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Monika Langmeier, Emmanuel Frossard, Michael Kreuzer, Paul Mäder ...+2 more authors

Institutions: École Polytechnique Fédérale de Lausanne, Research Institute of Organic Agriculture

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Monika Langmeier, Emmanuel Frossard, Michael Kreuzer, Paul Mäder, David Dubois, Astrid Oberson

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Original article

Nitrogen fertilizer value of cattle manure applied on soils originating from organic and conventional farming systems

Monika LANGMEIER[a], Emmanuel FROSSARD[a], Michael KREUZER[b], Paul MÄDER[c], David DUBOIS[d], Astrid OBERSON[a]*

[a]Institute of Plant Sciences, Plant Nutrition, Swiss Federal Institute of Technology (ETH), Eschikon 33, 8315 Lindau, Switzerland
[b]Institute of Animal Science, Animal Nutrition, Swiss Federal Institute of Technology (ETH), ETH Centre, 8092 Zurich, Switzerland
[c]Research Institute of Organic Agriculture, Ackerstrasse, 5070 Frick, Switzerland
[d]Swiss Federal Research Station for Agroecology and Agriculture (FAL), Reckenholzstrasse 191, 8046 Zurich, Switzerland

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Abstract – Nitrogen nutrition of plants in organic farming depends largely on animal manure. In a pot experiment the hypothesis was tested that on a long-term organically managed soil (ORG) characterized by higher soil microbial activity, a greater portion of N applied as cattle manure is mineralized and taken up by plants than on a conventionally managed soil that had received exclusively mineral fertilizers (MIN). Dry matter yields and N uptake by Italian ryegrass were higher by around 20% on ORG than MIN soil. The N utilization of ^{15}N labeled animal manure components and mineral N differed little between ORG and MIN. The major part of the increased N uptake on ORG compared with MIN was due to a significantly greater N supply from ORG soil. The increased capacity of the ORG soil to supply N to plants became more important at later cuts when N was severely limiting plant growth.

cattle manure / ^{15}N / organic farming / conventional farming / N fertilizer value

Résumé – Valeur fertilisante azotée d'un lisier de bovin appliqué sur des sols cultivés selon les règles de l'agriculture biologique ou conventionnelle. En agriculture biologique, la nutrition azotée des plantes dépend largement d'une utilisation efficace des engrais de ferme. L'objectif de ce travail était d'évaluer dans une expérience en pots l'aptitude de deux sols provenant d'un essai de longue durée, l'un cultivé selon les règles de l'agriculture biologique et n'ayant reçu que des engrais organiques (ORG) et l'autre cultivé selon les règles de l'agriculture conventionnelle n'ayant reçu que des engrais minéraux (MIN), à alimenter une culture de ray-grass italien après des apports de NO_3NH_4, de fèces avec/sans urine de bovin préalablement marqués à ^{15}N. Le sol ORG est caractérisé par une activité microbiologique plus élevée. Le prélèvement d'azote par le ray-grass était plus élevé d'environs 20 % pour le sol ORG que pour le sol MIN. Peu de différences furent observées entre ORG et MIN pour l'utilisation par la plante de l'azote issu des fèces (17 à 22 % du N apporté), de l'urine (62 à 66 % du N apporté) et de l'engrais minéral (75 à 76 % du N apporté). L'augmentation du prélèvement de N par le ray-grass dans le sol ORG s'explique donc par une plus grande minéralisation de l'azote du sol.

lisier de bovin / ^{15}N / agriculture biologique / agriculture conventionnelle / valeur fertilisante azotée

1. INTRODUCTION

Efficient use of animal manure N is crucial in organic farming systems because they exclusively depend on organic N sources. Promotion of soil microorganisms which mediate the complex mobilization-immobilization-turnover processes that finally determine the fertilizer value of organic fertilizers like manure [12, 15] is considered to be an integrated part of soil and nutrient management of organic systems [41]. It has often been shown that long-term manure application [10, 24, 43] or organic farming [4, 6, 7, 29] can lead to an increase in soil microbial biomass and activity, as suggested by higher activities of various enzymes, higher soil respiration rates, and higher microbial C contents. Soil respiration correlates well with N mineralization in incubation studies [8]. The increase in N mineralization of organically managed soils that was observed in incubation studies [4, 20] is consistent with reported higher biological activity for these soils.

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* Correspondence and reprints
astrid.oberson@ipw.agrl.ethz.ch

It has, however, never been shown whether this change in the microbial status of a soil has implications for the N use efficiency of freshly applied organic substrates. It is hypothesized that the N fertilizer efficiency for plants of freshly applied cattle manure is increased on long-term organically managed soils. The objective of this study was to test this hypothesis using soils from a long-term field experiment where different fertilization practices had resulted in different soil microbial properties. On long-term organically managed plots (ORG), microbial biomass and activity had increased compared with a conventionally managed soil receiving mineral fertilizers (MIN) [6, 22]. A pot experiment was conducted on these two soils, amended with ^{15}N labeled cattle manure, and Italian ryegrass was grown to determine the effect of the farming system (ORG vs. MIN) on N uptake from the manure components (feces, urine) by the grass.

2. MATERIALS AND METHODS

2.1. Soils

Soil samples were taken from a long-term field experiment located in Therwil near Basel (Switzerland), established on a loamy silt Typic Hapludalf developed on loess. In this experiment organic and conventional farming systems have been compared since 1978. The experiment is being carried out by the Swiss Federal Research Station for Agroecology and Agriculture (FAL), Zurich-Reckenholz and the Research Institute of Organic Agriculture (FiBL), Frick, Switzerland. The design and management practices of the trial have been described in detail by Besson and Niggli [3] and Siegrist et al. [32]. The main differences between the tested systems lie in fertilization and plant protection (Tab. I). Differences in soil cultivation are small. The seven-year crop rotation is uniform for all systems (Tab. I) [23, 32]. To test the hypothesis formulated previously, soils were sampled from two contrasting farming systems similar in chemical, but differing in microbiological soil properties (Tab. II). The ORG soils had been managed according to bio-organic guidelines [41] since 1978 and MIN soils were managed conventionally (Tab. I). During the first crop rotation from 1978 to 1984 the MIN plots were used as non-fertilized conventional control. After 1984, conventional management was continued with the application of exclusively mineral fertilizers at the rates indicated in Table I. The average annual N budgets, calculated as the difference between N input by fertilizers and N export from the field by harvested products [33], were similar for both systems (Tab. I). In both systems, N export exceeded N input by fertilizers.

