



## Article

# Nitrogen Supplying Capacity of Animal Manures to the Soil in Relation to the Length of Their Storage

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**Abstract:** The study estimated the relationship between the amount of nitrogen (N) that will become available to plants after incorporation of soil of sheep/goat, cattle, swine, and poultry manure and the duration of manure storage prior to soil addition. Manures were periodically sampled from 12 storage piles that were kept for 12 months each and mixed with soil before laboratory incubation for 83 days. The percentage of organic N mineralized after soil incorporation was clearly greater for poultry, ranging between 41 and 85%, in relation to the other three manure types, for which maximum mineralization ranged between 4.5 and 66%. For sheep/goat, cattle, and swine, the interaction between mineralization and immobilization processes showed a distinct pattern with two phases of net N release during the twelve months of storage. The first was separated from the second by a period where mineralization was zeroed and appeared at about six months after storage initiation. It was recommended that farmers should preferably use well-digested manures that have been aerobically stored more than six months to avoid materials that provoke intense immobilization, unless problems associated with the use of fresh manure are managed.

**Keywords:** nitrogen mineralization potential; animal manures; storage effect; nitrogen immobilization; goat manure; poultry manure

## 1. Introduction

The incorporation of animal manure in soil is one of the oldest and most geographically extended ways of maintaining soil fertility. Manures have been used in agricultural fields long before the appearance of the industrially manufactured fertilizers as they represent a valuable source of macro- and micro-nutrients for crops, while they ameliorate microclimatic conditions and increase soil microbial activity. Manures act also as soil improvers helping the prevention of clay dispersion and crust formation and increasing soil organic matter [1,2].

The appropriate use of manure to meet nutrient crop demands is, however, not straightforward. While the composition of chemical fertilizers, which are generally water soluble, can be manipulated to provide the required elements, organic substances need first to be processed by soil microorganisms before an eventual nutrient release takes place. As a typical microbiological process, therefore, nutrient mineralization during manure decomposition is controlled by many variables such as temperature, moisture, substrate quality, soil conditions etc. [3]. Moreover, manure is a material with an imbalanced content of elements compared with crop needs, so successive application to a field often results in involuntary buildup of some of these elements [4].

Health and environmental problems may also arise from their improper use. Manure that has not been appropriately digested may contain pathogens such as particular strains of *E. coli* that can cause illness to people, for example after consumption of fresh vegetables [5].

Difficulties in handling and particularly the risk of contamination of soil and crops with human pathogens have led authorities to recommend the use of well-digested manure as soil amendment. At the same time, application rates of manure should be such that the amount of N which will become available to plants would be the recommended one without causing nitrate pollution of surface or ground water. Hence, in nitrate vulnerable zones (NVZ) farmers are obliged to use digested manure, to avoid great rates of nitrogen release [6].

Existing information, though, on the relationship between the degree of digestion, which is closely linked with the duration of storage, and the N-releasing potential of manure after soil application is not extensive and usually limited to the comparison between fresh and composted materials [7,8]. Atallah et al. [9], for example, found that only the oldest of the manures they used in incubation trials of soil-manure mixtures induced net N mineralization while all the others immobilized N during the whole 76 day period of study. Moreover, chemical analysis would not be adequate to reveal storage effects as it has been shown that the availability of manure organic N cannot be predicted from simple compositional parameters [10].

Nevertheless, indirect information on manure N mineralization potential could be drawn from crop residue or leaf litter decomposition studies. These have shown a particular pattern of N dynamics consisting of two phases; an initial N immobilization phase followed by a net mineralization phase [11–13]. In some cases, immobilization was noticeably short or non-identified and in others it extended during the whole duration of the study [14]. There have been many efforts to correlate the magnitude and extent of immobilization with the chemical characteristics of litter and it seems that the best predictors are the C/N ratio and cellulose and lignin concentrations [14]. If there are analogies of manure incorporation in soil with litter decomposition studies it can be assumed that incorporation in soil of fresh material would probably result in soil N immobilization for some time before a net N release is observed, whereas incorporation of digested material due to storage in a pile would result directly in net mineralization.

Evidence on manure decomposition processes and associated N release could also be obtained from studies of compost use as soil amendment [15,16]. Composts have been proved to be extremely low in N fertilization value, releasing N rarely above 10% of their total organic N content and more frequently immobilizing soil inorganic N [17,18]. However, compost derived from animal manures has the highest rate of mineralization, compared to compost derived from source separated biowaste or green waste [17,19].

The proportion of manure's organic N that will be mineralized and eventually become available for crop uptake is a significant variable affecting sustainable management and is included in many manure management models (e.g., [20]). It is equivalent to soil N mineralization potential that has been defined as the quantity of soil organic N that is susceptible to mineralization [21]. As with soils, N mineralization potential of manures can be estimated by incubating soil-manure mixtures, without plants and fitting a mineralization curve to the obtained data. Although a time-consuming method, the incubation at controlled optimum conditions that typically extends for two to three months is a realistic and widely accepted approach to the N mineralization potential estimate [22].

The aim of the study was to estimate the N-supplying potential of manure in the growing season following application of sheep/goat, cattle, swine, or poultry manure in soil and to establish a relationship between N mineralization capacity and the duration of manure storage prior to soil addition. This relationship, which could be proved to be manure-specific, is of central importance for estimating the appropriate amount of manure that is to be incorporated in soil so that plant growth and environmental problems arising from N shortages or excesses are avoided. Moreover, this relationship is critical in considering the necessary compromises related to the selection of the optimal manure age for incorporation in soil, bearing in mind that the duration of storage is also linked with pathogen survival, its content in weed seeds, the loss of material and CO<sub>2</sub> emissions due to decomposition and N losses from storage sites.

