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ORIGINAL ARTICLE

Laboratory and greenhouse assessment of plant availability of organic N in animal manure

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Abstract Laboratory data (thermal fractionation, pepsin extraction, C:N$_o$ ratio) of dung and manure were mutually compared and contrasted with plant-availability of organic N (N$_o$) as found in a greenhouse experiment according to the double-pot technique. Two types of fresh cow dung (one with a relatively wide and the other with a relatively narrow C:N$_o$ ratio) and four types of manure (from poultry, sheep, pigs and cow) were compared with ammonium nitrate as chemical reference fertilizer. Relative effectiveness of organic N (RE$_o$) was used as characteristic; it was calculated as the fraction of organic N that has the same availability to plants as inorganic N. RE$_o$ for poultry and sheep manure could not be assessed, probably because of NH_3 volatilization causing direct damage to plants and N losses. RE$_o$ values decreased in the order: dung with narrow C:N$_o$ > dung with wide C:N$_o$ > pig manure > cow manure. Thermal fractionation did not provide a suitable index of plant-availability of organic N. Pepsin extracted organic N gave a positive, and C:N$_o$ ratio a negative relationship with RE$_o$. Also between pepsin extracted organic N and C:N$_o$ ratio a negative relationship was found. As C:N$_o$ ratio is relatively easy to determine, it is considered the most practical laboratory index for plant availability of organic N in animal manures low in ammonia. When using the double-pot technique, application rates of manure types high in ammonia should be restricted.

Keywords Animal manure · C:N$_o$ ratio · Double-pot technique · Dung · N-mineralization · Organic nitrogen · Pepsin · Relative effectiveness · Thermal fractionation

Introduction

It is generally recognized that integrated nutrient management for sustainable agriculture includes recycling of plant nutrients from animal manure (Gruhn et al. 2000; Groot et al. 2003; Yang 2006; Janssen and Oenema 2008). Use of N from such sources decreases the need for inorganic fertilizers. Nevens and Reheul (2003) could save about 80–90 kg fertilizer N per ha

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per year by applying about 40 Mg cattle slurry annually. In the tropics, manure is the key to restoration of depleted soils (Rowe et al. 2006; Zingore et al. 2008). However, the availability of manure N to plants is difficult to predict since a significant portion of manure N may be lost through NH_3—volatilization (Sommer and Olesen 1991) and more than 50% may be present as organic N (Kirchmann 1991). Organic N has to be transformed into inorganic N to become available to crops. That transformation usually is called mineralization. To be able to utilise manure N efficiently it is essential to estimate the quantity and the timing of mineralization (Trindade et al. 2001). The rate of N mineralization of dung and manure is affected by soil environmental conditions (Sørensen et al. 1994; Schils and Kok 2003), but depends most strongly on the carbon to nitrogen ($C{:}N_o$) ratio and the decomposability of the organic material (Janssen 1996), which in turn are related to the origin, treatment and straw content of the dung and manure (Castellanos and Pratt 1981; Kirchmann 1985; Van Faassen and Van Dijk 1987). The availability of N contained in manure has been of concern to soil scientists and agronomists for many decades. A number of investigations have tried to relate N release from organic materials to laboratory indices. Castellanos and Pratt (1981) reported that the best single chemical index for available N was the fraction of organic N extracted by digestion with pepsin; about 81% of the variation in available N was related to the variation in pepsin-extractable N. This method was also successfully used to estimate digestibility of forages (Cerda et al. 1989). Velthof et al. (1998) found that thermal fractionation provided a reasonable index of mineralizable N for processed organic materials low in inorganic N, and pepsin extraction for all organic materials except composted materials. In short, widely differing materials could be distinguished.

The present work was a sequel to the study by Raijmakers and Janssen (1993, 1995) and Velthof et al. (1998). They concluded that more research was needed to test the methods of thermal fractionation and pepsin extraction. In contrast to the preceding studies, the present one concentrated on animal manure of different origins and did not deal with compost and other strongly different types of organic materials. This restriction was made to examine whether the laboratory methods could also distinguish between organic materials that are not very far apart in nature. The major aim of this and the former studies was to test laboratory methods that could support decisions to optimize the management of organic nutrient sources in agriculture. The specific objectives of the present study were (1) to compare availability indices of organic N as obtained with different laboratory methods, (2) to assess the availability to plants of organic N in manures of various animals by determining the plant uptake of N in a greenhouse experiment, (3) to compare plant available organic N in manure as determined in the greenhouse experiment with availability indices obtained with different laboratory methods.

Materials and methods

Origin of manure

Fresh poultry manure, three types of farmyard manure from dairy cows, pigs and sheep, and two types of cow dung (one with a relatively wide and another with a relatively narrow $C{:}N_o$ ratio) were used. A description of the origin and codes of the products is presented in Table 1. Poultry manure was collected from the department of Animal Science of Wageningen University. The other manures were collected at the Minderhoudhoeve, an experimental farm of the Wageningen University in Oostelijk Flevoland (Lantinga et al. 2004). In all tables, manures and dung are arranged in the order of decreasing values of pH and NH_3–N which are given in Table 4.