Representative topsoil samples (0–20 cm) were taken from four field replicates per farming system under a decaying sunflower – vetch intercrop grown after winter wheat, before land preparation for red beet in February 1997. The plant residues were removed, and the soils were sieved at 5 mm and stored air-dried at room temperature until the setup of the pot experiment.

2.2. Manures

The ^{15}N labeled manure was obtained by feeding ^{15}N labeled hay as a one-day ration to a lactating cow (10.3 kg milk·day^{-1}, 738 kg live-weight) adapted for three weeks to the same kind of unlabeled hay [25, 27]. The labeled hay had an

Table I. Farming systems record of the investigated soils: average rate of N, P and K fertilization and input-output budgets of the three crop rotation periods, types of fertilizers added and plant protection strategy applied to the investigated soils from 1978–1996.

Soil name		ORG				MIN		
Farming system	Crop rotation	Bio-organic				Conventional		
Nutrient management	period	Total N	Organic N	P	K	Total N	P	K
Average nutrient input kg·ha^{-1}·yr^{-1}	1978–1984	113	71	29	124	0	0	0
	1985–1991	94	63	25	125	102	46	225
	1992–1996	72	45	25	121	163	38	293
Average budget kg·ha^{-1}·yr^{-1}	1978–1984	–71	–	–2	–43	–176	–27	–114
	1985–1991	–158	–	–10	–78	–153	11	4
	1992–1996	–134	–	–10	–28	–92	–5	7
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 norm		
Plant protection								
Weed control		Mechanical				Mechanical and herbicides		
Disease control		Indirect methods, copper 1978–91 in potatoes				Chemical		
Insect control		Plant extract, bio-control				Chemical		
Crop rotation	1978–1984	spring barley, two years of grass-clover mixture, potatoes, winter wheat, white cabbage, winter wheat						
	1985–1991	winter barley, two years of grass-clover mixture, potatoes, winter wheat, red beet, winter wheat						
	1992–1996	three years of grass-clover mixture, potatoes, winter wheat						

Table II. Characteristics of the investigated soils. Available P is presented by the quantity of isotopically exchangeable P within the first minute [5], available K by K extractable in saturated CO_2 water [1]; microbial C and N determined by chloroform fumigation [40] using k_{EC} and k_{EN} factors [13, 14].

Soil	pH (H_2O)	Total C	Total N	Available nutrients		Microbial biomass	
				P	K	C	N
		$g \cdot kg^{-1}$		$mg \cdot kg^{-1}$			
ORG	6.1 a	15 a	1.5 a	6.4 a	0.45 a	352 a	44 a
MIN	5.8 b	14 b	1.3 a	6.5 a	0.48 a	264 b	32 b

Data followed by different letters within a parameter indicate significant differences ($P < 0.05$) between the two soils (t-test).

Table III. Characteristics and rates of the fertilizer treatments applied in the pot experiment, with details given for single components in the case of composed fertilizer treatments. FecminN = feces with additional mineral N; Feces(minN) = feces N within the feces-mineral N-mixture; MinN = mineral N within the feces-mineral N-mixture; Feces(slu) and Urine(slu) = feces and urine, respectively, as components of the slurry; MinN1 and MinN2 = lower and higher level of mineral N treatment.

Treatment	Fertilizer characteristics			Applied rates			
Components	Dry matter content	Total N	^{15}N atomic enrichment	Total N	(NH_4+NO_3)–N	Total P	Total C
	%	% of dry matter	%	$mg \cdot kg^{-1}$ soil			
Control	–	–	–	0	0	0	0
Feces	11.6	2.5	0.962	55.6	2.7	30	893
FecminN				99.0	46	30	893
Feces(minN)	11.6	2.5	0.962	55.7	2.7	30	893
MinN†			0	43.3	43.3		
Slurry	8.9‡/8.8§	3.7‡/3.9§		108	35	31	995
Feces(slu)			0.962	56.4	2.7	30.2	905
Urine(slu)			0.555 / 0¶	51.2	32.5#	0.4	90
MinN1†	–	–	7.761	60	60	30††	0
MinN2†	–	–	7.761	101	101	30††	0

†Mineral N applied as solution of NH_4NO_3.
‡Slurry composed of labeled feces and labeled urine (feces N/slurry N = 0.52).
§Slurry composed of labeled feces and unlabeled urine (feces N/slurry N = 0.52).
¶Slurry treatment applied in the two equivalent variants, see footnotes ‡, § and text.
#Difference between mineral N determined in slurry and mineral N in feces.
††As solution of KH_2PO_4.

average N content of 1.9% and a ^{15}N atomic enrichment of 2.632%. It was obtained from a permanent grassland plot previously fertilized with $^{15}NO_3{}^{15}NH_4$ (10.5% ^{15}N atomic enrichment) at a rate of 150 kg $N \cdot ha^{-1}$. For eight days after feeding ^{15}N labeled hay, feces and urine were collected separately by the use of urinals [18] two to three times per day and immediately frozen. Feces samples collected 35 and 47 h after starting to feed the ^{15}N labeled hay were highest in ^{15}N enrichment (0.961 and 0.953% ^{15}N atomic enrichment). The ^{15}N enrichment in urine was also highest 35 h after the start of feeding (0.555% ^{15}N atomic enrichment). These samples (pooled for the two feces subsamples) were used for the manure treatments in the pot experiment. Manure characteristics are indicated in Table III.