## 2. Materials and Methods

### 2.1. Approach

Manure was collected from animal farms and stockpiled in conical piles at the premises of the Agricultural Research Institute (ARI). Every three months samples were taken from piles to the lab, where they were mixed with soil and incubated at controlled conditions. This experimental approach aimed to imitate one common management practice and final use of manure. In places where an intensive type of animal farming prevails, manure is accumulated in open-air piles close to the livestock houses and transported to the fields for incorporation in soil, usually a little before the beginning of the growing season of crops, after having been stored for varying durations of time. Apart from farms found in NVZ areas manure storage is carried out in an unsystematic way in diverse sizes of heaps. The stockpiling in this study corresponded to manure storage in the farm and soil incubations for about three months at optimum conditions intended to reveal processes occurring in fields for the growing season following application. It is necessary to emphasize that local extension services recommend the analysis of manure and if possible incubation tests for each load of manure that is to be applied to the soil.

### 2.2. Manure Description and Storage

Solid manures in this study refer to animal excreta, urine, and significant amounts of feed refuse, especially straw. In the case of pig manure, it refers to the solid fraction of mechanical slurry separators. These manures come from the periodic cleaning of animal houses, so they contain also abraded soil. A significant uncontrolled source of variation for our study was the frequency of stable cleaning and the time elapsed between cleaning and delivery to our premises. Sheep/goat and cattle manure of the first repetition (see below) came from the farm of the ARI.

The almost one year storage of manure that followed took place under sheltered conditions and was repeated three times (from now on referred to as repetitions), at three consecutive years, each time with a new set of four piles for each of the domesticated animal species of the study: sheep/goat (sheep and goats are usually kept in mixed farms), cattle, swine, and poultry. Water content of the materials was regularly measured gravimetrically and kept at the level appropriate for microbial activity by spraying water and turning the piles. At the piles of the 1st repetition, water was added after eight months of storage and at the 3rd repetition after two months of storage. During storage of the second set of manures, piles were moistened 50 days after transfer to the ARI but oblique rains accidentally dampened piles between the second and third sampling and shelters were ameliorated.

### 2.3. Soil Used in Incubations

In total, ten soils were used at the 15 incubations of the study as in some cases soil of the same field was sampled again and used in consecutive trials. Soils used were air dried after sampling from fields and screened through a 2 mm mesh to remove coarse particles. Their basic properties such as C and N content, pH, Electrical Conductivity (EC) and texture are shown in Table 1. They significantly varied in terms of their  $\text{CaCO}_3$  and total organic C content but not so much in terms of their other characteristics.

### 2.4. Manures Used in Incubations

Manures were sampled by discarding the outer 5 cm and collecting material from at least three different locations of their storage piles. They were sieved (2 mm sieve) and transferred to the lab, where one part was immediately extracted for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N determination, a second part was used for the determination of their total nitrogen content by the Kjeldahl digestion method, a third was used for incubations after mixing with soil and a fourth for water content, pH, total C, and ash determinations. Ash content of manures and soils was determined by igniting the oven-dried sample used for moisture content determinations in a muffle furnace at 450 °C for 12 h. Total C was measured

using a CHN elemental analyzer (EA 3000 Eurovector SpA, Milan, Italy) and pH was estimated on a 1:5 soil to water proportion.

**Table 1.** Some of the initial properties of the ten soils involved in the study. The last column indicates in which of the 15 laboratory incubations each soil was used. mos: months of storage.

	pH	EC	Sand	Silt	Clay	CaCO <sub>3</sub>	C	N	C:N	
		dS/m	%	%	%	%	%	%		
1	7.96	0.35	49.9	33.5	16.6	0	0.83	0.09	9.6	1st rep 0 mons
2	7.75	0.56	49.9	31.5	18.6	0.2	1.11	0.11	9.7	1st rep 3, 6, 9 mons
3	8.06	0.3	61.9	23.5	14.6	1.7	0.91	0.08	11.7	1st rep 12, 2nd rep 0 mons
4	8.04	0.51	61.9	21.5	16.6	0.4	1.16	0.10	11.5	2nd rep 3, 6, mons
5	7.55	3.01	63.9	21.5	14.6	5.1	0.91	0.08	12.0	2nd rep 9, 12 mons
6	7.95	0.47	64.0	19.8	16.1	12.4	0.39	0.05	8.6	3rd rep 0 mons
7	8.11	0.36	64.0	19.8	16.1	8.1	0.66	0.06	10.5	3rd rep 3 mons
8	8.08	0.26	64.0	21.8	14.1	2.1	1.88	0.16	11.4	3rd rep 6 mons
9	8.16	0.34	60.0	23.8	16.1	19.2	0.45	0.05	9.8	3rd rep 9 mons
10	8.17	0.28	66.0	21.8	12.1	18.8	0.40	0.04	9.1	3rd rep 12 mons

## 2.5. Nitrogen Mineralization Potential Estimates

At each of the fifteen 83-day incubation trials of this study, which overall lasted for almost four years, five sets of soil–manure mixtures (four sets for the species of manure and one for soil alone) were used to estimate N mineralization potential in controlled moisture and temperature conditions. Small plastic 50 mL vials, filled either with 20 g of soil and 0.1 g of manure or with 20 g of soil only, were used in these incubations. The vials were placed inside glass jars with fittings to reduce evaporation water losses and a small hole in the lid to replenish oxygen. Water initially added in containers corresponded to 70% of the Water Holding Capacity (WHC) of soils and never dropped below 60% till the end of the incubation. Throughout the incubation the temperature was kept at  $25 \pm 2$  °C. At regular, predefined and always the same intervals, three samples per treatment, contained in three different jars were removed from the incubation chamber to measure mineral N in the soil.