Table 1 Names and description of tested types of manure and dung

Name	Description
Manure of	
Sheep	Mixture of sheep faeces, urine and wheat straw
Poultry	Mixture of poultry faeces and urine
Cow	Mixture of cow dung, urine and wheat straw
Pig	Mixture of pig faeces, urine and wheat straw
Cow dung with	
Narrow $C{:}N_o$	From animals grazing on protein-rich grass/clover pastures day and night
Wide $C{:}N_o$	From animals grazing on grass/clover pastures, but during night in stable and fed with a mixture of protein-poor maize silage, beet pulp and straw

Laboratory studies

Chemical analysis

Table 2 gives a summary of the analyses carried out. For some analyses fresh manure and dung was used, for others manure and dung having been dried at 40°C. A separate sub-sample of both, fresh and dry material, was dried at 105°C for 24 h, to assess the dry matter (DM) content (Anonymous 1998). Osmolarity, water-soluble NH_4–N and NO_3–N, and pH were measured in fresh manure samples on the same day the manures were collected. The pH was determined in extracts obtained after shaking 20 g samples with 100 ml de-mineralized water for 2 h. Osmolarity and water soluble NH_4–N (i.e., the sum of NH_4–N(aq) and NH_3–N(aq)) and NO_3–N were measured in supernatants of centrifuged suspensions of fresh manure in de-mineralized water (1:10 mass ratio, shaking for 2 h) by using Osmometer 030 (Gonotec), and standard auto-analyser, respectively (Houba et al. 1989).

The samples dried at 40°C were ground to pass a 0.5 mm sieve in a hammer mill. Three sub-samples were taken. In one sub-sample, organic carbon (C) was determined by dichromate-oxidation (Houba et al. 1989). A second sub-sample was shaken for 2 h in 0.01 M $CaCl_2$ (mass ratio 1:10), centrifuged at 48,000*g* (Houba et al. 1986, 1990); in the supernatant of the centrifuged suspension NH_4–N and NO_3–N were determined by using standard auto-analyser (Houba et al. 1989). The third sub-sample was digested in a mixture of H_2SO_4 and salicylic acid and Se, to which H_2O_2 was added (Walinga et al. 1989). Total N in the digest was determined colorimetrically (Houba et al. 1989). Organic N (N_o) was calculated as the difference between total N and the inorganic N (NH_4–N plus NO_3–N).

Table 2 Laboratory studies in fresh and dried manure samples

Type of sample	Initial treatment	Analyses
Fresh	Dried at 105°C	Dry matter
	Centrifuge, supernatant	NH_4, NO_3, pH, osmolarity
Dried at 40°C	Dried at 105°C	Dry matter
	Wet oxidation	C
	$CaCl_2$ extraction	NH_4, NO_3
	Digestion	N, P, K, Na, Ca, Mg
	Heating, digestion	Total N and C in remaining residues
	Incubation with pepsin/HCl and centrifugation	Total N in supernatant and residue

Thermal fractionation

When organic material is heated till temperatures between about 200 and 500°C, a certain fraction of C and also a certain fraction of N are released. In their study on processed organic materials, Velthof et al. (1998) tried to relate the ratio of the N and C fractions released during heating (FN_{rel}/FC_{rel}) to the relative effectiveness of organic N (RE_o), as determined in the greenhouse experiments according to the double-pot technique (see below). The fractions of C and N released during heating were assessed as the difference between the fractions before heating and the fractions found in the remaining residue after heating:

$$\begin{aligned}
FN_{rel} &= (N_{ini} - N_{rem})/N_{ini} \\
FC_{rel} &= (C_{ini} - C_{rem})/C_{ini} \\
FN_{rel}/FC_{rel} &= (N_{ini} - N_{rem})/N_{ini} \cdot C_{ini}/(C_{ini} - C_{rem}) \\
&= (1 - FN_{rem})/(1 - FC_{rem}) \qquad (1)
\end{aligned}$$

where

$N_{ini}; C_{ini}$ = initial amounts of total N and C present in theproduct

$N_{rem}; C_{rem}$ = amounts of total N and C that remain after heating

$FN_{rem} = N_{rem}/N_{ini}$

$FC_{rem} = C_{rem}/C_{ini}$.

The effect of heating on the release of C and N from manure was studied with manure and dung samples dried at 40°C and ground to 0.5 mm. Three grams of a manure sample were heated for 6 h at 200, 300, 400 and 600°C, respectively. Thereafter, the mass, and total N and C mass fractions of the residues were determined. In all cases triplicate measurements were carried out.

Extraction with pepsin

The release of organic N by pepsin was studied in a number of laboratory experiments according to a standard procedure developed earlier (Raijmakers and Janssen 1993; Velthof et al. 1998). Also for these analyses, manure dried at 40°C and ground to 0.5 mm was used. Samples equivalent to 200 mg organic N were incubated for 48 h at 21°C in solutions of 0 and 625,000 units pepsin in 200 ml of 0.1 M HCl. After incubation and centrifugation, the supernatant was digested and N was determined (N_{super}). The difference between N_{super} and N in HCl alone (N_i) represents pepsin extracted organic N (N_{pep}). The ratio N_{pep}/N_o is the pepsin extracted fraction of organic N.

Greenhouse experiment according to the double-pot technique

Calculation of the relative effectiveness of organic N

The availability of organic N from manure was found by comparing the N uptake from organic materials with the N uptake from a reference fertilizer. Ammonium nitrate acted as reference. The relationship between N uptake by plant and application of available N usually is linear at low application rates but often levels off at high application rates (e.g., Van Erp and van Dijk 1992).

