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Nitrogen Availability in Organic Fertilizers from Tannery and Slaughterhouse By-Products

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Abstract: Agriculture can play a primary role in the context of nutrients recovery by promoting the use of organic fertilizers (OFs). In order to use them efficiently, it is necessary to predict the nitrogen (N) bioavailability, which is a challenging matter due to the different physical-chemical characteristics of commercially available OFs. This study aims to evaluate hot-water extractable N as a rapid and cheap chemical indicator of bioavailable N. The trial was conducted on nine animal-based OFs obtained from different raw materials and treatment processes. They were fully characterized and the bioavailable N was determined by a 7-week soil incubation experiment. The results showed that hot-water extractable N underestimated bioavailable N in the case of leather meal based OFs; however, a significant linear regression fitting was achieved ($R^2 = 0.53$). The C:N ratio was also assessed, which showed a negative correlation (−0.87) and a better linear regression fitting ($R^2 = 0.76$) with the bioavailable N, but manifested some limitations in the prediction of leather meal based products. This experiment showed that both hot-water extractable N and C:N ratio can provide useful information for farmers in managing this class of OFs.

Keywords: potentially mineralizable organic nitrogen; fertilizer efficiency; bio-waste recycling; soil fertility; circular economy

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1. Introduction

The use of organic fertilizers (OFs) represents a common agricultural practice to increase both nutrients and organic matter (OM) content in the soil [1]. This class of fertilizers contributes to alleviation of problems related to desertification and soil erosion, improving physical, chemical and biological properties of depleted soils by supplying OM [2–5]. They also play an important role in the reduction of environmental impact, minimizing the use of chemical fertilizers, which create concerns both in their (mis)use and in their production [3–6]. Furthermore, using OFs partially permits solving problems related to the disposal of biomasses, usually destined to landfill or incineration creating potential risks for the environment [7].

Despite their "green attitude", OFs, as well as the chemical ones, must be managed with awareness, since excessive and misleading use could lead to environmental contamination or pollution due to the dispersion of nutrients in other parts of the ecosystem [8]. In particular, nitrogen (N) represents the most crucial macronutrient for plant nutrition. Still, at the same time, the amount that is not absorbed by plants impacts not only on the soil itself, leading to acidification process, but also on the other environmental compartments in terms of coastal eutrophication, air pollution, increase of greenhouse gas emissions, and loss of biodiversity [9–12].

The OFs are typically divided, by source, into: (i) animal-based, such as blood meal, fish meal, leather meals, horn and hoofs meal, slaughterhouse by-products, and manure and (ii) plant-based, such as crop residues and seaweed extracts. Unfortunately, we have only partial data about the amount of global animal by-products available for OFs production; for example, in the European Union and UK about 1.4 billion tonnes of manure are generated annually [13], and the global slaughterhouse by-products are estimated at about 150 million tonnes per year [14]. Therefore, considering that the global production of meat is projected to progressively grow until 2050 [15], we expect that the amount of animal by-products available for OFs productions will increase. The recycling of this huge amount of materials containing N in agriculture as fertilizers is an opportunity, but also a matter of concern considering the lack of knowledge about the availability of N to plants.

Organic N fertilizers are generally constituted by two different pools of N consisting in inorganic (N_{in}) and organic (N_{org}) forms (Figure 1). While the N_{in} compounds, ammonium (NH_4^+) and nitrate (NO_3^-), are readily available to plants [16], the N_{org} component is characterized by a wide spectrum of organic substances [16], which, according to their chemical characteristics, have a different behavior in the soil and can be readily as well as scarcely bioavailable. In fact, the N_{org} fraction can be immediately available or speedily mineralizable by microorganisms (i.e., amino acids and small peptides), or recalcitrant and slowly mineralizable (i.e., polypeptides and heterocyclic N compounds). For these reasons, OFs management is still a challenge for agronomists and the lack of knowledge of potentially mineralizable organic N (N_0) and of the mineralization rate of N_{org} leads to low OFs' N efficiency use. Since these pools are strictly related to the raw materials and the transformation process from which OFs derive, the amount of N_0 as well as the rate at which it becomes bioavailable in soils (Figure 1) is highly affected by OFs' origin [17].

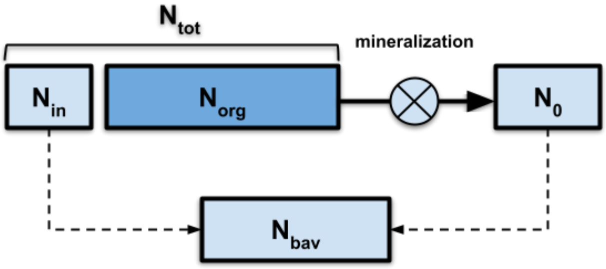


Figure 1. Schematic representation of the bioavailable fraction of nitrogen in organic fertilizers (N_{tot} = total N; N_{in} = inorganic N; N_{org} = organic N; N_0 = potentially mineralizable organic N; N_{bav} = bioavailable N).

In order to optimize N efficiency, it is therefore important to know the amount of N_{in}, as well as of N_0, since these pools are potentially available for crops and their sum can be defined as bioavailable N (N_{bav}). While N_{in} is obtainable by straightforward chemical analysis (i.e., extraction with 1 M KCl and determination of ammonium and nitrate in the extract), the determination of N_0 requires a long-time incubation essay, resulting in a time- and reagents-consuming experiment [18,19]. Therefore, developing a rapid method capable of giving a reliable indication of the N_0 content in both soils and fertilizers has always aroused great interest. For instance, several authors [20–22] found satisfying methods to estimate the organic N mineralization capacity of the soil through a single chemical extraction using hot water, potassium permanganate and warm water, respectively. Dell'Abate et al. [23] conducted a study on leather meal-based fertilizers, determining the faster and slower soluble N pools using phosphate buffer extractions and correlating them with the fertilizers' particle sizes. Cassity-Duffey et al. [17] stated that the total N (N_{tot}) content of the fertilizers could be used as an estimate of the N_0, while according to other authors [24,25] the C:N ratio is the most indicative parameter.

The aims of this study were (i) to determine the bioavailable N in organic fertilizers from animal by-products subjected to different treatment such as anaerobic digestion, thermobaric hydrolysis, chemical and biological stabilization, (ii) to determine the hot-water extractable N in the organic fertilizers, and (iii) to study the relation between bioavailable N and chemical indicators of N availability, thus assessing the possibility to predict the bioavailable N starting from these indicators.

2. Materials and Methods

2.1. General Description of Organic Fertilizers

Nine organic fertilizers (OFs) obtained from the manufacturer or from the market were codified by an increasing numbering from OF01 to OF09 (Table 1). OF01 and OF07 were anaerobic digestates obtained from slaughterhouse by-products, and were classified as nitrogen-phosphorus (NP) OFs. Similarly, OF02 was a NP OF derived from chemical stabilized tannery sludge mixed with meat and bone meals. OF03 and OF05 were N OFs resulting from mixing chemical stabilized tannery sludge and leather meal. OF04, OF08 and OF09 were N OFs obtained from leather by-products after thermobaric hydrolysis. OF06 was a bio-solid derived from the biological stabilization of tannery sludge.

Table 1. Codes, raw materials and transformation processes of the organic fertilizers collected.