Soil inorganic N ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) was extracted at each time point with 2N KCl by shaking samples for 30 min and filtering through a No. 2 Whatman filter paper. The extract was stored frozen until measurement, which was carried out by colorimetric methods. Nitrate determination involved the reduction of nitrate to nitrite through a copper–cadmium column. The nitrite was then reacted with sulfanilamide and the diazonium ion formed was reacted with a coupling reagent (*N*-(1-naphthyl)-ethylenediamine dihydrochloride). The purple color developed was measured at 540 nm. The estimation of  $\text{NH}_4^+$ -N was based on the emerald green color formed when ammonia and sodium salicylate react in the presence of sodium hypochlorite at high pH. The color reaction was catalyzed by the presence of sodium nitroprusside.

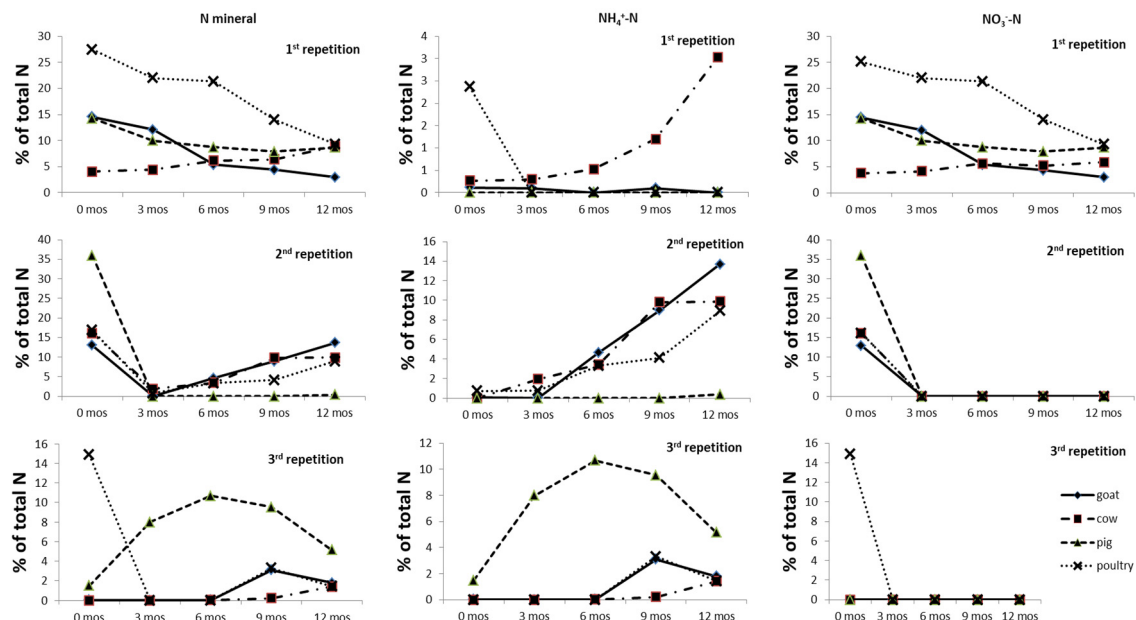
## 2.6. Calculations and Statistical Analysis

The sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in the soil–manure mixtures minus mineral N in the soil alone treatment was calculated and expressed as percentage of initial organic N mineralized to estimate the net N mineralization:

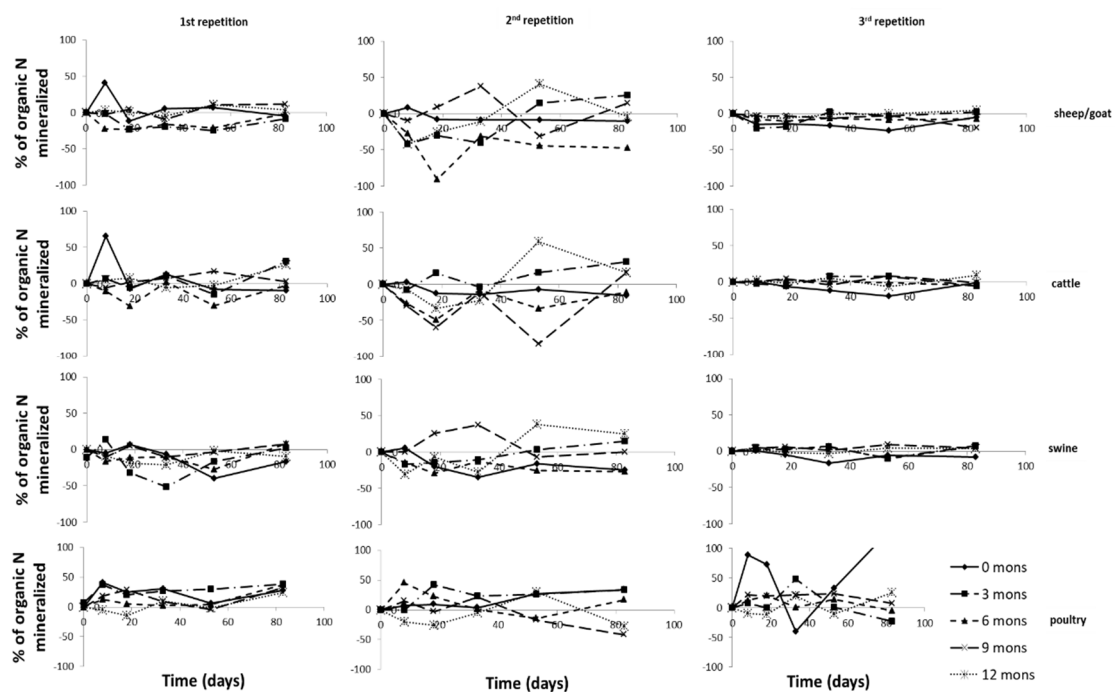
$$\% \text{ N mineralized} = 100 \times (\text{Mixture mineral N} - \text{Soil mineral N}) / \text{Manure N added} \quad (1)$$

The surface of the area formed between the line connecting cumulative mineral N data and the time axis was calculated, as described more extensively below, using the software PRISM (GraphPad Software Inc., San Diego, CA, USA). These areas were compared between them to reveal statistically significant effects of the type of manure or time of storage using repeated measures two-way ANOVA, repeated measures ANOVA, paired *t*-tests (PRISM, GraphPad), and matching values across repetitions.

