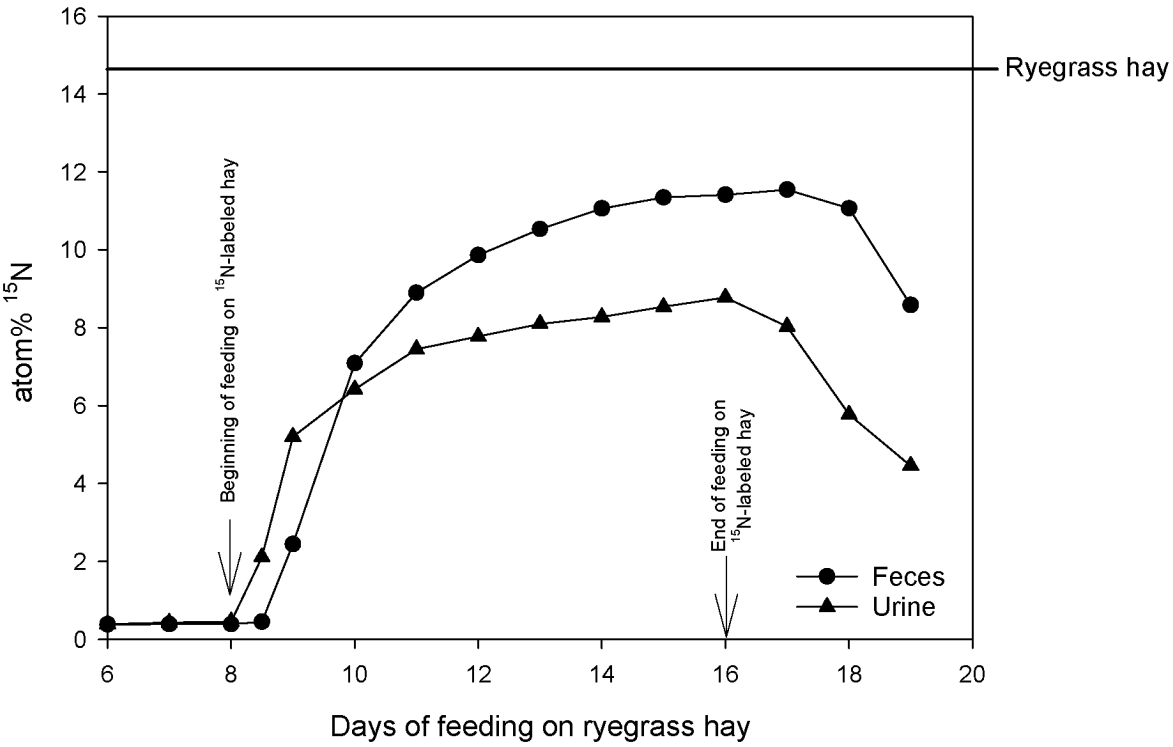

Figure 1.1. Evolution of ^{15}N-abundance in sheep feces and urine during the process of ^{15}N feeding over 19 experimental days (n = 3).

Checking the homogeneity of ^{15}N-labeling of feces

a) ^{15}N-abundance of feces fractions

The BEDN accounted for 53.1%, UDN for 33.0% and WSN for 13.6% to total fecal N (Table 1.2). While the proportion of BEDN in the feces was in the same range as obtained from sheep also fed only with ryegrass hay (Mason 1969; Sørensen et al., 1994b), WSN was only half as much. The UDN was slightly higher than from a sheep that was fed during the same period with ryegrass hay (Sørensen et al., 1994b). The proportion of fecal N fractions is dependent on the type of the diet and on the metabolism of the individual animal (Powell et al., 2004; Powell and Wu, 1999) and the distribution of ^{15}N in these fractions is moreover dependent on the duration of feeding ^{15}N-labeled forage to the animal. The ^{15}N-abundance of the feces fractions decreased

as follows: BEDN ≥ UDN > WSN (Table 1.2). These results are contradictory to Langmeier et al. (2002) who, after a pulse labeling of cow urine and feces, found no significant differences between the enrichment of WSN and BEDN that were more enriched than UDN. Furthermore, Sørensen et al. (1994b) reported a higher enrichment in WSN than non-WSN after feeding an adult castrated sheep during the same period as in our study with ^{15}N-labeled ryegrass hay. Powell and Wu (1999) showed that the ^{15}N-enrichment of endogenous and undigested feed-N in feces was similar 60 h after initiating the feeding of a ^{15}N-enriched mixed diet to cows. Isotopic fractionation in the plant may also contribute to different enrichments of the feces fractions, e. g. explaining the lower abundance of the fecal UDN compared to total N of the ryegrass hay and the higher abundance of the BEDN than the UDN. Given that isotopic fractionation provokes the formation of ^{15}N-enriched and ^{15}N-depleted N compounds, BEDN may become more enriched than UDN as the rumen-degradable dietary protein is mainly used by the microbes in the rumen and the hindgut and BEDN mainly consists of microbial protein. The low abundance of WSN may be due to a dilution by unlabeled N from endogenous digestion secretions of the animal.
However, as no study used exactly the same combination of forage type, ^{15}N-feeding period, animal breed and fractionation method as we did, the results cannot be compared directly.

b) Proportion of ^{15}N-enriched N-containing pyrolysis products in feces

Of the N-containing compounds in feces obtained by Py-GC/MS only one-fifth were ^{15}N-enriched (Table 1.3) supporting the results obtained from the fractionation.

c) Comparison of the ^{15}N-abundance of mineralized fecal N with the ^{15}N-abundance of total feces-N

By adding soil microorganisms to the system, assimilation and nitrification of fecal NH_4-N was accelerated compared to a system containing only animal-derived fecal microorganisms (data not shown) as 12 days after starting the incubation no more NH_4-

N was detectable. Between 5-11% of the organic feces-N was mineralized during the incubation period. The BEDN which accounted for more than half of total fecal N was higher enriched in ^{15}N than UDN and WSN indicating that N from the less enriched WSN was mineralized. Assuming that during incubation mineralization from UDN did not occur due to recalcitrant N-components, WSN and BEDN contributed 39.8% and 60.2%, respectively, to N mineralized from the feces. Despite that feces fractions were differently labeled, ^{15}N-abundance of total feces N could be used to estimate N use efficiency of feces in slurry, because abundance of mineralized fecal N did not vary significantly during the whole incubation period of 112 days (temporal fluctuation < 4.5%) and was not significantly different from abundance of total fecal N (except at day 47 and 97 of incubation) (Table 1.4).

Table 1.4. ^{15}N-abundance of mineralized fecal N incubated in quartz sand inoculated with soil and amount of mineralized fecal N at different incubation times. Standard deviation is shown in brackets. (n = 3).

	atom % ^{15}N of mineralized fecal N		mineral N (NO_3^-)	
	……%.....		…..µg g^{-1} fresh feces….	
Incubation time (days)				
11	10.76 (0.1)	ns	1.4 (0.2)	b
47	11.34 (0.1)	ns	2.1 (0.2)	ab
75	11.30 (0.1)	ns	2.6 (1.0)	ab
97	11.80 (0.2)	ns	3.3 (1.2)	a
112	10.58 (0.3)	ns	1.8 (0.9)	b

Within a parameter means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test. ns = not significant.

Composition of ryegrass hay and feces

In the chromatograms of the pyrolyzed ^{15}N-enriched ryegrass 15 N-containing compounds and of the pyrolyzed ^{15}N-enriched feces 14 N-containing compounds were assigned (Table 1.3), whose relative peak areas to the complete chromatogram accounted for 11.4% and 11.2%, respectively. Some of the N-containing pyrolysis products pyrroles, pyridines and pyrazoles were assigned in the ryegrass hay and in feces (Table 1.3). There are two possible origins of these molecules: i) they can be genuine constituents of the organic materials as all living biomass contains heterocyclic N, and ii) they can be formed due to the flash pyrolysis and electron impact ionization in Curie point Py-GC/MS. On the basis of the (flash) Curie point Py-GC/MS alone it is impossible to distinguish in between these two possible origins (Hatcher et al., 2001; Kögel-Knabner, 2000; Schulten and Leinweber, 2000). The signal assignments (Table 1.5) in the ^{15}N-CPMAS-NMR spectrum (Figure 1.2) call for the predominance of proteinaceous material. Pyrroles were shown to be thermal degradation products of hydroxyproline (Chiavari et al., 1998). Other N-containing pyrolysis products such as pyrimidines and piperdines which also originate from protein-derived amino acids (Niemann et al., 1995; Schulten, 1996; Van Arendonk et al., 1997) were exclusively assigned in the forage and pyrrazine only in feces (Table 1.3). In addition the relative peak area of pyrroles in the NMR spectrum (6.7%) was similar to the relative peak area of pyrroles in the chromatogram (5.3%).The N-containing compounds imidazole and methylimodazol-ethanone are known to derive from cell wall components (Niemann et al., 1995; Reeves and Francis, 1997; Van Arendonk et al., 1997) and were exclusively assigned in the forage (Table 1.3). Furthermore various non N-containing pyrolysis products (data not shown) originating from cytoplasm (e. g. proteins, lipids), vacuoles (e. g. phenolics and alkaloids, organic acids and sugars) and cell wall (e. g. cellulose, lignin, hemicellulose, pectin) of the ryegrass were identified in the hay whereas in feces mainly pyrolysis products originating from cell wall components of the forage were detected.

The NMR spectrum of the sheep feces was dominated by a signal between -220 to -285 ppm, peaking at -262 ppm (Figure 1.2) and assigned to amide N most likely in

proteinaceous material (Knicker et al., 1997) (Table 1.5). It comprises more than 70% of total ^{15}N signal intensity. Solid-state ^{15}N-CPMAS-NMR analysis of ^{15}N-enriched degrading plant residues (incubated for 4 years) demonstrated that despite microbial degradation amide-N remained the main organic form (Knicker, 2000). Apparently, some proteinaceous microbial biomass material was resynthesized but some peptide-like structures were also able to persist microbial degradation possibly by association with refractory biopolymers (Knicker, 2004). This might also apply for peptide N in forage which might either have resisted microbial degradation in the digestive tract of the sheep or originated from resynthesized microbial biomass and was than excreted in feces. The low-field shoulder of the main peak at -262 ppm may cover signals from heterocyclic-N in histidine, nucleic acid derivates and substituted pyrroles in the chemical shift range of -145 to -220 ppm (Knicker et al., 1996). Within this range, a weak signal was detected at a chemical shift of -204 ppm which most likely can be assigned to substituted pyrroles (Knicker et al., 1997) (Table 1.5) and which accounted for 5.3% of total peak area. The resonance line between -300 to -325 ppm and between -325 to -370 ppm is most tentatively assigned to NH_2-groups of basic amino acids which accounted for 2.2% of total peak area and NH_4^+-ions (3.2% of total peak area), respectively (Knicker et al., 1997; Kögel-Knabner, 1997) (Table 1.5).

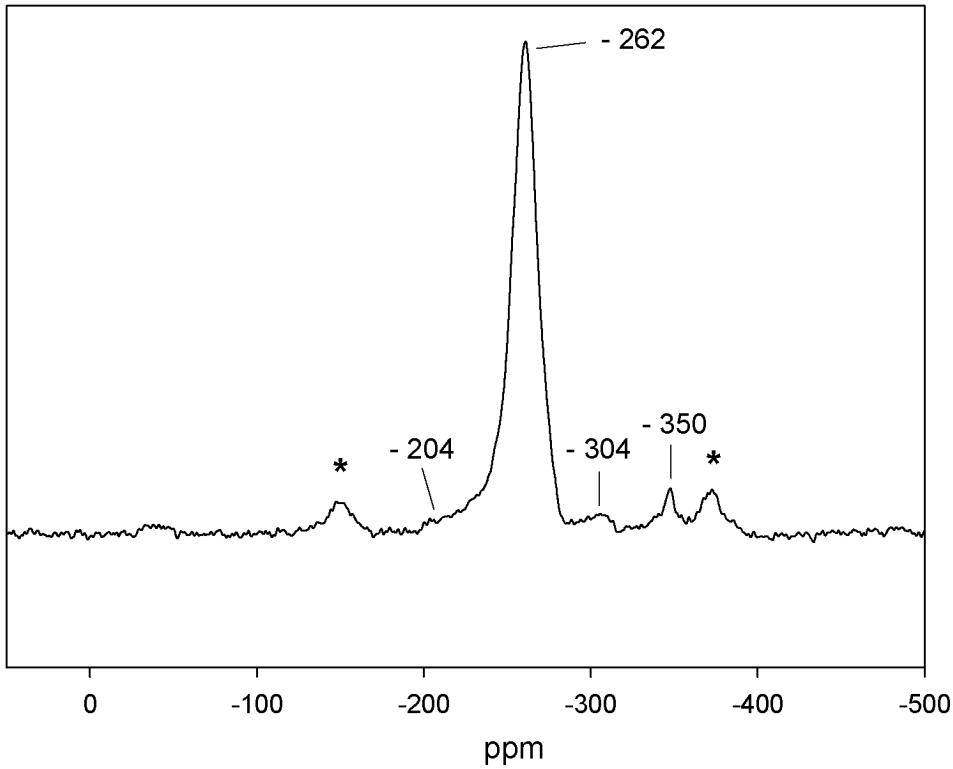

Figure 1.2. Solid-state ^{15}N-CPMAS-NMR spectrum of ^{15}N-enriched sheep feces. The spectrum is referenced to external nitromethane (= 0 ppm). Asterisks (*) indicate spinning side bands.

Table 1.5. Possible assignments for the solid-state ^{15}N-CPMAS-NMR spectrum of the ^{15}N-enriched sheep feces.

Chemical shift range (ppm)	Possible assignment	Reference
-145 to -220	pyrrole, purine/pyrimidine, indole, imidazole, uric acid	Kicker et al., 1997; Knicker and Lüdemann, 1995
-220 to -285	amide/peptide proline peptide bonds	Knicker et al., 1997 Kögel-Knabner, 1997 Knicker et al., 1993
-300 to -325	NH_2 and NR_2 groups	Kicker et al., 1997; Knicker and Lüdemann, 1995
-325 to -370	NH_4^+ free amino groups in amino acids	Knicker et al., 1997 Kögel-Knabner, 1997

Pathways of ^{15}N-enrichment of feces

Analyses of ryegrass and feces by Py-GC/MS gave indications about the metabolic pathways through which dietary N may have been excreted in feces. Of the N-containing pyrolysis products in the ryegrass hay eight were found to be enriched with ^{15}N and thereof two (1H pyrrole,1-methyl- and 1H pyrrole, 2,5-dimethyl-) were also recovered in feces (Table 1.3). Dietary N enters the digestive tract of the sheep as non-protein-N (e. g. nitrate, nucleic acids, urea, amines, amides) and true protein-N (Leng and Nolan 1984). Most non-protein N is converted to ammonia in the rumen whereof a part is taken up by rumen bacteria (Leng and Nolan, 1984). Feed N compounds that are non-degradable in the rumen may also be resistant to digestion in the small intestine (van der Walt, 1993). This insoluble feed N will pass the digestive tract and be recovered as undigested plant N in the UDN fraction of feces. True plant proteins are degraded in the rumen by a two-step process where extracellular proteases, produced by proteolytic microorganisms, break down the protein chains by hydrolysis of the peptide bonds (amide bonds in peptides) resulting in lower molecular weight peptides and amino acids which are then deaminated to ammonia by microbial deaminases (Tamminga, 1979; Zhu et al., 1999). This dietary N will be recovered in the BEDN fraction of feces. The ^{15}N-enriched compound 1H-pyrrole, 1-methyl- which was identified in ryegrass hay and feces was described to originate from protein (Simmonds, 1970) and specified as to be derived from proline by Nguyen et al. (2003). The ^{15}N-enriched 1H pyrrole, 2,5-dimethyl- may originate from hydroxyproline (Chiavari and Galletti, 1992). Ruminal degradation of proline-containing peptides is known to be low (Alcaide et al., 2003; Depardon et al., 1998; Wallace et al., 1993). Apparently certain peptide bonds are highly resistant to ruminal degradation (Yang and Russell, 1992). The occurrence of peptides was confirmed by the high ^{15}N signal intensity in the NMR spectrum of feces at a chemical shift of -262 ppm (Figure 1.2). Thus ^{15}N-enriched proline-containing peptides might have passed the digestive tract unchanged due to the highly resistant peptide bonds and thus will have been recovered in the UDN fraction of feces.

In contrast protein-derived pyridine, 4-methly- was present in the ryegrass hay and in the feces as well, but was found to be ^{15}N-enriched only in feces (Table 1.3). The

occurrence of pyridine, methyl- in the pyrolyzates is described as possibly being derived from cysteine (Chiavari and Galletti, 1992). Cysteine and methionine are sulphur (S)-containing amino acids found in most proteins and S is essential for microbial protein synthesis (Bird and Thornton, 1972). From an isotope study using ^{35}S Bird and Thornton (1972) concluded that up to 24% of fecal ^{35}S could result from the transfer of ^{35}S from the blood into the large intestine where it is then incorporated into cysteine (and methionine) by bacteria present in the large intestine and excreted as BEDN. A significant proportion of S in feces may be thus deriving from endogenous secretion (Kennedy, 1974). Thus pyridine, 4-methyl- may have become ^{15}N-enriched due to de novo synthesis of cysteine (and methionine) by microorganisms and then be excreted either in BEDN or WSN. De novo synthesis of proteins could explain the higher enrichment of BEDN and/or WSN than UDN. The remaining six N-containing pyrolysis products in the ryegrass hay that were also found to be ^{15}N-enriched (Table 1.3) were not identified in feces. These components probably were absorbed and incorporated in the body tissue of the sheep (e. g. wool, muscle) or excreted with urine.

Conclusions

Homogeneous ^{15}N-labeling of ruminant feces is difficult to achieve. First, the ryegrass hay fed to the animal was not homogenously labeled. Second, the inhomogeneity of ^{15}N-labeling of the sheep feces caused by metabolic processes in the animal was shown by unequal ^{15}N-abundance of physico-chemically separated feces fractions and by Py-GC/MS where ^{15}N-enriched N-containing compounds in feces accounted only for 21%. However, ^{15}N-feeding over a nine days period produced feces that could be used in an N-cycling study in the field, because the abundance of mineralized fecal N was not significantly different from the ^{15}N-abundance of total feces-N during an incubation time relevant for the field study. Concerning the level of the abundance of feces and urine the period of ^{15}N-feeding could have been reduced, as six days after having started feeding ^{15}N the abundance increased only slightly until the end of ^{15}N feeding.

Curie-point Py-GC/MS and solid-state ^{15}N-CPMAS-NMR spectroscopy proved to be valuable tools to gain information about the composition and homogeneity of ^{15}N-

labeling of feces. Certainly more detailed information could be achieved by carrying out Py-GC/MS and solid-state ^{15}N-CPMAS-NMR on different N-fractions of the forage and the feces.

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Chapter 2: Nitrogen use efficiency of animal manure and mineral fertilizer applied to long-term organic and conventional cropping systems

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submitted to Nutrient Cycling in Agroecosystems (note: description of DOC field experiment included)

Abstract

Nitrogen (N) utilization by crops has to be improved to minimize losses to the environment. We investigated N use efficiency of animal manure and mineral fertilizer and the fate of fertilizer N not taken up by crops in a conventional (CONMIN) and a bio-organic (BIOORG) cropping system of a long-term field experiment over three vegetation periods (wheat-soybean-maize). Microplots planted with wheat received a single application of ^{15}N-labeled slurries (either urine or feces labeled) or mineral fertilizer. At the end of each vegetation period we tested whether higher microbial activity and larger microbial biomass in BIOORG than CONMIN soils and lower long-term N input level in BIOORG affected use efficiency and fate of fertilizer N not taken up by crops. Recovery of ^{15}N in wheat was 36.6%, 10.0% and 47.1% from urine, feces and mineral fertilizer, respectively, and decreased strongly in the residual years. In total 40.5%, 14.8 % and 50.3% of ^{15}N applied as urine, feces and mineral fertilizer was recovered by the three crops. ^{15}N recovered from originally applied urine, feces and mineral fertilizer in the topsoil (0-18 cm) at the end of the third vegetation period was 19.4%, 24.5% and 20.3%, respectively. Of urine-, feces- and mineral fertilizer-^{15}N 40.1%, 60.7% and 29.4% was not recovered by the three crops and in topsoil suggesting significant transport of ^{15}N-labeled components to deeper soil layers. CONMIN and BIOORG neither affected ^{15}N recovery in soil nor fertilizer N use efficiency by crops which was predominantly affected by the form of fertilizer N applied.

Introduction

The intensification of agricultural production has increased nitrogen (N) fluxes between the different compartments of the biosphere and the emission of N compounds from agroecosystems to the environment (Tilman et al., 2001). Hence N use efficiency of fertilizers by crops has to be improved to minimize losses to the environment (Matson et al., 1997). Organic production systems are generally assumed to be an environmental-friendlier alternative to conventional systems (Pang and Letey, 2000; Reganold et al., 2001) as production is based on the reduction of inputs by recycling of

nutrients in order to conserve natural resources (IFOAM, 2005). The use of synthetic fertilizers is prohibited in organic farming (FAO, 2003). Therefore organic fertilizers (e. g. animal manure, plant residues, composts) are the most important N sources along with the soil organic matter (SOM) and biological N_2-fixation. The organic compounds present in the organic fertilizers and in native SOM have first to be mineralized before getting plant available, rendering their availability dependent on microbial mineralization and immobilization processes (Mary et al., 1996; Rasmussen et al., 1998). Greater soil microbial activity in organically than conventionally cropped soils has been frequently reported (Gunapala and Scow, 1998; Mäder et al., 2002). Nitrogen inputs to organic systems are usually lower than for conventional systems (Kirchmann and Bergström, 2001; Mäder et al., 2006) and a larger N retaining capacity in organically than conventionally cultivated soils has been suggested from long-term observations (Kramer et al., 2002). However, detailed comparison of N use efficiency of freshly applied ^{15}N-labeled mineral and organic fertilizers by crops and the fate of fertilizer N not taken up by the crops in the field under identical climatic and pedological conditions in conventional and organic cropping systems is rare.

The stable isotope ^{15}N is a useful tool to investigate N use efficiency of organic and mineral fertilizer and to trace the fate of N applied to cropping systems (Hauck and Bremner, 1976; Hopkins et al., 1998) provided that organic fertilizers are homogenously labeled (Powell et al., 2005; Sørensen et al., 1994a). However, the use of ^{15}N-labeled animal manure is limited by the high costs and high expenditure of time to produce homogenously labeled manures (Powell et al., 2004; Sørensen et al., 1994b). This limits the surface that can be fertilized in the field.