Code	Raw Materials	Transformation Process
OF01	Slaughterhouse by-products	Anaerobic digestion
OF02	Tannery sludge + meat and bone meals	Chemical stabilization
OF03	Tannery sludge + leather meal	Chemical stabilization
OF04	Leather meal	Thermobaric hydrolysis
OF05	Tannery sludge + leather meal	Chemical stabilization
OF06	Tannery sludge	Biological stabilization
OF07	Slaughterhouse by-products	Anaerobic digestion
OF08	Leather meal	Thermobaric hydrolysis
OF09	Leather meal	Thermobaric hydrolysis

2.2. Characterization of Organic Fertilizers

All the OFs were ground in a ball mill (Mixer mill MM400, Retsch GmbH, Haan, Germany) and sieved at 0.5 mm before analysis. The chemical reaction (pH) was measured in 1.5:25 mass-to-water ratio using a platinum electrode (5261 electrode, Crison Instruments SA, Alella, Spain) connected to a pH-meter (Compact titrator, Crison Instruments SA, Alella, Spain). The electrical conductivity (EC) was measured in 1:5 mass-to-water ratio using an electrical conductivity meter (Radiometer Analytical CDM 210, Hach Company, Loveland, CO, USA) equipped with a conductivity cell (Radiometer Analytical CDC 749, Hach Company, Loveland, CO, USA). Total solids (TS) and ashes were determined reaching the constant weight at 105 and 650 °C, respectively, using an oven (FD 23 model, Binder GmbH, Tuttlingen, Germany). Total organic carbon (TOC) was determined according to the dichromate method (Springer and Klee, 1954). Total Kjeldahl nitrogen (TKN) was determined after acid digestion with sulfuric acid and selenium-potassium persulfate as catalyzer, using a Kjeldahl automatic instrument (KjelFlex K360, BUCHI Labortechnik AG, Flawil, Switzerland). The ammonium N (NH_4^+-N) was determined according to the 920.03 A.O.A.C. method [26]. Briefly, OFs were extracted with 1 M potassium chloride (KCl) at 1:50 mass-to-volume ratio for 2 h, then an aliquot of filtrate (20 mL) was dispensed into a digestion tube and analyzed in the Kjeldahl automatic instrument after adding 1 g of magnesium oxide (MgO). The nitrate N (NO_3^--N) was determined with the same method, after reduction with 0.5 g of Devarda alloy (Carlo Erba, Italy). The total organic nitrogen (N_{org}) was calculated by the difference between TKN and NH_4^+-N. The total N was considered equal to TKN because the NO_3^--N was under the detection limit of the method for all the OFs. Total phosphorus (P) was determined by an inductively coupled plasma optical emission spectrometer (Spectro Arcos ICP-OES Analyzer, Spectro Analytical Instrument GmbH, Kleve, Germany) after flame digestion with 96% nitric acid.

2.3. Soil Description and Characterization

The soil used in the incubation test was sampled from the surface layer (0–0.2 m) of a cultivated field from the University of Bologna experimental farm located in the southern part of Po Valley, Italy (45.53° N, 11.38° E, 28 m a.s.l.). Once arrived in the laboratory, the soil was air dried, sieved at 2 mm and cleaned from plant debris. The main soil physical and chemical properties were determined according to the Soil Science Society of America methods [27] and the results are shown in Table 2. The soil was classified as a fine silty, mixed mesic Udic Ustochrept [28], and it was a sandy-loam soil characterized by a sub-alkaline pH with an average content of TOC and TN.

Table 2. Main characteristics of the soil used in the trial.

Parameters	**Unit**	**Value**
Texture (U.S.D.A.)		sandy-loam
Sand	g kg^{-1}	700
Silt	g kg^{-1}	260
Clay	g kg^{-1}	140
pH (in water)		7.8
Electrical conductivity	dS m^{-1}	0.17
Cation exchange capacity	$cmol_+$ kg^{-1}	23.4
Total carbonates	g $CaCO_3$ kg^{-1}	65
Total organic carbon	g C kg^{-1}	21.6
Total nitrogen	g N kg^{-1}	2.15
C:N ratio		10.1
Available phosphorous	mg P kg^{-1}	64
Exchangeable calcium	mg Ca kg^{-1}	5150
Exchangeable potassium	mg K kg^{-1}	430
Exchangeable magnesium	mg Mg kg^{-1}	280
Exchangeable sodium	mg Na kg^{-1}	32

2.4. Incubation of Organic Fertilizers into Soil

Aliquots of 200 g of dry soil were placed into cylindrical plastic pots and pre-incubated for 15 days in controlled conditions of temperature (20 °C) and moisture (30% of full water holding capacity) in a growth chamber in the dark. During this period, moisture was checked and restored when needed. OFs were added to the pots in a weight equivalent to 100 mg N kg^{-1} of dry soil (ds), corresponding approximately to 250–300 kg N ha^{-1}. The test was carried out in triplicate, according to a complete randomized design. In addition to the nine OFs, a negative control (CK) with no fertilizer added was included in the trial. The experiment, conducted under the same temperature and moisture conditions as the pre-incubation, lasted 7 weeks. A weekly soil sampling was carried out to determine the inorganic N (N_{in}) according to the ISO 14256-2 protocol [29]: aliquots of 5 g of soil were extracted in 50 mL of 1 M KCl for one hour, then the filtrate solution was analyzed by means of an automated flow analyzer (AutoAnalyzer 3, Bran Luebbe GmbH, Norderstedt, Germany).

The data obtained from the analysis of N_{in} were used to calculate the mineralized organic N (N_{min}) using the following equation:

$$N_{min\,(OF,t)} = [(N_{in})_{t=n} - (N_{in})_{t=0}]_{OF} - [(N_{in})_{t=n}]_{CK}, \tag{1}$$

where t is the sampling time, OF is the organic fertilizers added, CK is the control treatment.

The N_{min} values calculated for each product and for each sampling time were processed through a first order kinetic model:

$$N_{min} = N_0 \cdot (1 - e^{-kt}), \tag{2}$$

where N_0 is the potentially mineralizable organic N, k is the first order kinetic constant and t is the time. This equation provided qualitative and quantitative information on N mineralization, both in quantity and kinetic terms and it widely used in similar cases [19].

Finally, using the data obtained from Equations (1) and (2), it was possible to calculate the N fraction made bioavailable by the OFs added to the soil (N_{bav}). It corresponds to the sum of the N_{in} at time zero and the N_0:

$$N_{bav\,(OF)} = [(N_{in})_{t=0}]_{OF} - [(N_{in})_{t=0}]_{CK} + N_{0\,(OF)}, \tag{3}$$

2.5. Hot-Water Extractable Nitrogen

The determination of hot-water extractable nitrogen in OFs was performed following the procedure adopted by Curtin et al. [21] to evaluate the soil nitrogen availability. Briefly, 5 g of product was added to 100 mL of deionized water and incubated in a water bath at 80 °C under agitation for 16 h. The sample was subjected to centrifugation (6000 rpm, 15 min, 20 °C) and filtration using Whatman No. 42 paper filters. The filtered extract was analyzed for N_{tot}, NH_4^+-N and NO_3^--N as described in Section 2.2.

The hot-water extractable N was then calculated by discriminating the hot-water total nitrogen (HWTN) and the hot-water organic nitrogen (HWON), according to the following equations:

$$\text{HWTN } (\%\ N_{tot}) = (TN_{HW}/N_{tot}) \times 100, \tag{4}$$

$$\text{HWON } (\%\ N_{org}) = (TON_{HW}/N_{org}) \times 100, \tag{5}$$

where TN_{HW} (hot-water total N) = (TN_{HW} + NO_3^--N_{HW}) and TON_{HW} (hot-water total organic N) = (TN_{HW} − NH_4^+-N_{HW}); TN_{HW}, NO_3^--N_{HW} and NH_4^+-N_{HW} are the total N, the nitrate N and the ammonium N extracted in hot-water; N_{tot} and N_{org} are the total N and the organic N of OFs.