Although the three repetitions represent the three replications that were used in ANOVA analysis they are shown separately in Figures 1 and 2 to highlight the great effect of manure origin on the results even for the same manure type and at the same time to emphasize the significance of the eventual common trends.



**Figure 1.** Mineral forms of N extracted from manure samples in relation to time of storage at the three repetitions of the study.  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  and their sum (mineral N) were expressed as percentages of total manure N. mos: months of storage.



**Figure 2.** Cumulative net N mineralized during 83-day incubations expressed as percentage of organic manure N. Each graph shows results of one type of manure mixed with soil after five manure storage times. Incubations were repeated three times with different sets of manures. mos: months of storage.

### 3. Results

#### 3.1. Manure Characteristics in Relation to Storage

Manure storage exhibited signs that composting processes were occasionally taking place in piles. Some of them, especially of the 1st repetition were hot and steaming and sporadic measurements showed elevated temperatures, up to 53 °C for sheep/goat and up to 67 °C for cattle manure. During sampling, grey spots of ash were also evident.

Table 2 presents a general description of manures of all three repetitions after each sampling occasion. Manure water content results indicated the general tendency of decrease that is expected after storage in a sheltered area. This was reversed by water additions, either intentional as in the 1st repetition or accidental as in the case of the 2nd repetition described in the Materials and Methods. The most humid samples contained 65–80% water and the driest 19–20%. Changes of ash content in relation to storage showed that at the 1st and 2nd repetition manures showed an increasing trend in relation to storage but at the 3rd repetition materials were relatively stable. Bedding material influenced ash content estimates, which in one case (poultry of 3rd repetition) was gravel and resulted in large values of ash. The median values measured were 32.7, 41.9, 34.8, and 68.0% for sheep/goat, cattle, swine, and poultry manure respectively. Decreasing trends at the 1st and 2nd repetition and relative stability at the 3rd was exhibited by total C, C:N ratio, and pH values excluding poultry pH at the 1st and 2nd repetition, which showed a tendency to increase with storage.

**Table 2.** Water content and chemical properties of the manures used in incubations at the five successive sampling times of the storage piles. Mos: months of storage.

Mos	N (%)				C (%)				C/N			
	Sheep/Goat	Cattle	Swine	Poultry	Sheep/Goat	Cattle	Swine	Poultry	Sheep/Goat	Cattle	Swine	Poultry
0	1.45	1.99	2.02	3.89	35.3	33.1	41.4	34.6	24.3	16.7	20.5	8.9
3	2.26	2.03	2.09	5.11	28.7	24.3	39.8	29.6	12.7	12.0	19.0	5.8
6	1.48	1.06	2.04	5.08	28.3	23.4	35.8	19.2	19.1	22.0	17.6	3.8
9	2.51	1.99	2.30	2.56	27.0	23.2	32.5	17.3	10.8	11.6	14.1	6.8
12	2.98	2.22	2.87	2.16	25.7	22.2	30.7	15.6	8.6	10.0	10.7	7.2
0	2.05	1.94	1.98	2.82	32.6	28.8	38.3	27.8	15.9	14.8	19.3	9.8
3	2.20	2.52	2.07	2.79	28.2	24.0	35.0	24.5	12.8	9.5	16.9	8.8
6	2.61	2.38	3.32	2.19	27.3	24.3	33.2	19.1	10.5	10.2	10.0	8.7
9	2.51	2.22	2.21	2.09	25.4	21.9	27.5	16.1	10.1	9.8	12.4	7.7
12	2.68	2.26	2.30	2.46	23.2	21.1	26.3	15.3	8.7	9.3	11.4	6.2
0	2.10	3.23	3.09	2.00	24.3	27.3	22.4	19.6	11.6	8.4	7.2	9.8
3	2.10	2.81	3.01	1.97	28.8	26.3	22.2	13.9	13.7	9.3	7.4	7.0
6	2.12	2.91	2.51	1.87	31.5	25.5	25.6	15.0	14.9	8.8	10.2	8.0
9	2.49	2.79	2.91	1.60	28.0	26.3	25.7	12.7	11.2	9.4	8.8	7.9
12	2.45	2.07	2.77	2.12	25.1	26.9	23.3	15.0	10.2	13.0	8.4	7.1
	pH (1:5)				Water Content							
	Sheep/Goat	Cattle	Swine	Poultry	Sheep/Goat	Cattle	Swine	Poultry	Sheep/Goat	Cattle	Swine	Poultry
0	8.88	9.12	7.10	7.73	18.2	31.3	9.8	13.1	23.2	42.7	57.6	24.4
3	8.90	9.16	7.09	7.78	21.3	34.0	11.9	16.9	13.1	13.1	11.9	15.5
6	8.99	9.10	7.15	7.51	19.1	39.3	9.4	17.5	12.4	12.3	13.3	9.9
9	8.92	9.15	7.65	9.19	29.1	55.3	25.0	69.9	29.8	49.1	41.7	33.1
12	8.20	8.29	6.51	9.55	38.4	60.6	41.4	67.5	43.7	43.9	56.2	28.2
0	8.89	9.01	7.8	7.91	26.5	35.0	6.7	36.8	9.3	26.3	26.5	20.1
3	9.20	8.91	7.16	8.38	30.5	41.6	14.3	45.2	29.5	37.8	24.8	16.1
6	8.01	8.06	5.92	8.94	48.1	52.5	41.0	74.4	48.5	43.5	58.5	24.3
9	8.50	8.66	7.25	9.01	41.5	43.1	26.7	61.8	34.1	19.2	23.3	20.2
12	8.11	8.56	7.03	8.97	41.2	59.8	32.8	68.5	31.2	24.2	9.6	20.8
0	8.98	8.99	6.47	9.31	35.6	36.1	38.9	60.4	21.9	43.7	45.1	68.6
3	8.97	8.93	6.45	9.13	27.6	33.3	47.1	75.8	19.3	30.7	36.9	24.1
6	8.78	8.9	7.14	9.2	27.8	35.0	38.8	71.7	11.7	14.9	14.0	11.9
9	8.87	8.99	6.79	9.05	34.9	42.2	36.8	73.0	8.1	11.5	9.1	10.7
12	8.90	8.88	6.59	9.03	39.7	43.1	41.4	72.4	10.7	7.2	9.3	8.7