We carried out a microplot study in cropping systems that are conventionally (CONMIN) or bio-organically (BIOORG) managed since 1978. Because of higher microbial activity and biomass in BIOORG than CONMIN and lower amount of mineral N received with organic fertilizers in BIOORG, we hypothesized different N use efficiency of fertilizer N by the crops and different N recovery in soils of the two cropping systems. To study N use efficiency we assessed the proportion of N in the plant derived from the fertilizer and calculated the recovery of fertilizer-N in the plant biomass. A single application of ^{15}N-labeled animal manure or ^{15}N-labeled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) was carried out at

tillering of winter wheat in 2003 to microplots installed in CONMIN and BIOORG. The fate of this N was studied for three consecutive years. Unfertilized microplots (0N) served as control treatment. All fertilizer treatments, including 0N, were applied to CONMIN and BIOORG.

Materials and Methods

Description of the microplot study

Microplots were installed in December 2002 in the BIOORG and CONMIN plots (main plots) of the rotation unit b of the DOC long-term field experiment. Selected soil properties are given in Table 2.1.

Table 2.1. Selected properties of the topsoil (0-18 cm) of the investigated soils in 2003. (n = 4).

Soil	pH (H_2O)	Total N	Total C	Available nutrients		Microbial biomass[c]		Daily[d] respiration
						March 03		
				P[a]	K[b]	N	C	C
		……g kg^{-1}….....		----------mg kg^{-1}----------				….mg kg^{-1} d^{-1}…..
CONMIN	6.2 b	1.4 a	13 a	9.5 a	106 a	12.5 b	101 a	2.5 b
BIOORG	6.6 a	1.5 a	14 a	5.4 a	111 a	21.8 a	113 a	3.8 a

Data followed by different letters within a parameter indicate significant differences ($P \leq 0.05$) between the two soils (t-test).
[a] Quantity of isotopically exchangeable P within the first minute (Fardeau, 1993).
[b] Extraction with ammonium acetate EDTA (Cottenie et al., 1982).
[c] Microbial biomass N and C were determined by chloroform fumigation (Vance et al., 1987); no conversion factors applied.
[d] Average over a 60 days incubation experiment (Alef and Nannipieri, 1995).

The microplot study started in 2003 (year of fertilizer application) and was continued in 2004 (first residual year) and 2005 (second residual year). Winter wheat (*Triticium aestivum* var. Titlis) was cultivated in 2002/2003 followed by soybean (*Glycine max* var. Mapple Arrow) in 2004 and maize (*Zea mays* var. Gavott) in 2005. Between winter wheat and soybean a green manure mixture mainly consisting of *Phacelia tanacetifolia*

was sown which froze during winter and was incorporated into the soil in April 2004 before sowing of soybean. After harvest of soybean a rye (*Secale cereale*) intercrop was sown, which was mulched before sowing of maize in May 2005. Yields of winter wheat, soybean and maize were assessed in selected areas of the CONMIN and BIOORG main plots defined here as harvest plots (Figure 2.1).

The microplots were defined by frames with a length of 33 cm, a width of 14 cm and a height of 23 cm, and were open at the top and the bottom. The size and location of the microplots within the main plots represented a compromise between making use of ^{15}N-labeled fertilizers and keeping disturbance of the long-term field experiment minimal. It enabled to remove at the end of the three-year study all soil contained in the microplots (about 10 kg per microplot) without seriously affecting the main plots (e. g. without removing too much soil which would have affected overall nutrient stocks). Size and location also were chosen to permit work with natural ^{15}N abundance in the same field experiment (Oberson et al. 2007). In December 2002 the microplots were driven into the soil of the four field replicates of CONMIN and BIOORG main plots to a depth of 18 cm. Within the main plots they were placed at the fifth and sixth row of winter wheat in a distance of 75 cm from the main plot borders to avoid border effects and with a distance of 50 cm between the microplots to avoid cross effects (Figure 2.1). Location of the microplots was optimized with regard to the wheat crop, resulting in about 20 wheat plants per microplot. The microplot location also matched to soybean growing during the following year, since the microplots also encompassed two rows, resulting in two soybean plants per microplot. During the third year of the study when maize was grown, the microplots (containing one maize plant) lay between the maize rows of the main plots because microplots could not be moved. Each fertilizer treatment was repeated twice within each main plot, with one microplot being destined for destructive sampling (disturbed microplots) during the vegetation period of winter wheat while the other was used for plant and soil sampling at maturity of the crops (undisturbed microplots) (Figure 2.1). The disturbed microplots were further used for sampling of green manure in 2003, of soybean at flowering in 2004 to estimate N_2-fixation (Oberson et al., 2007), and of the rye intercrop in 2004.

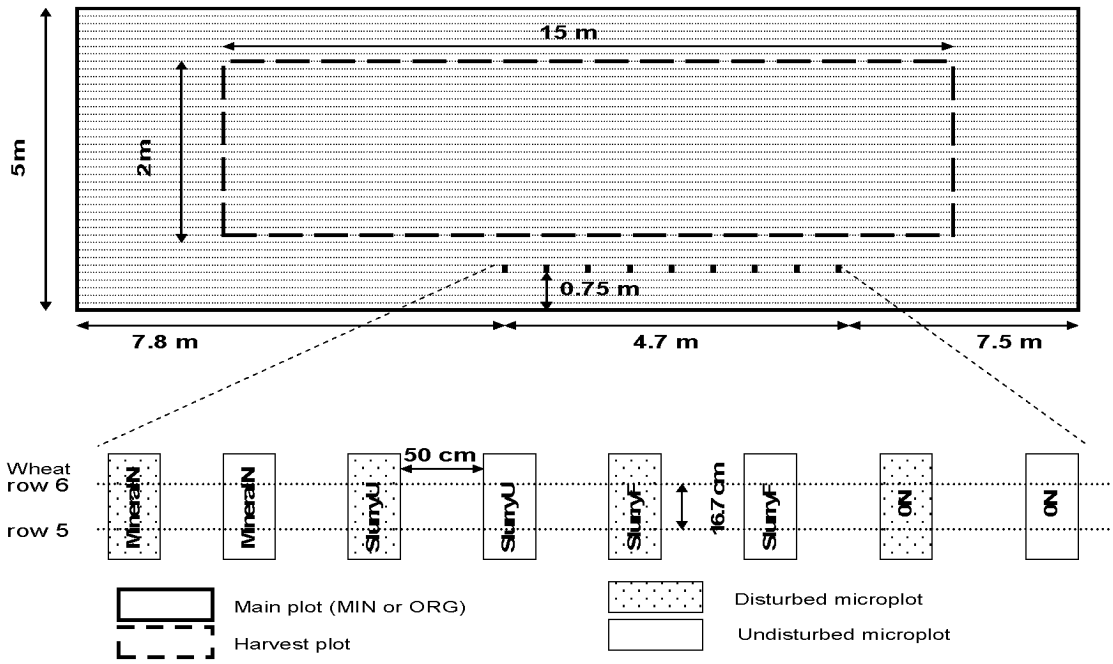

Figure 2.1. Arrangement of the microplots in the main plots of the long-term field experiment.

To account for the fertilization practice in CONMIN and BIOORG we evaluated the fate of both, mineral fertilizer and animal manure-N. To compare the effect of long term organic vs. conventional cropping on N use efficiency, we applied both, animal manure and mineral fertilizer to the microplots installed in both, BIOORG and CONMIN. Four different fertilizer treatments were tested using two ^{15}N-labeled animal manures and one ^{15}N-labeled mineral fertilizer as a single application to the microplots in March 2003 at beginning of tillering of winter wheat. The animal manure was feces-urine mixtures (slurries) just differing in the labeled component. One contained ^{15}N-labeled sheep urine (SlurryU) while the other contained ^{15}N-labeled sheep feces (SlurryF). Mineral fertilizer (MineralN) was applied in form of $^{15}NH_4{}^{15}NO_3$ as aqueous solution (109.7 mmol N l^{-1}). Microplots receiving no fertilizer at all (0N) served as control. The design resulted in four replicates per cropping system-fertilizer treatment combination for undisturbed and disturbed microplots, respectively (i. e. totally 64 microplots). The ^{15}N-labeled fertilizers were applied as single dose to wheat in 2003 because soybean as following crop anyhow received no N fertilizer and because the residual ^{15}N could be used to estimate

symbiotic N_2-fixation by the enriched ^{15}N dilution method (Oberson et al., 2007). In 2005 we deliberately added no fresh fertilizer to study the residual fertilizer-N use by maize under conditions of N demand.

The ^{15}N-labeled urine and feces were obtained by feeding a male sheep (101 kg live weight) with ^{15}N-labeled ryegrass hay for nine days and collecting urine and feces separately. Production of the labeled sheep excrements is described in detail in Chapter 1. For the study, urine and feces with the highest enrichment excreted on the ninth day were used. The amount of urine and feces excreted on the last day of ^{15}N-feeding also limited the area of the microplots that could be fertilized. Testing the homogeneity of the labeling of feces revealed that the ^{15}N-enrichment in physico-chemically separated feces fractions was slightly different. However, the enrichment of mineralized N released from feces during an incubation period of 112 days was not significantly different from the ^{15}N-enrichment of total feces-N, showing that the ^{15}N-enrichment of total feces-N could be used for N use efficiency calculation (Chapter 1). To reduce gaseous N losses and for easier application both slurries were diluted 1:1 with water. To minimize disturbance of young winter wheat plants, slurries and mineral fertilizer were distributed into three about 5 cm deep and 14 cm long narrow channels located between the wheat plants. The channels were covered with soil immediately after application of the fertilizers to minimize gaseous N losses, simulating direct injection. The applied rates and characteristics of the slurries and mineral fertilizer are shown in Table 2.2.

Table 2.2. Selected characteristics and applied rates of the different fertilizers.

Treatment	Fertilizer characteristics			Applied rates			
	DM content	Total N	^{15}N abundance	Total N	$NH_4 + NO_3$-N	Total P	Total K
	%	% of DM	%	..... $g\ m^{-2}$			
SlurryU	7.4	6.4	5.9797	17.6	4.6	6.1	24.9
Urine	8.4	11.2	8.5127	12.7	4.5[b]		
Feces	38.1	3.2	0.3767	4.9	0.1		
SlurryF	5.9	6.9	3.6700	15.9	4.6	7.9	29.5
Urine	8.7	12.6	0.4487	11.1	4.4[c]		
Feces	34.3	3.5	11.2770	4.8	0.2		
MineralN[a]	---	---	9.8685	5.0	5.0	0	0
0N	---	---	---	0	0	0	0

[a] Applied as a solution of NH_4NO_3.
[b] Difference between mineral N determined in SlurryU and feces of SlurryU.
[c] Difference between mineral N determined in SlurryF and feces of SlurryF.

The original aim was to apply the same amount of ^{15}N-labeled components which would have resulted with total N applied with slurry being twice as much as with mineral fertilizer. This would have corresponded to the usual strategy in studies using ^{15}N-labeled fertilizers (Langmeier et al., 2002; Thomsen et al., 1997). Because of an initial analytical problem finally more than twice as much urine-N than feces-N or mineral fertilizer-N was applied. Still, the N use efficiency of the different fertilizer components can be tested because the main aim of this study is to compare the N use efficiency between CONMIN and BIOORG and not to compare the fertilizers among each other. Additionally none of the fertilizer treatments resulted in significant added N interaction (ANI) (see discussion below). During fertilization of the main plots the microplots were covered to avoid additional N input. All other crop cultivation measures (e. g. application of pesticides and plant growth regulator, ploughing, mechanical weeding) conducted in the main plots of CONMIN and BIOORG were also carried out at the same time in the microplots. Because of the small area of the microplots (0.0462 m^2) ploughing and mechanical weeding were done by manual work with hand-held tools.

Plant and soil sampling and sample preparation

The above-ground plant biomass of the microplots was removed completely at physiological maturity of the crops, i. e. of winter wheat in July 2003, of soybean in September 2004 and of maize in September 2005, when also the main plots were harvested. At harvests a part of root biomass of winter wheat and soybean was collected down to about 10 cm and for maize down to 18 cm using a small spade to loosen the soil structure such that the roots could be removed from the microplots. Winter wheat was divided into stem, leave, chaff, grain and roots, soybean into stem, leave, pod, grain and roots and maize into stem, leave, tassel, silk, husk, cob, grain and roots. Additionally plant parts according the growth stage of winter wheat from two wheat plants were collected from the disturbed microplots 11, 47, 75, 97 and 112 days after fertilizer application which corresponds to the following growth stages of winter wheat: tillering, stem elongation, flowering, dough stage and physiological maturity. All plant material was dried at 45° C for 48 hours.
Six randomly distributed soil cores were collected from the microplots with an auger (Ø 2.5 cm, Eijkelkamp, Netherlands) at maturity of winter wheat and soybean, respectively, down to 28 cm. The soil cores were then divided into the 0-18 cm and 18-28 cm soil layer and air dried. After harvest of maize all microplots including the topsoil (0-18 cm) were removed from the main plots and soil was then air dried, thoroughly mixed and prepared for analyses. ^{15}N removed from the microplots with the soil sampled at harvest of winter wheat and soybean accounted for 1.1-1.4% and 1.5-3.7%, respectively, of the initially added total ^{15}N and was taken into account for the calculation of the ^{15}N-recoveries. For nitrate-^{15}N determination soil samples taken from destructive microplots 11 and 112 days after fertilizer application were used.

Analyses

Total N, ^{15}N and mineral N in animal manures

Feces, urine and slurries were freeze-dried and finely ground (only feces and slurries) using a ball mill (Retsch, Haan, Germany) prior to total N and ^{15}N abundance analysis

on a continuous flow Roboprep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England). Mineral N (NH_4-N and NO_3-N) in feces and slurries was extracted as described in Langmeier et al. (2002) except for filtering the KCl solutions through Whatman No.1 filters (Davidson et al. 1991). NH_4-N and NO_3-N were colorimetrically analyzed on a SAN^{plus} Analyzer (Skalar, Netherlands).

Total N and ^{15}N in plants

The dried plant parts from winter wheat, soybean and maize were homogenized separately by cutting the plant materials into small pieces with a centrifuge mill (Granomat, Fuchs Maschinen AG, Switzerland) and then finely ground using the same ball mill as for feces and slurries. The dried fine roots, tassel and silk from maize were directly ground with the ball mill. Total N and ^{15}N abundance analysis was carried out on the mass spectrometer previously mentioned.

Total N, ^{15}N and mineral N in soil

The air-dried soil samples were finely ground with a ball mill previous to total N and ^{15}N analyses. NO_3-N and soluble and exchangeable NH_4-N was determined using a 2 *M* KCl extraction as described in Davidson et al. (1991). Analysis for NH_4-N and NO_3-N was conducted colorimetrically using the SAN^{plus} Analyzer. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene (PTFE) trap was used to determine NH_4-^{15}N and NO_3-^{15}N as described by Sørensen and Jensen (1991). For the soil extract 12.5 g of soil dry weight was extracted with 2 *M* KCl. The trap consisted of a glass filter (Whatman, GF/C, 25 mm) strip that was wetted with 15 µl of 2 *M* sulphuric acid and enclosed in a piece of PTFE tape (Angst + Pfister). For the diffusion 250 ml polyethylene bottles with screw caps were used. For NH_4-^{15}N determination a PTFE trap and 0.2 g of magnesium oxide were added to 40 ml soil extract. The bottle was immediately closed and shaken for 72 h at 25℃ on a horizontal shaker (100 rpm). After removal of the PTFE trap a new trap and 0.4 g of Devarda's

alloy were added to the soil extract for NO_3-^{15}N determination. The bottle was again shaken for 72 h at 25℃. The glass filters were then removed from the PTFE tape using tweezers, dried in ammonia-free air and prepared for ^{15}N analysis on a continuous flow Roborep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England).

Atom% ^{15}N excess

The atom% ^{15}N excess of each sample denotes the ^{15}N abundance of the sample minus the natural abundance of its reference sample. For reference, soil or plant material obtained under identical experimental conditions in the microplots of the 0N treatment was used. Plant and soil sampling from the 0N treatment was carried out alike as for the fertilized treatments. The natural abundance of the reference plant and soil material was 0.37 atom% ^{15}N.

Calculations

N in the crop derived from the labeled component of the fertilizers (Ndflc)

The proportion of N derived from the ^{15}N-labeled component of the fertilizers – urine for SlurryU, feces for SlurryF, and $^{15}NH_4{}^{15}NO_3$ for MineralN – in the different plant parts ($Ndflc_{plp}$) of winter wheat during different growth stages was calculated according to equation (1) using isotope dilution principles (Hauck and Bremner, 1976):

$$Ndflc_{plp}\ (\%) = (^{15}Nex_{plp}/^{15}Nex_{lc}) \times 100 \qquad (1)$$

where $^{15}Nex_{plp}$ is the atom% ^{15}N excess of the plant part (%) and $^{15}Nex_{lc}$ is the atom% ^{15}N excess of the labeled component of the fertilizer (%).

The absolute amount of N derived from the ^{15}N-labeled component of the fertilizers in the different plant parts ($Ndflc_{plp}$) of winter wheat, soybean and maize, respectively, was calculated according to equation (2):

$$Ndflc_{plp}\ (g\ m^{-2}) = N_{plp}(^{15}Nex_{plp}/^{15}Nex_{lc}) \quad (2)$$

where N_{plp} is the total N amount (g m^{-2}) in the corresponding plant part, $^{15}Nex_{plp}$ is the atom% ^{15}N excess of the plant part (%) and $^{15}Nex_{lc}$ is the atom% ^{15}N excess of the labeled component of the fertilizer (%).

If the $Ndflc_{plp}$ resulting from equation (2) was obtained for *n* different plant parts the N derived from the labeled component of the fertilizer in the whole plant (Ndflc) can be calculated as follows for the respective crop:

$$Ndflc\ (g\ m^{-2}) = \sum_{i=1}^{n} Ndflc_{plp\ i} \quad (3)$$

where $Ndflc_{plp\ i}$ denotes the N derived from the labeled component of the fertilizer in a plant part of a crop.

N in the crop derived from the complete fertilizer (Ndff)

Because of the almost identical composition of the two slurries (Table 2.2) we assumed that the percentage of N derived from the feces in SlurryU was the same as in SlurryF. This assumption is considered to be also valid for the urine in SlurryF. The Ndff (g m^{-2}) was then assessed as follows: N derived from the unlabeled manure component in each plant part was calculated by multiplying the proportion of $Ndflc_{plp}$ from the labeled treatment with the N amount of the plant part from the treatment with the unlabeled manure component. For MineralN the Ndff corresponds to the Ndflc.

N in the crop derived from soil N (Ndfs)

For winter wheat and maize the N derived from soil was calculated by subtracting the N derived from the complete fertilizer (Ndff, g m^{-2}) from the total N amount in the winter wheat plant and the maize plant, respectively:

$$Ndfs\ (g\ m^{-2}) = N_{plant} - Ndff \quad (4)$$

where N_{plant} denotes total N in winter wheat or maize.

Because of the ability of soybean to fix N_2 from the atmosphere the Ndfs of soybean can only be calculated by knowing the N derived from the atmosphere (Ndfa). The Ndfa of soybean grown in the microplots was estimated by the ^{15}N dilution method in a study evaluating the N_2-fixation of soybean (Oberson et al., 2007).

Knowing the Ndfa, the Ndfs for soybean can then be calculated:

$$Ndfs\ (g\ m^{-2}) = N_s - Ndff - Ndfa \quad (5)$$

where N_s denotes total N in soybean.

^{15}N recovery

The ^{15}N recovered in each crop (Rec_{crop}) was calculated using equation (6):

$$Rec_{crop}\ (\%) = Ndflc/Nlc \times 100 \quad (6)$$

where Nlc (g m^{-2}) denotes total N applied with the labeled component and Ndflc (g m^{-2}) is obtained from equation (3) for wheat, soybean and maize, respectively.

For ^{15}N recovery in the soil the content of soil in a microplot was calculated by multiplying the volume of the microplot with the soil density of 1.3 kg dm^{-3} (no significant differences between CONMIN and BIOORG) for the 0-18 cm soil layer and 1.4 kg dm^{-3} for the 18-28 cm soil layer (Oehl et al., 2002). This results in 10.8 kg soil per microplot for the soil layer 0-18 cm and 6.5 kg underneath the microplot for the soil layer 18-28 cm. The ^{15}N recovery in the soil was then calculated by dividing the amount of ^{15}N remaining in the soil through the amount of ^{15}N applied to the microplots with the fertilizers. The calculated content of 10.8 kg soil per microplot to a depth of 18 cm could be confirmed by determining the effective soil weight in the microplots after harvest of maize when the microplots were removed from the main plots. The average variation between the actual and the calculated weight was 2.9%.

Because of technical reasons the recovery of ^{15}N in the 18-28 cm soil layer was only assessed after harvest of winter wheat and soybean. Thus, unaccounted ^{15}N at the end of the three vegetation periods was calculated as the difference between the amount of ^{15}N added with the labeled component of the fertilizer and the amount of ^{15}N removed by the three crops plus the ^{15}N recovered in the soil (0-18 cm) after harvest of maize.

Statistical analysis

Analysis of variance was performed by using the statistical analyses package SYSTAT 11 (Systat Software Inc., USA) to test the effects of the cropping systems and fertilizer treatments on the fertilizer N use efficiency. In case of significant effects separation of means was tested using Tukey's HSD (honestly significant difference) test with a significance level of $P \leq 0.05$.

Results

Soil characteristics

Soil microbial biomass and activity were higher in BIOORG than CONMIN in March 2003 (Table 2.1). This confirms several previous measurements on microbial biomass and activity in the same soils (Fliessbach and Mäder, 2000; Mäder et al., 2002). Soil pH was lower in CONMIN than BIOORG, probably due to the acidifying effect of mineral fertilizers (Mäder et al., 2006). In spite of long term organic fertilization in BIOORG, total C and N concentrations in soils were not significantly different between CONMIN and BIOORG (Table 2.1). This agrees with Fliessbach et al. (2007) who did an extended study on soil organic C in the same field experiment. The average N budgets of CONMIN and BIOORG, calculated as difference between N input by fertilizers and N removed with harvested products, are negative (Table I1).