Manures contain both, organic and inorganic, forms of N. Organic N becomes available only after mineralization. Mineralized organic N as well as inorganic N from manure was assumed to be available to the plant and to behave in the same way as the inorganic N of the reference AN. So, the mineralized fraction of organic N represents the relative efficiency of organic N (RE_o). The applied organic N is indicated by AN_o, and the applied organic N that is available to the plant is indicated by AAN_o. So, AAN_o is $RE_o \cdot AN_o$. In this study, the total application of available N (AAN_t) was partitioned into applications of N from seeds and solution (AN_{ss}), of inorganic N (AN_i), and of mineralized organic N ($RE_o \cdot AN_o$):

$$AAN_t = AN_{ss} + AN_i + RE_o \cdot AN_o. \qquad (2)$$

We assumed that AN_{ss} (see below) was as available as AN_i. The relationship between applied available N and N uptake in the pots that received ammonium nitrate was used as a calibration curve to find AAN_t in the pots receiving manure or dung. This relationship was fitted to a parabolic expression:

$$AAN_t = b \cdot UN + c \cdot UN^2 \qquad (3)$$

where UN = measured uptake of N; UN and AAN_t were expressed in mg N per pot.

Next AAN_o and RE_o were calculated by application of Eq. 2 in a reverse way:

$$AAN_o = AAN_t - AN_{ss} - AN_i \qquad (4)$$

$$RE_o = AAN_o/AN_o. \qquad (5)$$

Technical procedure

A greenhouse experiment according to the double-pot technique (Janssen 1990) was carried out to compare the plant availability of N in the various organic materials with that of N in a reference chemical fertiliser, for which ammonium nitrate was used. Raijmakers and Janssen (1993, 1995) gave a detailed description of the modifications made to use the double-pot technique for examining the nutrient availability of organic products. In brief, plants are grown in two pots piled on top of each other. The plant roots can take up nutrients from both pots. The upper pot contains the substrate to be tested and the lower pot contains a nutrient solution in which all nutrients except the tested nutrients are present. There is an air space between the upper and lower pots, preventing direct transport of water and nutrients between the pots. In the present experiment, a 150-l tank was used, instead of individual lower pots, on top of which 28 upper pots could be placed. Below each upper pot a small plastic bucket with holes was placed to avoid interaction among the roots of separate upper pots. The crop was perennial ryegrass (*Lolium perenne* L.). The experiment comprised three tanks, each representing a completely randomized block of 28 treatments (7 fertilizers × 4 levels, see below). The upper pots (effective volume 2.1 l) were filled with a mixture of inert quartz sand and fresh animal manure or ammonium nitrate. The upper pots were weighed and watered with demineralised water every other day. The volume of moisture per pot was kept at 330 ml, corresponding to field capacity. The 150-l tank contained a nutrient solution without N, made up of the following (mM/l): $MgSO_4$ 0.75, KH_2PO_4 1.0, K_2SO_4 1.0, K_2SO_4 0.5, $CaCl_2$ 0.5, and trace elements (mg/l) B 0.5, Mn 0.5, Zn 0.05, Cu 0.02, Mo 0.01 and Fe 1.0 (as Fe-EDTA). The nutrient solutions were refreshed every 2 weeks.

Ammonium nitrate was applied in the upper pots at N rates of 100, 200, 300 and 400 mg per pot, but initially all ammonium nitrate pots received 100 mg N per pot to avoid salt damage. After the first grass cut (see below), dissolved ammonium nitrate was applied to reach the total application rates of 100, 200, 300 and 400 mg/pot. It was the intention to apply dung and manure at the same N rates. Calculations of required quantities of dung and manure were based on literature data about their N mass fractions. Later, the real application rates were calculated based on measured N contents in manure. These actual rates were close to the intended rates except in the cases of dung where the amounts of N present were lower than we had assumed. Table 3 shows how much N was applied at the lowest N rate, and the corresponding quantities of fresh manure or dung and dry matter. The applied N has been subdivided into inorganic and organic N, based on the data of Tables 4 and 5.

Further some N was supplied through the seeds (15 mg per pot, Raijmakers and Janssen 1993), and a little bit from Fe-EDTA and ammonium molybdate in the nutrient solution. The sum of the applications of N from seeds and solution (AN_{ss}) was calculated at 22 mg N per pot.

Grass was sown on 28 May 1998, at a rate of 0.75 g/pot. Grass was cut three times on 17 June, 13 July and 6 August, depending on the growth rate. At the end of the experiment, roots in the tank solution and roots (including stubbles) in the upper pots were harvested, as well as the shoots. Roots and stubbles in the upper pots were separated from sand-manure or sand-AN mixture by sieving and washing with demineralised water. Dry matter yields of shoots and roots were determined after drying the plant materials at 70°C, and analyzed for Total N (Walinga et al. 1989). Total accumulated dry matter was calculated as the sum of the shoots of all cuts, and the roots and stubbles at the final harvest. Total N uptake

Table 3 Applied fresh manure or dung (FM, g/pot), dry matter (DM, g/pot), osmolarity (Osmo, mosmol/pot), Total N (AN_t), inorganic N (AN_i), organic N (AN_o), and estimated NH_3–N (all in mg N/pot) at the lowest application level of manure and dung

	FM	DM	Osmo	AN_t	AN_i	AN_o	NH_3–N
Sheep	9.76	4.00	11.51	117	26	91	8.57
Poultry	8.55	2.56	7.92	144	26	118	1.51
Cow	31.75	6.35	11.02	156	24	132	1.05
Pig	19.23	5.58	7.50	137	12	125	0.37
Narrow C:N_o	7.59	0.76	1.59	27	3	25	0.03
Wide C:N_o	11.10	1.66	1.22	43	0.3	43	0.00

Table 4 Dry matter (DM, g per kg fresh material), N_i (mg per kg fresh material, mg per kg dry matter), pH, calculated (see text) ratio NH_3/NH_4 (R, mol/mol), estimate of NH_3–N (mg per kg fresh material), and osmolarity (Osmo, mosmol per kg fresh material) in fresh manure and dung