### 3.2. Evolution of Mineral Forms of N during Storage

The ammonium ( $\text{NH}_4^+\text{-N}$ ) concentration in the manure piles in relation to storage either decreased slowly, as at the 1st repetition, or rapidly, as at the 2nd repetition, or was always zero, as at the 3rd repetition (Figure 1). The general pattern of nitrate ( $\text{NO}_3^-\text{-N}$ ) concentration dynamics showed a progressive increase during the 12-month storage. Nitrates in swine manure at the 3rd repetition dropped after six months of increase (Figure 1).

### 3.3. Cumulative Mineral N Release after Incubation at Controlled Conditions

Changes of extractable mineral N with time of incubation did not show a common pattern either in relation to the species of animal or in the degree of manure storage, although the latter was a stronger source of variation than the former (Figure 2). Very often extractable N showed periods of net immobilization followed by periods of net mineralization and vice versa. On many occasions, an initial short net mineralization phase was followed by N immobilization, which was followed again by net N release. Net mineralization was maintained during the whole incubation period in very few cases especially with poultry manure but even in these cases there were fluctuations. On the contrary, sheep/goat manure showed immobilization that lasted for the whole duration of the incubation (83 days) after six months of storage at all three repetitions similarly with cow and pig manure after six month storage at the second repetition.

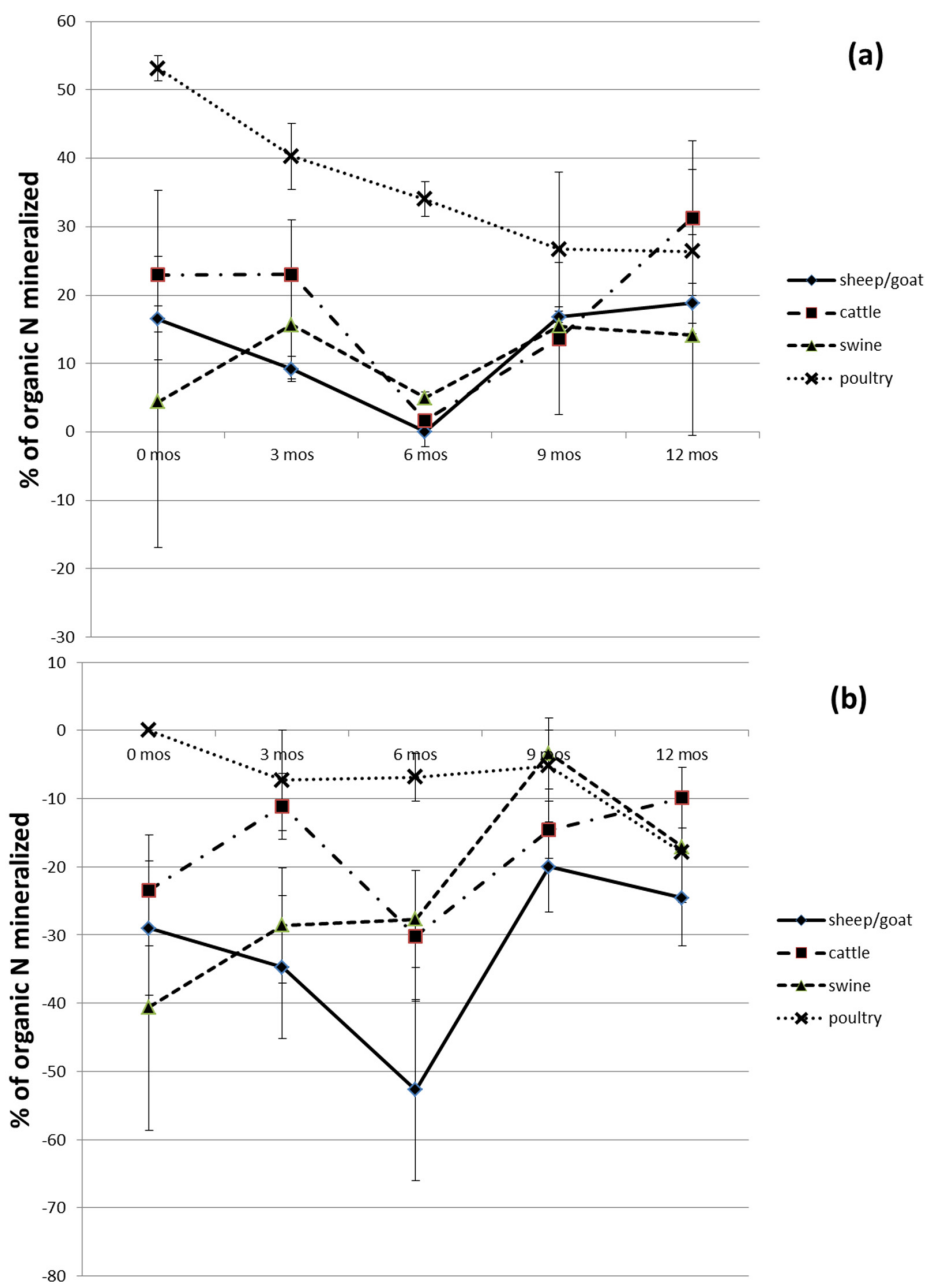
Incubations after six and nine months of storage using sheep/goat, cattle, and swine manure samples taken after accidental rain dampening of the 2nd repetition piles, as described in the Materials and Methods section, showed exceptionally high values of N immobilization and a respective decrease of net N release (Figure 2).