Weather conditions

In 2003 precipitation usually was lower than the long-term (1864-2004) mean values, except in October. In March 2003 when the fertilizers were applied to the microplots precipitation was very low. From April 2004 until September 2004 the monthly rainfall was below the long-term mean values (Figure 2.2a). Exceptionally high air temperatures which resulted in high soil temperatures were measured from June 2003 until August 2003 (Figure 2.2b). From September 2003 until September 2004 the measured air temperatures agreed with the long-term mean air temperature curve (Figure 2.2b).

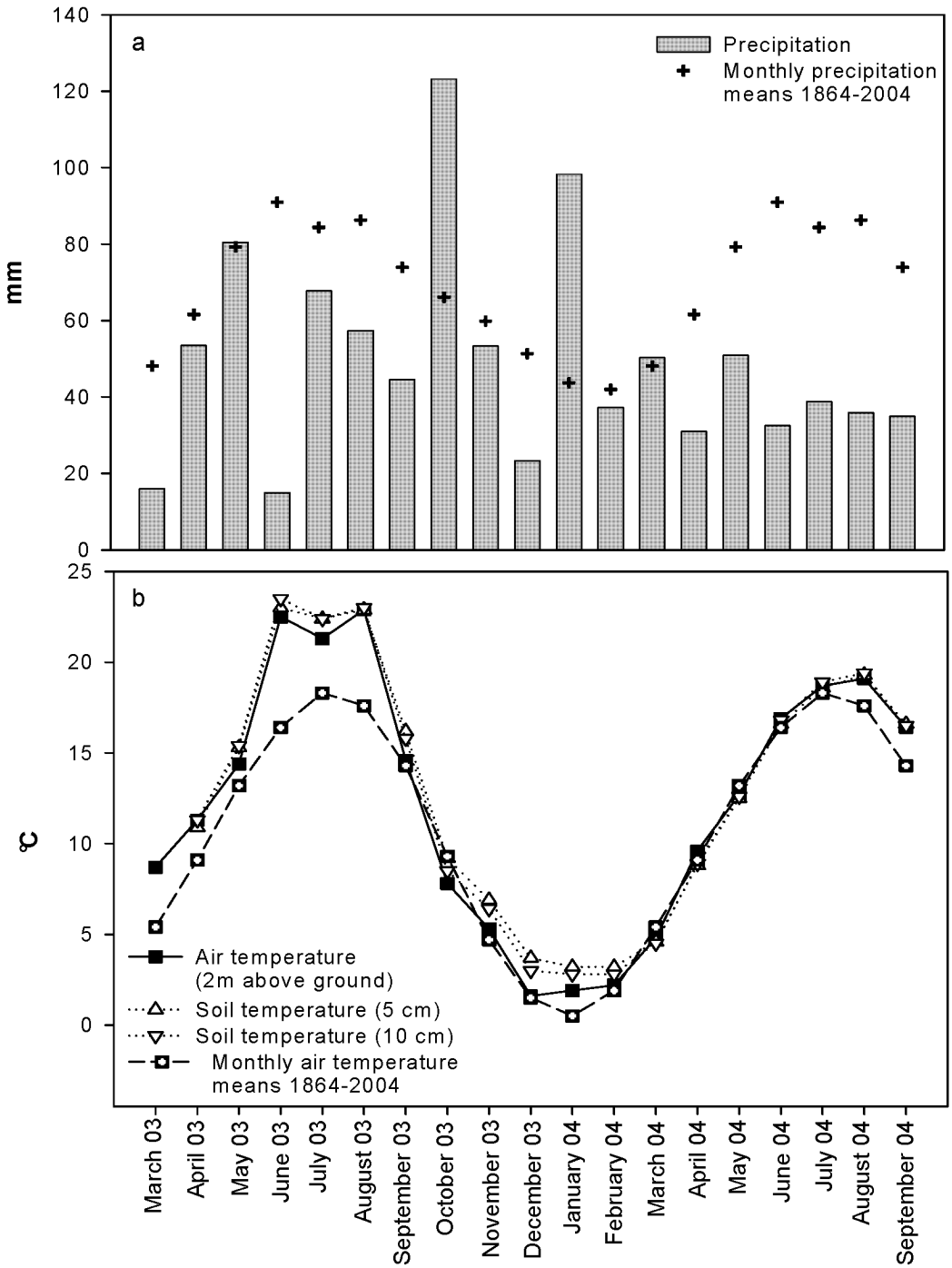

Figure 2a + b. Precipitation and air and soil temperature measured by the meteorological station installed in the long-term field experiment and monthly long-term (1864-2004) precipitation and air temperature means measured at the meteorological station Basel-Binningen (7° 34' E, 47° 32' N).

Grain yields and total N uptake

Winter wheat

The grain dry matter (DM) yield of winter wheat grown in the microplots in July 2003 did not differ between the two cropping systems and was comparable to yields from the harvest plots (460 g DM m^{-2} for CONMIN and BIOORG) (Table 2.3). Grain yield was affected by the fertilizer treatment: SlurryU ≥ SlurryF = MineralN ≥ 0N (Table 2.3). Grain yield was strongly linearly correlated to total N input with the fertilizers (R^2 = 0.84). Total N taken up by winter wheat at maturity ranged from 12.8 g N m^{-2} up to 19.5 g N m^{-2} (Table 2.4).

Table 2.3. Grain dry matter (DM) yields of winter wheat, soybean and maize of the fertilizer treatments. Because of no significant differences between CONMIN and BIOORG mean values are shown. (n = 8).

Year	2003	2004	2005
Crop	Winter wheat	Soybean	Maize
Fertilizer treatment[a]:		g DM m^{-2}	
SlurryU	547 a	585 ns	1211 ns
SlurryF	457 ab	623 ns	1015 ns
MineralN	421 ab	495 ns	996 ns
0N	338 b	543 ns	909 ns
Standard error	46	130	257

Within columns, means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test; ns = not significant.

[a] SlurryU = ^{15}N-labeled urine + unlabeled feces, SlurryF = unlabeled urine + ^{15}N-labeled feces, MineralN = $^{15}NH_4{}^{15}NO_3$, 0N = unfertilized control.

Table 2.4. Total N uptake by winter wheat, soybean and maize, N derived from the labeled component of fertilizer (Ndflc), N derived from the complete fertilizer (Ndff), N derived from the soil (Ndfs) in crops and N derived from the atmosphere (Ndfa) in soybean of the fertilizer treatments at the year of fertilizer application (2003) and the residual years (2004 and 2005). Because of no significant differences between CONMIN and BIOORG mean values are shown. (n = 8).

	Nuptake	Ndflc	Ndff	Ndfa	Ndfs
	……g m^{-2}……				
Winter wheat (July 2003)					
Fertilizer treatment[a]:					
SlurryU	19.5 a	4.64 a	5.2 a	---	14.4 ns
SlurryF	16.6 ab	0.48 c	4.5 a	---	12.1 ns
MineralN	15.1 ab	2.36 b	2.4 b	---	12.8 ns
0N	12.8 b	---	---	---	12.8 ns
Standard error	1.7	0.2	0.5	---	1.5
Soybean (September 2004)					
Fertilizer treatment[a]:					
SlurryU	40.1 ns	0.33 a	0.5 a	19.5 ns	20.1 ns
SlurryF	39.9 ns	0.16 a	0.5 a	18.0 ns	21.3 ns
MineralN	33.0 ns	0.10 b	0.1 b	10.1 ns	22.8 ns
0N	37.8 ns	---	---	nd	nd
Standard error	9.1	0.03	0.06	7.2	7.0
Maize (September 2005)					
Fertilizer treatment[a]:					
SlurryU	21.5 ns	0.17 a	0.3 a	---	21.3 ns
SlurryF	19.9 ns	0.07 b	0.2 a	---	19.6 ns
MineralN	21.3 ns	0.05 b	0.1 b	---	21.2 ns
0N	16.4 ns	---	---	---	16.4 ns
Standard error	3.8	0.02	0.03	---	3.8

Within columns and crop means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test; ns = not significant; nd: not defined.
[a] For explanation see Table 2.3.

Soybean

The grain yield (500-620 g DM m^{-2}) of soybean harvested from the microplots in September 2004 did neither differ between the two cropping systems nor between the fertilizer treatments (Table 2.3) and was higher than in the harvest plots (280-330 g DM m^{-2}). Total N contained in mature soybean ranged from 33.0 g N m^{-2} up to 39.9 g N m^{-2} (Table 2.4) and was not correlated with the amount of N applied with the fertilizers in 2003.

Maize

The grain yield of maize harvested from the microplots in September 2005 was not significantly different between the two cropping systems and the fertilizer treatments (Table 2.3) but on average 40% lower than in the harvest plots (CONMIN: 1900 g DM m^{-2}, BIOORG: 1500 g DM m^{-2}). Total N uptake by maize at maturity ranged from 16.4 g N m^{-2} to 21.5 g N m^{-2} and was not correlated to the total N input with the fertilizers in 2003 (Table 2.4).

N derived from the labeled component of the fertilizers (Ndflc) in winter wheat, soybean and maize

Neither the $Ndflc_{plp}$ (%) for winter wheat (Figure 2.3) nor the absolute Ndflc (g m^{-2}) in winter wheat, soybean and maize (Table 2.4) were significantly affected by the cropping system. At harvest of winter wheat 23.8%, 2.9% and 15.6% of total N taken up by winter wheat derived from urine, feces and mineral fertilizer, respectively. The course of $Ndflc_{plp}$ (%) during wheat growth shows that $Ndflc_{plp}$ (%) was greater for urine and mineral fertilizer than for feces from the earliest sampling onwards (Figure 2.3). However, while $Ndflc_{plp}$ (%) remained stable for urine-N (SlurryU), it slightly decreased for mineral fertilizer-N (MineralN) and slightly increased for feces-N (SlurryF).

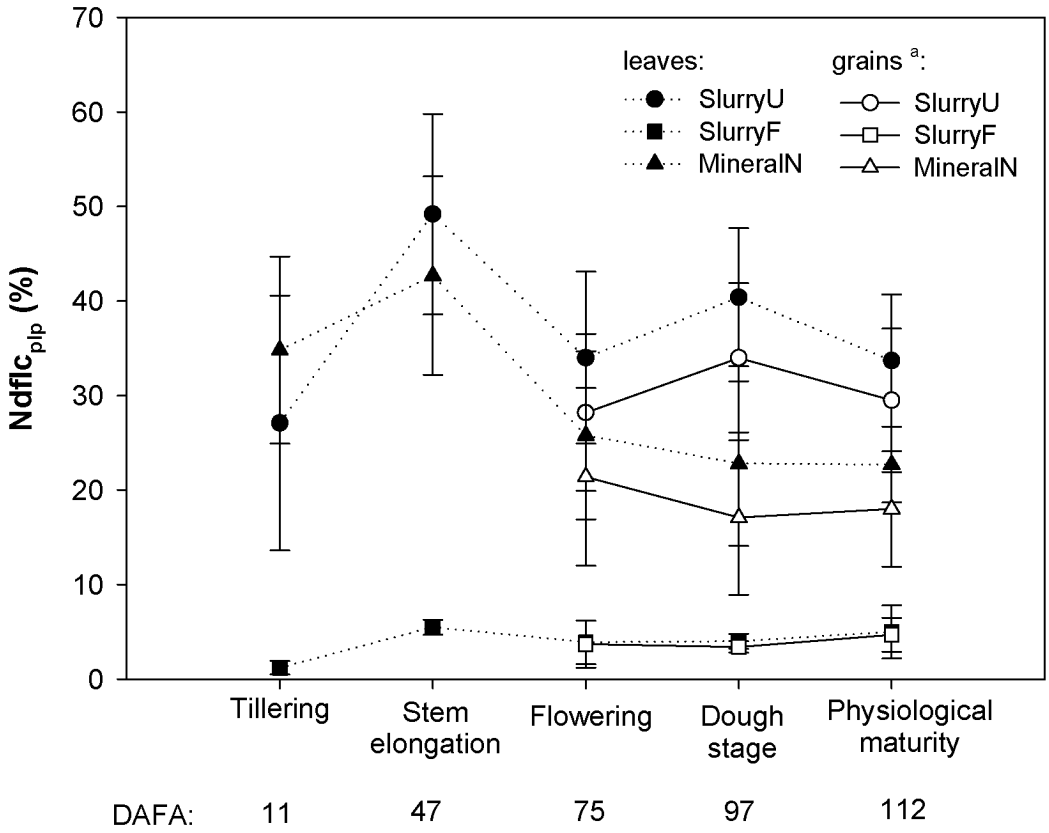

Figure 2.3. Proportion of N derived from the labeled component of the fertilizers in selected winter wheat plant parts ($Ndflc_{plp}$) at different growth stages. DAFA = days after fertilizer application. [a] At flowering ears and at dough stage and physiological maturity only grains were used. Because of no significant differences between CONMIN and BIOORG mean values are shown. (n = 8).

There were only minor differences in the N content (mg N g^{-1} DM) in wheat during its growth (data not shown), which was at a level of 27 mg N g^{-1} grain DM at physiological maturity suggesting that none of the fertilizer treatments resulted in N limiting concentrations (Walther et al. 2001). The high availability of urine-N and mineral fertilizer-N was also reflected in the soil nitrate N pool. At eleven days (tillering of winter wheat) after fertilizer application, nitrate-N deriving from urine, mineral fertilizer and feces accounted for 75.7%, 64.6% and 7.5% of the soil nitrate, respectively, and decreased to 9.1%, 3.3% and 2.3%, respectively, at 112 days (harvest of winter wheat) (Table 2.5).

Table 2.5. Total NO_3-N and NO_3-N derived from the labeled component of fertilizer (NO_3dflc) in the topsoil (0-18 cm) of the fertilizer treatments 11 days (tillering of winter wheat) and 112 days (physiological maturity of winter wheat) after fertilizer application. Because of no significant differences between CONMIN and BIOORG mean values are shown. Standard deviation is shown in brackets. (n = 8).

	11 days after fertilizer application		112 days after fertilizer application	
Fertilizer treatment[a]:	Total NO_3	NO_3dflc	Total NO_3	NO_3dflc
	..... mg kg^{-1} soil			
SlurryU	70.9 (33.4) ns	55.2 (30.0) a	6.7 (2.0) ns	0.6 (0.3) a
SlurryF	55.1 (16.8) ns	4.2 (2.2) b	5.7 (1.2) ns	0.1 (0.08) b
MineralN	40.2 (14.5) ns	26.6 (12.3) b	6.0 (2.0) ns	0.2 (0.1) b

Within columns, means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test; ns = not significant.
[a] For explanation see Table 2.3.

Compared to the year of fertilizer application the Ndflc of the slurries and the mineral fertilizer decreased strongly in the residual years. Of total N taken up by soybean in the first residual year 0.8% derived from urine, 0.4% from feces, and 0.3% from the mineral fertilizer (Table 2.4). The Ndlfc of all fertilizers remained on a low level in the second residual year with 0.8% of total N taken up by maize deriving from residual urine-N, 0.4% from residual feces-N and 0.2% from residual mineral fertilizer-N (Table 2.4).
From total N taken up by winter wheat, by soybean and by maize 27.0%, 1.3% and around 1.2% derived from the complete slurries (Ndff), the remaining N taken up by the crops originated from the soil (Table 2.4). Furthermore about 50% of total N taken up by soybean derived from the atmosphere (Table 2.4).

Fertilizer ^{15}N recovery

No significant differences in ^{15}N recovery were found between the two cropping systems. At harvest 36.6%, 10.0% and 47.1% from urine, feces and mineral fertilizer was recovered in winter wheat (Table 2.6). At the first residual year ^{15}N recovered from the originally applied labeled fertilizers by soybean decreased to 2.6%, 3.3% and 2.1% for urine, feces and mineral fertilizer, respectively (Table 2.6). In the second residual year 1.3% of urine-^{15}N, 1.5% of feces-^{15}N and 1.1% of mineral fertilizer-^{15}N was recovered in maize (Table 2.6). Fertilizer-derived ^{15}N recovered in the topsoil (0-18 cm) was each year between 20-25% except for feces-^{15}N in 2004 where it was about twice as high, but at high variation (Table 2.6). At harvest of winter wheat between 2-3% and at harvest of soybean 7-9% of fertilizer-^{15}N was recovered in the 18-28 cm soil layer (Table 2.6). Thirty months after application of the labeled fertilizers - at harvest of maize - unaccounted fertilizer-^{15}N not taken up by the crops or recovered in the 0-18 cm soil layer amounted to 40.1%, 60.7% and 29.4% for urine-, feces- and mineral fertilizer-N, respectively.

Table 2.6. Fertilizer-^{15}N recovery in winter wheat, soybean, maize and the soil of the fertilizer treatments at the year of fertilizer application and the residual years. Because of no significant differences between CONMIN and BIOORG mean values are shown. Standard deviation is shown in brackets. (n = 8).

Fertilizer treatment[a]:	SlurryU	SlurryF	Slurry (weighted)[c]	MineralN
	……^{15}N recovery (% of applied ^{15}N)……			
Year of fertilizer application 2003				
winter wheat	36.6 (0.01) b	10.0 (0.02) c	29.2 (3.1)	47.1 (0.05) a
soil (0-18 cm)	25.1 (10.9) ns	20.1 (6.3) ns	23.7 (8.7)	21.8 (6.5) ns
(18-28 cm)[b]	2.0 (1.7) ns	2.4 (2.7) ns	2.2 (1.6)	2.5 (2.1) ns
First residual year 2004				
soybean	2.6 (0.01) b	3.3 (0.01) a		2.1 (0.003) b
soil (0-18 cm)	25.2 (3.8) b	47.1 (19.6) a		22.4 (4.0) b
(18-28 cm)	7.1 (1.1) ns	8.6 (4.0) ns		7.2 (2.8) ns
Second residual year 2005				
maize	1.3 (0.003) ab	1.5 (0.002) a		1.1 (0.003) b
soil (0-18 cm)	19.4 (3.2) ns	24.5 (5.9) ns		20.3 (11.9) ns
(18-28 cm)	nd	nd		nd

Within rows, means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test; ns = not significant; nd = not defined.
[a] For explanation see Table 2.3.
[b] Significant effect of cropping system (BIOORG > CONMIN).
[c] Weighted recovery from SlurryU and SlurryF.

Discussion

Dry matter production

Grain dry matter production of winter wheat grown in the microplots at harvest in July 2003 was comparable to grain yield of winter wheat in the harvest plots, whilst grain yields from soybean harvested from the microplots in September 2004 was higher than

in the harvest plots. This probably can be ascribed to the fact that grains of soybean in the microplots were harvested manually and thus losses were reduced compared to the harvest plots where soybean was harvested with a combined harvester. Furthermore, only two soybean plants could be grown per microplot hampering extrapolation of the grain yield to larger areas. Total dry matter production of soybean in the microplots was lower than of soybean sampled in areas of the main plots corresponding to the microplot area, suggesting that the growth of soybean was restricted by the microplot frames (Oberson et al., 2007). The 40% lower grain yield of maize harvested in September 2005 from the microplots compared to the harvest plots could be explained by the following facts: i) the microplots remained unfertilized since March 2003 and, besides N (Walther et al., 2001) soil P- and K-availability might have limited plant growth and thus dry matter production as shown for soybean (Oberson et al., 2007), ii) light conditions for maize in the microplots were not optimal during the vegetation period as the microplots were located between two rows of maize of the main plots and maize in the microplots was growing slower than in the main plots due to no addition of fresh fertilizer and iii) restriction of maize root growth by the microplot frames down to a depth of 18 cm.

N use efficiency of animal manure and mineral fertilizer

a) Impact of cropping system

Despite the differences in microbial activity, biomass and long-term fertilization between CONMIN and BIOORG no differences in fertilizer N use efficiency were found. The fertilizer N use efficiency of mineral and organic fertilizer N seems not to be affected by the activity and the size of the microbial biomass initially present in the soil. This finding is consistent with results reported by Langmeier et al. (2002) who conducted a pot experiment using the same soils and applied ^{15}N-labeled cattle manure and mineral fertilizer to ryegrass. They reported a similar response of CONMIN and BIOORG to fertilization. In contrast to our results, however, they found BIOORG to have a greater soil N mineralization capacity than CONMIN which may be ascribed to the fact that several cuts of ryegrass were grown on a limited soil volume under N limiting conditions.

Also no effect of long-term organic versus conventional cropping on total N recovery (in crop and soil) from different ^{15}N sources (urea, vetch residues, mineral fertilizer) were reported by Kramer et al., (2002), Glendining et al., (1997) and Glendining et al., (2001).

b) Impact of fertilizer type

Direct comparison of the N use efficiency of urine with feces and mineral fertilizer might be hampered as a higher amount of total N and thus ^{15}N was applied with urine in SlurryU compared to feces in SlurryF or mineral fertilizer in MineralN. However, other studies have indicated that the percentage of ^{15}N recovery in crops is only slightly influenced by the amount of labeled N applied (Glendining et al., 1997). Added N interaction (ANI) might also obscure the interpretation of results (Jenkinson et al., 1985; Kuzyakov et al., 2000), e. g. if the application of labeled N influences the plant uptake of soil-derived N. In our study ANI can be neglected for several reasons. First, the Ndfs was not significantly different between the 0N and the fertilized treatments, and the Ndfs was only slightly and not significantly greater in SlurryU than the other fertilized treatments. Also, the recovery of slurry-N and mineral fertilizer-N by winter wheat was similar for the ^{15}N method or when calculated by the difference method (Muñoz et al., 2004) where the apparent recovery is defined as (N uptake in fertilized treatment - N uptake in 0N treatment)/ total N applied x 100. The ^{15}N method resulted in 29% and 47% ^{15}N recovery, respectively (Table 2.6), and apparent recoveries for slurry and mineral fertilizer were 31% and 46%, respectively.

Starting conditions for winter wheat were similar for each fertilizer treatment as similar amounts of directly plant available mineral N (NH_4- and NO_3-N) were applied with the slurries and the mineral fertilizer. From total N taken up by the crops the proportion of urine-derived N was highest over all three vegetation periods. The higher proportion of urine-derived N in the crops was due to the higher amount of N applied with urine, which mostly consists of urea (Bristow et al., 1992). The availability of urinary N depends on the hydrolysis of urea to ammonium which is catalyzed by urease (Antil et al., 1993). Hydrolysis of urea to ammonium was shown to be completed within few days after addition of urine to soil (Antil et al., 1993; Whitehead and Bristow, 1990). This was

confirmed by our own observations as about two weeks after application of SlurryU a large proportion of soil nitrate was derived from urine.