	DM	N_i		pH	R	NH_3–N[a]	Osmo[b]
		Fresh	Dry				
Sheep	413	2672	6469	8.89	0.490	878.3	1180
Poultry	296	3045	10286	7.99	0.062	176.8	927
Cow	196	755	3854	7.86	0.046	33.0	347
Pig	287	634	2210	7.69	0.031	19.0	390
Narrow C:N_o	102	339	3324	7.27	0.012	3.9	210
Wide C:N_o	147	25	167	7.09	0.008	0.2	143
VC, %		6.5[c]		1.0	NA[d]	NA[d]	3.8[b]

In all materials NO_3–N was 0. Variation coefficients (VC) were averaged over the six materials

[a] NH_3–N was calculated as $N_i \cdot R/(1 + R)$

[b] Osmolarity refers to osmols per liter; osmolality to osmols per kg. We use here osmolarity and not osmolality because the analysis was made in the supernatant

[c] The VC of Wide C:N_o was much higher (101.7 and 10.7% for NH_4–N and osmolarity, respectively, and therefore it was not included in the average value

[d] NA, not applicable

Table 5 Organic carbon, total N, NO_3–N and NH_4–N, organic N(N_o), C:N_o ratio in manure and dung dried at 40°C

	C	Total N	NO_3–N	NH_4–N	N_o	C:N_o
Sheep	435	23.50	0.01	6.74	22.81	19.0
Poultry	396	50.68	0.01	4.79	45.88	8.6
Cow	407	21.70	0.11	0.81	20.77	19.5
Pig	443	23.41	0.03	0.98	22.40	19.7
Narrow C:N_o	489	32.77	0.00	0.22	32.54	15.0
Wide C:N_o	444	26.01	0.01	0.17	25.84	17.2
VC, %	3.4	1.2	66.5	5.2	1.5	

All quantities are expressed in g per kg dry matter (105°C). Variation coefficients (VC) were averaged over the six materials was calculated as the sum of N in all cuts and finally harvested material.

Results

Laboratory studies

Fresh manure

NO_3–N is not included in Table 4, as it was not found in any of the fresh materials. The table shows that sheep and poultry manure had the highest fractions of dry matter in fresh manure. The lowest values were in dung, lower in dung with a narrow C:N_o than in dung with a wide C:N_o ratio. Pig and cow manure took a middle position. A similar division in three categories holds for the values of pH, osmolarity and NH_4–N, but these were in dung with a narrow C:N_o ratio higher than in dung with a wide C:N_o ratio. The values of pH and osmolarity were connected with the dry matter content in the fresh manure; in other words they were inversely proportional to the degree of dilution in the 1:10 mass ratio suspensions of fresh manure, as is shown in Fig. 1. Because no NO_3–N was present, N_i is the sum of NH_4 and NH_3(aq). N_i was calculated twice, as fraction of fresh material and as fraction of the dry matter in fresh material. The value of K_b, standing for $[NH_4^+][OH^-]/[NH_3]$ is $1.8 \cdot 10^{-5}$ (Atkins and Jones 1997), and hence R, the ratio $[NH_4^+]/[NH_3]$ in fresh material, could be calculated as $10^{(-9.2)}/(10^{(-pH)})$ and NH_3–N as $N_i \cdot R/(1 + R)$.

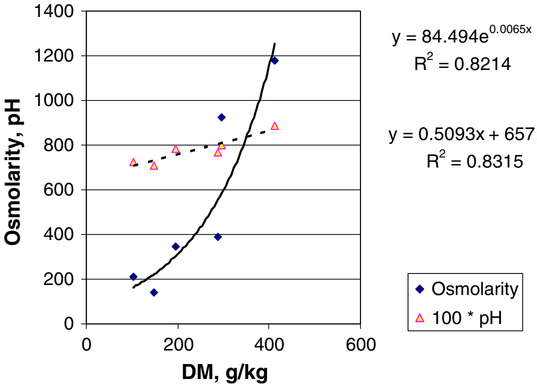

Fig. 1 Osmolarity (mosmol per kg fresh material) and (100 times) pH in relation to dry matter fraction (g per kg fresh material) of fresh manure and dung

Manure dried at 40°C

Chemical analysis The C mass fraction in Table 5 may be seen as an index of “organic purity” of the manure. It was highest in cow dung with narrow C:N_o ratio, and lowest in poultry manure, which may have contained silicate fragments. On the other hand, poultry manure was by far the highest in total and organic N, and had the lowest C:N_o ratio. The manures containing straw (from sheep, pig, cow) were low in N and had high C:N_o ratios, while the two types of dung took a position in between. The VC of NO_3–N was high because the mass fraction of NO_3–N was very low.

Table 6 Remaining fractions of N and C (FN_{rem}, FC_{rem}), expressed as % of initial N and C, and the ratio of the fractions of released N and C(FN_{rel}/FC_{rel}), as calculated with Eq. 1, after heating at 200, 300, 400 and 600°C

	Temperature, °C			
	200	300	400	600
FN_{rem}				
Sheep	83.3	15.3	1.57	0.05
Poultry	66.8	43.6	0.55	0.32
Cow	91.2	23.5	0.83	0.19
Pig	76.2	21.6	0.83	0.16
Narrow C:N_o	85.9	28.1	1.78	0.44
Wide C:N_o	86.5	23.4	1.44	0.30
VC, %	3.3	10.7	14.3	16.7
FC_{rem}				
Sheep	81.4	14.3	1.98	0.23
Poultry	63.2	33.5	0.68	0.30
Cow	79.8	8.9	0.84	0.38
Pig	71.4	12.5	1.38	0.36
Narrow C:N_o	73.7	12.5	1.13	0.32
Wide C:N_o	76.9	8.6	0.72	0.24
VC, %	5.7	15.6	34.4	18.5
FN_{rel}/FC_{rel}				
Sheep	0.91	0.99	1.00	1.00
Poultry	0.90	0.85	1.00	1.00
Cow	0.45	0.84	1.00	1.00
Pig	0.88	0.89	1.00	1.00
Narrow C:N_o	0.55	0.82	0.99	1.00
Wide C:N_o	0.62	0.84	0.99	1.00
VC, %	9.7	2.5	0.4	0.1