2.6. Data Handling and Statistical Analysis

The data obtained were handled and analyzed using the environment for statistical computing R [30]. The first order kinetic model (Equation (2)) has two unknown parameters (N_0 and k), which are estimated using nonlinear regression procedure with the Marquardt–Levenberg algorithm and iterative method to find the parameters to minimize the residual sum of squares [31]. In some cases, manual adjustment of initial values was needed to obtain sensible results. The good-of-fitness of nonlinear models was estimated by the Pseudo-R^2 (P-R^2), calculated using the follow equation: P-R^2 = 1 − (RSS/TSS), were RSS is residual sum of squares and TSS is the total sum of squares [32]. The half-time of organic N mineralization ($t_{1/2}$) was determined using the following equation: $t_{1/2}$ = Ln(2)/k. The relationships between N mineralization (N_0 and N_{bav}) vs. hot-water extractable N (HWON and HWTN) and C:N ratio were assessed using simple linear regression analysis. The coefficient of determination (R^2 = SSreg/TSS, where SSreg is the sum of squares explained by regression and TSS is the total sum of squares) was calculated as an indicator of goodness of fit.

3. Results

3.1. Characterization of Organic Fertilizers

The main characteristics of the OFs used are reported in Table 3. In general, the physical-chemical characteristics of OFs strictly reflected the raw materials and the production processes undergone. The OFs obtained from leather by-products (OF04, OF08, OF09) have acidic pH (4.3–4.7), probably due to the use of acids (i.e., sulfuric acid) in the tanning treatment and in the leather by-products thermobaric hydrolysis [33]. The remaining OFs showed a sub-alkaline pH, ranging from 7.3 to 8.5, in agreement with previous research with similar materials [34–36]. The EC ranged between 0.9 to 5.3 dS m^{-1}, which represent typical values for this type of organic fertilizers [34]. Clearly, the EC depends on total soluble salts present in OFs, and the fact that OF04 showed the highest value is probably linked to the use of huge quantities of salts (i.e., NaCl) during the tanning process. However, high EC values were recorded in OFs subjected to chemical stabilization (OF02, OF03 and OF05).

All samples were in dried form and contained approximately 90% of total solids (TS). Differently, the volatile solids (VS) were found in a wide range, from 44 to 91%. In particular, OFs derived from leather (OF04, OF08, OF09) and slaughterhouse by-products (OF01, OF07) showed higher VS, up to 70%, clearly due to the high content of OM of the raw materials. The percentage of ashes was inversely proportional to the content of VS, and was higher in OFs derived from tannery sludge (OF02, OF03, OF05 and OF06), probably due to inorganic compounds (i.e., inorganic salts) present in these materials.

Table 3. Main physical-chemical characteristics of the organic fertilizers (fw = fresh weight, dw = dry weight).

Parameters	OF01	OF02	OF03	OF04	OF05	OF06	OF07	OF08	OF09
pH	7.7	7.9	7.6	4.3	7.5	8.5	7.3	4.7	4.3
Electrical cond. (dS m^{-1})	0.9	5.1	4.4	5.3	3.53	2.75	2.5	2.4	3.8
Total solids (% fw)	90.7	91.5	93.4	96.6	93.3	90.3	88.5	84.6	91.7
Volatile solids (% dw)	83	43	57.6	90.7	55.1	44.7	81.4	91.1	89.4
Ash (% dw)	17	57	42.4	9.3	44.9	55.3	18.6	8.9	10.6
Total organic C (% dw)	44.2	14.3	31.6	46.2	34.9	32.4	40.2	47.6	47.1
Total Kjeldahl N (%)	4.47	4.11	5.74	14.8	4.27	2.78	5.03	15.7	13.5
C:N ratio	9	5.2	5.5	3.1	8.4	12	8	3	3.5
Ammonium N (% dw)	0.17	0.51	0.14	0.25	0.1	0.07	0.60	0.05	0.03
Nitrate N (% dw)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Organic N (% dw)	4.3	3.6	5.6	14.6	4.17	2.71	4.43	15.7	13.5

Total organic carbon (TOC) of OFs ranged from 14 to 47% (Table 3). This large range of TOC is due to the different chemical characteristics of raw materials used for OFs production [34–36] and represents an important indicator for OFs quality. Total Kjeldahl nitrogen (TKN), as well as total organic nitrogen (N_{org}), was significantly higher in OFs made from leather by-products (OF04, OF08 and OF09), presenting values ranging from 13 to 15%, clearly due to the large amounts of animal proteins (or polypeptides) in the raw material. The lowest value of TKN was found in OF06 (2.78%), the only sample obtained from tannery sludge that does not include the addition of other raw materials, such as OF02, OF03, and OF05. As expected, the higher the TKN the lower the C:N ratio, which showed values approximately of 3 for OF04, OF08 and OF09, and of 12 for the OF06.

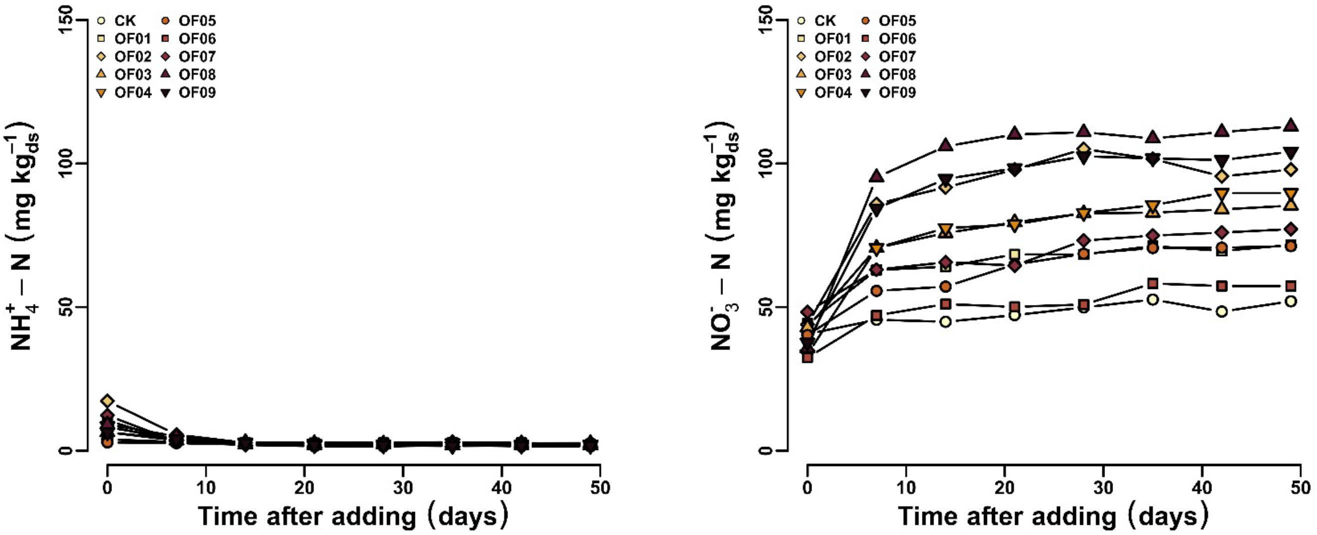
While the nitrate N (NO_3^--N) content was below the detection limit of the method (<100 ppm), the ammonium N concentration (NH_4^+-N) ranged from 0.03 to 0.60%. In this case, there was no discrimination based on raw materials; the differences probably depend from the specific productive processes used. In any case, the amount of inorganic (or ammonium) N was a small fraction of TKN and the organic N was always higher than 95% of TKN.