The recovery of mineral fertilizer-derived ^{15}N in the crops declined strongly from 47.1% in the year of fertilizer application to below 2.5% in the residual years. This low recovery in the second year is in accordance with the uptake in cereals found in other studies (Glendining et al., 2001; Sørensen and Thomsen, 2005a; Thomsen et al., 1997). This might be due to an exhaustion of this N source as nearly half of the originally applied mineral fertilizer-N was taken up by winter wheat in the year of fertilizer application and around 30% remained unaccounted.

Probably because of the same reasons as for mineral fertilizer the recovery of urine-^{15}N in the crops declined strongly from 36.6% in the year of fertilizer application to less than 3% in the residual years. This agrees with the recovery of urine-N in sheep slurry found by Thomsen et al. (1997) after application to barley.

At same amounts of total N applied with feces and mineral fertilizer the N use efficiency of feces-N in the year of fertilizer application was only 10% and significantly lower than that of mineral fertilizer and urine. Thomsen et al. (1997) similarly found 12-14% recovery of fecal N in barley in the first year. Of total fecal N organic N forms accounted for the largest fraction and thereof about 30% were undigested feed N (Chapter 1). Organic N compounds in feces that remained undigested after having passed the animal are supposed to mineralize very slowly in soil (Muñoz et al., 2003; Sørensen and Jensen, 1998). In the year of fertilizer application a small amount of organic feces-N was mineralized at a slow rate as the amount of feces-derived N in winter wheat exceeded the amount of mineral-N applied with the labeled feces and as also suggested by the increasing proportion of $Ndflc_{plp}$ during wheat growth. Low recovery in soybean and maize suggests that mineralization of feces-N was also low during the residual years.

The availability of manure-N can be compared to the availability of mineral fertilizer-N by calculating the mineral fertilizer equivalent (MFE) (Sørensen and Jensen, 1998). The MFE is defined as % recovery of ^{15}N from manure x 100 divided by % recovery of ^{15}N from mineral fertilizer (Sørensen and Thomsen, 2005b). The MFE of feces- and urine-N for the first crop was 21% and 78%, respectively, which is comparable with previous studies (Sørensen and Jensen, 1998). Likewise the MFE of the complete slurry (61.4%)

was in the range of MFE obtained by Thomsen et al. (1997) in spite that they applied twice as much N with cattle slurry than with mineral fertilizer. The MFE obtained from the ^{15}N method in our study was also comparable to the MFE (67.4%) obtained from the difference method calculated from the apparent recovery of slurry-N.

Fate of fertilizer N not taken up by crops

Urine- and mineral fertilizer-derived ^{15}N remaining in the soil after each vegetation period was comparable to results found in other studies applying ^{15}N-labeled animal manure to soils, whilst the recovery of feces-^{15}N was about three times lower (Muñoz et al., 2003; Sørensen et al., 1998; Thomsen et al., 1997). Only the recovery of feces-^{15}N in soil in 2004 was comparable to results in other studies. Because of the recalcitrant N compounds in feces, feces-N is expected to accumulate in the soil as shown by Sørensen et al. (1998) and Thomsen et al. (1997). However, size density fractionation conducted with the same soil samples to evaluate incorporation of fertilizer-^{15}N into different soil organic matter fractions confirmed the low soil ^{15}N recovery for the SlurryF treatment (Chapter 3). The low recovery of feces-N in the soil is difficult to explain. Gaseous N losses, i. e. ammonia volatilization, denitrification and nitrate leaching require mineralization of applied N. However, as indicated by the low availability to crops, and as confirmed in incubation studies (Chapter 1), mineralization of feces-N was low. Furthermore, slurry was applied to a depth of 5 cm and then covered with soil to minimize ammonia volatilization. The low recovery of feces-derived ^{15}N suggests that non-mineralized feces-N would have been lost from the microplots for example by leaching of dissolved organic N (DON) or by redistribution of feces-N outside the microplots by earthworms that were present numerously in the microplots, e. g. into deeper soil layers by anecic earthworms. The recovery in the 18-28 cm soil layer suggests ^{15}N displacement into deeper layers. It ranged from 2.0 to 2.5% in the year of fertilizer application and increased to 7.1 to 8.6% in the first residual year. Sørensen and Thomsen (2005b) similarly found 10-13% of applied fecal ^{15}N in the 20-40 cm soil layer one year after application and Muñoz et al. (2003) found 4-8% in the 30-60 cm soil layer and another 1-4% in the 60-90 cm soil layer. These studies indicate that the transport of

labeled N below 18 cm depth could be around 20%, but the low recovery in the present study indicates an even higher transport of labeled fecal N to deeper soil layers. Dissolved organic N (DON) is composed of material resulting from breakdown of SOM that is soluble but recalcitrant and thus not readily available to soil microorganisms (Smolander et al., 1995; Yu et al., 2002). Siemens and Kaupenjohann (2002) showed that it contributes significantly to N leaching from arable soils and that DON leaching was higher in manured plots than plots that received mineral fertilizer-N. In addition earthworms can have a significant influence on the disappearance of sheep dung as they can assimilate slurry-derived N (Hirschberger and Bauer, 1994; Schmid and Ostle, 1999).

Unaccounted ^{15}N 30 months after application of the labeled fertilizers amounted to 40.1%, 60.7%, and 29.4% for urine, feces and mineral fertilizer, respectively. Despite higher microbial activity in BIOORG than CONMIN soils the fate of fertilizer-N was the same in both systems for a same fertilizer applied at same rate as shown by similar fertilizer N use efficiency by crops, ^{15}N recovery in the soil and unaccounted ^{15}N. This suggests that the two cropping systems have the same potential to emit N compounds to the environment. Differences in the local emissions from cropping systems seem to be determined by form and level of inputs rather than by intrinsically different use efficiencies of inputs.

Conclusions

Higher microbial activity and biomass in BIOORG than CONMIN soils was expected to affect the fate of added fertilizer-N through mineralization-immobilization processes. However, the cropping system had no significant impact on the N use efficiency of animal manure or mineral fertilizer and on the recovery of fertilizer-N in the soil over a 3-year period. The N use efficiency was affected by the type of fertilizer. Recalcitrant N-compounds as contained in feces were mineralized at a low rate in the year of fertilizer application which was expressed in lower Ndflc and lower recovery of feces-N than urine- or mineral fertilizer-N in winter wheat. The residual fertilizer N effect was very low for all tested fertilizers but comparable to other studies. Especially in the residual years

the main N source for the crops was the soil and for soybean additionally the atmosphere, again without significant effects of the cropping systems. In spite of lower recovery of feces-N in the crops at the end of the third vegetation period, ^{15}N-recovery in the 0-18 cm soil layer was similar for urine, feces and mineral fertilizer. As recalcitrant feces-derived compounds are expected to accumulate in the soil, ^{15}N recovered from feces was unexpectedly low. The low ^{15}N-recovery may be explained by the transport of feces-^{15}N to deeper soil layers by earthworm activity and/or leaching of DON.

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Chapter 3: Incorporation of ^{15}N-labeled amendments into physically separated soil organic matter fractions

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Abstract

Physically separated soil organic matter (SOM) fractions may take different functions in soil nitrogen (N) dynamics. We studied the effect of long-term organic matter (OM) management and different soil biological activity on the incorporation of N added with organic and mineral amendments into aggregate fractions and size density fractions. We applied ^{15}N-labeled sheep feces, urine and mineral fertilizer to microplots installed in plots of conventional (CONMIN) and bio-organic (BIOORG) cropping systems. Soil sampled 112 days after amendment was separated into macro-, micro-aggregates and microstructures. Aggregates were then fractionated into free light fraction (LF), intra-aggregate particulate organic matter (iPOM) and the mineral-associated organic matter fraction (MF). Of total soil N, 67% was contained in macro-aggregates. Size density fractionation of aggregates revealed that about 60% of soil N was stored in MF while LF and iPOM contained together less than 3% of soil N. Despite long-term OM input and higher soil biological activity in BIOORG than CONMIN the two soils did not differ in the distribution and content of N in aggregate and size density fractions. Recovery of ^{15}N in non-fractionated soil ranged from 20% (SlurryF) to 25% (SlurryU) of originally applied ^{15}N. The small macro-aggregates were for each amendment the major sink (7-12 % of applied ^{15}N). In all aggregates and for all amendments, MF was the most important ^{15}N sink, totally containing between 6.6% (SlurryF) to 11.6% (SlurryU) of applied ^{15}N. Less than 1% of applied ^{15}N was recovered in LF, and even less (<0.5%) in iPOM. The proportion of amendment-derived N in aggregate fractions and in several size density fractions (LF, fine iPOM, MF) was higher for urine than for feces and mineral fertilizer. Recovery of urine-derived ^{15}N was greater in aggregate fractions of BIOORG than CONMIN soil. During dispersion of aggregates to obtain iPOM and MF, about 27% of total soil N and between 37 and 55% of ^{15}N contained in non-fractionated soil was lost, showing the importance of aggregation to protect N.

Introduction

Soil organic matter (SOM) is the most important nitrogen (N) reservoir in soils. The amount and quality of SOM present and the rate of SOM turnover are influenced by agricultural management practices, which, in turn, may also affect amounts and forms of N retained in the soil. Repeated manure application and incorporation of animal and crop residues usually increase SOM content and affect its quality (Aoyama et al., 1999; Kong et al., 2007). Soil organic matter and associated N is very heterogeneous and consists of fractions differing in composition, biological function and stabilization by chemical and physical mechanisms. Different SOM fractions can be obtained by aggregate separation and size density fractionation (Six et al., 2000; von Lützow et al., 2007). Aggregate separation assesses the distribution of organic matter (OM) among large macro-aggregates (> 2000 µm), small macro-aggregates (250-2000 µm), micro-aggregates (50-250 µm) and microstructures (< 50 µm) (Six et al., 1998). Macro-aggregates consist of micro-aggregates, primary organo-mineral complexes and uncomplexed particulate OM (POM). The POM is neither present as readily recognizable litter components (typically > 2 mm) nor incorporated into primary organo-mineral complexes. Micro-aggregates are composed of primary organo-mineral complexes and clay microstructures (Christensen, 2001; Tisdall and Oades, 1982). In combined aggregate and size density separation schemes (Six et al., 1998), size density fractionation is then used to divide the aggregates into the free light fraction (LF; inter-aggregate OM) and the heavy fraction (HF). After dispersion the HF is separated into coarse and fine intra-aggregate particulate organic matter (iPOM) and the mineral-associated organic matter fraction (MF). Free LF consists mainly of partially decomposed plant material, animal residues or manure, microbial debris which are not associated to the mineral soil particles and of older uncomplexed material previously occluded within aggregates (Christensen, 2001; Fliessbach and Mäder, 2000). Free LF is more closely related to plant residues and often responds more sensitively towards changes in soil and/or fertility management than iPOM, but both free LF and iPOM fractions are known to be sensitive indicators of the effects of agricultural management practices on SOM (Gregorich et al., 2006).

Crop N supply in organic farming relies on the mineralization of SOM and organic N sources such as animal manure. Therefore, organic farming aims at maintaining or increasing SOM content. Manure N not taken up by the crop shall be retained in the soil to avoid N losses and conserve it as N source for subsequent crops. Few studies are concerned with the long-term effect of animal manure and mineral fertilizer on SOM fractions (Christensen, 1988; Aoyama et al., 1999; Wander et al., 2007). Only few of them used the stable isotope ^{15}N as tracer to follow the incorporation of ^{15}N-labeled mineral fertilizer (Compton and Boone, 2002; Ladd et al., 1977; Monaghan and Barraclough, 1995) or ^{15}N-labeled plant residues (Kölbl et al., 2006; Moran et al., 2005; Vanlauwe et al., 1998; Kong et al., 2007) into different SOM fractions. However, incorporation of ^{15}N-labeled animal manure has not been investigated. Also, we know no study where incorporation of ^{15}N from same amendments applied either to organically or conventionally cropped soils has been followed.

We applied sheep slurries (urine-feces mixture) of which either urine or feces were labeled with ^{15}N, and ^{15}N-labeled mineral fertilizer to microplots installed in plots of a field experiment. These plots were either managed according to conventional cropping practices and had received only mineral fertilizer since 1985 (CONMIN), or were managed according to bio-organic cropping practices and had received only farmyard manure and slurry since 1978 (BIOORG). Four months after application of the amendments, at harvest of mature wheat grown in the microplots, we studied the recovery of ^{15}N in physically separated SOM fractions in soil sampled from the 0-18 cm layer in the microplots. Soil microbial biomass and activity are higher in the BIOORG than the CONMIN soil (Fliessbach and Mäder, 2000; Mäder et al., 2006). As OM management, e.g. through manure amendment, and soil biological activity may affect the incorporation of OM into different SOM fractions we hypothesized that i) the distribution of N among the SOM fractions differs between CONMIN and BIOORG soils due to the long-term manure input in BIOORG and ii) the incorporation patterns of freshly applied ^{15}N-labeled animal manure N and mineral fertilizer N differ because of different forms of N applied with the amendments and because of OM input with animal manure.

Materials and Methods

Design of the microplot study and soil sampling

Microplots were installed in December 2002 in plots of a long-term field experiment located in Therwil (7°33' E, 47°30' N) near Basel (Switzerland) managed by Acroscope Reckenholz-Tänikon (ART), Zurich, and the Research Institute of Organic Farming (FiBL), Frick, Switzerland. The soil is a loamy silt Typic Hapludalf (USDA, 1999) developed on loess in a temperate climate. Selected soil properties are given in Table 3.1.

Table 3.1. Selected soil properties of the topsoil (0-18 cm) from soil samples collected in March 2003. (n = 4).

	Cropping system	
	CONMIN	BIOORG
Soil properties		
pH (H_2O)	6.2 b	6.6 a
Total N (g kg^{-1})	1.4 ns	1.5 ns
Total C (g kg^{-1})	13.3 ns	13.6 ns
Microbial biomass N (mg kg^{-1})[a]	12.5 b	21.8 a
Microbial biomass C (mg kg^{-1})[a]	100.5 ns	112.6 ns
Daily respiration (mg C kg^{-1} day^{-1})[b]	2.5 b	3.8 a
Texture[c]: clay (%)	15.3 ns	15.5 ns
silt (%)	70.6 ns	69.0 ns
sand (%)	14.1 ns	15.5 ns

Within rows means followed by different letters are significantly different ($P \leq 0.05$) between the cropping systems (t-test); ns = not significant.
[a] Microbial biomass N and C determined by chloroform fumigation (Vance et al., 1987); no conversion factors applied.
[b] Average over a 60 days incubation experiment (Alef and Nannipieri, 1995).
[c] Soil samples collected from the 0-20 cm soil layer in 2002.

The microplots were defined by frames with a length of 33 cm, a width of 14 cm and a height of 23 cm, and were open at the top and the bottom. In December 2002 they were driven into the soil of CONMIN and BIOORG plots to a depth of 18 cm. In March 2003,

at beginning of tillering of winter wheat, two ^{15}N-labeled animal manures and a ^{15}N-labeled mineral fertilizer were deployed as a one-time application. The two animal manures were feces-urine mixtures (slurries) just differing in the labeled component. One contained ^{15}N-labeled sheep urine (SlurryU) while the other contained ^{15}N-labeled sheep feces (SlurryF). Mineral fertilizer-N (MineralN) was applied in form of $^{15}NH_4{}^{15}NO_3$ as aqueous solution (109.7 mmol N l^{-1}). A non fertilized treatment was included as control (0N). Each of the four amendment treatments (SlurryU, SlurryF, MineralN, 0N) was applied to microplots installed in CONMIN and BIOORG, resulting in four replicates per cropping system - amendment combination.

The ^{15}N-labeled urine and feces were obtained by feeding a sheep with ^{15}N-labeled ryegrass hay for nine days and collecting urine and feces separately. For the study, urine and feces with the highest enrichment excreted on the ninth day were used. Non-labeled urine and feces were collected from the same sheep at day six of the initial seven days lasting feeding period with non labeled ryegrass hay. The non labeled ryegrass hay was obtained under identical conditions as the labeled hay (Chapter 1).

To reduce gaseous N losses and for easier application both slurries were diluted 1:1 with water. To minimize disturbance of young winter wheat plants, slurries and mineral fertilizer were distributed into three about 5 cm deep and 14 cm long narrow channels located between the wheat plants. Channels were covered with soil immediately after application of the amendments to minimize gaseous N losses, simulating direct injection. The applied rates and characteristics of the slurries and mineral fertilizer are shown in Table 3.2. Slurries contained similar amounts of mineral N (NH_4 and NO_3) as the mineral fertilizer.

For aggregate separation and size density fractionation six soil cores were randomly collected from each microplot to a depth of 18 cm with an auger (Ø 2.5 cm, Eijkelkamp, Netherlands) immediately after harvest of mature winter wheat in July 2003. At harvest also the wheat straw had completely been removed. The mature shoots of wheat contained 37, 10 and 47% of ^{15}N applied with urine, feces and mineral fertilizer, respectively (Chapter 2). The six soil cores from the same microplot were pooled and air dried. Soil samples were not sieved before drying.

Table 3.2. Characteristics and applied rates of the different fertilizers.

	Fertilizer characteristics			Applied rates			
	Dry matter content	Total N	^{15}N abundance	Total N	NH_4 + NO_3-N	Total P	Total K
	%	% of DM	%	..... $g\ m^{-2}$			
SlurryU	7.4	6.4	5.9797	17.6	4.6	6.1	24.9
Urine	8.4	11.2	8.5127	12.7	4.5[b]		
Feces	38.1	3.2	0.3767	4.9	0.1		
SlurryF	5.9	6.9	3.6700	15.9	4.6	7.9	29.5
Urine	8.7	12.6	0.4487	11.1	4.4[c]		
Feces	34.3	3.5	11.2770	4.8	0.2		
MineralN[a]	-	-	9.8685	5.0	5.0	0	0
0N	-	-	-	0	0	0	0

[a]Applied as a solution of $NH_4^+NO_3^-$.
[b] Difference between mineral N determined in SlurryU and feces of SlurryU.
[c] Difference between mineral N determined in SlurryF and feces of SlurryF.

Aggregate separation

100 g of air dried soil was capillary rewetted allowing trapped air to escape with minimal disruption of soil structure (Cambardella and Elliott, 1993). Subsequently, soil was wet sieved through a series of three sieves (2000 µm, 250 µm and 50 µm), exactly as described by Six et al. (1998). Briefly, the soil was submerged for 5 min in deionized water. Aggregate separation was then achieved by manually moving the 2000 µm sieve up and down. The stable aggregate fraction remaining on the sieve was gently washed off the sieve into a pan. Water plus soil that went through the sieve was poured onto the next sieve and the sieving was repeated. The method results in four aggregate fractions: large macro-aggregates (> 2000 µm), small macro-aggregates (250-2000 µm), micro-aggregates (50-250 µm) and microstructures (< 50 µm) (Figure 3.1). After aggregate separation all aggregate samples were air dried.

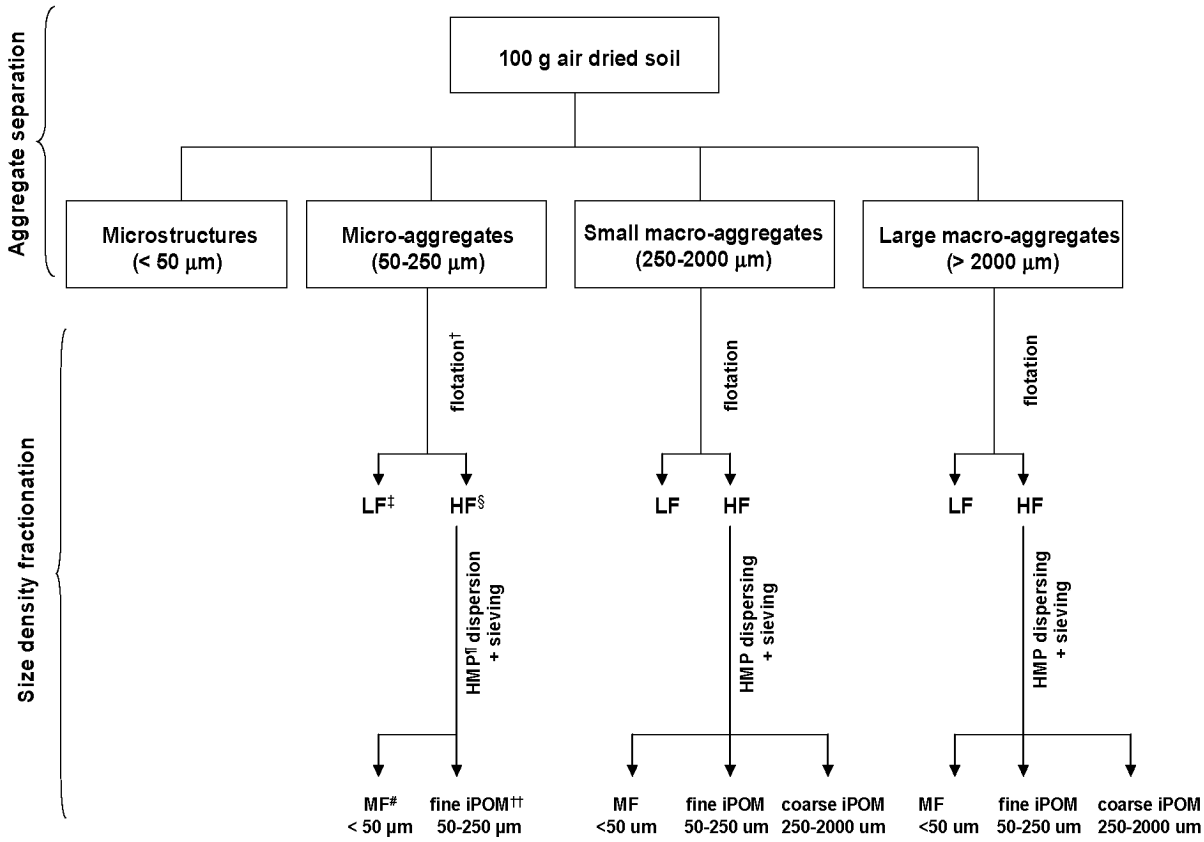

Figure 3.1. Aggregate separation and size density fractionation (Six et al., 1998). †using sodium polytungstate, ‡free light fraction, § heavy fraction, ¶ hexametaphosphate, #mineral- associated organic matter fraction, †† intra-aggregate particulate organic matter.