In order to test the homogeneity of the ^{15}N labeling, the ^{15}N atomic enrichment of physico-chemical fractions of feces N was compared. The fractionation method described by Mason [19] and simplified by Kreuzer and Kirchgessner [16] distinguishes mainly three N fractions, differing in their origin from the cows' metabolism: (i) undigested feed N (UDN) containing mainly celluloses, hemicelluloses, lignified materials and some denatured proteins of the cell walls of the plant tissue ingested by the animal, (ii) N compounds derived from intact or disrupted microbial cells of the rumen and hind gut or from abrasions from the digestive tract tissue (bacterial and endogenous debris N, BEDN), and (iii) water-soluble N (WSN), mainly derived from endogenous sources in the animal. The amount and enrichment of the UDN fraction can be directly determined, whereas the values for BEDN and WSN are calculated from the determination of total feces N and total water-insoluble N. As the water-insoluble N contains UDN and BEDN, the BEDN fraction is calculated as the difference between water-insoluble N and UDN. The WSN is the difference between total feces N and water-insoluble N. The fractions were determined in separate subsamples of a homogenate of 350 g fresh feces and distilled water containing 3 to 6% dry matter (DM). The UDN fraction was obtained by boiling 30 g of the homogenate with a neutral detergent solution [39] for 1 h in a Fibretec System M (Tecator Ltd., Hønegäs, Sweden) after shaking of the homogenate

subsample for 24 h at 4 °C and 200 rpm in a water bath in order to remove microbial matter from truly undigested material [17]. The detergent-insoluble fibrous material was washed with boiling distilled water and dried overnight at 103 °C prior to total N and ^{15}N analysis. The water-insoluble N associated with the particulate matter of the feces was determined by centrifuging another 30 g of the homogenate twice at 27 000 *g* for 30 min at 4 °C. The supernatant was carefully discarded, the sediment containing the water-insoluble N recovered, lyophilized and analyzed for total N and ^{15}N. All fractions were analyzed in triplicate subsamples of the homogenate. Total feces N was determined in lyophilized undiluted feces (see Chap. 2.5).

2.3. Pot experiment

The air-dried soils were re-wetted to 44% water-holding capacity (i.e. 242 and 229 g $H_2O \cdot kg^{-1}$ soil DM for MIN and ORG, respectively), sieved at 2 mm and pre-incubated for 14 days at 20 °C and 80–85% atmospheric humidity.

Fertilizer treatments included feces alone (feces), a mixture of feces and urine (slurry: feces N/slurry N = 0.52 according to excretion), feces with additional mineral N (fecminN), mineral N fertilizers as references at two levels (minN1 and minN2) and an unfertilized control. In order to separate the N derived from the two slurry components (feces(slu), urine(slu)), two equivalent slurries with different ^{15}N labeling were prepared. One slurry was composed of 2.9 kg labeled feces (0.962% ^{15}N atomic enrichment) and 1.8 L of labeled urine (0.555% ^{15}N atomic enrichment). The other was composed of the same amount of labeled feces but 1.5 L of an unlabeled urine portion taken immediately before feeding ^{15}N labeled hay to the cow. The urine volumes differed slightly in order to provide equal amounts of urine N to both slurries. After mixing, the treatments were refrozen until use, a procedure which has been shown not to alter their mineralization characteristics [38].

A detailed overview of the treatments and their characteristics is given in Table III. Manure rates were calculated on the basis of an equal P level of 30 mg $P \cdot kg^{-1}$ soil DM. The elements K, Ca and Mg were added to reach the same level in all treatments. Each fertilizer treatment was mixed with a batch of 3.6 kg soil DM by adding the required volumes of mineral solutions or manures and careful stirring to obtain a homogenous distribution of the amendments. Thereafter, each fertilizer treatment was distributed into 4 replicate pots at 900 g soil $DM \cdot pot^{-1}$. Soil moisture was controlled by weighing and readjusted to 60% of total water-holding capacity throughout the experiment by daily watering. The pots were sown with 0.9 g of Italian ryegrass seeds (*Lolium multiflorum*, var. Axis) and placed in a growth chamber (65% atmospheric humidity, photoperiod 16 $h \cdot d^{-1}$, temperature 22 °C and 18 °C during day and night, respectively). The ryegrass was cut six times at 19, 36, 64, 96, 134 and 162 days after sowing. After the second, third and fourth cut, K, Ca, Mg and some microelements were re-amended in amounts equivalent to the estimated removal by previous cuts. The elements N and P were not re-amended during the experiment. Shoot P concentrations, measured after each cut, were at least 0.34%, showing that P was never a growth-limiting factor [2].

At each cut, DM yield was determined and samples for analysis of total N and ^{15}N content of the shoots were obtained. At the final cut, the soils and roots from each pot were recovered as well and separately subjected to the analyses.

2.4. Determination of nitrogen fertilizer value and nitrogen-15 balances

The N in the plants derived from the ^{15}N labeled fertilizers (Ndff, $mg \cdot kg^{-1}$ soil) was calculated based on isotopic dilution principles [9] according to equation (1):

$$Ndff = N_{pl} (^{15}Nen_{pl} / ^{15}Nen_f) \quad (1)$$

where N_{pl} is the total N content of the plant ($mg \cdot kg^{-1}$ soil), $^{15}Nen_{pl}$ is the ^{15}N enrichment in the plant (%) and $^{15}Nen_f$ is the ^{15}N enrichment in the fertilizer (%). The real coefficient of utilization of the fertilizer N (CUN,%) was calculated according to equation (2):

$$CUN = Ndff / N_f \times 100 \quad (2)$$

where N_f denotes the amount of fertilizer N applied ($mg \cdot kg^{-1}$ soil).

The N derived from the slurry had to be calculated as the sum of the N derived from the two separate slurry components [feces ($Ndf_{feces(slu)}$) and urine ($Ndf_{urine(slu)}$)], because equation (1) is only valid for homogeneously labeled fertilizers. The $Ndf_{feces(slu)}$ was directly calculated according to equation (1) from the slurry treatment composed of labeled feces and unlabeled urine. The $Ndf_{urine(slu)}$ was deduced by combining this $Ndf_{feces(slu)}$ value with the data obtained in the slurry treatment which was composed of both labeled slurry components. The total excess ^{15}N taken up from this slurry consisted of the portions of excess ^{15}N taken up from labeled feces and from labeled urine as given in equation (3):

$$^{15}Nen_{pl} \times N_{pl} = {}^{15}Nen_{feces} \times Ndf_{feces(slu)} + {}^{15}Nen_{urine} \times Ndf_{urine(slu)}. \quad (3)$$

Since the two slurries were similar in their N composition, it was assumed that the percentage of $Ndf_{feces(slu)}$ of the total plant N uptake was equal in both slurry treatments. Thus, equation (3) could be solved for $Ndf_{urine(slu)}$ using the known ^{15}N atomic enrichments of the feces and urine components. The ^{15}N recovery of slurry N over the whole pot experiment was based on the same assumption, i.e., that the percentage of feces N recovered was equal in both slurry treatments.