Variation coefficients (VC) were averaged over the six materials

Thermal fractionation The remaining fractions of N and C after heating, and the ratio of the N and C fractions released during heating (FN_{rel}/FC_{rel}) at the four temperatures are shown in Table 6. The major loss in both N and C occurred between 200 and 300°C. At 400°C, more than 98%, and at 600°C practically all C and N had been released. At 200 and 300°C, relatively less N than C was released. At these temperatures, cow manure and the two types of cow dung had lower values of FN_{rel}/FC_{rel} than the manure of pigs, sheep, and poultry. At 400 and 600°C, there were hardly any differences, because practically all N and C had disappeared.

Extraction with pepsin Poultry manure had by far the highest values of N extracted by HCl plus pepsin (N_{super}), and by HCl alone (N_{HCl}) (Table 7). As a result, its value for N_{pep} was relatively low, and its ratio N_{pep}/N_o was lowest of all materials. Dung had higher values of N_{pep} and N_{pep}/N_o than manure. In the manure–straw mixtures of sheep, pig and cow, the ratio N_{pep}/N_o was intermediate.

Greenhouse experiment according to the double-pot technique

Table 8 presents data on dry matter yields, N uptake and N mass fraction, all in relation to total applied N. Total dry matter yield is the sum of three cuts of grass shoots and the final yield of stubbles and roots in the upper pots and of roots in the nutrient solution (in the tank). The dry matter yields of the first cut are also presented in Table 8 in order to show that poultry and sheep manure initially had negative and finally positive effects on grass growth.

Table 7 N extracted by HCl plus pepsin (N_{super}), and by HCl alone (N_{HCl}), both expressed in g per kg dry (105°C) manure or dung. N_{pep} is calculated as the difference between N_{super} and N_{HCl}, Pepsin N is also calculated as fraction of organic N (N_{pep}/N_o), using data of N_o as given in Table 5

	N_{super}	N_{HCl}	N_{pep}	N_{pep}/N_o
Sheep	8.32	2.80	5.52	0.24
Poultry	22.03	14.61	7.43	0.16
Cow	6.17	1.22	4.95	0.24
Pig	10.32	2.61	7.71	0.34
Narrow $C:N_o$	16.66	2.82	13.84	0.43
Wide $C:N_o$	12.54	2.43	10.11	0.39
VC, %	4.05	11.71	8.96	

Variation coefficients (VC) were averaged over the six materials

Table 8 Dry-matter yields of shoots of the first cut of grass (DMC1) and of total plant (DMtot), total uptake of nitrogen (UN), and nitrogen mass fraction (NMF) of whole plants (= UN/DMtot) for the four levels of applied total N (AN_t)

	AN_t (mg/pot)	DMC1 (g/pot)	DMtot (g/pot)	UN (mg/pot)	NMF (mg/g)
Sheep	117	1.32	6.57	80	12.2
	234	1.08	6.97	83	11.9
	351	0.99	7.90	96	12.1
	468	0.97	8.52	108	12.7
Poultry	144	0.68	5.56	62	11.2
	288	0.82	7.03	88	12.5
	432	0.77	8.67	107	12.3
	576	0.78	10.20	131	12.8
Cow	156	1.70	8.37	102	12.2
	313	1.84	8.95	122	13.6
	469	2.09	9.96	142	14.3
	626	2.10	10.94	169	15.4
Pig	137	1.27	7.96	92	11.6
	275	1.36	10.00	123	12.3
	412	1.40	12.28	150	12.2
	549	1.44	13.82	168	12.2
Narrow $C:N_o$	27	0.69	4.32	44	10.2
	54	1.01	5.79	68	11.7
	82	1.23	6.86	82	11.9
	109	1.33	7.22	89	12.3
Wide $C:N_o$	43	0.81	5.45	59	10.8
	87	0.94	6.03	68	11.3
	130	1.16	7.44	88	11.8
	173	1.24	7.64	92	12.0
Ammonium nitrate	100	1.88[a]	7.40	118	15.9
	200		8.51	209	24.6
	300		9.86	278	28.2
	400		11.64	363	31.2
VC, %		12.1	18.38	26.0	

Variation coefficients (VC) were averaged over the six organic materials

[a] During the first period all AN pots only received 100 mg N per pot

The total amount of N taken up by the crop is the sum of N in the three cuts of grass and in the finally harvested stubble and root material from tank and upper pots. The maximum N uptake of the plants in the pots with manure or dung was always below 200 mg/pot (Table 8).

Discussion

Relationships among laboratory data

The data of poultry and cow manure and of dung narrow C:N$_o$ in Table 5 correspond to those found by Kirchmann and Witter (1992) in material dried at 60°C. The NH_4–N fractions in the material dried at 40°C (Table 5) do not exactly reflect the N$_i$ fractions in the dry matter of the fresh materials (Table 5). A part of NH_3 present in fresh material might have been lost through volatilization during drying at 40°C. As a result the ratio of NH_4–N in 40°C dried material to N$_i$ in fresh material (both expressed in mg per kg dry matter) would be less than one. The ratio was one for Dung wide C:N$_o$ ratio, probably because NH_3 in this material was too low to find measurable losses. For the other materials, the ratio proved related to the dry-matter fraction in fresh material with the highest value of one for sheep manure (Fig. 2). Apparently NH_3 volatilized the easier the more diluted the materials were.