3.2. Incubation of Organic Fertilizers in the Soil

The dynamics of NH_4^+-N showed significant differences only at the beginning of incubation (t_0) since this form of N is immediately released in soil by the input of OFs (Figure 2a and Table 4). In fact, OF02 and OF07 presented the highest values of NH_4^+-N released, being the two characterized by the highest percentage of NH_4^+-N content (Table 3). In the subsequent samplings, all OFs showed values close to zero and equal to the untreated soil (CK) demonstrating that NH_4^+-N was subjected to soil nitrification process.

Consequently, analysis of nitrate manifested an opposite trend to that of ammonium (Figure 2b). In fact, an increase was observed in the first period, from the beginning to the 14th day, followed by a general stabilization phase until the end of the incubation. In particular, OF08 was the sample that released the most nitrate N, showing values above 100 mg N kg^{-1} d.s. starting from the fourteenth day. OF09 and OF02 showed approximately the same trend, achieving values around 90 mg N kg^{-1} d.s. in the same period, followed by a slight decrease of OF02 in the last two samplings. A value of 75 mg N kg^{-1} d.s. was reached and exceeded by both OF04 and OF03 during the incubation. In particular, the fertilizer deriving from leather meal (OF04) showed slightly higher values than OF03 in the last three sampling dates (t_{35}, t_{42}, t_{49}). OF07 displayed an increase between the 3rd and the 4th week of incubation (respectively t_{21} and t_{28}), reaching values above 60 mg N kg^{-1} d.s., as well as OF05 and OF01. OF06 was the sample that presented the lowest values (50 mg N kg^{-1} d.s.), but showed a positive trend compared to the unfertilized control.

As expected, the kinetic curves are very similar to those obtained with the NO_3^--N analysis, since the NH_4^+-N release showed no differences between the fertilized samples and the unfertilized control, except for t_0. OF08 and OF09 showed the greater ability to mineralize, reaching values of mineralization of 63 and 54% of the total N added, respectively (Table 4). The mineralization percentage ranged from 29.6 to 36% for OF04, OF02, OF05 and OF03, while OFs deriving from anaerobic digestion, OF01 and OF07, attained 16 and 13%, respectively (Table 4). As confirmed by the NO_3^--N analysis, OF06 is the one that manifested the lowest ability to mineralize the organic N (6.8%). These results were quite expected, as OF01, OF07 and OF06 showed a consistent loss of organic matter during the digestion and the biological treatment.



(**a**) (**b**)

Figure 2. Inorganic N release during the incubation of organic fertilizers in the soil: (**a**) ammonium N, and (**b**) nitrate N (ds = data expressed on dry soil basis).

Table 4. Fitting of inorganic N release in the soil after incubation with organic fertilizers: N_{in} is the inorganic N at the beginning of incubation, N_0 is the potentially mineralizable organic N, N_{bav} is the sum of N_{in} and N_0, k is the first order kinetic constant, $t_{1/2}$ is the half-time of organic N mineralization and P-R^2 is the Pseudo R-squared coefficient of the first order kinetic model. Data are reported as mean ± error standard from the mean.

Fertilizers	**N_{in} (%N_{tot})**	**N_0 (%N_{tot})**	**N_{bav} (%N_{tot})**	**k (days^{-1})**	**$t_{1/2}$ (days)**	**P-R^2**
OF01	7.9 ± 4.1	16.1 ± 1.0	24.0	0.177 ± 0.066	3.9	0.887
OF02	16.7 ± 5.5	36.1 ± 1.4	52.8	0.165 ± 0.035	4.2	0.959
OF03	4.8 ± 2.7	29.6 ± 1.9	34.4	0.162 ± 0.056	4.3	0.895
OF04	4.3 ± 2.9	36.0 ± 2.2	40.3	0.110 ± 0.023	5.8	0.951
OF05	2.8 ± 1.5	32.0 ± 1.0	34.8	0.173 ± 0.035	9.8	0.962
OF06	7.3 ± 3.2	6.8 ± 0.6	14.1	0.294 ± 0.252	28	0.766
OF07	12.0 ± 0.3	13.1 ± 1.4	25.1	0.037 ± 0.020	8.8	0.865
OF08	1.5 ± 0.5	63.1 ± 0.3	64.6	0.211 ± 0.016	3.6	0.996
OF09	0.5 ± 0.2	54.1 ± 0.6	54.6	0.260 ± 0.010	3.9	0.999

Figure 3 shows the curves of mineralization of the organic N during the incubation period. The asymptote of these first kinetic order curves is equal to N_0, the potentially mineralizable organic N, results for which are reported in Table 4.

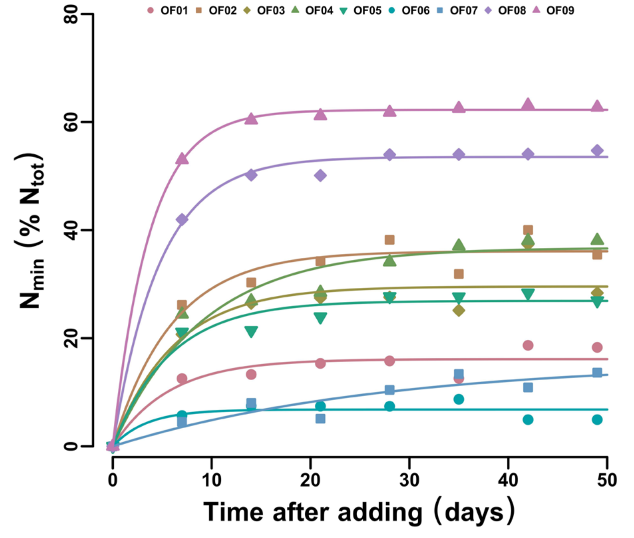


Figure 3. First order kinetic model fitting of organic N mineralized in the soil incubated with the organic fertilizers.

3.3. Hot-Water Extractable Nitrogen

The results of HWON and HWTN are reported in Table 5. The values of HWON ranged between 10.5 (OF06) and 46.3% (OF02) of N_{org}, a wide range which, however, agrees with the results of N_0 previously reported (Table 4). The higher HWON was observed for OF02, OF03, OF04, OF08 and OF09 with values ranging between 35 to 45% of N_{org}. These OFs were characterized by leather and bone meals as raw materials and chemical or thermobaric hydrolysis as transformation process (Table 1). Otherwise, OF01 and OF06 showed the lowest HWON, ranging between 10 to 20% of N_{org}. These fertilizers were made from slaughterhouse by-products and tannery sludge, respectively. The other OFs, from the same by-products, showed an intermediate HWON, with values ranging from 25 to 30% of N_{org}. The values of HWTN were slightly higher than HWON (Table 5), but closely related. Only OF02 and OF07 showed a difference between HWTN and HWON higher than 5%, due to their greater N_{in} content (Table 4).

Table 5. Results of hot-water organic N (HWON) and hot-water total N (HWTN) analysis of the organic fertilizer.