The ^{15}N balances were calculated for each pot as the difference between ^{15}N added and ^{15}N removed by the six cuts, ^{15}N contained in the roots and ^{15}N remaining in the soil at the end of the pot experiment.

2.5. Analyses

2.5.1. Total N, organic C, nitrogen-15 and mineral N in manures

Feces, slurries and sediments obtained from the N fractionation described previously were lyophilized and finely ground using a ball mill (Retsch, Haan, Germany) prior to

total N, 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) [21]. Comparison of these total N determinations with total N measured in fresh feces samples analyzed on a macro CN analyzer (Leco CN-2000, St. Joseph, Michigan) showed that N loss from feces during lyophilization was only 5% on average. The urine portions were centrifuged at 500 *g* in a cooled centrifuge for 10 min, an aliquot of 40 ml of the supernatant acidified with 1.5 ml of 10 M H_2SO_4 and, after foaming had ceased, analyzed for total N on the Leco macro CN analyzer. For ^{15}N analysis, centrifuged urine samples were acidified to a pH between 3 and 5, lyophilized and analyzed for ^{15}N abundance as described previously for the feces samples. The ^{15}N atomic enrichment was calculated by subtracting the natural ^{15}N abundance, determined in unlabeled feces (0.371%) and urine (0.365%) collected immediately before the feeding of ^{15}N labeled hay, from measured values.

The mineral N (NH_4 and NO_3) in feces and slurries was extracted by shaking 5 g of fresh material, acidified by four drops of concentrated H_2SO_4, with 100 ml of 2 M KCl for 1 h and filtering through filter papers (Schleicher and Schuell, Dassel, Germany) [35]. Nitrate and ammonium N were colorimetrically analyzed on an Evolution II continuous flow autoanalyzer (Alliance Instruments, Nanterre, France).

2.5.2. Total N and nitrogen-15 in plant and soil materials

The plant shoot and root material was dried at 80 °C for 48 h and cut into small pieces. Soil samples were dried at 105 °C for 24 h. Both materials were then finely ground using the ball mill mentioned previously and analyzed for total N and ^{15}N as described for feces samples. The ^{15}N enrichments were calculated by subtracting the natural ^{15}N abundance (0.366%). Unlabeled samples were analyzed for total N on a Carlo Erba flash combustion CN-analyzer (NA1500) (Carlo Erba, Milano, Italy).

2.5.3. Statistical analysis

The GLM procedure [30] was used for analysis of variance to detect the effects of fertilizer treatments and soils and to calculate least significant differences at the $P = 0.05$ level. Means were compared with the Tukey's studentized range (HSD) test or the Bonferroni multiple comparison t-tests for the fertilizer treatments and with two sample t-tests for the soils at the $P = 0.05$ level.

3. RESULTS

3.1. Homogeneity of nitrogen-15 labeling in feces

Of the total N present in the feces used for the pot experiment, 10.7% was undigested feed N (UDN) (Tab. IV). The bacterial and endogenous debris N (BEDN) made up the major fraction, amounting to 76.9% of total feces N. The remaining 12.5% of total feces N was water-soluble N (WSN).

Table IV. Percentage of total N and ^{15}N atomic enrichment of different N fractions of the cow feces applied in the pot experiment.

N fraction	Fraction N in total N		^{15}N atomic enrichment	
	%			
Undigested N	10.7	(0.44)	0.648	(0.028) a
Bacterial and endogenous debris N	76.9	(1.24)	0.997	(0.011) b
Water-soluble N	12.5	(1.43)	1.014	(0.040) b
Total N	100		0.962	(0.001) b

Mean and standard deviation (in brackets). Data followed by different letters within a parameter indicate significant differences at $P < 0.05$ (Bonferroni multiple comparison t-tests, SAS Institute [30]).

The ^{15}N atomic enrichment was significantly lower in the UDN than in the other N fractions. However, as the UDN was only a small proportion of the total N, the overall ^{15}N atomic enrichment was not significantly influenced by this low value. Using the overall ^{15}N atomic enrichment to calculate the fertilizer value of feces reflected the N from the BEDN and the WSN fractions quite accurately, as the ^{15}N atomic enrichment of these two fractions was similar to the overall ^{15}N atomic enrichment (Tab. IV).

3.2. Total yield and nitrogen uptake

The DM yield and N uptake of the Italian ryegrass shoots were significantly higher on the ORG than on the MIN soil (Tab. V). On both soils, the highest yield and N uptake was obtained under the high level of mineral N fertilizer and the lowest in the unfertilized control. The feces alone only slightly increased shoot DM yield and N uptake compared with the control in both soils. Nitrogen uptake from fertilizers applied was similar on both soils and was well related to the proportion of mineral N contained in the fertilizers (Tab. III). Differences between treatments were more pronounced for N uptake than for DM yield.

The higher root N uptake on ORG compared with MIN soil (Tab. VI) was due not only to the higher DM yield but also to a significantly higher root N concentration (overall mean 9.3 and 8.7 mg $N \cdot g^{-1}$ DM on ORG and MIN soils, respectively). The lowest root DM yield was observed in the unfertilized control on both soils. For root N uptake, differences between the treatments were greater on ORG than MIN soil, but were generally small. The DM shoot-to-root ratio ranged from 1.6 to 2.1 and was generally higher on MIN than ORG soil except in the feces treatment. Considering the composition of the total N uptake as the sum of N uptakes of shoots and roots, a greater proportion of N was allocated to the roots on ORG than MIN soil. The total DM yield as the sum of shoot and root DM was highest for treatment minN2 on both soils, followed by slurry and fecminN, minN1, feces and the control.

Table V. Total dry matter yield, total N uptake and N derived from fertilizer (Ndff) and from soil (Ndfsoil) by the six cuts of Italian ryegrass shoots, and real coefficient of utilization of applied N (CUN) in the different fresh fertilizer treatments on the long-term organically (ORG) and exclusively minerally (MIN) fertilized soil. For abbreviations of treatments and components see Table III.