Thermal fractionation showed major losses of both N and C between 200 and 300°C (Table 6). This suggests that if FN_{rel}/FC_{rel} is to act as an index for N mineralisation, the most appropriate heating temperature would be somewhere between 200 and 300°C, as was found earlier by Velthof et al. (1998). Comparing the ratio FN_{rel}/FC_{rel} at 200°C with C:N$_o$ (from Table 5) or with N_{pep}/N_o (from Table 7) did not yield significant relationships. Also the mutual relationships between C:N$_o$ and N_{pep}/N_o were not significant, if poultry manure was included. If poultry manure was not included, a weak negative relationship was found: $N_{pep}/N_o = -0.0353 \cdot C{:}N_o + 0.9654$, $R^2 = 0.6551$, but C:N$_o$ range only from 15.0 to 19.7. In view of its low C:N$_o$ (8.6), poultry manure could be expected to have the highest N_{pep}/N_o ratio of the tested materials. Comparison with the study by Velthof et al. (1998), is not possible because poultry manure was not included in their study.

Greenhouse experiment according to the double-pot technique

Dry matter yields

Figure 3 shows the relationship between total dry-matter yield and total applied N for the various manures and dung. Two groups may be distinguished: pig manure, and the two types of cow dung with a clear response to applied N, and sheep, poultry and cow manure with a weak response. The latter group had higher values of pH and far more calculated NH_3–N (Tables 3, 4), suggesting that NH_3–N could have played a role. Possible volatilization of NH_3–N may have had two effects: (1) direct damage to the plants resulting in retarded initial growth, especially in the pots with sheep and poultry manure (Table 8, DMC1), (2) loss of N, with the consequence that applied inorganic N and hence applied total N must have been lower than shown in Table 8 (AN$_t$) and Fig. 3. The more NH_3–N was applied the greater the risk of volatilization. The lowest point of cow manure in Fig. 3 is among the points for pig manure, and the

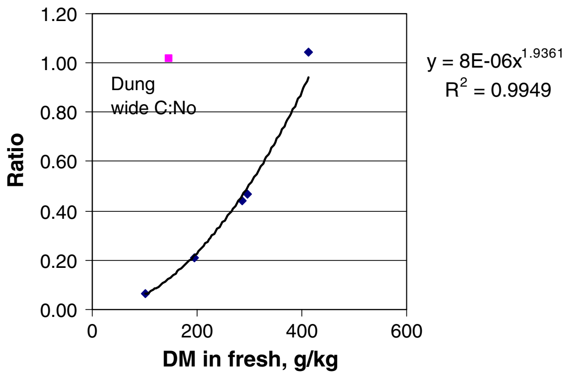

Fig. 2 Relationship between the ratio of NH_4–N in 40°C dried material to N$_i$ in fresh material (both expressed in mg per kg dry matter) and the dry-matter fraction in fresh material

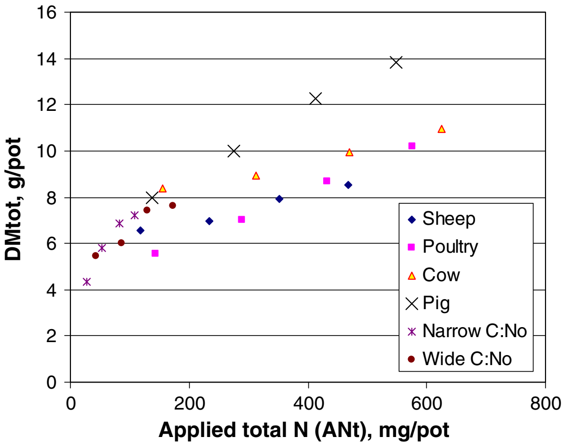

Fig. 3 Dry-matter yield in relation to total applied nitrogen with manure and dung

two types of cow dung. These pots with cow manure had received 1.05 mg NH_3–N (Table 3). We decided to make a distinction between the treatments on the basis of applied NH_3–N. Pots were considered to have little risk of NH_3 volatilization if they had received less than 1.15 mg NH_3–N, and great risk if they had received more than 1.45 mg NH_3–N. Following this criterion, the pots with the highest levels of pig manure and with the second and higher levels of cow manure, and all pots with poultry and sheep manure belonged to the risky group.

Uptake of nitrogen and relative effectiveness (RE_o) of organic N

Only the treatments with less than 1.15 mg NH_3–N (Table 9) were used to estimate the amounts of applied available N (AAN_t) and of organic available N (AAN_o). The maximum UN in these pots was 150 mg (Table 8). Therefore, only the rates of 100 and 200 mg N applied with AN were used in Fig. 4 for the assessment of the relationship between available N applied ($AN_{ss} + AN_i$) and Total N uptake. The regression line was forced through the origin, because the N from seed and other sources was taken into account along the *X*-axis via AN_{ss}. The fit to Eq. 3 resulted in $AAN_t = 0.9928 \cdot UN + 0.0003 \cdot UN^2$, and this equation was used to calculate the total amount of applied available N (AAN_t) of the pots with little risk of NH_3 volatilization (Table 9). Applied available organic N (AAN_o) was found with Eq. 4 where 22 mg N per pot was taken for AN_{ss}. Data of ANH_3, and AAN_o are also presented in Table 9.