### 3.4. Maximum Net N Mineralization and Immobilization

Figure 3a shows the maximum values of net N mineralization expressed as percentages of initial manure organic N mineralized during incubation. Maximum net N immobilization estimates (Figure 3b) and “integrated net immobilization”, which will be presented below, were estimated not from results shown in Figure 2, as percentages of organic manure N immobilized, but as percentages of mineral N immobilized in relation to the available soil mineral N. Mineral N availability was given by “soil only” treatments.

Figure 3a shows the clear superiority of poultry manure in relation to the other three kinds of manure in releasing mineral N to the soil. The latter showed similar “behavior”. Lowest maximum net mineralization potential was shown at six months of storage and peaks at the first 0–6 and the last 6–12 month intervals of storage. Peak values varied very much between repetitions. For sheep/goat manure they ranged between 4.4 and 41.2%, for cattle between 8.9 and 65.5%, for swine between 9 and 38%, and for poultry between 41 and 84.8%. On the contrary, the dip of Figure 3a, at six months of storage was characterized by a much smaller variation of maximum values between repetitions.

Poultry differed also from the other manures as far as the maximum net N immobilization was concerned, which remained very small and in many cases zero during the 12 months of storage at all repetitions. For the other manures, immobilization as was revealed by maximum values (Figure 3b) seemed negatively related with net mineralization but often both processes existed in parallel.



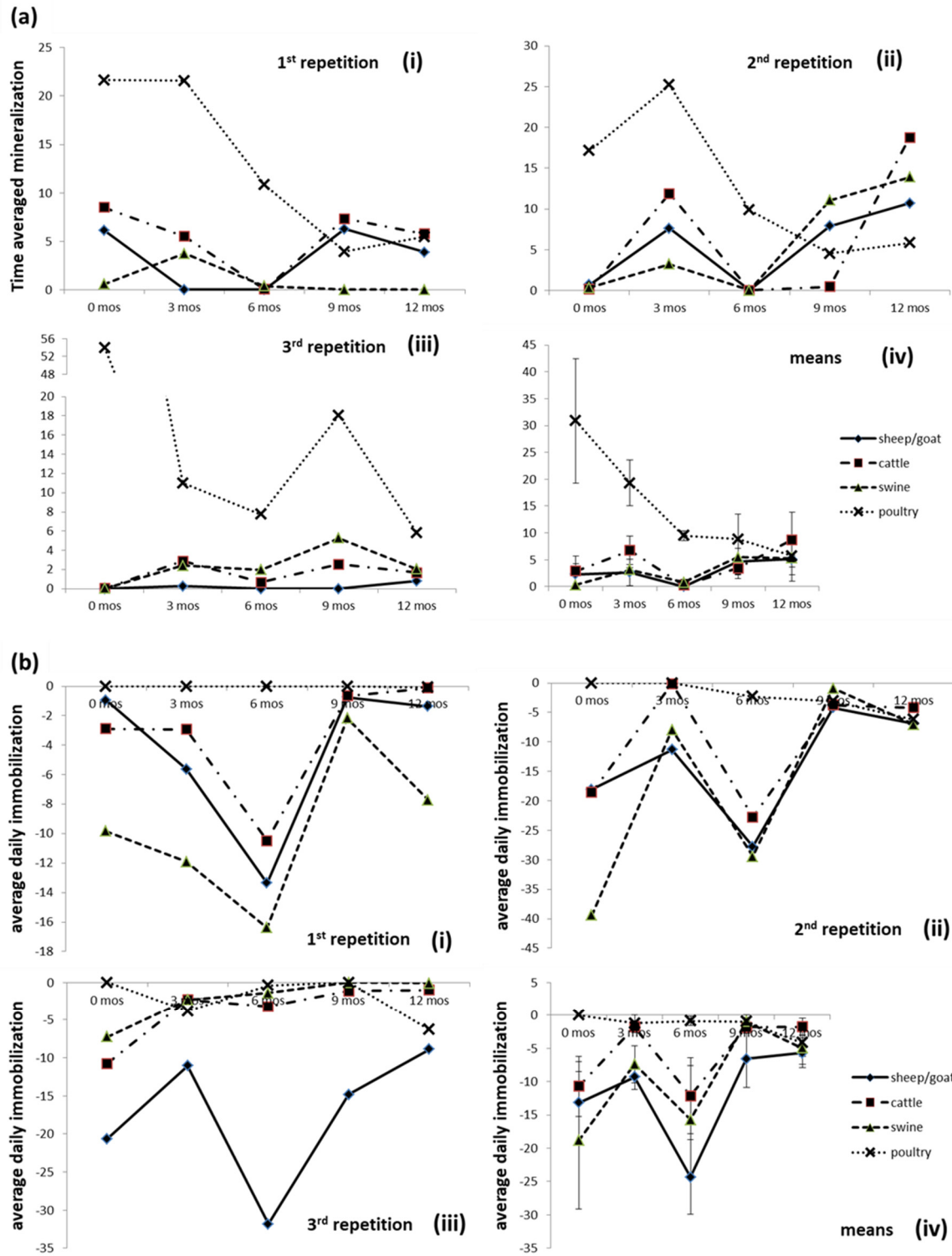
**Figure 3.** (a) Maximum net N mineralized and (b) maximum net N immobilized during incubations in relation to storage time for each of the manure types used. Error bars indicate standard errors of the mean of the three repetitions. mos: months of storage.