Treatments Components	Yield	N uptake	Ndff	Ndfsoil	CUN
	$g \cdot kg^{-1}$ soil		$mg\ N \cdot kg^{-1}$ soil		%
Soil ORG					
Control	8.4 d A	122.8 d A		122.8 b A	
Feces	9.4 c A	126.6 d A	10.6 d A	116.0 b A	19.1 d A
FecminN	10.7 ab A	167.0 bc A	nd‡	154.7† A	nd
Feces(minN)			12.4 d A		22.2 d A
MinN			nd		nd
Slurry	10.3 ab A	159.9 c A	43.7 b A	116.2 b A	40.7 c A
Feces(slu)			12.3 d A		21.8 d A
Urine(slu)			31.5 c A		61.6 b A
MinN1	10.0 bc A	174.9 b A	44.6 b A	130.3 a A	74.8 a A
MinN2	10.9 a A	208.8 a A	77.1 a A	131.7 a A	76.4 a A
Soil MIN					
Control	6.6 b B	97.7 d B		97.7 b B	
Feces	7.4 b B	104.1 d B	9.3 d A	94.8 b B	16.8 d B
FecminN	8.8 a B	145.9 b B	nd	135.6† B	nd
Feces(minN)			10.3 d A		18.5 d B
MinN			nd		nd
Slurry	8.6 a B	129.4 c B	44.1 b A	85.4 c B	41.1 c A
Feces(slu)			10.3 d A		18.3 d B
Urine(slu)			33.8 c A		66.2 b B
MinN1	8.8 a B	150.5 b B	44.6 b A	105.9 a B	74.7 a A
MinN2	9.1 a B	186.7 a B	76.8 a A	109.9 a B	76.1 a A
			ANOVA		
Source of variation					
Soil (S)	***	***	ns	***	ns
Treatment (T)	***	***	***	***	***
S × T	ns	ns	ns	*	ns

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively. Different lower case letters within a parameter and a soil indicate significantly different values at $P < 0.05$ level (Tukey's studentized range (HSD) test [30]). Different capital letters denote significant differences at the $P < 0.05$ level of the respective parameter between the two soils (t-test).
†Sum of Ndfsoil and N derived from minN.
‡Not determined since non-labeled minN not distinguishable from non-labeled soil N.

3.2.1. Nitrogen uptake from fresh fertilizers

Total N from different fertilizer treatments (Ndff) taken up by the shoots and the respective real coefficients of utilization of the fertilizer N (CUN) were similar on both soils (Tab. V). The Ndff was highest for minN2 and lowest for feces alone treatments. Slurry N was less available to plants than the same dose of N applied in mineral form (minN2). The average relative N efficiency of slurry N, calculated as the CUN of slurry N divided by the CUN of minN2, was 54%. The CUN of the urine component in the slurry was on average 64%, which is lower than the CUN of the mineral fertilizer. The CUN of the mineral fertilizer was similar for both application rates (average 75.5%, Tab. V).

The Ndff of the feces applied alone or as a slurry component exceeded the amount of N initially present as mineral N (Tab. III) or as total water-soluble N (Tab. IV), indicating that a portion of organically-bound feces N was mineralized during the experiment on both soils.

When the CUN of the manure components were considered separately (t-test), statistically significant differences between the soils were detected. Less urine N and more feces N was taken up on ORG than MIN soil. In the slurry, the resulting overall CUN, however, was similar on both soils. The CUN of feces N was increased by 3% on ORG and 1.5% on MIN when feces were applied with additional N (urine or mineral N). These differences were, however, very small compared with the differences between applied fertilizer types.

The CUN of the different fertilizers in the roots (Tab. VI), indicating the percentage of the respective fertilizers present in the roots at the end of the pot experiment, showed a different situation. Significantly more manure N was recovered in roots on ORG than MIN soil, and on both soils, the CUN of

Table VI. Dry matter yield, N uptake and real coefficient of utilization of applied N (CUN) in the different fresh fertilizer treatments of the Italian ryegrass roots at the end of the pot experiment, and the resulting total CUN of shoots and roots, on the long-term organically (ORG) and exclusively minerally (MIN) fertilized soil. For abbreviations of treatments and components see Table III.

Treatment	Roots			Shoots + Roots
components	Yield	N uptake	CUN	Total CUN
	$g \cdot kg^{-1}$ soil	$mg\ N \cdot kg^{-1}$	%	%
Soil ORG				
Control	4.9 b A	47.1 b A		
Feces	5.8 ab A	55.4 a A	10.9 a A	30.0 d A
FecminN	6.1 ab A	56.4 a A		
Feces(minN)			10.2 a A	32.5 d A
Slurry	6.5 a A	62.1 a A	9.8 a A	50.5 c A
Feces(slu)			9.8 a A	31.6 d A
Urine(slu)			9.8 a A	71.4 b A
MinN1	6.1 ab A	54.3 ab A	5.8 b A	80.6 a A
MinN2	6.8 a A	59.6 a A	6.3 b A	82.8 a A
Soil MIN				
Control	3.8 b B	35.6 ab B		
Feces	4.7 ab B	42.0 ab B	8.5 a B	25.3 c B
FecminN	4.8 ab B	40.9 ab B		
Feces(minN)			8.3 a A	26.8 c B
Slurry	4.9 a B	42.6 a B	8.0 a B	49.0 b A
Feces(slu)			7.5 ab B	25.8 c B
Urine(slu)			8.4 a A	74.6 a A
MinN1	4.1 ab B	35.1 b B	6.1 bc A	80.7 a A
MinN2	4.4 ab B	37.0 ab B	5.2 c B	81.2 a A
			ANOVA	
Source of variation				
Soil (S)	***	***	***	*
Treatment (T)	***	***	***	***
S × T	ns	*	*	ns

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively. Different lower case letters within a parameter and a soil indicate significantly different values at the $P < 0.05$ level (Tukey's studentized range (HSD) test [30]). Different capital letters denote significant differences at the $P < 0.05$ level of the respective parameter between the two soils (t-test).

the manures were higher than that of the mineral N fertilizers. The highest root CUN was found for feces. Expressed as Ndff, most fertilizer N found in roots was in the slurry treatment on both soils. Considering the total utilization of the fertilizers as the sum of the CUN in shoots and roots, the influence of soil, including all treatments, was statistically significant. More feces N was utilized on ORG than MIN, and the influence of additional N sources on feces N utilization observed in the shoots nearly disappeared on both soils. Total urine N utilization was similar on ORG and MIN soils, and was lower than the utilization of mineral N fertilizers.