Fertilizers	**HWON (%N_{org})**	**HWTN (%N_{tot})**
OF01	16.9	19.2
OF02	46.3	53.9
OF03	39.7	41.5
OF04	43.1	44.1
OF05	31.1	32.4
OF06	10.5	10.9
OF07	28.6	35.7
OF08	39.6	40.8
OF09	34.8	36.0

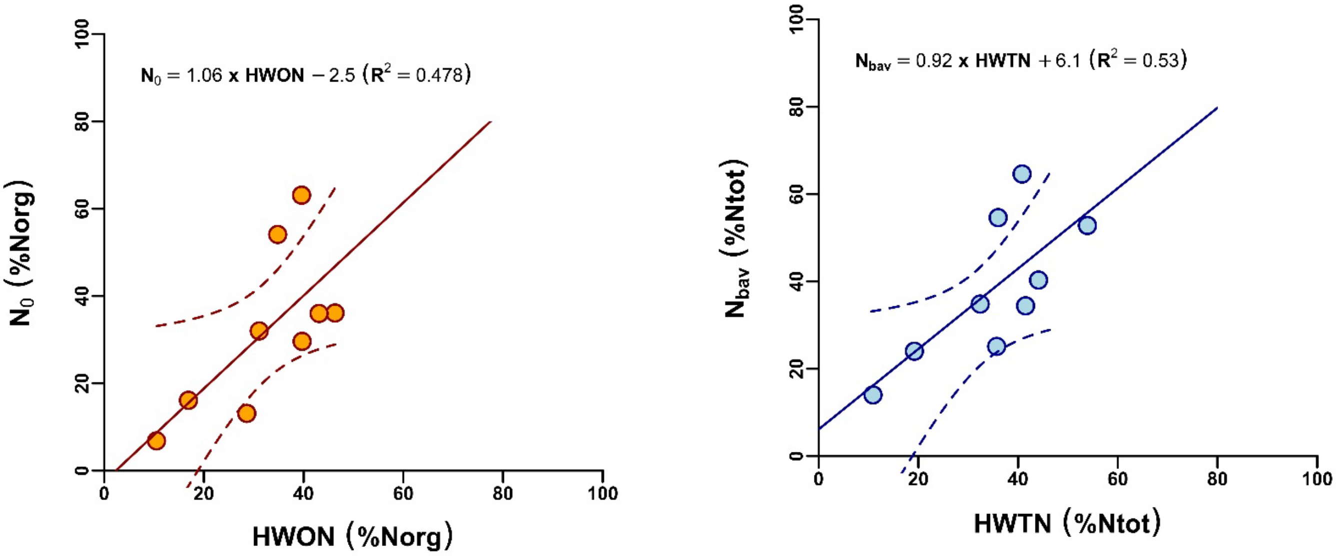
3.4. Correlation and Linear Regression between Indicators of Available Nitrogen

The data concerning indicators of N availability in OFs were analyzed for correlation using the Pearson coefficient. The results obtained are shown in Table 6. The N_0, N_{bav}, HWON and HWTN were significantly and positively correlated (p-value < 0.001). On the other hand, N_0 and N_{bav} were significantly but negatively correlated with C:N ratio (p-value < 0.05).

Table 6. Correlation between indicators of bioavailable nitrogen in organic fertilizers.

Correlation	Pearson Coefficient	Degree of Freedom	*p*-Value
N_0 vs. N_{bav}	0.962	7	<0.001
HWON vs. HWTN	0.980	7	<0.001
N_{bav} vs. HWTN	0.728	7	0.027
N_0 vs. HWON	0.691	7	0.039
N_{bav} vs. C:N ratio	−0.873	7	0.002
N_0 vs. C:N ratio	−0.863	7	0.003

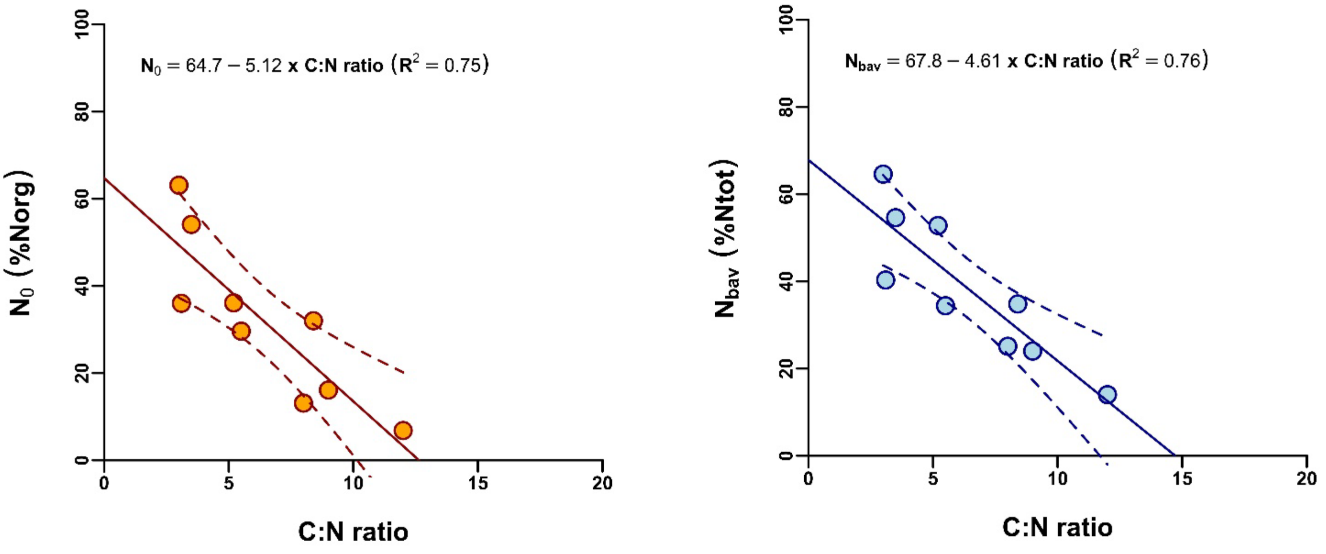
The capacity of HWON, HWTN and C:N ratio to estimate N_0 and N_{bav} was assessed using the linear regression approach. The results are shown in Figures 4 and 5. The HWON showed a significant (*p*-value = 0.039) linear regression with the N_0 (Figure 4a) and a $R^2 = 0.478$. Even if the slope of the straight line was 1.065 ± 0.420 (*p*-value = 0.039), with a quite perfect linearity between the two indicators, the samples OF08 and OF09 deviated from linearity and fell outside the 95% confidence limit. HWTN and N_{bav} showed similar results (Figure 4b), with a significant linearity ($R^2 = 0.53$, *p*-value = 0.027), a slope of 0.921 ± 0.328 (*p*-value = 0.027) and the same samples outside of the 95% confidence limits. In both cases the estimated intercept was not significantly different from zero (respectively: −2.5 ± 14.4 (*p*-value = 0.87) and 6.1 ± 12.1 (*p*-value = 0.63)).



(**a**) (**b**)

Figure 4. Linear regression between: (**a**) potentially mineralizable organic N (N_0) with hot-water organic N (HWON), and (**b**) bioavailable N (N_{bav}) with hot-water total N (HWTN). The straight lines indicate the linear regression model and the dotted lines the 95% confidence limits.

The C:N ratio showed (Figure 5a,b) a significant linearity with N_0 ($R^2 = 0.75$, *p*-value = 0.003) and N_{bav} ($R^2 = 0.76$, *p*-value = 0.002). The slope was negative in both cases and significantly different from zero: −5.12 ± 1.13 (*p*-value = 0.003) and −4.61 ± 0.97 (*p*-value = 0.002), respectively, for N_0 and N_{bav}. The intercepts were higher than zero and corresponded to 64.7 ± 8.0 (*p*-value <0.001) and 67.8 ± 6.9 (*p*-value <0.001) for N_0 and N_{bav}, respectively.



(**a**) (**b**)

Figure 5. Linear regression between: (**a**) potentially mineralizable organic N (N_0) with C:N ratio, and (**b**) bioavailable N (N_{bav}) with C:N ratio. The straight lines indicate the linear regression model and the dotted lines the 95% confidence limits.

4. Discussion

4.1. Characterization and Soil Incubation of the Organic Fertilizers

After the physical-chemical characterization and incubation in soil, it is possible to classify the studied animal-based OFs in four groups: (i) leather meal from thermobaric hydrolysis, (ii) slaughterhouse by-products from anaerobic digestion, (iii) tannery sludge + animal based by-products from chemical stabilization, and (iv) tannery sludge from biological stabilization. In fact, this confirms that the source materials and treatment processes largely affected OFs characteristics and the N release in the soil.