### 3.5. Integrated Net N Mineralization and Immobilization

The sum of trapezoids formed between the mineralization connecting lines and the time axis of Figure 2, was used to estimate the surface of the areas under or above these lines and offered a means to characterize the mineralization potential of the manures that would be less influenced by eventual experimental inaccuracies. Estimates of these areas, which are referred to as “integrated net N mineralization” and “integrated net N immobilization” indicated mineralization characteristics of materials not only as far as their maximum capacity to release N but also in terms of the persistence of this capacity in time. Hence, results became more robust and less influenced by eventual experimental imprecisions, and, more importantly, comparisons between manure species became more objective. Again, “integrated net N immobilization” was estimated not from results



shown in Figure 2, as percentages of organic manure N immobilized, but from the evolution with time of the percentage of mineral N immobilized in relation to the available soil mineral N. Areas under or below the lines of cumulative mineral N were divided by the number of incubation days to present results as Time-Averaged Mineralization (TAM) or Time-Averaged Immobilization (TAI—Figure 4).



**Figure 4.** (a) Average daily mineralization (ADM) and (b) average daily immobilization (ADI) against time of storage for the four types of manure used. Graphs (iv) show means and standard errors of the mean of the three repetitions. mos: months of storage.

First repetition results show that highest TAM for sheep/goat and cattle were shown either by “fresh” or by well-digested manure (Figure 4a(i)). Initial elevated mineralization was very much reduced after three or six months of storage and increased again when materials had been stored for nine or twelve months. Poultry TAM was always high with a slight decrease towards the end of the storage period, while swine manure always showed a small TAM with the greatest value at three months of storage. The highest values of TAM estimated across the five storage times were 6.3, 8.5, 3.7, and 21.6% of manure organic N for sheep/goat, cattle, swine, and poultry respectively. Apart from poultry, sheep/goat, cattle, and swine manures showed also high values of TAI that peaked at six months of storage (Figure 4b(i)). This dropped significantly at nine months of storage.

Second repetition results showed a peak of TAM after three months of storage, lowest values at six months (nine months for poultry) and increased values again towards the end of storage (Figure 4a(ii)). For this repetition, the greatest TAM estimates were 10.7, 18.8, 13.9, and 25.2% of manure organic N for sheep/goat, cattle, swine, and poultry respectively. Immobilization peaked at intermediate stages of storage and was also strong when “fresh” materials were incorporated in the soil (Figure 4b(ii)).

Third repetition results showed generally lower values of TAM than the previous two. Apart from the poultry treatment, values of TAM of the other three manures never exceeded 5.3% (Figure 4a(iii)), while net immobilization of sheep/goat showed the highest values among manures that peaked after six months of storage (Figure 4b(iii)).

Estimates from all three repetitions were grouped and two-way ANOVA was used for manure and storage time effects on mineralization and immobilization. Poultry showed statistically significant differences from the other manures both in relation to net N mineralization after incorporation in soil and in relation to its response to time of storage. The group of data for sheep/goat, cattle, and swine was further analyzed for storage time effects using ANOVA and t-tests. It was shown that N release processes were significantly affected by the duration of stay in piles, with TAM and TAI after six months of storage to be significantly smaller or greater respectively than three and nine months.

## 4. Discussion

### 4.1. Incubations in Soil

Manure incubations were not always carried out in the same soil. Keeping a large amount of soil stable to be used in all incubations that lasted overall for three years would be practically difficult and probably not efficient. This unavoidable drawback may have resulted in some cases of increased variability between repetitions. However, since the experiment was not designed to reveal soil type effects, eventual common trends appearing in the results would also have greater generalization validity.

Modelization efforts of manure application consider that the effect of soil type on N mineralization potential is either insignificant, outweighed by other more significant variables [20], or it is taken under consideration using a coefficient (e.g., pH and soil organic matter coefficient, NDICEA model, [23]). The introduction of a soil coefficient in manure application models is not only the best possible approach but is founded on decomposition studies, where the same material is incorporated in different soils [24–26]. These works indicated that soil type shifted mineralization or nutrient release curves without altering the general form of these curves. Therefore, in the present work it was assumed that soil type may have affected the rates of N transformation and the estimated percentages of organic manure N mineralized, but not whether manure incorporation resulted in net mineralization or immobilization. This result was considered to be ultimately controlled by the quality of the decomposing organic additive, an assumption which is fundamental to the formulation of Plant-Available Nitrogen Tables used in organic fertilizer recommendation guides (e.g., [27]). Nevertheless, since the magnitude of mineralization or immobilization is reasonably expected to be influenced by soil type, current related results should be considered as indicative.

#### 4.2. Net N Mineralization and Immobilization

The study showed that besides the already known effect of animal species [8], the duration of manure storage is an important variable controlling the N mineralization processes after incorporation in the soil. Some common characteristics and general trends of this effect are discussed below and the values of net mineralization and immobilization can be drawn from the present results and be used in crop N fertilizer recommendations. However, the great variability of results and the lack of common mineralization dynamics that could be described by a simple function, strengthened the irreplaceable worth of incubations at controlled conditions, as a means to evaluate mineralization potential and the N fertilizer value of manures before soil application. Moreover, the utilization of simple hyperbolic or first order kinetic models [28] that are widely used to estimate the N mineralization potentials of soils and decomposing litter seemed not to be suitable for describing the present results. Mineralization dynamics did not follow a recurrent pattern that could be described by a single model. This inadequacy makes the prospect of shortening the incubation time [29] not advisable.