Table 9 Applied organic N (AN_o) and inorganic N (AN_i), calculated quantities of applied NH_3 (ANH_3), applied available total N (AAN_t), and applied available organic N (AAN_o)

	AN_o	AN_i	ANH_3	AAN_t	AAN_o
Cow	132	24	1.05	104	58
Pig	125	12	0.37	94	60
	251	24	0.74	126	80
	376	36	1.11	156	97
Narrow C:N_o	24	3	0.03	44	20
	48	6	0.06	69	42
	73	9	0.09	83	53
	97	12	0.12	90	58
Wide C:N_o	43	0.3	0	60	37
	86	0.6	0	69	47
	129	0.9	0	90	67
	172	1.2	0	94	70

All values are in mg/pot

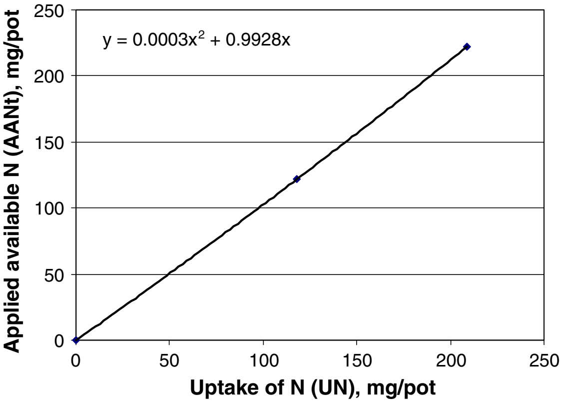

Fig. 4 Calibration line for the estimation of total applied available N (AAN_t) on the basis of measured total uptake of N (UN) in pots with ammonium nitrate

In Fig. 5, applied available organic N (AAN_o) is plotted against applied organic N (AN_o, Table 9). The data of pig manure and cow dung with narrow C:N_o follow one curvilinear line, while the points of cow manure and cow dung with wide C:N_o lie somewhat below these data. Expolinear functions were fitted through the points (Table 10). Because the ratio (AAN_o)/(AN_o) or RE_o is not constant, the various organic materials must be compared at fixed amounts of AN_o. The data of the two types of dung have an overlap between 43 and 97 mg AN_o, and those of pig manure and cow dung with wide C:N_o

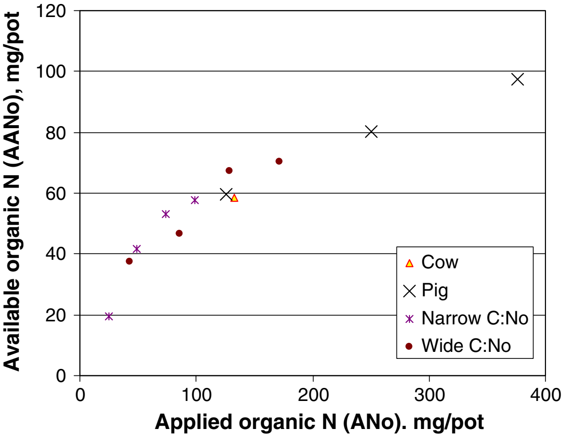

Fig. 5 Comparison of calculated applied available organic N (AAN_o) and applied organic N (AN_o) for the treatments with less than 1.20 NH_3 per pot. Explanation is given in text

Table 10 Regression equations for the relationship between available (y) and applied organic N (x), pertaining to Fig. 5, and RE_o at 50, 80 and 132 mg organic N applied

Manure	Regression equation	R^2	RE_o at		
			50	80	132
Cow manure			ND[a]	ND[a]	0.44
Pig manure	$y = 104.1 \cdot (1-\exp(-0.0065x)$	0.97	0.58[b]	0.53[b]	0.45
Narrow $C{:}N_o$	$y = 78.1 \cdot (1-\exp(-0.0148x)$	0.98	0.82	0.68	0.51[b]
Wide $C{:}N_o$	$y = 79.2 \cdot (1-\exp(-0.0127x)$	0.92	0.74	0.63	0.49

[a] ND, not determined

[b] Found by extrapolation

between 125 and 172 mg AN_o, while AN_o of cow manure was 132 mg/pot. Therefore RE_o was calculated with the expolinear equations for AN_o of 50, 80 and 132 mg/pot (Table 10). The RE_o values for cow manure and pig manure were about equal and lower than those for dung. Dung with narrow $C{:}N_o$ has higher values than dung with wide $C{:}N_o$. The differences in RE_o values between the materials decreased with increasing rates of AN_o.

Relationships between laboratory tests and relative effectiveness (RE_o) of organic N

For the evaluation of the laboratory indices, RE_o was compared with FN_{rel}/FC_{rel}, N_{pep}/N_o and $C{:}N_o$. Poultry and sheep manure were not considered. No clear relationship between RE_o and FN_{rel}/FC_{rel} was found. The relationships between RE_o and N_{pep}/N_o and between RE_o and $C{:}N_o$ were consistent, even for the extrapolated values of RE_o (Fig. 6). The relationship between RE_o and N_{pep}/N_o is in agreement with the findings of Velthof et al. (1998) and Castellanos and Pratt (1981).

A classical linear negative relationship between RE_o and $C{:}N_o$ was found (Fig. 6, bottom). The $C{:}N_o$ ratio is often considered as an index for N mineralization, but this is only realistic for materials with equal decomposability, as outlined by Castellanos and Pratt (1981) and Janssen (1996). Figure 6 suggests that cow manure is a little less decomposable than pig manure.