The OFs obtained from leather meals (OF04, OF08 and OF09) showed higher N_{tot} (12–14%) than other OFs, in agreement with the greater protein content of leather [23,33,37]. These OFs are characterized also by lower C:N ratio (3–3.5:1), higher volatile solids (77–88%) and lower ash contents (7.5–10%) than other OFs. Soil incubation indicated that this class of OFs mineralized more organic N, with a N_0 ranging from 36 to 63% of N_{tot}, resulting in 40–64% of bioavailable N (N_{bav}). The rate of mineralization was also higher than in other OFs studied, with a half-life time of 3.6–5.8 days at the experimental conditions applied in this study. Practically, the release of the bioavailable N in soil from these OFs proceeded intensely and quickly, similar to inorganic N fertilizers [23,38], due to the thermobaric hydrolysis that guaranteed a high availability of N_{org} for soil microorganisms, and to the lower C:N ratio that results in a lower N use efficiency by soil microorganisms and consequently a net N release into the soil [39].

Slaughterhouse by-products (OF01 and OF07) after anaerobic digestion have been poorly studied as fertilizers [14,40], and they are characterized by a relatively high N_{tot} (4–5%) and low C:N ratio (8–9:1). Therefore, it is not surprising that these OFs released as N_{bav} only a quarter of the N_{tot} added to the soil. As previously discussed [17,39], the net mineralization of N_{org} in soil depends on the C:N ratio and on the quality or availability of OM for soil microorganisms. Regarding this class of OFs, a large part of the N_{bav} was directly released as N_{in} probably due to anaerobic digestion, which is well known to generate digestates that often contain important amounts of ammonium N [35,41,42].

The tannery sludge-based OFs (OF02, OF03 and OF05) showed a N_{tot} content similar to the slaughterhouse by-products (3.5–5%), but with a lower C:N ratio (5–8:1). These OFs were obtained by mixing tannery sludge with other animal-based fertilizers (bone, meat and leather meals), thus increasing the N_{tot}. This reduced the C:N ratio compared to a pure tannery sludge from biological stabilization (OF06), which instead showed the lowest N_{tot} (2.5%) and the highest C:N ratio (16:1). The tannery sludge has been proposed as organic N fertilizer considering its interesting N content [43–45], but it is often mixed with other OFs to meet commercial and agronomical requirements. Indeed, mixing tannery sludge with meat, bone and leather meal makes it possible to modulate N_{tot} and the C:N ratio of the final OF. This induces a distinct behavior of the OFs in soil and a different release of bioavailable N: for example, OF02 characterized by lower C:N ratio (5.2:1), showed higher N_{org} mineralization (36% of N_{tot}). The N_{bav} released by this group of OFs showed a wide range of values (34–52%) which is largely due to the different N_{in} content. Indeed, the high N_{in} (16%) in OF02 corresponded to the high N_{bav} (52%), and this probably derived from the raw materials (i.e., meat meal) or from the hydrolysis during the transformation process.

The characteristics of pure tannery sludge (OF06) were less interesting from the agronomical point of view, with the lower N_{tot} (2.5%), the higher C:N ratio (16:1) and the lower volatile solids (40%), compared to the other OFs. OF06 released only the 16% of N_{tot} in available form, a value much lower compared to those obtained testing sewage sludge deriving from different raw materials [46–48]. This outcome is probably related to the more intensive biological treatment to which the tannery wastewater was subjected [49] due to the high load of OM and stabilizing compounds, such as chromium, aluminum and glutaraldehyde [45,50]. Practically, this product is more like an amendment than a OF; indeed, the mineralization of organic N in soil was lower than for the other OFs (7%). For these reasons, the tannery sludge is generally mixed with other OFs to make it a marketable OF [51].

4.2. Relation between Indicators of Available Nitrogen

Three indicators were used to estimate the available N in animal-based OFs: HWON, HWTN and C:N ratio. All the indicators are relatively easy to determine and standardized methods are available, making them a valid alternative to estimate the bioavailable N in OFs.

However, in some cases these indicators under- or overestimate the bioavailable N. In particular, OF08 and OF09, based on leather meal, mineralized the highest amount of organic N, but these results do not agree with those found in the analysis of HWON and HWTN that underestimate N_0 and N_{bav}. Conversely, in the case of OF02 and OF03, based on tannery sludge mixed with other materials, the hot-water indicators slightly underestimate only the N_{bav}. The reason for these discrepancies seems to be connected to the quality of N_{org}, which appears, in some cases, to be more hot-water extractable than mineralizable, and in other cases less so. In the case of OFs based on leather meal, the hot water may not be a strong enough extractant to guarantee the optimal solubility of collagen, the main component of leather meal [23], whereas it is well hydrolyzed by collagenase and protease in the soil [52]. In the case of tannery sludge, the hot water seems to extract the organic N fraction that is less accessible to or less degradable by the soil microbiota.

The C:N ratio of OFs was, as expected [17,25,42], a good indicator of organic N mineralization in soil. The C:N ratio was negatively correlated with bioavailable N; in fact, fertilizers with a low ratio induce high bioavailable N in soil [39]. The linear regression analysis between bioavailable N and C:N ratio was more significant ($R^2 = 0.75$) than that of hot-water indicators. Interestingly, the linear models estimate a maximum available N for the animal-based OFs near to 65–68% of N_{tot} (Figure 5, calculate for C:N = 0), and a C:N ratio limit between mineralization-immobilization of N near to 12–15:1 (Figure 5, calculate for N_0 and $N_{bav} = 0$), in agreement with findings of other authors [17,25]. Indeed, a C:N ratio less than 15–25:1 generally results in net N mineralization, whereas a C:N ratio greater than 15–25:1 can lead to net N immobilization [25,53,54], and all OFs studied fall in the first case. The C:N ratio is, unlike HWON and HWTN, an eco-stoichiometric indicator or an indicator that predicts the N availability on the basis of N and C use efficiency by soil microorganisms. When the C:N ratio is less than 15–25:1, the soil microorganisms face a substrate with an excess of N with respect to their needs, and this N is released in the soil as inorganic (available for plant) form. Nevertheless, this is valid only theoretically, as in real cases the quality of the OM added with the OF and the presence of other elements or compounds able to inhibit or stimulate the microbial metabolism can modulate the N mineralization. For example, the OFs based on leather meal (OF04, OF08 and OF09) have a similar C:N ratio, 3–3.5:1, but show three different N_{bav}, 40.3% (OF04), 64.5% (OF08) and 54.6% (OF09), that probably are due to differences in the treatment process (i.e., more or less hydrolysis conditions) or to a residual presence of stabilizing agents used for tanning the leather.

5. Conclusions

The results of this research can inform farmers and fertilizer producers about the N availability in organic fertilizers from animal by-products and the relationship with chemical indicators of N availability. It was demonstrated that it is difficult to predict N mineralization solely on the basis of their total N content; in fact, raw materials and treatment processes influence the ability of OFs to release N in inorganic forms. The hot-water extractable N proved to be a good indicator of $N_{bav,}$ but underestimated it in the case of OFs based on leather meal. The C:N ratio provided the best linear fitting with N_{bav}, but also showed some limitations in estimating the N_{bav}.

This study has demonstrated that OFs from animal by-products are potentially useful as organic N fertilizers. However, to use them consciously and efficiently, the evaluation of C:N ratio and the hot-water extractable N analysis should be considered, as they are rapid and inexpensive methods that can provide a reliable indication of N availability in the soil.