3.2.2. Nitrogen uptake from soil

Significantly more N was taken up from ORG than from MIN soil in all fertilizer treatments (Tab. V). The average difference was 24.7 $mg\ N \cdot kg^{-1}$ soil and did not differ much between the treatments. The mineral N additions caused a slight but significant increase in N uptake from soil compared with the unfertilized control, suggesting that mineral N addition had increased mineralization of soil N. In contrast, manure addition reduced Ndfsoil on both soils compared with the controls, indicating that a slight soil N immobilization had been induced [15].

3.3. Temporal pattern of nitrogen uptake

3.3.1. Total nitrogen uptake and nitrogen uptake from soil

For all fertilizer treatments, more than 50% of the final total N uptake in shoots was taken up by the first cut (Fig. 1). Because no N was subsequently added during the experiment, N conditions became limiting for all fertilizer treatments on both soils after the first cut. The N concentration declined from an overall average of 26.5 $g \cdot kg^{-1}$ plant DM

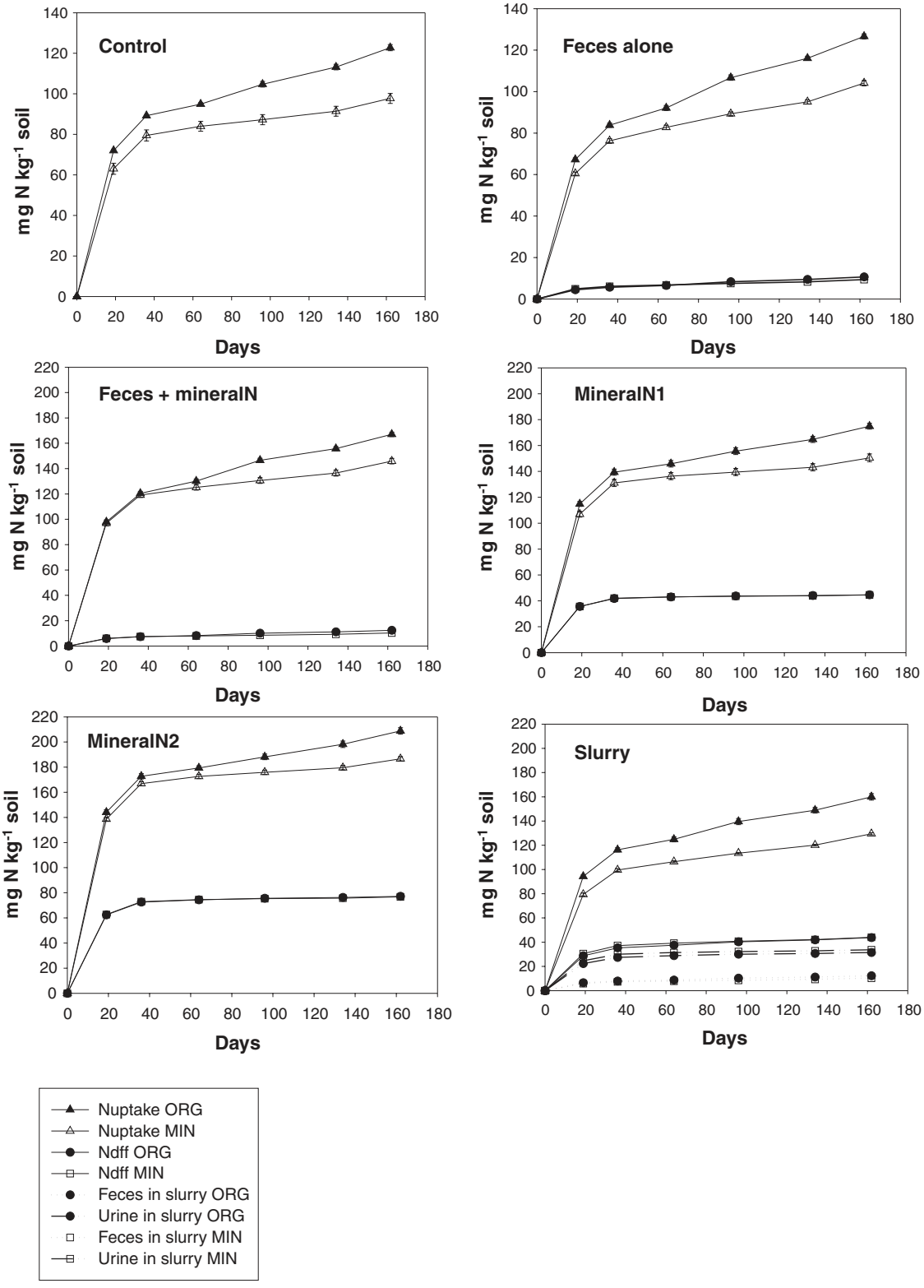

Figure 1. Cumulative total N uptake and N taken up from fertilizers (Ndff) over time by the Italian ryegrass shoots under different fertilizer treatments applied to the long-term organically (ORG) or conventionally (MIN) cultivated soil in a pot experiment. Means and SEM (bars) of 4 replicates; consider the different scales of the y axis, and that curves of Ndff for various treatments are almost overlaying. For abbreviations of treatments and components see Table III.

measured for plant material harvested at the first cut to 10.5 g·kg^{-1} for the second cut, and was always around 10 g·kg^{-1} for the following cuts. This is clearly below the 30–42 g N·kg^{-1} DM indicated as sufficient for the growth of *Lolium ssp.* [2].

The increased N uptake on ORG soil was for most treatments manifested from the beginning of the experiment. In all cases it was due to greater N uptake from soil N (Ndfsoil). Thus, the evolution of the cumulative total N uptake reflects the uptake of cumulative Ndfsoil (Fig. 1; Tab. VII). This shows that from the second cut onwards, mineralizable soil N reserves determined N uptake by the ryegrass. Fertilizer N remaining in the soil after the first cut contributed, in spite of N-limiting conditions, very little to plant N uptake during the following cuts.