Usefulness of the double-pot technique

In our study, the relative effectiveness (RE_o) of organic N in manures and dung was calculated on the basis of N uptake by plants. The double-pot technique (Raijmakers and Janssen 1993; Velthof et al. 1998) was used to assess N uptake. A major advantage of this method is that differences among the organic fertilizers with regard to the supply of other nutrients than N have little consequences as the plants can

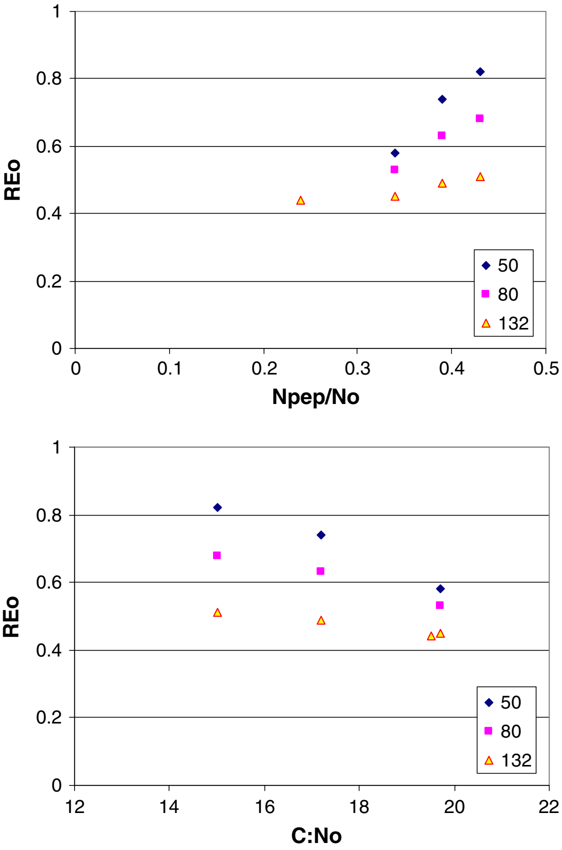

Fig. 6 RE_o calculated for applications of 50, 80 and 132 mg organic N per pot (Table 10), in relation to N_{pep}/N_o (top) and to $C{:}N_o$ (bottom)

easily take up these nutrients from the minus-N nutrient solution in the lower pots or tanks. In the pots with the reference fertilizer, ammonium nitrate, an almost linear relationship was found between AAN_t (amount of applied available N) and UN (N uptake). The regression lines were $AAN_t = 0.9928 \cdot UN + 0.0003 \cdot UN^2$, when only the applications of 100 and 200 mg N per pot were considered (Fig. 4), and $AAN_t = 0.9779 \cdot UN + 0.0005 \cdot UN^2$ ($R^2 = 0.9977$, $P = 0.04$), when also the rates of 300 and 400 mg/pot were included. They point to a nearly complete uptake of all available N; only at high N application rates the uptake slightly levelled off. It was assumed that the plant availability of inorganic N and of mineralized organic N in manure and dung was the same as that of ammonium nitrate. From Table 8 it follows that the N mass fraction (NMF) of whole plants, i.e., the ratio UN/DM total, was on average 12.2 mg/g for the pots with organic materials, and 15.9 mg/g and higher for the pots with ammonium nitrate. This leads to the conclusion that plant growth in the pots with organic materials obviously was limited by N-deficiency. Also in the pots with poultry and sheep manure, N mass fractions in the plants were around 12.2 mg/g. So, the relatively low N uptake in pots receiving poultry, sheep and cow manure was not only due to poor growth conditions probably caused by NH_3 damage but also to low availability of N, which in its turn must have been caused by loss of inorganic N through volatilization and by limited mineralization of organic N. Such problems would have been prevented if lower applications of manure had been used. With the here used equipment application rates of fresh manure from sheep, poultry, cows and pigs should not exceed 1, 5, 8 and 15 g/pot, respectively. On the contrary, the rates of dung could have been higher than used in the present experiment, e.g., up to 30 g/pot for dung with narrow $C{:}N_o$ and even more for dung with wide $C{:}N_o$.

With regard to mineralisability of organic N, the organic materials can be divided into three classes: poultry and sheep manure, cow and pig manure, fresh cow dung. The lowest values were observed in case of poultry and sheep manure (Tables 7, 8). Poultry manure contains, apart from protein, either undigested or partially digested during transit through the gut, a mixture of NH_4, urea, uric acid (Chescheir et al. 1986; Kirchmann and Witter 1992), compounds that are not extracted by pepsin. Organic N in fresh cow dung was mineralised a little easier than organic N in cow and pig manure (higher N_{pep}/N_o and RE_o in Tables 7 and 10), probably because manure contains straw besides dung. Another reason might be that very easily decomposable materials which are present in dung are not present anymore in manure as they have already been decomposed during storage of manure.

Conclusions

Organic N in cow dung as well as in pig and cow manure was more available to plants and microorganisms than organic N in poultry and sheep manure. Thermal fractionation proved not useful as an index of plant-available N in manure and dung.

Plant availability of organic N was positively related to the fraction of organic N extracted by pepsin, and negatively to the $C{:}N_o$ ratio of manure and dung of cows and pigs. Pepsin extraction of organic N can be recommended as laboratory method for determining mineralisable organic N in manure and dung of cows and pigs, but as $C{:}N_o$ ratio is more easily determined, it is considered the most practical laboratory index for plant availability of organic N in animal manure.

When using the double pot technique for the evaluation of plant available N in organic material, application rates should be rather low to avoid damage to plants and loss of N by NH_3 volatilization. Hence, the major conclusion of this study on the plant availability of organic N in organic materials that are not very far apart in nature is that the information provided by the simple $C{:}N_o$ ratio is at least as reliable as the information provided by more sophisticated analytical methods. The restriction of this conclusion to organic materials not very far apart in nature refines the existing general knowledge found in literature since the classical article by Jensen (1929).

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