Nitrogen mineralization data as those shown in Figure 2 can be seen as indicative of the amount of mineral N that would eventually become available for uptake by plant roots. These data confirmed the N fertilizing effect of manure, which is, however, much smaller than implied by its total N content.

Considering that the essential variables controlling microbial decomposition and mineralization processes are the same, the effects of manure application at the non-optimum conditions of the field could be revealed by incubation results when time was “normalized” to days of optimum temperature and moisture [30]. Hence, the incubation approach apart from the simplification of the experimental approach enables a significant shortening of “real time” [31]. It is acknowledged, however, that the field conditions are much more complex than those of the incubations. The presence of roots and the exudation of energy-rich organic compounds associated with them, may modify microbial processes [32]. Thus, synchrony issues should be taken into account, since matching availability with crop demand is for both the environmental and agronomic perspective of manure N exploitation very important. Despite these restrictions it is believed that the incubation approach gives a good insight of the trends that experimentation in field conditions could not reveal.

Secondary immobilization phases were common in this study as well as in other published work [33]. However, decreases of cumulative net N release have not always been discussed (see [34–37]), since they were probably considered as non-significant, or as measurement inaccuracies or denitrification losses.

A unifying interpretation scheme of current results would be that manures are composed of two chemical fractions characterized by contrasting N dynamics, one promoting mineralization and another promoting immobilization. Due to microbial decomposition processes these fractions are continuously diminished but at the same time one supplies material to the other. When N demands of the microbial community are fully satisfied by the N content of the decomposing material then net N mineralization is manifested, whereas when microbial needs are not covered by the organic N content of the substrate, either due to inadequacy or due to physical or chemical inaccessibility, the available mineral N in soil is consumed and immobilization is shown. Successive microbial communities may have different capacities for decomposing detrital material or feed on dead microbial biomass, secondary products of metabolism, or recondensed compounds, thus secondary immobilization or mineralization stages may appear. Assuming that manures are mixtures of mineralizing and immobilizing substances, the magnitude of the extracted mineral N would be the result of the synergistic effect of the substance categories. The work of [38] where poultry manure, which can be considered as a typical material promoting net N mineralization [39] and wheat straw, which promotes immobilization, were incubated in soil separately or mixed, provides a good example of immobilization at late stages of incubation. When the easily decomposable fraction of the poultry + wheat mixture was exhausted then N dynamics were dominated by mineral N capturing processes lead by microbial straw decomposition.

Immobilization can reduce the amount of nitrogen available to support plant growth but should not be considered as a permanent loss of N available to crops, as a significant part of immobilized N will

be re-mineralized later. In some cases, management practices even seek to achieve N immobilization in order to reduce the high concentration of mineral N in soil solution and so prevent leaching [40]. Immobilized N will be probably not be available in the first year of manure application but microbial decomposition of dead organic biomass will render some of it available in subsequent rainy seasons. This is probably the reason why manures are considered as long-lasting fertilizing materials.

#### 4.3. Effect of Storage

The interaction between mineralization and immobilization processes showed a distinct pattern, which should not be considered as coincidental since it has been manifested by different kinds of manure, decomposition, or composting procedures that were carried out at different seasons of the year with independent incubation trials. This pattern points out two phases of net N release during the twelve months of storage. The first is separated from the second by a period in which mineralization is zeroed and appears at about six months after storage initiation. The wave like form of this pattern probably implies a non-continuous causal process either of an explicit succession of decomposing microbial communities feeding on dead microbial biomass and secondary products of metabolism, or of the need for the completion of the transformation of certain nitrogenous compounds before a sequential biochemical process is initiated.

Ammonium- and nitrate-N constitute the readily available forms of manure N and the fractions that can be managed similarly with inorganic fertilizers if their magnitude is known. They may represent a significant fraction of total N especially for fresh manures, which is however, vulnerable to losses or immobilization and strongly influenced by the duration of storage. The dynamics of initial mineral N in this study varied a lot between repetitions but resembled the evolution of these forms of N during composting and compost maturation [41]. Nitrate concentration increase and progressive ammonium decrease designate the transition to the curing phase of composting [42,43]. Results of initial content in mineral forms of N, but also changes of ash and pH values, suggest that sheep/goat, cattle, and swine manures of the 2nd and particularly the 3rd repetition did not come from recently cleared stables and they were not as fresh as the materials of the 1st repetition, despite the assurance of the farm owners to the contrary. Ammonium production soon ceased (2nd) or never appeared (3rd repetition) and ash content was almost stable (3rd repetition) implying small rates of decomposition and C losses. Consequently, manures of the 1st, 2nd, and 3rd repetition, apart from poultry, were ranked in decreasing order in terms of freshness. It is emphasized, however, that despite these initial quality differences, the general characteristics of the relationship between duration of storage and mineralization/immobilization dynamics were shown by materials of all three repetitions.

## 5. Conclusions

Taking into account the increased likelihood for pathogens to be present in fresh manure together with the odor and greater handling difficulties, it would be reasonable to recommend that farmers and manure managers should aim to exploit the second peak of net mineralization that appears after six months of storage and to avoid the period of storage when intense N immobilization is manifest. Similar conclusion can be drawn from poultry manure results, which, however, showed different mineralization dynamics than the other three kinds of manure. Nevertheless, fresh manure was shown to be equally valuable to well digested material to provide nitrogen. Hence, if appropriate sanitation is achieved, eventual odor and handling problems overcome, and phytotoxicity concerns associated with a less stable material prove not to be important, the application of fresh manure in the fields would be more profitable since significant C and N losses during storage or composting would be avoided [44].

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