3.3.2. Nitrogen uptake from fertilizers

As seen for the total fertilizer N taken up by all cuts (Tab. V), the cumulative temporal uptake pattern of N from the fertilizers (Ndff) was not significantly influenced by the soils (Fig. 1). The slightly higher urine N uptake on MIN than ORG soil (Tab. V) was due to the urine N uptake during the first cut, while the increased feces N uptake on ORG compared with MIN soil developed in the course of the experiment in all the treatments containing feces (Fig. 1).

Table VII. Soil N uptake (Ndfsoil) by the Italian ryegrass of the first cut and as sum of the following five cuts in the different fresh fertilizer treatments on the long-term organically (ORG) and exclusively minerally (MIN) fertilized soil. MinN1 and MinN2 = lower and higher level of mineral N treatment.

Treatment	Ndfsoil	
components	mg N·kg^{-1} soil	
Cut	1st	2nd–6th
Soil ORG		
Control	72 b A	51 a A
Feces	63 c A	53 a A
Slurry	66 c A	51 a A
MinN1	79 a A	51 a A
MinN2	82 a A	50 a A
Soil MIN		
Control	63 b B	35 bc B
Feces	56 c B	39 a B
Slurry	49 d B	37 b B
MinN1	71 a B	35 bc B
MinN2	76 a B	34 c B
Source of variation	ANOVA	
Soil (S)	***	***
Treatment (T)	***	***
S × T	***	ns

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level respectively. Different lower case letters within a parameter and a soil indicate significantly different values at $P < 0.05$ level (Tukey's studentized range (HSD) test [30]. Different capital letters denote significant differences at the $P < 0.05$ level of the respective parameter between the two soils (t-test).

In all treatments, most of the fertilizer N was taken up by the first cut (Fig. 1). For the mineral N, about 80% of the total Ndff was contained in the first cut. For the feces N, this portion was 56% on MIN soil and 47% on ORG soil, indicating that the feces as an organic N fertilizer was effective over a longer period, although at a low N release rate. This was also manifested when the contribution of the various fertilizers to plant nutrition was expressed as the percentage of the Ndff in the total N uptake (%Ndff). The importance of the mineral N fertilizer (initially representing over 40% of the plant N uptake) decreased rapidly with time, whereas feces N continued to contribute to plant N uptake at a low efficiency during the experiment. Slurry, being a mixture of readily and slowly available N components, resulted in the relatively highest Ndff found in the roots, suggesting that this fertilizer continued the longest to deliver N to the grass. As more soil N was taken up by the grass on ORG than MIN soil, the %Ndff was generally lower on ORG than MIN soil.

3.4. Nitrogen-15 balances

As all applied fertilizer treatments except the mineral N added to the fecminN treatment were ^{15}N labeled, balances could be established to account for the fertilizer N losses occurring during the five-months duration of the experiment. The average ^{15}N recovery in six cuts, in roots and remaining in the soil at the end of the pot experiment was 96.5%, indicating that only minor losses had occurred (Fig. 2). These

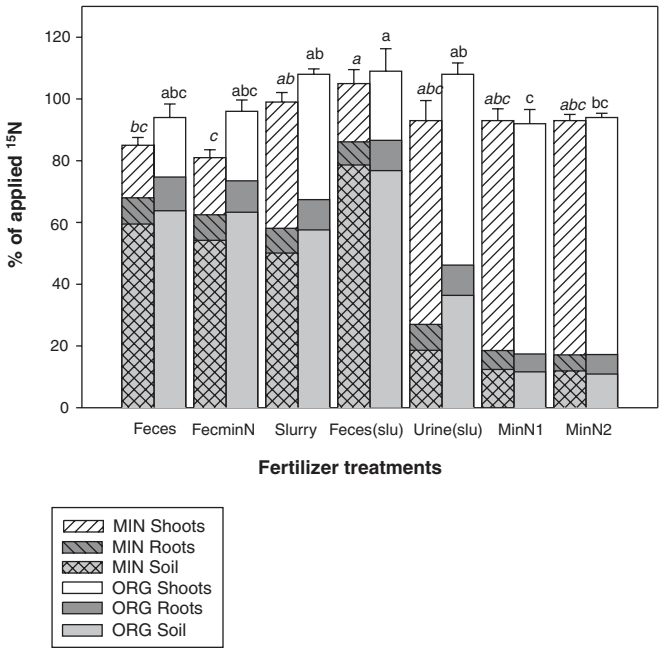

Figure 2. Recoveries of ^{15}N applied in different fertilizer treatments on the long-term organically (ORG) and conventionally cultivated (MIN) soil. Values are means of 4 replicates. Bars indicate the standard errors of the total recoveries. Means of total N recoveries carrying different letters are significantly different at $P < 0.05$ within one soil. For abbreviations of treatments and components see Table III.

losses were mainly attributed to denitrification since closed pot bottoms prevented N leaching and ammonia volatilization was minimized by incorporating well the fertilizers into the soils. The total ^{15}N recovery tended to be greater for the slurry and its components, followed by mineral fertilizers and feces applied alone or with additional mineral N.

Only about 11% of ^{15}N from the applied mineral fertilizer remained in the soil after the final cut, while this portion was about 60% for feces N, and 50 to 60% for slurry N on MIN and ORG soils, respectively (Fig. 2). The mean ^{15}N recovery over all treatments was significantly higher for ORG than MIN soil. This was due to a higher recovery of N from the manure treatments, especially as N remaining in soil and contained in roots. Soil aggregate stability was lower in MIN than ORG [32], resulting in a crusted soil surface after the setup of the pot experiment. Therefore, some areas of higher water saturation could have developed temporarily in MIN after watering, thus enhancing denitrification.