Size density fractionation

Size density fractionation was used to separate each of the aggregate fractions > 50 µm into three POM fractions (free light fraction = LF, coarse and fine intra-aggregate POM = iPOM) and the mineral-associated soil organic matter fraction (MF) (Figure 1; Six et al., 1998). Size density fractionation was conducted with 10 g aggregate subsamples. First free LF was separated from the heavy fraction (HF) by density flotation in 1.85 g cm^{-3} sodium polytungstate. After dispersion with hexametaphosphate the HF was wet sieved through a series of sieves (2000 µm and/or 250 µm and 50 µm depending on the aggregate fraction) resulting in the following iPOM fractions: coarse iPOM (250-2000 µm) and/or fine iPOM (50-250 µm). Material passing

the 50 µm sieve was assigned to MF. All size density fractions were air dried after separation.

N and C analyses

N, ^{15}N and C analyses

Air-dried soil, aggregate fractions and size density fractions were finely ground using a ball mill (Retsch, Haan, Germany) prior to total N and C and ^{15}N abundance analysis on a continuous flow Roboprep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England).

Atom% ^{15}N excess

The atom% ^{15}N excess of each sample denotes the ^{15}N abundance of the sample minus the natural abundance of its reference sample. The natural abundance of reference soil samples taken from the 0N microplots where no ^{15}N-labeled amendment was applied was 0.3684 atom% ^{15}N for CONMIN and 0.3693 atom% ^{15}N for BIOORG.

N derived from the labeled component of the amendments (Ndflc)

The proportion of N derived from the ^{15}N-labeled component of the amendments - urine for SlurryU, feces for SlurryF, and $^{15}NH_4{}^{15}NO_3$ for MineralN - in the aggregate fractions or the size density fractions, was calculated according to equation (1) using isotope pool dilution principles (Hauck and Bremner, 1976):

$$\% \text{ Ndflc} = (^{15}\text{Nex}_{\text{fractions}}/^{15}\text{Nex}_{\text{lc}}) \times 100 \quad (1)$$

where $^{15}\text{Nex}_{\text{fractions}}$ denotes the atom% ^{15}N excess of the aggregate fractions or size density fractions and $^{15}\text{Nex}_{\text{lc}}$ denotes the atom% ^{15}N excess of the labeled component of the amendment (Table 3.2). Analysis of the homogeneity of ^{15}N labeling of fecal N by

physico- and biochemical techniques revealed that the excess of total fecal ^{15}N can be used for calculations in spite of small deviations in excess among fecal N fractions (Chapter 1).

^{15}N recovery

For ^{15}N recovery in the aggregate fractions or the size density fractions the amount of soil in a microplot was calculated by multiplying the volume of the microplot with the soil bulk density. Soil bulk density for the 0-18 cm soil layer was 1.3 kg dm^{-3} and was not significantly different between CONMIN and BIOORG. It resulted in 10.8 kg soil dry matter per microplot. For the aggregate fractions their proportion on dry matter in the soil, and for the size density fractions their proportion on dry matter in the aggregate fractions and subsequently in the soil were used to calculate their respective dry weight per microplot. From the dry weight, the N concentration and the ^{15}N excess of aggregate fractions and size density fractions, respectively, the amount of ^{15}N recovered in each of these fractions was calculated. The amount divided through the amount of ^{15}N applied to the microplots multiplied by 100 is the recovery in each fraction as percentage of applied ^{15}N.

Statistical analysis

Analysis of variance was performed by using the statistical analyses package SYSTAT 11 (Systat Software Inc., USA). Effects of the main factors cropping system and amendment and of the secondary factors aggregate fraction and size density fraction were tested using a split-plot design. For analysis of variance percentage data was transformed using arcsin-transformation. In case of significant effects separation of means was tested using Tukey's HSD (honestly significant difference) test with a significance level of $P \leq 0.05$.

Results and Discussion

Soil characteristics

Soil microbial biomass and activity were higher in BIOORG than CONMIN in March 2003 (Table 3.1). This confirms several previous measurements on microbial biomass and activity in the same soils (Fliessbach and Mäder, 2000; Mäder et al., 2002). Soil pH was lower in CONMIN than BIOORG, probably due to the acidifying effect of mineral fertilizers (Mäder et al., 2006). In spite of long term organic fertilization in BIOORG, total C and N concentrations in soils were not significantly different between CONMIN and BIOORG (Table 3.1). This agrees with Fliessbach et al. (2007) who did an extended study on soil organic C in the same field experiment and who report a decreasing trend in soil organic C concentrations in BIOORG and CONMIN since starting the field experiment in 1977 (-15% in CONMIN and -9% in BIOORG). Also Wander et al. (2007) found no accumulation of soil organic C under organic cropping. In contrast, several studies suggest that the repeated application of animal manure increases soil N content (Glendining et al., 1997; Kong et al., 2007).

Aggregate fractions

Distribution and total N concentration

Distribution of the aggregate fractions was not affected by the cropping system. The sum of the different aggregate fractions represented 98.5% of the non-fractionated soil dry matter (DM). The macro-aggregates (> 250 µm) represented 68.6% of total soil DM, with the greatest contribution from the small macro-aggregates (Table 3.3).

Table 3.3. Distribution of aggregate fractions in the soil, total N concentration in the aggregate fractions, and contribution of aggregate fraction N to total soil N. Standard deviation is shown in brackets. Because of no significant differences between CONMIN and BIOORG and between amendments, mean values are shown (n = 24).

	Aggregate fractions				
	Large macro-aggregates (> 2000 µm)	Small macro-aggregates (250-2000 µm)	Micro-aggregates (50-250 µm)	Micro-structures (< 50 µm)	Total
Distribution (% of soil dry weight)	28.9 (5.3)	39.7 (3.1)	17.8 (5.2)	12.1 (2.7)	98.5
Total N concentration (g kg^{-1} aggregate)	1.5 (0.2) a	1.5 (0.2) a	1.6 (0.3) a	1.0 (0.1) b	
Total N concentration (g kg^{-1} soil)	0.4	0.6	0.3	0.1	
% of total soil N	26.7	40.0	20.0	8.1	94.8

Within a row means followed by same letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test.

Total N concentration (g kg^{-1} aggregate) in the macro- and micro-aggregates was higher than in the microstructures, and was also not significantly affected by the cropping system (Table 3.3). Calculated on a non-fractionated soil basis, the small macro-aggregates accounted for 40.0%, the large macro-aggregates for 26.7%, the micro-aggregates for 20.0% and the microstructures for 8.1% of total soil N (Table 3.3). This confirms the importance of the macro-aggregates for N storage (Green et al., 2005). Total recovery of soil N in all aggregate fractions was 94.8%. This suggests that some N was lost during aggregate separation, probably by leaching of soluble N compounds during wet sieving and/or gaseous losses during air drying of the aggregate fractions.

N derived from the labeled component of the amendments (Ndflc)

At 112 days after application of the amendments, ^{15}N applied with the amendments was detected in all aggregate fractions (Table 3.4). Between 0.09 and 1.5% of N

contained in the aggregates derived from the labeled components of the amendments. The proportion of N derived from the labeled amendments tended to increase with decreasing aggregate size (Table 3.4). The proportion of N derived from ^{15}N-labeled urine was higher than N derived from labeled feces and mineral fertilizer. For the ^{15}N-labeled urine, the Ndflc was for all aggregate fractions greater in BIOORG than CONMIN soils. The cropping system had no significant effect on N derived from labeled feces or labeled mineral fertilizer. The availability of urinary N to soil microorganisms and crops largely depends on the hydrolysis of urea to ammonium which was shown to be completed within a few days after addition of urine to soil (Whitehead and Bristow, 1990). This observation was confirmed by a companion study where the ^{15}N enrichment of nitrate showed that a large proportion of soil nitrate derived from urine at 11 days after slurry application (Chapter 2). The higher proportion of urine-derived N in all aggregate fractions compared to the proportion of N derived from the mineral fertilizer, which contained directly available N, may result from the higher amount of total N applied with urine. In addition, urine N was coupled with organic carbon contained in feces and urine (Ditter et al., 1998). This carbon probably stimulated microbial activity and might have accelerated microbial immobilization and incorporation of urine N into the aggregates. Thus, the higher proportion derived from urine N in aggregates of BIOORG than CONMIN soils could result from the higher microbial activity of the BIOORG soil. At similar amount of total N applied with feces and mineral fertilizer (Table 3.2), the proportion of feces-derived N was similar to the proportion of mineral fertilizer-derived N, the only exception being the large macro-aggregates of the CONMIN soil where the Ndflc is lower for SlurryF than for MineralN. Of total fecal N, organic N forms in feces were the largest fraction (Table 3.2) and thereof about 30% were undigested feed N (Chapter 1). Organic N compounds in feces that remained undigested after having passed the animal are supposed to mineralize very slowly in soil (Muñoz et al., 2003; Sørensen and Jensen, 1998). Our results obtained four months after amendment application suggest that the presence of organic compounds does not affect the incorporation of ^{15}N into the aggregates if the ^{15}N is contained in recalcitrant forms.

Table 3.4. Proportion of N derived from the labeled component of the amendment (Ndflc) in the aggregate fractions and the non-fractionated soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system (CS). Standard deviation is shown in brackets (n = 4).

		Amendment† (Amd)					
		SlurryU		SlurryF		MineralN	
	CS‡	CONMIN	BIOORG	CONMIN	BIOORG	CONMIN	BIOORG
Aggregate fractions (AF)							
		……%……					
large macro-aggregates (> 2000 µm)		0.35 (0.21) a	0.77 (0.46) a	0.09 (0.04) b	0.15 (0.09) b	0.20 (0.07) b	0.14 (0.04) b
small macro-aggregates (250-2000 µm)		0.60 (0.29) a	0.91 (0.25) a	0.22 (0.10) ab	0.23 (0.07) ab	0.21 (0.07) b	0.21 (0.07) ab
micro-aggregates (50-250 µm)		0.70 (0.32) a	1.10 (0.33) a	0.28 (0.14) ab	0.26 (0.05) ab	0.31 (0.07) ab	0.30 (0.07) a
microstructures (< 50 µm)		0.95 (1.1) a	1.50 (0.52) a	0.36 (0.16) a	0.33 (0.08) a	0.36 (0.08) a	0.34 (0.07) a
Ndflc non-fractionated soil		0.68 (0.3) A	1.1 (0.4) A	0.28 (0.1 A	0.26 (0.04) A	0.32 (0.1) A	0.31 (0.1) A
Source of variance:							
CS	**						
Amd	***						
AF	***						
CSxAmd	***						
CSxAF	ns						
AmdxAF	*						

* P = 0.01-0.05, ** P = 0.01-0.001, *** P = < 0.001, ns = not significant.

Within one column means of aggregate fractions followed by same lowercase letters, and within one row and within amendment, means of non-fractionated soil followed by same uppercase letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test.

† For both CONMIN and BIOORG SlurryU > SlurryF = MineralN (SlurryU = ^{15}N-labeled urine + unlabeled feces, SlurryF = unlaeled urine + ^{15}N-labeled feces, MineralN = $^{15}NH_4{}^{15}NO_3$).

‡ BIOORG > CONMIN over complete dataset.

Recovery of ^{15}N derived from the labeled amendments

At 112 days after application, the recovery of urine-derived ^{15}N was in the non-fractionated soil and in aggregate fractions higher for BIOORG than CONMIN soil (Table 3.5). Nearly three times more of urine-derived ^{15} N was recovered in the large macro-aggregates of BIOORG than CONMIN soils (7.6 vs. 2.7%). Also in the small macro-aggregates, the recovery was higher for BIOORG than CONMIN. In contrast, the recovery of feces- and mineral fertilizer-derived ^{15}N was not affected by the cropping system.

Most of ^{15}N applied with the labeled amendments was recovered in the small macro-aggregates (Table 3.5), confirming their importance as N sink. In spite of their highest Ndflc, the proportion of fertilizer ^{15}N recovered in the microstructures < 50 µm was lowest because they contain only 8% of total soil N (Table 3.3).

The ^{15}N recovered in the non-fractionated soil accounted on average for 25.1%, 20.1% and 21.8% of originally applied ^{15}N in the SlurryU, SlurryF and MineralN treatment, respectively (Table 3.5). While recovery of mineral fertilizer N is in the range usually reported for topsoil layers, the recovery of feces is lower (Jensen et al., 1999; Thomsen et al., 1997). The sum of ^{15}N recovered in all aggregate fractions in the SlurryU treatment amounted to 22.4%, in the SlurryF treatment to 15.0% and in the MineralN treatment to 14.8% (Table 3.5). Hence between 11 (SlurryU) and 32% (MineralN) of ^{15}N contained in non-fractionated soil was lost during aggregate separation. These losses are higher than losses of about 5% derived from the total N recovery in the aggregates. The greatest proportion of ^{15}N was lost from the MineralN treatment, suggesting that at four months after applications, part of the applied ^{15}N was still present in water soluble form and was leached out of the soil during aggregate separation. In contrast, losses were lowest for the SlurryU treatment of BIOORG. For this soil - amendment combination, microbial immobilization may have increased N retention.

Table 3.5. Recovery of ^{15}N in the aggregate fractions and the non-fractionated soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system (CS). Standard deviation is shown in brackets (n = 4).

		Amendment† (Amd)					
		SlurryU		SlurryF		MineralN	
	CS‡	CONMIN	BIOORG	CONMIN	BIOORG	CONMIN	BIOORG
Aggregate fractions (AF)							
		..... ^{15}N recovery (% of originally applied ^{15}N).....					
large macro-aggregates (> 2000 µm)		2.7 (1.7) a	7.6 (5.2) ab	2.2 (1.9) ab	3.1 (1.7) b	4.0 (1.6) ab	3.3 (0.4) b
small macro-aggregates (250-2000 µm)		6.8 (4.9) a	11.2 (2.5) a	7.1 (4.3) a	6.6 (1.5) a	5.9 (1.9) a	5.6 (1.5) a
micro-aggregates (50-250 µm)		4.1 (2.0) a	6.3 (0.8) ab	4.2 (2.4) ab	3.5 (0.8) b	2.4 (1.0) b	3.5 (1.1) ab
microstructures (< 50 µm)		2.2 (1.1) a	3.9 (0.6) b	1.5 (0.5) b	1.8 (0.4) b	2.3 (1.0) b	2.5 (0.4) b
Total recovery in AF		15.8 (8.8)	29.0 (7.9)	15.0 (8.0)	15.0 (3.0)	14.6 (3.1)	14.9 (4.0)
Recovery non-fractionated soil		19.6 (11.3) A	30.6 (8.4) A	21.2 (9.2) A	19.1 (2.4) A	22.0 (9.3) A	21.6 (3.3) A
Source of variance:							
CS	**						
Amd	**						
AF	***						
CSxAmd	**						
CSxAF	ns						
AmdxAF	ns						

* P = 0.01-0.05, ** P = 0.01-0.001, *** P = < 0.001, ns = not significant.

Within one column means of aggregate fractions followed by same lowercase letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test. Within one row and within amendment, means of non fractionated soil followed by same uppercase letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test.

† For CONMIN no significant differences, for BIOORG: SlurryU > SlurryF = MineralN (SlurryU = ^{15}N-labeled urine + unlabeled feces, SlurryF = unlabeled urine + ^{15}N-labeled feces, MineralN = $^{15}NH_4{}^{15}NO_3$).

‡ BIOORG > CONMIN over complete dataset; within single amendment - aggregate fractions combinations only significant for microstructures of SlurryU: BIOORG > CONMIN.

Size density fractions

Distribution and total N concentration

Neither the distribution nor the N and C concentrations and resulting contents in the size density fractions were affected by the cropping system (Table 3.6, Table 3.7). With a proportion of more than 90% of aggregate dry weight the MF was the dominant fraction in all aggregate fractions (Table 3.6). Fine and coarse iPOM as well as free LF accounted for less than 1% of aggregate dry weight of the respective aggregate fractions.

Table 3.6. Distribution of size density fractions (LF = light fraction; iPOM = intra-aggregate particulate organic matter, MF = mineral-associated organic matter fraction) in soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system. Standard deviation is shown in brackets. Because of no significant differences between CONMIN and BIOORG and between amendments, mean values are shown (n = 24).

	Size density fractions			
Aggregate fractions	LF	coarse iPOM	fine iPOM	MF
	.....% of aggregate dry weight.....			
Large macro-aggregates (> 2000 µm)	0.4 (0.3)	0.1 (0.04)	0.8 (0.5)	90.7 (6.1)
Small macro-aggregates (250-2000 µm)	0.3 (0.1)	0.2 (0.08)	0.6 (0.3)	94.7 (3.3)
Micro-aggregates (50-250 µm)	0.3 (0.2)	---	0.7 (0.2)	92.8 (3.8)

However, total N and C concentration (g kg^{-1} size density fraction dry weight) was for all aggregate fractions higher in free LF and iPOM than in the MF (Table 3.7), as shown in other studies (Besnard et al., 1996; Pulleman et al., 2005; Six et al., 2001). Coarse iPOM of the large macro-aggregates had significantly higher total N and C concentrations than coarse iPOM of the small macro-aggregates (Table 3.7).

N and C contained in all LF and iPOM fractions accounted for 2.7% of total soil N and 4% of total soil C (Table 3.7). This is relatively low when compared to a number of studies of agricultural soils where on average 14% of total soil N and 19% of total soil C were found in POM fractions (Gregorich et al., 2006). It is, however, comparable to POM recovered from the same soils by Fliessbach and Mäder (2000) although they used a different size density fractionation method. Low contents of LF and iPOM suggest a rapid decomposition of these fractions, thus preventing their accumulation (Wander et al., 2007). Despite having the lowest N and C concentration the MF accounted for about 60% of total soil N due to its big proportion in all aggregate fractions and thus plays an important role for N and C storage. Comparison of aggregate fractions shows that the greatest proportion of N was found in the LF, fine iPOM and MF of the small macro-aggregates.

Only 63.2% of total soil N was recovered in the size density fractions resulting in an overall N recovery of 71.3% as 8.1% of total soil N was recovered in the microstructures. About 10% of total soil N was lost from the small macro-aggregates and about 7% each from the large macro-aggregates and micro-aggregates. This suggests that soluble N must have been protected in the aggregates and released during dispersion of the heavy fraction and its separation into iPOM and MF. Also only 60% of total soil C was recovered in the size density fractions, suggesting that during dispersion of the soil also significant amount of C was lost.

Table 3.7. Total N and total C concentration in the size density fractions (LF = light fraction; iPOM = intra-aggregate particulate organic matter, MF = mineral-associated organic matter fraction), and contribution of size density fraction N and C to total soil N and C of the soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system. Standard deviation is shown in brackets. Mean values of CONMIN and BIOORG and fertilizer treatments are shown (n = 24).

	Aggregate fractions (µm)								
	> 2000	250-2000	50-250	> 2000	250-2000	50-250	> 2000	250-2000	50-250
	N								
	g kg^{-1} size density fraction dry weight			mg kg^{-1} soil			% of total soil N or C		
LF	7.4 (1.8) a	10.9 (2.7) a	13.3 (2.3) a	6.8 (2.6) b	10.9 (13.3) b	5.5 (4.1) b	0.5	0.7	0.4
coarse iPOM	3.4 (1.2) b	2.2 (0.9) b	---	1.2 (0.7) b	1.5 (0.9) b	---	0.1	0.1	---
fine iPOM	2.6 (0.9) c	2.6 (0.7) b	2.2 (0.8) b	4.9 (2.5) b	5.7 (1.9) b	2.8 (1.0) b	0.3	0.4	0.2
MF	1.1 (0.3) d	1.1 (0.2) c	1.2 (0.3) c	294.4 (103.1) a	427.9 (70.6) a	186.2 (44.2) a	19.6	28.5	12.4
	C								
LF	136.5 (43.8) a	158.3 (42.8) a	161.6 (26.6) a	119.8 (43.7) b	148.8 (33.8) b	55.7 (21.0) b	0.9	1.1	0.4
coarse iPOM	53.1 (18.9) b	33.8 (12.4) b	---	18.2 (9.9) b	23.5 (13.0) b	---	0.1	0.2	---
fine iPOM	25.2 (10.1) c	29.7 (6.9) b	26.2 (8.5) b	49.5 (25.9) b	65.4 (19.6) b	33.5 (11.7) b	0.4	0.5	0.4
MF	8.9 (2.1) c	9.3 (1.3) c	10.0 (2.1) c	2405.6 (792) a	3493.1 (557) a	1584 (384) a	18.1	26.3	11.9

Within a column means for N and C, respectively, followed by same letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test.

C/N ratios

The C/N ratios decreased in the order free LF ≥ coarse iPOM > fine iPOM > MF (Table 3.8) suggesting an increased degree of decomposition from free LF to the MF. Decreasing C/N ratios with diminishing particle size were also found by Gregorich et al. (2006), Pulleman et al. (2005) and Six et al. (1998) and might be attributed to a reduction of C content due to exhaustion of easily decomposable OM (Kanazawa and Filip, 1986). The wider C/N ratios of free LF and iPOM compared to the MF suggest that these fractions are richer in recent plant residues (Jastrow, 1996; Willson et al., 2001) whereas the MF is dominated by microbial products (Christensen, 2001). Wheat roots and senescent leaves which might have fallen from wheat plants before harvest and which may subsequently have been incorporated into SOM fractions (straw was removed at harvest) had a much larger C/N ratio (senescent leaves: 60, roots: 47) than the density size fractions indicating that this material has been microbially degraded (Six et al., 2001). The C/N ratio of free LF decreases with decreasing aggregate size confirming that LF related to smaller aggregates is more decomposed (Gregorich et al., 2006).

Table 3.8. C/N ratios of the size density fractions (SDF) (LF = light fraction; iPOM = intra-aggregate particulate organic matter, MF = mineral-associated organic matter fraction)of the soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system (CS). Standard deviation is shown in brackets. Because of no significant differences between CONMIN and BIOORG and between amendments, mean values are shown (n = 24).

	Size density fractions (SDF)			
	free LF	coarse iPOM	fine iPOM	MF
Aggregate fractions (AF)				
large macro-aggregates (> 2000 µm)	18.8 (3.0) a A	15.9 (0.9) b A	11.4 (0.9) c B	8.2 (0.3) d B
small macro-aggregates (250-2000 µm)	15.2 (2.3) a B	15.8 (1.5) a A	11.6 (0.9) b AB	8.2 (0.2) c B
micro-aggregates (50-250 µm)	12.1 (1.2) a C	---	12.0 (0.6) a A	8.5 (0.2) b A

Source of variance:

CS	ns
Amendment (Amd)	ns
AF	***
SDF	***
CSxAmd	ns
CSxAF	ns
AmdxAF	ns
CSxSDF	ns
AmdxSDF	ns

* P = 0.01-0.05, ** P = 0.01-0.001, *** P = < 0.001, ns = not significant.