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References

1. Luo, G.; Li, L.; Friman, V.-P.; Guo, J.; Guo, S.; Shen, Q.; Ling, N. Organic Amendments Increase Crop Yields by Improving Microbe-Mediated Soil Functioning of Agroecosystems: A Meta-Analysis. *Soil Biol. Biochem.* **2018**, *124*, 105–115. [CrossRef]
2. Carbonell, G.; de Imperial, R.M.; Torrijos, M.; Delgado, M.; Rodriguez, J.A. Effects of Municipal Solid Waste Compost and Mineral Fertilizer Amendments on Soil Properties and Heavy Metals Distribution in Maize Plants (*Zea Mays L.*). *Chemosphere* **2011**, *85*, 1614–1623. [CrossRef] [PubMed]
3. Spångberg, J.; Hansson, P.-A.; Tidåker, P.; Jönsson, H. Environmental Impact of Meat Meal Fertilizer vs. Chemical Fertilizer. *Resour. Conserv. Recycl.* **2011**, *55*, 1078–1086. [CrossRef]
4. Svanbäck, A.; McCrackin, M.L.; Swaney, D.P.; Linefur, H.; Gustafsson, B.G.; Howarth, R.W.; Humborg, C. Reducing Agricultural Nutrient Surpluses in a Large Catchment—Links to Livestock Density. *Sci. Total Environ.* **2019**, *648*, 1549–1559. [CrossRef]
5. Zhang, G.; Sun, B.; Zhao, H.; Wang, X.; Zheng, C.; Xiong, K.; Ouyang, Z.; Lu, F.; Yuan, Y. Estimation of Greenhouse Gas Mitigation Potential through Optimized Application of Synthetic N, P and K Fertilizer to Major Cereal Crops: A Case Study from China. *J. Clean. Prod.* **2019**, *237*, 117650. [CrossRef]
6. Ott, C.; Rechberger, H. The European Phosphorus Balance. *Resour. Conserv. Recycl.* **2012**, *60*, 159–172. [CrossRef]
7. Sharma, B.; Vaish, B.; Singh, U.K.; Singh, P.; Singh, R.P. Recycling of Organic Wastes in Agriculture: An Environmental Perspective. *Int. J. Environ. Res.* **2019**, *13*, 409–429. [CrossRef]
8. Song, K.; Xue, Y.; Zheng, X.; Lv, W.; Qiao, H.; Qin, Q.; Yang, J. Effects of the Continuous Use of Organic Manure and Chemical Fertilizer on Soil Inorganic Phosphorus Fractions in Calcareous Soil. *Sci. Rep.* **2017**, *7*, 1164. [CrossRef]

9. Billen, G.; Garnier, J.; Lassaletta, L. The Nitrogen Cascade from Agricultural Soils to the Sea: Modelling Nitrogen Transfers at Regional Watershed and Global Scales. *Phil. Trans. R. Soc. B* **2013**, *368*, 20130123. [CrossRef]
10. Galloway, J.N.; Aber, J.D.; Erisman, J.W.; Seitzinger, S.P.; Howarth, R.W.; Cowling, E.B.; Cosby, B.J. The Nitrogen Cascade. *BioScience* **2003**, *53*, 341–356. [CrossRef]
11. Lassaletta, L.; Billen, G.; Grizzetti, B.; Anglade, J.; Garnier, J. 50 Year Trends in Nitrogen Use Efficiency of World Cropping Systems: The Relationship between Yield and Nitrogen Input to Cropland. *Environ. Res. Lett.* **2014**, *9*, 105011. [CrossRef]
12. Sutton, M.A.; Bleeker, A. The Shape of Nitrogen to Come. *Nature* **2013**, *494*, 435–437. [CrossRef] [PubMed]
13. Köninger, J.; Lugato, E.; Panagos, P.; Kochupillai, M.; Orgiazzi, A.; Briones, M.J.I. Manure Management and Soil Biodiversity: Towards More Sustainable Food Systems in the EU. *Agric. Syst.* **2021**, *194*, 103251. [CrossRef]
14. Limeneh, D.Y.; Tesfaye, T.; Ayele, M.; Husien, N.M.; Ferede, E.; Haile, A.; Mengie, W.; Abuhay, A.; Gelebo, G.G.; Gibril, M.; et al. A Comprehensive Review on Utilization of Slaughterhouse By-Product: Current Status and Prospect. *Sustainability* **2022**, *14*, 6469. [CrossRef]
15. Bustillo-Lecompte, C.F.; Mehrvar, M. Slaughterhouse Wastewater Characteristics, Treatment, and Management in the Meat Processing Industry: A Review on Trends and Advances. *J. Environ. Manag.* **2015**, *161*, 287–302. [CrossRef]
16. Whalen, J.K.; Thomas, B.W.; Sharifi, M. Novel Practices and Smart Technologies to Maximize the Nitrogen Fertilizer Value of Manure for Crop Production in Cold Humid Temperate Regions. In *Advances in Agronomy*; Elsevier: Amsterdam, The Netherlands, 2019; Volume 153, pp. 1–85. ISBN 978-0-12-817404-3.
17. Cassity-Duffey, K.; Cabrera, M.; Gaskin, J.; Franklin, D.; Kissel, D.; Saha, U. Nitrogen Mineralization from Organic Materials and Fertilizers: Predicting N Release. *Soil Sci. Soc. Am. J.* **2020**, *84*, 522–533. [CrossRef]
18. Stanford, G.; Smith, S.J. Nitrogen Mineralization Potentials of Soils. *Soil Sci. Soc. Am. J.* **1972**, *36*, 465–472. [CrossRef]
19. Honeycutt, C.W.; Zibilske, L.M.; Clapham, W.M. Heat Units for Describing Carbon Mineralization and Predicting Net Nitrogen Mineralization. *Soil Sci. Soc. Am. J.* **1988**, *52*, 1346–1350. [CrossRef]
20. Bremner, J.M.; Keeney, D.R. Steam Distillation Methods for Determination of Ammonium, Nitrate and Nitrite. *Anal. Chim. Acta* **1965**, *32*, 485–495. [CrossRef]
21. Curtin, D.; Wright, C.E.; Beare, M.H.; McCallum, F.M. Hot Water-Extractable Nitrogen as an Indicator of Soil Nitrogen Availability. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1512–1521. [CrossRef]
22. Hussain, F.; Malik, K.A.; Azam, F. Evaluation of Acid Permanganate Extraction as an Index of Soil Nitrogen Availability. *Plant Soil* **1984**, *79*, 249–254. [CrossRef]
23. Dell'Abate, M.T.; Benedetti, A.; Trinchera, A.; Galluzzo, D. Nitrogen and Carbon Mineralisation of Leather Meal in Soil as Affected by Particle Size of Fertiliser and Microbiological Activity of Soil. *Biol. Fertil. Soils* **2003**, *37*, 124–129. [CrossRef]
24. Delin, S.; Stenberg, B.; Nyberg, A.; Brohede, L. Potential Methods for Estimating Nitrogen Fertilizer Value of Organic Residues: Methods for Estimating Nitrogen Fertilizer Value of Organic Residues. *Soil Use Manag.* **2012**, *28*, 283–291. [CrossRef]
25. Lazicki, P.; Geisseler, D.; Lloyd, M. Nitrogen Mineralization from Organic Amendments Is Variable but Predictable. *J. Env. Qual.* **2020**, *49*, 483–495. [CrossRef] [PubMed]
26. A.O.A.C. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemist: Washington, DC, USA, 1990.
27. *Methods of Soil Analysis: Part 3 Chemical Methods*; Sparks, D.L.; Page, A.L.; Helmke, P.A.; Loeppert, R.H.; Soltanpour, P.N.; Tabatabai, M.A.; Johnston, C.T.; Sumner, M.E. (Eds.) SSSA Book Series; Soil Science Society of America, American Society of Agronomy: Madison, WI, USA, 1996; ISBN 978-0-89118-866-7.
28. Soil Survey Staff. Soil Survey Staff. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. In *U.S. Department of Agriculture Handbook*, 2nd ed.; Natural Resources Conservation Service: Washington, DC, USA, 1999.
29. *ISO Standard No 14256-2*; International Organization for Standardization Soil Quality—Determination of Nitrate, Nitrite and Ammonium in Field-Moist Soils by Extraction with Potassium Chloride Solution—Part 2: Automated Method with Segmented Flow Analysis. ISO: Geneva, Switzerland, 2005.
30. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022.
31. Benbi, D.K.; Richter, J. A critical review of some approaches to modelling nitrogen mineralization. *Biol. Fertil. Soils* **2002**, *35*, 168–183. [CrossRef]
32. Schabenberger, O.; Pierce, F.J. *Contemporary Statistical Models for the Plant and Soil Sciences*; CRC Press: Boca Raton, FL, USA, 2001.
33. Ciavatta, C.; Manoli, C.; Cavani, L.; Franceschi, C.; Sequi, P. Chromium-Containing Organic Fertilizers from Tanned Hides and Skins: A Review on Chemical, Environmental, Agronomical and Legislative Aspects. *JEP* **2012**, *3*, 1532–1541. [CrossRef]
34. Kataki, S.; Hazarika, S.; Baruah, D.C. Assessment of By-Products of Bioenergy Systems (Anaerobic Digestion and Gasification) as Potential Crop Nutrient. *Waste Manag.* **2017**, *59*, 102–117. [CrossRef]
35. Moukazis, I.; Pellera, F.-M.; Gidarakos, E. Slaughterhouse By-Products Treatment Using Anaerobic Digestion. *Waste Manag.* **2018**, *71*, 652–662. [CrossRef]
36. Prado, J.; Ribeiro, H.; Alvarenga, P.; Fangueiro, D. A Step towards the Production of Manure-Based Fertilizers: Disclosing the Effects of Animal Species and Slurry Treatment on Their Nutrients Content and Availability. *J. Clean. Prod.* **2022**, *337*, 130369. [CrossRef]
37. Corte, L.; Dell'Abate, M.T.; Magini, A.; Migliore, M.; Felici, B.; Roscini, L.; Sardella, R.; Tancini, B.; Emiliani, C.; Cardinali, G.; et al. Assessment of Safety and Efficiency of Nitrogen Organic Fertilizers from Animal-Based Protein Hydrolysates-a Laboratory Multidisciplinary Approach. *J. Sci. Food Agric.* **2014**, *94*, 235–245. [CrossRef]