4. DISCUSSION

4.1. Homogeneity of nitrogen-15 labeling in feces

Calculation of N fertilizer value using ^{15}N labeling is based on the assumption that the fertilizer N is homogeneously labeled [12, 27]. In addition, the highest possible ^{15}N atomic enrichment of feces N is desirable for reliable detection of ^{15}N in the subsequently collected plant and soil samples. The amount and distribution of ^{15}N in ruminant feces depend on the type and ^{15}N atomic enrichment of the feed, on the length of the ^{15}N feeding period and on the time elapsed between feeding and collecting the excreta [27, 36]. The feces pooled for use in this study were the portions showing the highest ^{15}N atomic enrichment, collected 35 and 47 h after the beginning of a one-day ^{15}N feeding period on labeled hay. Peschke [25] observed the peak in ^{15}N enrichment in a similar feeding trial after the same period. When cows were fed on ^{15}N labeled feed for 36 h, the highest ^{15}N atomic enrichment was detected in feces collected 60 h after the ^{15}N feeding, and feces were homogeneously labeled at this time [27].

In the feces collected for this study, the ^{15}N atomic enrichments of the analyzed feces' N fractions (undigested N (UDN), bacterial and endogenous debris N (BEDN), water-soluble N (WSN); Tab. IV) were not identical at any time during the 8-day collecting period (data not shown). However, the ^{15}N atomic enrichment of the BEDN was similar to the ^{15}N enrichment of the total feces N throughout the collection period, as the BEDN constituted about three quarters of the total feces N. Assuming that the UDN contributes little to plant-available N because it is most resistant to microbial and metabolic turnover in the digestive tract, it was considered important that the ^{15}N enrichment of the WSN and the BEDN were similar and well represented by the total feces N enrichment (Tab. IV).

Most urinary N is in the form of urea, and urea N is rapidly hydrolyzed to NH_3. Sørensen and Jensen [34] found the ^{15}N enrichment of urinary total N and NH_4-N produced after urine storage to be similar, indicating that the urine was uniformly labeled with ^{15}N.

4.2. Fertilizer value of cattle manure on soils of different farming systems

The hypothesis was tested whether freshly applied manure N was mineralized and thus rendered plant-available to a greater extent on a long-term organically (ORG) than a long-term conventionally, exclusively minerally fertilized soil (MIN) due to ORG's higher microbial activity. The results indicate that this applies for slowly mineralizable manures like feces, but only to a limited degree. Great differences were found in the amount of soil N mineralized during the experiment, resulting in significantly higher plant yields and N uptakes on the ORG compared with the MIN soil regardless of the type of fresh fertilization. Application of fresh fertilizers thus seemed to induce a similar response of N turnover processes in both soils whereas the soils differed in the release of soil N.

4.2.1. Nitrogen fertilizer value of freshly applied cattle manures

The N fertilizer values of the different fertilizers, expressed as the utilization of applied fertilizer N by the plants (CUN), were slightly affected by the long-term fertilization history. Response to the different fertilizers was similar for ORG and MIN soils, leading to similar ranking of total N uptakes among the treatments (Tab. V) according to the initial mineral N content of the different fertilizers (Tab. III).

Many studies have been conducted to assess the fertilizer efficiency of cattle slurry, but most of them are based on the difference method since the ^{15}N labeling of ruminant manures is time-consuming and costly. Reported values range from 30 to 50% of apparent slurry N utilization [11, 31]. Peschke [26] found between 30 and 40% of slurry N utilized by maize based on ^{15}N techniques, which is congruent with a CUN of 41% for slurry N found in this study (Tab. V).

The CUN of urine N and mineral fertilizer N (Tab. V) were generally high in this study compared with reported values, which might be due to the high N demand of the ryegrass and specific experimental conditions (small pots, N deficiency). The average CUN of feces of 18% was similar to that found (17%) by Rauhe et al. [28] for maize grown in a field experiment and slightly higher than that of sheep feces (12–14%) in a lysimeter experiment growing spring barley [37]. In the presence of additional N as mineral N or urine N, the utilization of the feces N was increased, suggesting an interaction between the N turnovers of fertilizer components. Some N of the additional source might have been immobilized instead of feces N.

The similar responses to fresh fertilization of the two soils suggest that the reactions of the soil microbial biomass to application of a given fertilizer were independent of the size and activity of the microbial biomass initially present in the soils.

4.2.2. Nitrogen supply from soil as affected by the long-term fertilization history

Much more soil N was mineralized and thus taken up by the plants from the long-term organically than from the long-term minerally fertilized soil (Tab. V, Fig. 1). For soil N mineralization, therefore, results obtained in the presence of ryegrass confirm the greater N mineralization capacity [20] and the higher soil respiration [22] measured during incubation experiments without fresh substrate additions in ORG than MIN soils. Thus, ORG soil seems to have a greater mineralizable soil N reserve than MIN, which is probably due to the fertilization history [42], i.e. the repeated organic inputs to the ORG soil amounting to on average 61 kg organic $N \cdot ha^{-1} \cdot yr^{-1}$ (Tab. I). As shown in Figure 2, about 60% of the applied feces N and on average 28% of the urine N remained in the soil at the end of the pot experiment. Under field conditions, the portion contained in the roots would additionally contribute to the fertilizer N remaining in the soil. A portion of this N would become available for subsequent crops. Field studies based on ^{15}N labeled animal manures showed residual N fertilizer values in the season after manure application of 4 and 3% for sheep feces and urine [12, 37] and of 4 to 7% for cattle slurry [26, 28]. The portions of manure N still remaining in soil two or three years after application reported by these studies range from 60 to 80% for feces and from 40 to 60% for urine. Thus, especially the residual organic substances of feces will accumulate in soils receiving repeated applications of animal manures and become significant [42]. This increase in mineralizable soil N reserves is not significantly reflected in total soil N contents (Tab. II), but differences in organic matter quality are reported for ORG and MIN soils. Microbial biomass C and N as well as their ratios to the total and light fraction C and N pools were higher in ORG than MIN [6]. Furthermore, increased soil N supply in ORG might be due to higher mineralization-immobilization-turnover in ORG than MIN.

5. CONCLUSIONS

Short-term N fertilizer efficiency of manure assessed in a pot experiment was only slightly altered through long-term application of organic fertilizers in organically managed soils compared with long-term minerally fertilized soils. However, the capacity of the soil to sustain plant N nutrition was enhanced by 19 years of organic farming practices. Quantitative and temporal patterns of this different soil N supply should be evaluated under field conditions to determine (i) whether the N release is synchronized with the demand of growing annual crops, and (ii) to which extent soil N release can overcome N limitations. In addition, the potential risk of N losses in different farming systems has to be assessed.

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