Within rows, means followed by same lowercase letters and within columns, means followed by same uppercase letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test.

N derived from the labeled component of the amendments (Ndflc)

The long term cropping system did not significantly affect the incorporation of the amendments into the size density fractions. However, the Ndflc was significantly higher for urine than feces or mineral fertilizer. The Ndflc shows that a ^{15}N inflow occurred into each size density fraction during the four months after amendment application. A higher proportion of amendment-derived N was associated with free LF and coarse iPOM than

fine iPOM and MF, except for SlurryF where the greatest proportion of amendment-derived N was found in coarse iPOM (Table 3.9).

Table 3.9. Proportion of N derived from the labeled component of the amendment (Ndflc) in the size density fractions (SDF) (LF = light fraction; iPOM = intra-aggregate particulate organic matter, MF = mineral-associated organic matter fraction) of the soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system (CS). Standard deviation is shown in brackets. Because of no significant differences between CONMIN and BIOORG and between aggregate fractions (AF), mean values are shown (n = 24).

	Amendment† (Amd)		
	SlurryU	SlurryF	MineralN
Size density fractions			
free LF	1.9 (1.5) a	0.4 (0.3) b	1.1 (1.0) a
coarse iPOM	1.5 (0.7) a	1.4 (1.7) a	0.8 (0.3) a
fine iPOM	0.6 (0.3) b	0.3 (0.2) b	0.2 (0.1) b
MF	0.7 (0.3) b	0.2 (0.1) b	0.2 (0.1) b

Source of variance:

CS	ns
Amd	***
AF	ns
SDF	***
CSxAmd	ns
CSxAF	ns
AmdxAF	**
CSxSDF	ns
AmdxSDF	***

* P = 0.01-0.05, ** P = 0.01-0.001, *** P = < 0.001, ns = not significant.

Within columns, means followed by the same letters are not significantly different ($P \leq 0.05$) by Tukey's multiple range test.

† SlurryU > SlurryF = MineralN (SlurryU = ^{15}N-labeled urine + unlabeled feces, SlurryF = unlabeled urine + ^{15}N-labeled feces, MineralN = $^{15}NH_4{}^{15}NO_3$) except for coarse iPOM with no significant difference between amendment treatments.

As a part of ruminant feces enters soil as particulate material, and as stabilization of iPOM within aggregates requires time, this comparatively high Ndflc in iPOM suggests that particulate feces N compounds may have been incorporated into aggregates (Aoyama et al., 1999). In contrast, urine- and mineral fertilizer-derived N may have entered the LF through plant debris. Besides these indications, we cannot clearly separate the different pathways such as ^{15}N incorporation through microbial or abiotic processes, or via exudates from wheat roots or wheat residues. Soil bacteria were found to be mostly located in the silt- and clay-fraction where also enzyme activity was higher than in the sand-sized fraction (Blackwood and Paul, 2003; Kandeler et al., 2000; Kirchmann et al., 2004). Therefore, the incorporation of ^{15}N into MF may mainly have been mediated through microbial processes (Christensen, 2001). The importance of abiotic processes, in our soils, was demonstrated by ^{15}N isotopic pool dilution experiments where within the first 30 minutes after addition of highly enriched $(^{15}NH_4)_2SO_4$ to sterilized soil more than 30% of the $^{15}NH_4^+$ underwent physico-chemical binding processes (data not shown). Therefore, part of NH_4 derived from mineral fertilizer and urine may undergo fixation to clay and might through this process end up in the MF.

Recovery of ^{15}N derived from the labeled amendments

The recovery of amendment ^{15}N in the size density fractions did not differ significantly between CONMIN and BIOORG but was significantly affected by the amendment and differed between aggregate fractions and size density fractions (Table 3.10). Recovery was higher for urine- than feces- or mineral fertilizer-derived ^{15}N, mostly because of greater recovery of urine-derived ^{15}N in the MF. For all amendments, significantly more ^{15}N was recovered in the MF of small macro-aggregates than in MF of large macro-aggregates and micro-aggregates (Table 3.10) because of the large size of this fraction (Table 3.6). More amendment ^{15}N was recovered in MF than in free LF and iPOM together, showing that MF acted as major N sink for all amendments. This agrees with Gerzabek et al. (2001) who deduced from ^{15}N natural abundance from soils fertilized differently since 1956 that most of applied fertilizer N was stored in the silt-sized fraction.

For SlurryU and MineralN, ^{15}N recovered in free LF generally decreased with decreasing aggregate size while for SlurryF recovery in free LF was highest in the small macro-aggregates (Table 3.10). Provided that free LF associated with large macro-aggregates is less protected and has a shorter turnover time than LF associated with small macro-aggregates (von Lützow et al., 2007), this observation also supports that urine- and mineral fertilizer-derived ^{15}N may have entered the free LF more recently through plant debris while ^{15}N in LF in small macro-aggregates derived from feces may have been deposited earlier, i.e. through particulate feces material at amendment application. Recovery was generally low in iPOM fractions, but there was a trend for greater recovery of feces N than urine- and mineral fertilizer- derived N in iPOM.

In total, between 7.4% (SlurryF) and 12.7% (SlurryU) of originally added ^{15}N was recovered in the size density fractions from all aggregate fractions (Table 3.10). When relating these recoveries to recoveries in aggregate fractions (Table 3.5), except the microstructures which were not used for size density fractionation, then the estimated ^{15}N losses during size density fractionation ranged between 33 and 47% of ^{15}N in aggregates or between 37 and 55% of ^{15}N contained in non-fractionated soil (Table 3.5). Estimated ^{15}N losses were higher for SlurryF (47%) than SlurryU (35%) and MineralN (33%). Thus dispersion of aggregates seems to release high amounts of soluble mineral and/or organic N which was previously protected in the aggregate structure and then washed out during wet sieving of the HF. While also mineral ^{15}N may have been lost from MineralN and SlurryU treatments, we assume that mostly organically bound ^{15}N was lost from the SlurryF treatment. Siemens and Kaupenjohann (2002) showed that soluble organic N contributes significantly to N leaching from arable soils. In their study, organic N leaching was higher in manured plots than in plots that received mineral fertilizer N.

Table 3.10. Recovery of ^{15}N in the size density fractions (SDF) (LF = light fraction; iPOM = intra-aggregate particulate organic matter, MF = mineral-associated organic matter fraction) of the soil of the conventional (CONMIN) and the bio-organic (BIOORG) cropping system (CS). Standard deviation is shown in brackets. Because of no significant difference between CONMIN and BIOORG mean values are shown (n = 8).

		Size density fractions (SDF)†				Total recovery in:	
		free LF	coarse iPOM	fine iPOM	MF	***SDF***	**non-fractionated soil**
	Aggregate fractions (AF)						
Amendment‡ (Amd)		……^{15}N recovery (% of originally applied ^{15}N)……..					
SlurryU	large maro-aggregates	0.46 (0.3) a	0.03 (0.02) a	0.04 (0.03) a	2.6 (1.4) b		
	small macro-aggregates	0.26 (0.1) ab	0.07 (0.05) a	0.07 (0.04) a	5.9 (2.7) a		
	micro-aggregates	0.15 (0.1) b	---	0.05 (0.03) a	3.1 (1.3) b		
	Total recovery in AF	*0.87 (0.2)*	*0.10 (0.02)*	*0.16 (0.1)*	*11.6 (3.9)*	***12.7* (4.1)**	**25.1 (10.9)**
SlurryF	large maro-aggregates	0.10 (0.02) ab	0.04 (0.04) a	0.04 (0.01) b	1.2 (0.4) b		
	small macro-aggregates	0.26 (0.2) a	0.15 (0.2) a	0.09 (0.05) a	3.4 (1.0) a		
	micro-aggregates	0.09 (0.05) b	---	0.06 (0.03) ab	1.9 (0.8) b		
	Total recovery in AF	*0.45 (0.2)*	*0.19 (0.1)*	*0.19 (0.1)*	*6.6 (1.2)*	***7.4* (1.6)**	**20.1 (6.3)**
MineralN	large maro-aggregates	0.68 (0.4) a	0.06 (0.05) a	0.03 (0.02) a	1.6 (1.0) b		
	small macro-aggregates	0.27 (0.1) b	0.03 (0.02) a	0.05 (0.02) a	3.5 (0.8) a		
	micro-aggregates	0.08 (0.03) b	---	0.03 (0.02) a	2.1 (0.6) b		
	Total recovery in AF	*1.03 (0.4)*	*0.09 (0.05)*	*0.11 (0.04)*	*7.1 (2.1)*	***8.3* (2.5)**	**21.8 (6.5)**

Source of variance: CS: ns; Amd: *; AF: ***, SDF: ***; CSxAmd: *; CSxAF: ns; AmdxAF: ns; CSxSDF: ns; AmdxSDF: ***.

* P = 0.01-0.05, ** P = 0.01-0.001, *** P = < 0.001, ns = not significant.

Within a column means of SlurryU, SlurryF and MineralN, respectively, followed by the same letter are nor significantly different ($P \leq$ 0.05) by Tukey's multiple range test.

† MF > free LF = coarse iPOM = fine iPOM for all amendments and all aggregate fractions.
‡ for free LF in large macro-aggregates: MineralN ≥ SlurryU ≥ SlurryF; for MF in large macro-aggregates: SlurryU ≥ MineralN ≥ SlurryF; for MF in small macro-aggregates: SlurryU > SlurryF = MineralN

Conclusions

Our first hypothesis that the distribution of N among SOM fractions differs between CONMIN and BIOORG soils due to long-term manure input in BIOORG had to be rejected. At the experimental site of this study, 25 years of organic farming induced no significant difference in total soil N or in N contained in any aggregate or size density fraction between BIOORG and CONMIN soils. Differences in fertilization strategy are probably overridden by crop rotation (including leys and green manures), residue management and ploughing, which are identical in BIOORG and CONMIN.

In contrast, incorporation of fresh amendment-derived N into different SOM fractions was affected by the amendment, confirming our second hypothesis. A higher proportion of urine-derived N than feces- or mineral fertilizer-derived N was found in the aggregate fractions as well as in free LF, fine iPOM and MF. These higher proportions of urine-derived N could be attributed to the amount and form of N added with urine and the coupling with carbon applied with the slurry.

The incorporation of urine-derived N was higher in aggregate fractions of BIOORG than CONMIN, suggesting that higher microbial activity of BIOORG than CONMIN may increase the potential of the BIOORG soil to retain N.

During aggregate separation between 20 and 30% of amendment ^{15}N contained in the non-fractionated soil was lost. During dispersion of aggregates for size density fractionation, another 37 to 55% of ^{15}N contained in non-fractionated soil was lost. This shows the importance of soil aggregation to protect N. As ploughing disrupts soil aggregates, regular ploughing may explain why the suggested potential of BIOORG soil to retain more N cannot be translated into a significant long term effect.

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Chapter 4: N budget and N stock in soils under organic and conventional farming

Abstract

Maintenance of soil N stock in cropping systems requires balanced nitrogen (N) budgets (N inputs equal N outputs). To assess the impact of cropping system management on soil N stock we calculated soil-surface N budgets and measured changes in soil N content of an organic (BIOORG) and a conventional (CONMIN) cropping system in a long-term field experiment. N budgets were calculated as difference between total N inputs (manure or mineral fertilizer N, atmospheric N deposition, symbiotic N_2-fixation, seed N) and total N outputs (N export by harvested products, N losses from manure or mineral fertilizer) over a 26-years period (1978-2003). Changes in soil N stock of both cropping systems were assessed by the difference in N content of the topsoil (0-20 cm) in 2003 and at establishment of the field experiment in 1977. Additionally soil N content of the topsoil was monitored based on total N measured in samples taken in 1977, 1984, 1987, 1991, 1994, 1998 and 2003. N budgets are negative for both BIOORG and CONMIN suggesting a yearly N deficit of -77 ±17 kg N ha^{-1} and -89 ±10 kg N ha^{-1}, respectively. Simple linear regression analyses showed a decrease in soil N content of the topsoil of both cropping systems between 1977 and 2003 which was only significant for CONMIN ($P < 0.05$). Measured yearly decrease in topsoil N stock (difference between soil N content in 2003 and 1977) was -29 kg N ha^{-1} for BIOORG and -39 kg N ha^{-1} for CONMIN. Despite inclusion of grass-clover mixtures and green manure crops in BIOORG and CONMIN and application of animal manure in BIOORG, the maintenance of soil N stock may be difficult due to the high N mineralization potential of the Loess soil at this experimental site. Ongoing decrease of soil N stock may only be countered by reducing soil tillage activities to minimize release of N protected in soil aggregates.

Introduction

Nitrogen (N) is the main yield-limiting nutrient in agricultural systems (Berry et al., 2002; Muñoz et al., 2003). To sustain soil N stock over long-term, N inputs into and N outputs from a cropping system have to be balanced. An N deficit (N input < N output)

may lead to mining of soil N stocks. An N surplus (N input > N output) holds the risk to pollute the environment due to N losses from the cropping system. Changes in soil N stock from a defined system can be estimated by calculating an N budget (i. e. inputs into minus outputs from the system) (Watson and Stockdale, 1999) or by direct measurements of soil N content over an extended period.

Soil organic matter (SOM) contains most of the soil N reserves (McNeill and Unkovich, 2007; Stockdale et al., 2002). The amount of organic N present in SOM and its turnover rate are influenced by the agricultural management practices. In contrast to conventional farming the use of mineral fertilizers is prohibited in organic farming (IFOAM, 2005). Therefore organic farming relies on the usage of organic fertilizers (e. g. animal manure, plant residues, composts). Repeated input of organic N sources through application of animal manure or incorporation of crop residues into the soil usually increase SOM content and in parallel organic N contained in SOM. In conventional cropping systems between 40 and 55% of N applied with mineral fertilizer is taken up by the crops (Carranca et al., 1999; Kramer et al., 2002; Limaux et al., 1999; Thomsen et al., 1997). In organic cropping systems in contrast N uptake by crops was between 20-30% of applied N with slurry (mixture of urine and feces) (Sørensen, 2004). Between 30-50% of urine-N and 40-70% of feces-N applied with slurry can be immobilized and accumulated in the soil (Sørensen and Jensen, 1998; Sørensen and Thomsen, 2005b). N which is retained in the soil is incorporated into SOM and physically protected by soil aggregates. Tillage breaks up the aggregates and exposes SOM to microbial attack inducing release of previously protected N and in parallel a reduction of SOM (Cambardella and Elliott, 1994; Pulleman et al., 2005).

The aim of this study was to evaluate the impact of long-term organic and conventional farming on soil N content. We calculated soil-surface N budgets for each of four crop rotations and a total N budget over a 26-year period (1978-2003) for an organically managed cropping system receiving farmyard manure and a conventionally managed cropping system that received exclusively mineral fertilizer. Soil surface budget takes into account all N entering the soil via the surface and leaving the soil via harvested

products (Oenema et al., 2003; Watson et al., 2002b). Estimated N losses from manure or mineral fertilizer where also included into the budgets. The estimated change in soil N stock obtained from the N budget was then compared to the measured change which was assessed by the difference in soil N content of the topsoil (0-20 cm) at establishment of the field experiment in 1977 and on soil sampled in 2003. Additionally soil N content of the topsoil was monitored based on total N measured in samples taken at different dates between 1977 and 2003. Change in soil N content of the subsoil layer (30-50 cm) was also assessed by the difference in soil N content at establishment of the field experiment in 1977 and on soil sampled in 1998.

Materials and Methods

Soil sampling

Soil was sampled in each of the four plots (field replicates) of BIOORG and CONMIN in the rotation unit b of the DOC long-term field experiment. Selected soil properties of BIOORG and CONMIN are shown in Table 4.1. Soil samples from the 0-20 cm soil layer, corresponding to the plough layer, were taken in 1977, 1984, 1987, 1991, 1994, 1998 and 2003. 1977 and at the end of the first, second and third crop rotation period (1984, 1991, 1998) the soil was additionally sampled from the 30-50 cm soil layer (subsoil). To avoid border effects the samples were taken over an inner 4 m x 16 m area of the 5 m x 20 m BIOORG and CONMIN plots of each of the four field replicates. Within each of the four field replicates of BIOORG and CONMIN 15-20 cores were sampled using a 3 cm diameter auger. The cores were separated into the 0-20 cm layer and the 30-50 cm layer.

Table 4.1. Selected soil properties of the topsoil (0-18 cm) of the bio-organic (BIOORG) and the conventional (CONMIN) cropping system in March 2003. (n = 4).

	Cropping system	
	BIOORG	CONMIN
Total N (g kg^{-1})	1.5 ns	1.5 ns
Total C (g kg^{-1})	13.6 ns	13.3 ns
Microbial biomass N (mg kg^{-1})[a]	21.8 a	12.5 b
Microbial biomass C (mg kg^{-1})[a]	113 ns	101 ns
pH (H_2O)	6.6 a	6.2 b
Texture[b]:		
Clay (%)	15.5 ns	15.3 ns
Silt (%)	69.0 ns	70.6 ns
Sand (%)	15.5 ns	14.1 ns

Within rows means followed by different letters are significantly different ($P \leq 0.05$) between the cropping systems (t-test); ns = not significant.

[a] Microbial biomass N and C determined by chloroform fumigation (Vance et al., 1987); no conversion factors applied.
[b] Soil samples collected from the 0-20 cm soil layer in 2002.

Soil-surface N budget

Average annual N budgets were calculated at the plot level (100 m^2) as difference between N inputs by fertilization (N_{fert}), atmospheric N deposition (N_{depo}), symbiotic N_2-fixation (N_{fix}) and seed N (N_{seed}) and N outputs by export with harvested products (N_{harv}) and losses of N added with manure or mineral fertilizer (N_{loss}) for the respective treatment during the respective period:

$$\text{Annual N budget [kg ha}^{-1}\text{ yr}^{-1}\text{]} = [(N_{fert} + N_{depo} + N_{fix} + N_{seed} - N_{harv} - N_{loss})/\text{years}] \quad (1)$$

Because CONMIN served as unfertilized control during the first seven years, two budgets were calculated, one with and one without the first crop rotation period.

Quantification of N inputs and outputs

Manure N

Total N in manure was determined in a subsample of 20-50 g farmyard manure and 20 ml slurry, respectively, taken from each manure application using Kjedahl procedure (Bremner and Mulvaney, 1982).

Atmospheric N deposition

Fields in the immediate surroundings of livestock production receive up to 50 kg N ha^{-1} yr^{-1} by dry deposition of ammonia (Janzen et al., 2003). Because of emissions from a hog house located nearby the DOC field experiment yearly N input during the first crop rotation period was assumed to be 50 kg ha^{-1}. Installation of a filter system in the hog house was expected to reduce N emission. Hence, for the following crop rotation periods N deposition was assumed to be 25 kg ha^{-1} yr^{-1} as obtained by Rihm and Dällenbach (1999) from modeled deposition of N compounds in the cantons of Basel-Stadt and Basel-Landschaft. They measured N deposition on individual locations and interpolated the values to a spatial reference unit of 100 m x 100 m. The modeled N deposition represents an average value of the annual deposition from 1995-1998.

Symbiotic N_2-fixation

Based on experiments using the ^{15}N dilution method to measure symbiotic N_2-fixation Boller et al. (2003) developed equations to estimate symbiotic N_2-fixation in grass-clover mixtures. For each meadow total symbiotic N_2-fixation (N_{sym}) was calculated by adding up N fixed by red clover (*Trifolium pretense* L.) and by white clover (*Trifolium repens* L.) using equation (2).

$$N_{sym}\ (kg\ ha^{-1}) = DM \times \%RC \times \%N_{RC} \times \%N_{symRC}/10\,000 + DM \times \%WC \times \%Nw_C \times \%N_{symWC}/10\,000 \quad (2)$$

DM (dt ha^{-1}) is the measured total dry matter yield of the grass-clover mixture (accumulated yield of five cuts) and %RC and %WC is the measured proportion of red clover and white clover, respectively, in the dry matter of the grass-clover mixture. The proportions of red and white clover used for calculation is shown in Table 4.2. $\%N_{RC}$ and $\%N_{WC}$ denotes the N content in dry matter of red clover and white clover, respectively. $\%N_{symRC}$ and $\%N_{symWC}$ is the proportion of symbiotically fixed N on total N in red clover and white clover, respectively. Estimation of $\%N_{RC}$ and $\%N_{WC}$ is based on linear multiple regression analyses. $\%N_{RC}$ was calculated using equation (3) and $\%N_{WC}$ using equation (4):

$$\%N_{RC} = 3.91\text{-}0.0222 \times DM \quad (3)$$

$$\%N_{WC} = 4.63 - 0.0171 \times DM - 0.0040 \times \%WC \quad (4)$$

To determine $\%N_{symRC}$ and $\%N_{symWC}$ equations (5) and (6), respectively, were used:

$$\%N_{symRC} = 91.0 + 0.103 \times DM - 0.144 \times \%RC - 0.191 \times N_{appl.} \quad (5)$$

$$\%N_{symWC} = 69.9 + 0.782 \times DM - 0.147 \times \%WC - 0.428 \times N_{appl.} \quad (6)$$

where $N_{appl.}$ denotes total N applied with manure or mineral fertilizer (kg ha^{-1}). Manure contains less direct plant available N than mineral fertilizer (Chapter 2). Hence, availability of manure-N compared to mineral fertilizer-N was assumed to be 61% (Sørensen and Jensen, 1998).

Table 4.2. Proportion of white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) in the dry matter of the grass-clover mixture of the four crop rotation periods (CRP) used for calculation of symbiotic N_2-fixation.

CRP	Year	Proportion of clover (%)			
		White clover		Red clover	
		BIOORG	CONMIN	BIOORG	CONMIN
1	1980[a]	15	15	30	46
	1981[a]	14	25	8	11
2[a]	1987[b]	15	11	30	34
	1988[a]	6	10	1	5
3[b]	1993[c]	30	8	56	19
	1994[c]	31	16	3	1
	1995[c]	11	2	0	0
4[b]	2000[c]	24	8	55	35
	2001[c]	34	11	8	13

[a] Only proportion of complete clover (white and red clover together) in the mixture available (Besson et al., 1992). Thus for the first year meadows average of ratio of white to red clover in the mixture in 1993 and 2000 and for the second year meadows average of ratio of white to red clover in the mixture in 1994 and 2001 used.
[b] Proportion of complete clover not determined. Thus for BIOORG proportion of complete clover in the first meadow of the first CRP used. For CONMIN proportion of complete clover in the first meadow of the first CRP of the other conventional cropping system in the DOC field experiment used (Besson et al., 1992).
[c] Proportion of white and red clover determined in the third of fifth cut.