38. Cayuela, M.L.; Velthof, G.L.; Mondini, C.; Sinicco, T.; van Groenigen, J.W. Nitrous Oxide and Carbon Dioxide Emissions during Initial Decomposition of Animal By-Products Applied as Fertilisers to Soils. *Geoderma* **2010**, *157*, 235–242. [CrossRef]
39. Mooshammer, M.; Wanek, W.; Hämmerle, I.; Fuchslueger, L.; Hofhansl, F.; Knoltsch, A.; Schnecker, J.; Takriti, M.; Watzka, M.; Wild, B.; et al. Adjustment of Microbial Nitrogen Use Efficiency to Carbon:Nitrogen Imbalances Regulates Soil Nitrogen Cycling. *Nat. Commun.* **2014**, *5*, 3694. [CrossRef] [PubMed]
40. Bhunia, S.; Bhowmik, A.; Mukherjee, J. Use of Rural Slaughterhouse Wastes (SHWs) as Fertilizer in Agriculture: A Review. In Proceedings of the 2019 International Conference on Energy Management for Green Environment (UEMGREEN), Kolkata, India, 25–27 September 2019; pp. 1–6.
41. Alburquerque, J.A.; de la Fuente, C.; Bernal, M.P. Chemical Properties of Anaerobic Digestates Affecting C and N Dynamics in Amended Soils. *Agric. Ecosyst. Environ.* **2012**, *160*, 15–22. [CrossRef]
42. Sigurnjak, I.; De Waele, J.; Michels, E.; Tack, F.M.G.; Meers, E.; De Neve, S. Nitrogen Release and Mineralization Potential of Derivatives from Nutrient Recovery Processes as Substitutes for Fossil Fuel-Based Nitrogen Fertilizers. *Soil Use Manag.* **2017**, *33*, 437–446. [CrossRef]
43. Araujo, A.S.F.; de Melo, W.J.; Araujo, F.F.; Van den Brink, P.J. Long-Term Effect of Composted Tannery Sludge on Soil Chemical and Biological Parameters. *Environ. Sci. Pollut. Res.* **2020**, *27*, 41885–41892. [CrossRef]
44. Giacometti, C.; Cavani, L.; Gioacchini, P.; Ciavatta, C.; Marzadori, C. Soil Application of Tannery Land Plaster: Effects on Nitrogen Mineralization and Soil Biochemical Properties. *Appl. Environ. Soil Sci.* **2012**, *2012*, 395453. [CrossRef]
45. Guo-Tao, F.; Zhi-Hua, S.; Shuqing, L.; Hui, C. The Production of Organic Fertilizer Using Tannery Sludge. *J. Am. Leather Chem. Assoc.* **2013**, *108*, 189–196.
46. Antil, R.S.; Bar-Tal, A.; Fine, P.; Hadas, A. Predicting Nitrogen and Carbon Mineralization of Composted Manure and Sewage Sludge in Soil. *Compos. Sci. Util.* **2011**, *19*, 33–43. [CrossRef]
47. Grigatti, M.; Cavani, L.; di Biase, G.; Ciavatta, C. Current and Residual Phosphorous Availability from Compost in a Ryegrass Pot Test. *Sci. Total Environ.* **2019**, *677*, 250–262. [CrossRef]
48. Sciubba, L.; Cavani, L.; Negroni, A.; Zanaroli, G.; Fava, F.; Ciavatta, C.; Marzadori, C. Changes in the Functional Properties of a Sandy Loam Soil Amended with Biosolids at Different Application Rates. *Geoderma* **2014**, *221–222*, 40–49. [CrossRef]
49. Zhao, J.; Wu, Q.; Tang, Y.; Zhou, J.; Guo, H. Tannery Wastewater Treatment: Conventional and Promising Processes, an Updated 20-Year Review. *J. Leather Sci. Eng.* **2022**, *4*, 10. [CrossRef]
50. Sundar, V.J.; Gnanamani, A.; Muralidharan, C.; Chandrababu, N.K.; Mandal, A.B. Recovery and Utilization of Proteinous Wastes of Leather Making: A Review. *Rev. Env. Sci. Biotechnol.* **2011**, *10*, 151–163. [CrossRef]
51. Tahiri, S.; De La Guardia, M. Treatment and Valorization of Leather Industry Solid Wastes: A Review. *J. Am. Leather Chem. Assoc.* **2009**, *104*, 52–67.
52. Harper, E. Collagenases. *Annu. Rev. Biochem.* **1980**, *49*, 1063–1078. [CrossRef] [PubMed]
53. Calderón, F.J.; McCarty, G.W.; Reeves, J.B. Analysis of Manure and Soil Nitrogen Mineralization during Incubation. *Biol. Fertil. Soils* **2005**, *41*, 328–336. [CrossRef]
54. Gale, E.S.; Sullivan, D.M.; Cogger, C.G.; Bary, A.I.; Hemphill, D.D.; Myhre, E.A. Estimating Plant-Available Nitrogen Release from Manures, Composts, and Specialty Products. *J. Environ. Qual.* **2006**, *35*, 2321–2332. [CrossRef] [PubMed]