N input by seeds

Seed N input (N_{seed}) over the 26-years period with potato tubers, cabbage, beetroots and grass-clover was less than 1 kg N ha^{-1} yr^{-1} and thus neglected. Seed N input by cereals was assessed as follows:

$$N_{seed} \text{ (kg ha}^{-1}\text{)} = N_{grain} \text{ x grain sown} \quad (7)$$

whereas N_{grain} (mg N $grain^{-1}$) denotes the N content in grain of winter wheat sampled in July 2003 from all four field replicates of CONMIN and BIOORG, and grain sown (grains

ha^{-1}) is the amount of grains sown per CONMIN and BIOORG plot in 2003 extrapolated to one hectare.

N exported by harvested products

For determination of N export by harvested products each crop in the crop rotation was yielded over an inner 2 m x 15 m area (harvest area) of the 5 m x 20 m BIOORG and CONMIN plots of each of the four field replicates. Until 2002 total N in plant material was determined by Kjedahl procedure. From 2002 onwards total N determination on 300-400 mg plant material was set by Dumas combustion (Bremner and Mulvaney, 1982) using a vario MAX CN (Fa. Elementar Analysesysteme GmbH, Hanau, Germany).

N losses from fresh applied manure or mineral fertilizer

N losses from manure and mineral fertilizer were obtained from the study where ^{15}N-labeled slurry and mineral fertilizer was applied to microplots installed in the BIOORG and the CONMIN soil to trace the fate of fertilizer-N (Chapter 2). N loss from slurry and mineral fertilizer was assumed to equal unaccounted slurry-^{15}N and mineral fertilizer-^{15}N, respectively. It was calculated by subtracting ^{15}N taken up by the crop and ^{15}N recovered in the 0-18 cm soil layer from the total amount of ^{15}N applied with slurry or mineral fertilizer. Resulting N losses from slurry and mineral fertilizer were 47% and 30%, respectively, of total N applied (Chapter 2).

Total soil N content

Soil sampled from the 0-20 cm and 30-50 cm soil layer of BIOORG and CONMIN, respectively, were sieved (2 mm), air dried and then finely ground with a ball mill (Retsch, Haan, Germany). Total N and C analysis of all soil samples was carried out on a Flash EA 1112 (Thermo Electron Corporation, Italy).

The N content in kg N ha^{-1} was calculated using a bulk density of 1.3 kg dm^{-3} for the topsoil and 1.5 kg dm^{-3} for the subsoil (Oehl et al., 2002). The change in soil N stock ($\Delta N_{topsoil}$) of the topsoil (0-20 cm) was determined according to equation (8) and the change in N stock ($\Delta N_{subsoil}$) of the subsoil (30-50 cm) was determined using equation (9).

$$\Delta N_{topsoil} \text{ (kg N ha}^{-1}\text{)} = \text{soil N content in 2003 - soil N content in 1977} \quad (8)$$

$$\Delta N_{subsoil} \text{ (kg N ha}^{-1}\text{)} = \text{soil N content in 1998 - soil N content in 1977} \quad (9)$$

Soil N mineralization

a) Isotope tracer technique

Isotope tracer technique based on the use of the stable isotope ^{15}N allows estimating soil N mineralization by determining N in the plant derived from soil (Ndfs) (Hauck and Bremner, 1976; Langmeier et al., 2002). In the study where ^{15}N-labeled mineral fertilizer and slurries were applied to winter wheat (*Triticium aestivum* var. Titlis) grown in microplots the Ndfs during the growth cycle of winter wheat (20 March until 10 July 2003) was calculated for BIOORG and CONMIN by subtracting N in plant derived from the ^{15}N-labeled fertilizers from total N content in the plant at harvest (Chapter 2).

b) Soil respiration

On March 2003 soil samples were taken from the four field replicates of BIOORG and CONMIN, respectively, down to a depth of 18 cm with an auger (Ø 2.5 cm, Eijkelkamp, Netherlands). Soil respiration was measured in analytical triplicates of fresh soil. CO_2 released from incubated soil (20 g) was trapped in 20 ml of 0.05 *M* NaOH and determined by titration with 0.1 *M* HCl (Alef and Nannipieri, 1995) at days 3, 10, 18, 24, 39, 52 and 60 of incubation. Gross N mineralization was calculated as average over three and 60 days of incubation using the C to N ratio of the microbial biomass (Table 4.1).

Statistics

The evolution of soil N content was tested on the four field replicates per treatment by simple linear regression analyses using the statistical analyses package SYSTAT 11 (Systat Software Inc., USA). According to the Durbin-Watson test values of the time series of N content between 1977 and 2003 were not autocorrelated. Means of soil N content of BIOORG and CONMIN at the establishment of the DOC field experiment in 1977 were compared using a two sample t-test at $P \leq 0.05$ level.

Results

N budget

The N budgets for each of the crop rotation period were negative for both cropping systems (Table 4.3). Over the 26 years period from 1978-2003 the estimated budget deficit was -77 ±17 kg N ha^{-1} yr^{-1} for BIOORG and -89 ±10 kg N ha^{-1} yr^{-1} for CONMIN (Table 4.3). With a proportion of over 60% in BIOORG and over 70% in CONMIN N applied with fertilizer was the major input parameter. Major output parameter in both cropping systems was N exported by harvested products.

Table 4.3. Average annual N input, N output and soil-surface N budgets of the soils of the bio-organic (BIOORG) and the conventional (CONMIN) cropping system for each crop rotation period (CRP) and as average of the four crop rotation periods (1978-2003).

	1. CRP (1978-1984)		2. CRP (1985-1991)		3. CRP (1992-1998)		4. CRP (1999-2003)		Total budget (1978-2003)	
	BIOORG	CONMIN	BIOORG	CONMIN	BIOORG	CONMIN	BIOORG	CONMIN	BIOORG	CONMIN[a]
	..... kg N ha^{-1} y^{-1}									
Input:										
N fertilizer	120	0	100	101	82	151	149	136	110	129 (94)
N deposition	50	50	25	25	25	25	25	25	32	25 (32)
N fixed	24	37	18	18	48 ± 3[b]	11 ± 4	53 ± 1	20 ± 4	34 ± 8	16 ± 2 (22)
N seed	2	2	2	2	2	2	2	2	2	2 (2)
Total Input	*196 ± 0.2*	*89 ± 0*	*145 ± 0.1*	*146 ± 0.2*	*157 ± 3*	*189 ± 4*	*229 ± 2*	*183 ± 4*	*178 ± 18*	*172 ± 12 (150 ± 23)*
Output:										
N export harvest	188 ± 7	172 ± 5	216 ± 6	233 ± 3	171 ± 3	220 ± 9	248 ± 5	224 ± 17	203 ± 16	226 ± 8 (211 ± 14)
N loss fertilizer	56	0	47	30	39	45	70	41	52	38 (28)
Total Output	*244 ± 8*	*172 ± 5*	*263 ± 6*	*263*	*210 ± 3*	*265 ± 7*	*318 ± 5*	*265 ± 10*	*255 ± 23*	*264 ± 1 (239 ± 23)*
N budget	**-48** ± *7*	**-83** ± *5*	**-118** ± *6*	**-117**	**-53** ± *3*	**-76** ± *8*	**-89** ± *6*	**-82** ± *8*	**-77** ± *17*	**-92** ± *11* **(-89** ± *10***)**

[a] Not fertilized during the first crop rotation period ; therefore averages shown for 19 and 26 (in brackets) years, respectively.

[b] Standard error of means (SEM) only calculated if separate values for all four field replicates available for BIOORG and CONMIN, respectively.

Evolution of total soil N content

N content in the 0-20 cm soil layer at establishment of the field experiment in 1977 was not significantly different between BIOORG and CONMIN. Regression analyses showed for both cropping system that the N content of the topsoil tends to decrease since the establishment of the experiment in 1977. The negative correlation was only significant ($P \leq 0.05$) for the CONMIN soil (Figure 4.1). Regression analyses showed a yearly decrease of 0.009 (± 0.0005) and 0.013 (± 0.002) mg N g^{-1} soil for BIOORG and CONMIN, respectively.

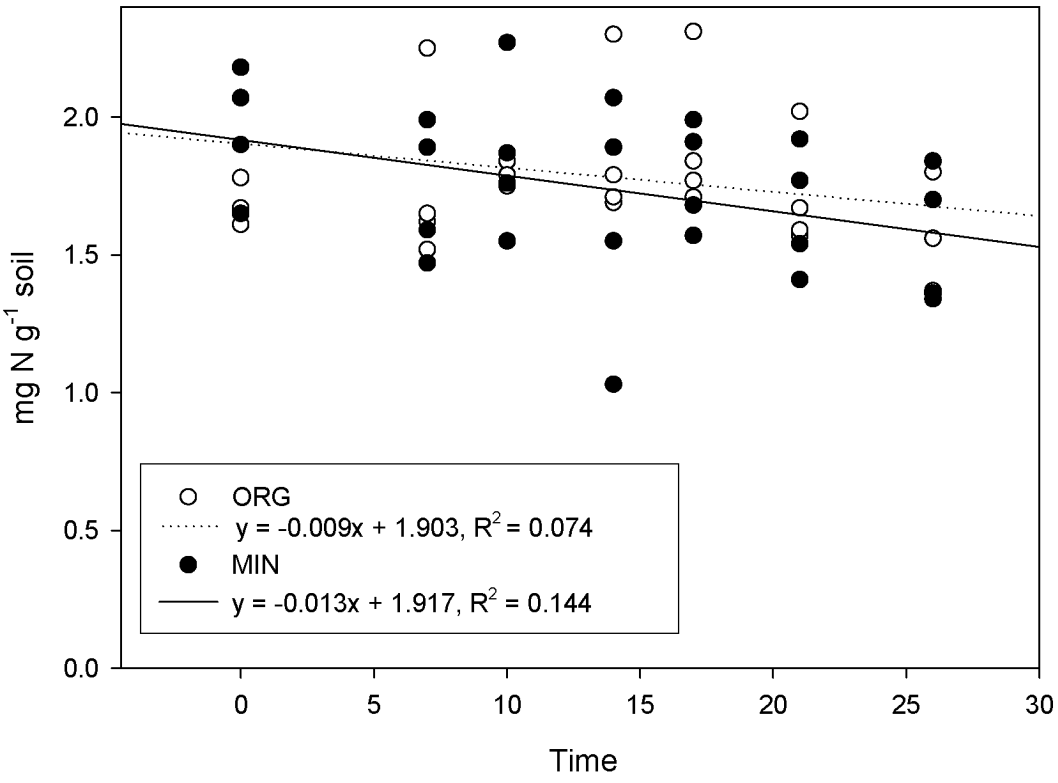

Figure 4.1. Evolution of soil N content in the 0-20 cm soil layer of the bio-organic (BIOORG) and conventional (CONMIN) cropping system over time (establishment of the field experiment in 1977 set zero). BIOORG: $P = 0.162$, CONMIN: $P = 0.046$. Data points are shown for all four field replicates of BIOORG and CONMIN, respectively.

Measured change in soil N stock of the 0-20 cm soil layer ($\Delta N_{topsoil}$) between 1977 and 2003 was -760 ±177 kg N ha^{-1} for BIOORG and -1014 ±188 kg N ha^{-1} for CONMIN (Table 4.4). Between 1977 and 1998 an accumulation of +383 ±148 kg N ha^{-1} and +412 ±136 kg N ha^{-1} was measured in the subsoil (30-50 cm) of BIOORG and CONMIN, respectively (Table 4.4).

Table 4.4. Comparison of total soil-surface N budget (expected change in soil N stock) with the measured change in total N content ($\Delta N_{topsoil}$ and $\Delta N_{subsoil}$) in the soils of the bio-organically (BIOORG) and the conventionally (CONMIN) managed cropping system during 26 years (kg N ha^{-1}).

	Topsoil (0-20 cm)				Subsoil (30-50 cm)		
Soil	N 1977	N 2003	$\Delta N_{topsoil}$	Total soil-surface N budget	N 1977	N 1998	$\Delta N_{subsoil}$
BIOORG	4706 ± 334	3946 ± 276	-760 ± 177	-2002	2475 ± 107	2858 ± 214	+383 ± 148
CONMIN	5070 ± 300	4056 ± 324	-1014 ± 188	-2314	2558 ± 240	2970 ± 354	+412 ± 136

Soil N mineralization

Daily soil N mineralization rates assessed by two different methods are shown in Table 4.5. From soil respiration a mineralization rate of 2.0 mg N kg soil^{-1} day^{-1} was obtained for the BIOORG soil for short-term incubation of three days, which was twice as much as for the CONMIN soil. Over the 60 days incubation period an average mineralization rate of about 0.5 mg N kg soil^{-1} day^{-1} (BIOORG: 0.8 mg N kg soil^{-1} day^{-1}, CONMIN: 0.3 mg N kg soil^{-1}day^{-1}) was calculated from soil respiration. Ndfs calculated with the isotope tracer technique was around 130 kg N ha^{-1} for both BIOORG and CONMIN soil during a 112 days period. This corresponds to an N mineralization rate of 0.5 mg N kg soil^{-1} day^{-1}.

Table 4.5. Daily soil N mineralization rates in the 0-18 cm soil layer of the organically (BIOORG) and conventionally (CONMIN) cropped soil assessed by different methods. (isotope tracer technique: n=4; soil respiration: n = 12).

Method	Soil N mineralization	
	BIOORG	CONMIN
	.....mg N kg^{-1} soil day^{-1}.....	
Isotope tracer technique[a]	0.5 (± 0.03)	0.5 (± 0.03)
Soil respiration		
day3[b]	2.1 (± 0.14)	0.9 (± 0.06)
day60[c]	0.8 (± 0.27)	0.3 (± 0.11)

[a] Assessed from N derived from soil (Ndfs) during the 112 days growth period of winter wheat which was calculated by subtracting N derived from the ^{15}N-labeled fertilizers (Ndff) from total N uptake by the crop (Chapter 2).
[b] Average N mineralization rate assessed as for three incubation days.
[c] Average N mineralization rate assessed as for 60 incubation days.

Discussion

Changes in soil N stock between 1977 and 2003

For both, BIOORG and CONMIN, soil-surface N budgets for each crop rotation period and total N budget are negative. Simple N budget for the 26-years period (1978-2003) calculated by the difference between N inputs with fertilization and N outputs exported with harvested products resulted in a yearly N deficit of -93 kg N ha^{-1} for BIOORG and -118 kg N ha^{-1} for CONMIN (Table I1). Atmospheric N deposition and symbiotic N_2-fixation can substantially contribute to the input of N into agroecosystems. Including these inputs into the N budgets reduced the yearly N deficit to -77 kg N ha^{-1} for BIOORG and -89 kg N ha^{-1} for CONMIN (Table 4.3). Assuming that the total soil-surface N budget would only affect the N stock of the topsoil (0-20 cm), then the estimated N deficits were still higher than the measured changes in soil N stock of the topsoil. The yearly decrease in soil N stock between 1977 and 2003 was -29 kg N ha^{-1} and -39 kg N ha^{-1} for BIOORG and CONMIN, respectively (Table 4.4). The difference between the N deficit resulting from the total soil-surface N budget and measured N deficit in the 0-20 cm soil layer between 1977 and 2003 was 1242 kg N ha^{-1} for BIOORG and 1300 kg N ha^{-1} for CONMIN (i. e. measured total N decrease less than expected from the budget deficit) (Table 4.4). The discrepancy between the estimated (N budget) and the measured ($\Delta N_{topsoil}$) change in soil N stock may be attributed to i) uncertainties in the soil-surface N budget caused by the underlying estimates and assumptions and sampling errors (Oenema and Heinen, 1999) and/or ii) N uptake from the subsoil (e. g. internal recycling of N transferred to deeper soil layers during the course of the experiment) or other N sources (e.g. nitrate from groundwater).

N input with fertilization and N exported with harvested products are based on measurements. The largest source of error in the N budget thus may come from underestimation of atmospheric N deposition and/or symbiotic N_2-fixation. In mixed farming systems of the temperate climate zone with a stocking rate comparable to the DOC field experiment N_2-fixation accounted for 50% or more of total N input (Watson et

al., 2002b). The contribution of N_2-fixation to total N input in BIOORG and CONMIN was less than 20% (Table 4.3). Estimated annual symbiotic N_2-fixation resulting from the soil-surface budget was about 100 kg ha^{-1} yr^{-1}. Annual amount of symbiotically fixed N by clover can range from 20-370 kg ha^{-1} yr^{-1}, depending on the amount of mineral N available and to the establishment of the meadow (i. e. seeding year or first year of production) (Boller and Nösberger, 1987). Proportion of red and white clover in the meadows of BIOORG and CONMIN was only determined in the third cut, disregarding that the composition of the meadow certainly alternate from the first to the fifth cut (i. e. change of the ratio red to white clover and clover to grass species). In addition total symbiotic N_2-fixation is underestimated by the equation of Boller et al. (2003) as it comprises only the above ground plant parts. The amount of N fixed in the belowground biomass can be up to 50% of N fixed in the above ground biomass (Carlsson and Huss-Danell, 2003; Poudel et al., 2001) which would result in an additional N input of around 15 kg N ha^{-1} yr^{-1} for BIOORG and CONMIN. On the other hand soils with a high N mineralization potential will reduce N_2-fixation as clover will fix N only after most of available soil N is used (Meisinger and Randall, 1991). N mineralization rate in the 0-18 cm soil layer of the DOC soil obtained by isotope pool dilution (Appendix) was more than twice as much as the mineralization rate from a Typic Hapludalf with similar soil properties (Recous et al., 1999). N mineralization from the DOC soil was shown to be up to 140 kg N ha^{-1} yr^{-1} and consequently symbiotic N_2-fixation by soybean was shown to be low (Oberson et al., 2007). However, measurements of symbiotic N_2-fixation by clover in the DOC field experiment are lacking. An improvement of the values used in this study based on values from literature is difficult. The amount of symbiotically fixed N reported in literature varies widely because N_2-fixation is dependent on many factors (e. g. environmental conditions, climate, genetic factors, amount of fertilizer N applied, amount of available soil N) (Carlsson and Huss-Danell, 2003; Watson et al., 2002a).

In addition to the above mentioned uncertainties in the soil-surface N budget sampling errors will make it difficult to draw conclusions and may also explain the discrepancy between estimated and measured changes in soil N stock. Sampling errors are provoked by heterogeneity within the plots and between the plot of the four field replicates as well as from spatial and temporal variations (Oenema and Heinen, 1999).

The range of $\Delta N_{topsoil}$ between the four plots (field replicates) of CONMIN and BIOORG was -598 to -1248 kg ha^{-1} and -286 to -1066 kg ha^{-1}, respectively (data not shown). $\Delta N_{subsoil}$ varied from +270 to +810 kg ha^{-1} in CONMIN and +30 to +750 kg ha^{-1} in BIOORG (data not shown). Hence, preparing N budgets for each single plot of BIOORG and CONMIN and comparison with the measured N deficit of each single plot may allow more differentiated conclusions.

N transported to soil layers deeper than 20 cm may be returned to the topsoil due to crop uptake of N by roots below the vertical system boundary of 20 cm. Of N applied with mineral fertilizer and slurry 30% and 47% remained unaccounted in CONMIN and BIOORG, respectively (Chapter 2). This unaccounted N either may have left the soil-plant system through leaching or gaseous losses (denitrification, volatilization) or be transported to deeper soil layers. N lost by volatilization and denitrification leaves the defined system boundaries (i. e. plot at 0-20 cm depth). Gaseous N losses are considered as well in the N budget as in the measured change of soil N stock. As shown in Table 4.4 between 1977 and 1998 383 kg N ha^{-1} and 412 kg N ha^{-1} accumulated in the subsoil (30-50 cm) of BIOORG and CONMIN, respectively. A part of this accumulated N probably is brought back from the subsoil to the topsoil. Internal recycling of N would have an impact on the measured change in soil N stock (decrease of N deficit) but not on the N budget. Internal recycling of N probably depends on the mobility of N and is thus affected by the type of fertilizer applied. N contained in mineral fertilizer (e. g. nitrate) is very mobile and thus can easily be transported to deeper soil layers and be taken up by plant roots. This also may be the case for urine-N contained in slurry as hydrolysis of urea to ammonium is completed within few days after addition of urine to soil (Antil et al., 1993; Whitehead and Bristow, 1990). Feces contained in slurry in contrast comprise only a low proportion of mineral N (Table 2.2). Organic N compounds derived from feces can also be transported to deeper soil layers by leaching of dissolved organic N (DON) or by anecic earthworms. But recycling of DON is rather expected to be low at it is composed of material resulting from breakdown of SOM that is soluble but recalcitrant and thus not readily available to soil microorganisms and crops (Smolander et al., 1995; Yu et al., 2002). Discrepancy between measured and estimated

changes of total phosphorus (P) in the DOC soil during 21 years was shown to be lower than for N most likely because of lower mobility of P (Oehl et al., 2002). However, establishing the vertical system boundaries to 50 cm would increase the discrepancy between measured and estimated changes in soil N stock because of a decrease of measured N deficit.

Impact of cropping system management on soil N stock

The main difference between BIOORG and CONMIN is the fertilization strategy besides plant protection (Table I1). Repeated application of animal manure is expected to increase SOM content and in parallel to contribute to the build up of the organic soil N pool due to accumulation of recalcitrant organic N compounds derived from feces contained in slurry and farmyard manure. Hence, organically managed soils often reveal an increase in soil N content (Haraldsen et al., 2000; Poudel et al., 2001; Watson et al., 2002b). However, in a study where ^{15}N-labeled urea was applied to a long-term conventionally and ^{15}N-labeled vetch to a long-term organically managed cropping system, respectively, total ^{15}N recoveries (crop and 0-15 cm soil layer) were shown to be similar, suggesting no difference between conventional and organic cropping systems to retain added N in the soil in short-term (Kramer et al., 2002). Results obtained from the microplot study carried out in the DOC field experiment where ^{15}N-labeled slurry and ^{15}N-labeled mineral fertilizer was applied to long-term organic and conventional cropping systems did not support these findings. Total ^{15}N-recovery (crop and 0-18 cm soil layer) of slurry-derived ^{15}N was significantly lower than of mineral fertilizer-derived ^{15}N (Table 2.6). In contrast to the study of Kramer et al. (2002) where retention of ^{15}N derived from vetch in the topsoil was significantly higher than retention of urea-derived N, similar amount of slurry-^{15}N and mineral fertilizer ^{15}N was retained in the topsoil of BIOORG and CONMIN. Following the incorporation of urine-N and feces-N contained in slurry and of mineral fertilizer-N into SOM may give more indication about the ability of a soil to sequester N. At same amount of organic and mineral ^{15}N applied to the DOC soil (feces vs. mineral fertilizer) total ^{15}N recovery in SOM of the topsoil was similar after four month (Table 3.5). ^{15}N recovery of urine-N was higher than of feces-N or mineral fertilizer-N,

suggesting that incorporation of fertilizer-N into SOM was rather affected by the amount (about twice as much urine applied) than by the form of N applied to the soil.
However, when changes in soil N content were monitored over long-term organic cropping systems in general showed an increase in soil N content (Benbi and Biswas, 1997; Kramer et al., 2002). Long-term manure application in BIOORG did not increase soil N stock. On the contrary, as compared to the initial value N content in the topsoil of BIOORG decreased by 16% between 1977 and 2003. During the same period a decrease of 20% (or 10% when first crop rotation is excluded) was assessed for CONMIN. Likewise a decrease in organic C of about 7% in BIOORG and 14% in CONMIN was observed (Fliessbach et al., 2007). These findings suggest a decrease in SOM of the topsoil of BIOORG and CONMIN. Long-term OM input through manure and higher activity and size of the microbial biomass in BIOORG than CONMIN seemed not to affect composition of SOM, as N and C content in different SOM fractions were shown to be similar for the two cropping system (Table 3.7). The expected build up of SOM and in parallel of soil N stock in BIOORG due to long-term fertilization with manure may have been prevented by tillage operations. Tillage breaks up soil aggregates and expose previously protect SOM to microbial attack (i. e. decomposition) resulting in reduction of SOM and coevally of soil N content. During dispersion (i. e. break up of aggregate structure) of soil aggregates with hexametaphosphate to obtain the intra-aggregate particulate organic matter (iPOM) about 27% of previously physically protect total soil N was released (Chapter 3). Improvement of the cropping systems towards preservation of soil N stock may thus only be achieved by drastically reducing (or even give up) soil tillage operations.

Conclusions

N budgets for each crop rotation period and the total N budget over the 26-years period (1978-2003) are negative for both, the organic and the conventional cropping system. Simple N budget (i. e. difference between N input with manure or mineral fertilizer and N exported with harvested products) overestimated N deficit from the cropping systems because N supply by atmospheric N deposition and symbiotic N_2-

fixation were ignored. Still, N deficits estimated from the soil-surface budget were higher than the measured change in soil N stock of BIOORG and CONMIN between 1977 and 2003. The discrepancy of about 50 kg N ha^{-1} yr^{-1} could partly be explained by i) uncertainties in the soil-surface N budget caused by underlying assumptions (e. g. underestimation of atmospheric N deposition and/or symbiotic N_2-fixation) and ii) recycling of N which left the defined vertical system boundaries (i. e. recycling of N transferred to deeper soil layers during the course of the experiment). However, improvement of the cropping systems towards preservation of soil N stock may be difficult due to a high N mineralization potential of the soil at this experimental site. Increasing or maintaining soil N stock may only be achieved by drastically reducing (or even give up) soil tillage operations and thus promoting build up of SOM.

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General conclusions

Intensification of agricultural production has greatly increased N fluxes between different compartments of the biosphere and the emission of N compounds from the agroecosystem with negative impact to the environment (e. g. emission of green house gases, contamination of surface and ground water) (Matson et al., 1997; Tilman et al., 2002). The need to make efficient use from any N source and thus to protect natural resources (e. g. soil N stock) and at same time reducing N losses to the environment is recognized in many industrial countries. Organic farming is seen as a possible way to reduce N losses from agroecosystems as production is based on the reduction of inputs by recycling of nutrients in order to conserve natural resources (IFOAM, 2005). The main objective of this thesis was to investigate the impact of long-term organic (BIOORG) and conventional (CONMIN) cropping on N dynamics after application of different N sources to the plant-soil system. The main findings concerning the impact of application of different N sources and of long-term organic and conventional cropping on N dynamics are discussed below.

Nitrogen release and uptake by crops from different N sources applied to the soil

To what extend N sources (e. g. slurry, farmyard manure, plant residues) applied to the soil are mineralized and immobilized by the microbial biomass and incorporated into the soil organic matter (SOM) depends on its composition (e. g. C/N ratio, mineral N content, biodegradability of organic N compounds). N sources with a low C/N ratio and a low amount of recalcitrant organic N compounds are rather mineralized. N sources with high C/N ratio and high organic N content on the other hand are rather immobilized. Biological and non-biological immobilization of N in the soil reduces N availability for crops. N use efficiency of mineral fertilizer that contained exclusively direct plant available mineral N forms (NH_4-N and NO_3-N) was higher than of urine or feces contained in slurry. In the first experimental year 47% of N applied with mineral fertilizer was taken up by the winter wheat (Table 2.6). Compared to mineral fertilizer N availability of urine (mineral fertilizer equivalent (MFE) of 78%) and feces (MFE of 21%) and consequently of complete slurry (MFE of 61%) was lower. Hence, of total N applied with urine and feces 37% and 10% was recovered in winter wheat resulting in a

recovery of slurry-N of 29%. Due to its low C/N ratio of 2 and due to the fact that hydrolysis of urine-N into plant available mineral form occurs fast much more N was released from urine than feces. Eleven days after application of slurry and mineral fertilizer to the BIOORG and CONMIN soil urine-derived and mineral fertilizer-derived N, respectively, contributed to about 70-80% of total nitrate in the topsoil (0-18 cm) whereas feces-derived N accounted only for 8% (Table 2.5). The high C/N ratio of 14 and the high proportion (> 95%) of organic N forms in feces rather favor immobilization of feces-N. Furthermore Py-GC/MS revealed that some of the organic compounds resisted digestion in the rumen and the small intestine of the animal and thus may also resist attack of soil microorganisms. Peptide-like structures, which were detected in feces by ^{15}N-CPMAS-NMR and Py-GC/MS, may persist microbial degradation possibly by association with refractory biopolymers (Knicker, 2004). Recalcitrant N compounds contained in feces are mineralized only slowly. Due to its composition fecal N is expected to accumulate in the soil over long-term. Thirty months after application of labeled slurry an accumulation of feces-N at least in the topsoil (0-18 cm) could not be approved in this study as recoveries of urine-, mineral fertilizer- and feces-N in the topsoil at harvest of maize were similar (Table 2.6). The low recovery of feces-N in the topsoil could not be explained conclusively. Weather conditions at application time and incorporation of slurry into the soil rather exclude large gaseous N losses suggesting that a considerable amount of fecal N has left the topsoil (e. g. displacement into deeper soil layers).

Fate of fertilizer nitrogen not taken up by crops

Retention of fertilizer N in the soil

Four months after application 25.1%, 20.1% and 21.8% of originally applied urine-, feces- and mineral fertilizer-^{15}N, respectively, were recovered in the topsoil layer (0-18 cm) (Table 2.6). Most of N applied with urine, feces and mineral fertilizer, respectively, was recovered in the macro-aggregates of the soil (Table 3.5). Incorporation of N into particulate organic matter (POM) which represents a fraction of SOM and consists of the

free light fraction (LF; occurring between aggregates) and the intra-aggregate POM (iPOM; occurring within aggregates) was expected to be affected by the N source applied. At same amount of different N form applied (feces vs. mineral fertilizer) total N recovery in POM was similar (Table 3.5). Apparently recalcitrant organic N compounds contained in feces did not affect the incorporation into POM. Higher recovery of urine-N than mineral fertilizer-N in POM most likely can be referred to the N amount applied with urine which was more than twice as high as with mineral fertilizer or feces (Table 2.2). N applied with urine may undergo the same physico-chemical reactions as mineral fertilizer-derived N due to rapid hydrolysis of urine-N into mineral N forms. However, major sink for fertilizer-N was the mineral-associated organic matter fraction (referred to as MF in Table 3.5) where most of urine-, feces- and mineral fertilizer-N was recovered.
Release of N incorporated into SOM depends on the composition of the SOM fractions and on the degree of stabilization of N (i. e. physical protection within the soil aggregates) in the respective fraction. Compared to the mineral-associated organic matter fraction free LF had a higher C/N ratio (Table 3.8). As not protected in the aggregate structure free LF represents a labile source of mineralizable N and C. Free LF thus can be regarded as a short-term N sink and source which is assumed to be decomposed and turned over more quickly than OM incorporated into the mineral-associated organic matter fraction. N content per kg dry weight of POM (i. e. free LF and iPOM) was higher than for the mineral-associated organic matter fraction (Table 3.7). Nevertheless POM may contribute less to N supply since the proportion of POM to total soil N in the 0-18 cm soil layer was less than 3% (around 100 kg N ha^{-1}) compared to a proportion of about 60% (around 2000 kg N ha^{-1}) of the mineral-associated organic matter fraction (Table 3.7). As soil fractionation and determination of the incorporation of fertilizer-^{15}N into the different fractions was carried out only for one sampling date it was not possible to make a statement about N turnover rates. Turnover time of POM was estimated to be about seven years (Gregorich et al., 2006). OM which is incorporated into the clay-sized fraction seems to be highly stabilized (Kirchmann et al., 2004) and thus only slowly released. Turnover rates of stable pools of SOM may be years or even centuries (Jarvis, 1996; Whalen et al., 2000). Stabilization of slurry- and mineral fertilizer-N in the mineral-associated organic matter fraction thus may explain the low

residual fertilizer-N effect in the second and third year after fertilizer application (Table 2.6). Residence time of mineral fertilizer N retained in the soil after the first season was estimated to be five years and of N retained after this period to be about 25 years (Kelley and Stevenson, 1995). Stabilization of N in SOM may thus increase or at least maintain soil N stock. Tillage physically disrupts the aggregates and previously protected OM is exposed to decomposition by soil microbial biomass. After dispersion of the heavy fraction with hexametaphosphate (Figure 3.1) a large amount of N was lost. Frequent physical disruption of aggregates caused by tillage and the subsequent release of previously protected N which then is exposed to attack by soil microorganisms may explain the decrease in N content of the DOC soil.

Losses of fertilizer N from the cropping systems

About the same proportion of mineral fertilizer-, urine- and feces-derived N was retained in the topsoil 30 months after application. Because N use efficiency of mineral fertilizer was higher than of urine and of urine higher than of feces, N losses consequently were lowest for mineral fertilizer (29%), followed by urine (40%) and feces (61%) (Table 2.6). Cropping system did not affect N losses. Hence, both BIOORG and CONMIN seems to have to same potential to emit N-compounds to the environment as differences in the local emissions from the cropping system seems to be determined by the form and level of N inputs rather than by intrinsically different N use efficiencies of inputs. However, it has to be taken into account that neither gaseous N losses nor leaching of N were directly measured. Moreover N transported to deeper soil layers than 18 cm could not be determined after harvest of maize. Unaccounted N thus not necessarily left the soil-plant system as a part of it may be transported to deeper soil layers and still be available to plant roots.

Independent if manure or mineral fertilizer is applied, the potential risk of N losses from a cropping system is high, when available N supply exceeds crop demand. Organic N retained in the soil can also be mineralized in periods without N uptake by crops, increasing the risk of N losses. N uptake by green manure sown after harvest of winter

wheat suggests that 7 kg N ha^{-1} was mineralized from slurry between August and October 2003 (Table A1 in Appendix). This mineralized N would have been susceptible to be lost if soil would have been left fallow. Inclusion of cover crops in the crop rotation helps reducing N losses from the cropping system. Incorporation of the cover crop into the soil will cause a release of N. Hence, cover crops only improve N utilization of manure and reduce N losses if the subsequent crop is in need of N (Schröder, 2005).

Impact of cropping system

Microbial biomass and microbial activity was shown to be higher in the BIOORG than the CONMIN soil (Fliessbach et al., 2007; Mäder et al., 2002), and soil bacterial community structures differs between the two cropping systems (Hartmann et al., 2006). Soil microbial biomass is a key agent in the release of N from fresh applied organic sources (e. g. slurry, farmyard manure, crop residues), thus one may expect differences in the rate and amount of N released from slurry (urine-feces mixture) applied to both BIOORG and CONMIN soils. But recovery of urine- and feces-derived N in crops over three consecutive years were not significantly different between BIOORG and CONMIN (Table 2.6), suggesting that the N release from this sources was not affected by the size and activity of microbial biomass initially present in the soil. Apparently enough N-metabolizing microorganisms were present and active in the soil of both cropping systems. Hydrolyzes of urine and transformation of organic N-compounds contained in feces into mineral forms was thus not limited by microbial activity but rather determined by the chemical nature of the N-components. Hence, size and activity of the soil microbial community may not be appropriate indicators to obtain some evidence about N release from fresh applied organic N sources. A more meaningful statement may by allowed by identifying functional groups of the soil microbial community.

Expected differences in the composition of SOM between the two cropping systems due to long-term organic matter (OM) input and higher microbial activity in BIOORG than CONMIN could not be found. Total N and C content in the SOM fractions was not significantly different between BIOORG and CONMIN (Table 3.7). This may be related

to the facts that i) soil microbial biomass which controls incorporation of N into SOM through mineralization and immobilization processes was not a limiting factor, ii) tillage, crop rotation and residue management were identical in BIOORG and CONMIN and iii) the amount of non-biological fixed NH_4^+-N could be expected to be similar for BIOORG and CONMIN because of no significant differences in the mineralogy of soil and thus in clay content.

Sustainability of the investigated cropping systems

In the context of this study a cropping system is sustainable when it is balanced (i. e. inputs into equals outputs from the system). As indicated by the negative N budgets N outputs exceeded N inputs in both BIOORG and CONMIN (Table 4.3). This imbalance may cause mining of soil N reserves. Possible exhaustion of soil N stock is suggested by a decrease in soil N content of the topsoil as compared to the initial value in 1977 before the start of the DOC field experiment. Likewise a decrease in organic C and total phosphorus content in the topsoil was observed (Fliessbach et al., 2007; Oehl et al., 2002). Long-term organic N input in BIOORG was expected to maintain or increase soil N stock. However, when applied at same amount incorporation pattern of organic N (feces-N) into SOM was similar to mineral N (mineral fertilizer-N) (Table 3.5), suggesting that organic N input would not increase soil N stock. Despite inclusion of meadows in the crop rotation of both cropping systems as additional N source it would be difficult to avoid mining of soil N stocks because of the high potential of the DOC soil to release N. Sustainability of both cropping systems may only be improved by reducing soil tillage activities to minimize release of N protected in SOM.

Agronomic perspective

Continuous increase of the global population and changes in eating habits (e. g. increase of meat consumption) requires an increase in food production (Brown et al., 1998). Furthermore world grain harvest area per person decreased since 1950 (Brown et al., 1998). Increased input of inorganic fertilizer (e. g. nitrogen fertilizer increased

eightfold between 1960 and 2000) enabled food production to be increased substantially during the past decades (Matson et al., 1997). But intensification of agricultural production has also increased emission of N compounds from agroecosystems with negative impact to the environment (e. g. emission of green house gases, contamination of surface and ground water) (Tilman et al., 2002). Reducing N losses is still a concern in many industrial countries. Hence, the question arises how to increase crop production and at same time minimize N losses to the environment? Undoubted many progress in nitrogen research has already been done to better understand N dynamics in agroecosystems. But the improvement of N use efficiency, the reduction of N losses to the environment and the maintenance of soil N resources are obviously still of important concern.

Organic farming may be the answer to this problem as it is stated to be sustainable and environmental-friendly because i) repeated input of organic N is expected to maintain soil N stocks and ii) N losses are estimated to be lower than in conventional farming as lower livestock and restriction in purchase of animal fodder limit the amount of available organic fertilizer (Gutser et al., 2005). But return of OM can not only be attributed to organic farming. On conventional mixed farms manure also is applied to the soil and meadows are part of the crop rotation. Risk of N losses from a cropping system is high when N supply exceeds N demand by crops, independently if N derives from an organic or mineral source. N use efficiency of any type of fertilizer can only be increased by better synchronizing N supply with crop demand. Hence, cropping system is of lesser importance than the timing, the placement and the amount of N application (Crews and Peoples, 2005; Schröder, 2005). Manure - an important organic fertilizer in organic farming - is a mixture of direct available N (e.g. NH_4-N), readily available N (e. g. urea) and hardly available N (e.g. undigested or partly digested complex organic N-compounds) (Chapter 1; Jarvis, 1996) making prediction of N release from this organic source much more difficult compared to mineral fertilizer. Release of manure N when not synchronized with crop demand can result in high nitrate leaching and ammonia volatilization occurs rather from manure than from mineral fertilizer. It thus may be reasoned that N losses from organically managed soils have not necessarily to be lower than from conventionally managed soil when best management practices are followed.

Moreover, it was shown that N use efficiency of manure is lower than of mineral fertilizer (Chapter 2; Muñoz et al., 2003; Sørensen, 2004) and that yields from organically managed cropping systems in general are 20-40% lower than from conventionally managed cropping systems (Berry et al., 2002; Mäder et al., 2006). Increasing crop production and decreasing negative impact to the environment can consequently not only be attained with organic farming. Cropping systems where manure and inorganic fertilizers were combined showed a better N utilization and thus lower N losses than when either source is applied alone (Korsaeth and Eltun, 2000; Oberson et al., 2007; Power and Doran, 1984). Application of fertilizers in high-intensity crop production can be improved and negative impacts to the environment reduced by using new technologies (e. g. global positioning systems (GPS), geographic information systems (GIS)) as in precision agriculture (Robert, 2002).

The following measures can be taken to better synchronize N supply with crop demand to reduce N losses and enhance N use efficiency: site-specific application rates, split-application, right fertilizer placement, assessment of the mineral N status of the soil before sowing, estimation of mineralization-immobilization turnover, better estimation of crop need for N, utilization of nitrification inhibitors, and inclusion of cover crops in the crop rotation (Crews and Peoples, 2005; Schröder, 2005).

Outlook

To improve fertilizer N use efficiency basic processes controlling N dynamics in acgroecosystems must fully be understood. Future research thus may comprise:

- improving the knowledge about fertilizer-N availability (i. e. short- and long-term N dynamics especially of organic fertilizers) under field conditions using ^{15}N as tracer and by better characterizing organic N forms using for example physico-chemical fractionation in combination with Py-GC/MS and solid-state ^{15}N-CPMAS-NMR spectroscopy
- improving the knowledge about mineralization-immobilization turnover rates of different soil types under different agricultural management

- quantifying gaseous N losses and N leaching from cropping systems under different management and different crops
- monitoring the impact of different management strategies on soil N reserves of different soil types over long-term
- improving knowledge about fluxes and interactions between all pools of the N cycle of agroecosystems

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Appendix

Measuring gross N mineralization using isotope pool dilution method

Objective

Gross soil N mineralization can be calculated from the decline in ^{15}N enrichment of the NH_4^+ pool during one day incubation (Davidson et al., 1991) using the equation of Kirkham and Bartholomew (1954).

Methods

From each of the four field replicates of BIOORG two soil cores (diameter 5.5 cm, depth 15 cm) were collected end of October 2003, and transported in cool boxes to the research station. Until use the soil cores were stored at 4.5℃ which corresponds to the soil temperature in the field at sampling. For labeling soil from each core was spread out in a pan before evenly applying 7 ml of a $(^{15}NH_4)_2SO_4$ solution, equivalent to 2.3 mg N kg soil^{-1} with 99 atom% ^{15}N, with a pipette. After application of the solution the soil was thoroughly mixed. Fifteen minutes after application of the $(^{15}NH_4)_2SO_4$ solution (time 0; t_o) half of the soil samples was analyzed for initial content of NH_4^+ and $^{15}NH_4^+$. The remaining soil samples were incubated for additional 24 hours (t_1) before sampling for determination of the post-incubation NH_4^+ and $^{15}NH_4^+$. At each sampling (t_o and t_1) 25 g soil (dry weight basis) was extracted for 1 h in 80 ml 2 *M* KCl in an over-head shaker. Subsequently all KCl extracts were filtered (Whatman no. 1). NH_4^+ in the soil extract was analyzed colorimetrically on a SAN^{plus} Analyzer (Skalar, Netherlands). ^{15}N abundance analyses of NH_4^+ was carried out on a continuous flow Roboprep CN Biological Sample Converter coupled to a Tracermass Mass Spectrometer (Europa Scientific, Crewe, England) after diffusion of $^{15}NH_4^+$ from the soil extracts to acidified glass filters (Goerges and Ditter, 1998; Sørensen and Jensen, 1991).

Results

Gross N mineralization from the BIOORG topsoil (0-18 cm) after short-term incubation of one day resulted in a mineralization rate of 2.1 mg N kg $soil^{-1}$ day^{-1}. From soil respiration the same mineralization rate (2.0 mg N kg $soil^{-1}$ day^{-1}) was obtained for the BIOORG soil after short term incubation of three days (Table 4.5). Gross mineralization rate of the BIOORG soil was more than twice as high as gross mineralization rate of a Typic Hapludalf with similar soil properties (Recous et al., 1999).

Conclusions

Isotope pool dilution method represents a valuable tool to assess the gross mineralization potential of a soil. But comparison of daily mineralization rates obtained from short-term incubation with daily mineralization rates determined over a longer period (e. g. incubation over 60 days or Ndfs after 112 days) is impossible as isotope pool dilution does not include re-mineralization.

Table A1. Total N uptake by green manure (mainly consisting of *Phacelia tanacetifolia*), N derived from the labeled component of fertilizer (Ndflc), N derived from the complete fertilizer (Ndff), N derived from the soil (Ndfs) and proportion of originally applied ^{15}N recovered in green manure between August and October 2003. Because of no significant differences between MIN and BIOORG mean values are shown. (n = 8).

Treatment	N uptake	Ndflc	Ndff[a]	Ndfs	^{15}N recovery
	……g m^{-2}……				% of applied ^{15}N
SlurryU	10.9 ns	0.6 a	0.7 a	10.2 ns	4.6 a
SlurryF	10.2 ns	0.1 b	0.7 a	9.5 ns	2.7 b
MineralN	8.2 ns	0.2 b	0.2 b	8.1 ns	3.0 b
0N	8.7 ns			8.7 ns	

Within columns, means followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple range test. ns = not significant.

[a] Ndf_{feces} + Ndf_{urine} for SlurryU and SlurryF; $Ndf_{Mineral\ fertilizer}$ for MineralN.

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