Nitrogen mineralization from digestate in comparison to sewage sludge, compost and urea in a laboratory incubated soil experiment

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Abstract

This paper evaluated, in a laboratory incubated soil, the properties of digestate as a nitrogen fertilizer in comparison with sewage sludge, compost and urea, this last as a typical mineral fertilizer. The incubation period lasted for 90 d and during this time, pH, CO_2 and NO_3^- evolution were measured. The maximum concentration of nitrate was reached in the incubated microcosm fertilized by urea (133 mg kg^{-1} after 62 d), and that of digestate was very similar (113 mg kg^{-1}). Soil treated with compost showed a slower nitrate evolution. A significantly negative correlation was detected between cumulative nitrogen nitrified at the end of the trial, and the values of the C:N ratio of the biomasses used (compost, sludge and digestate) (mg kg^{-1} N-NO$_3^-$ *vs.* C:N, $r = -0.94$, $n = 3$, $p < 0.05$), and between the alkyl-C content at the end of the experiment (mg kg^{-1} N-NO$_3^-$ *vs.* alkyl-C, $r = -0.95$, $n = 3$, $p < 0.05$). As expected, pH decreases and soil respiration (CO_2 evolution) were also well correlated with the content of nitrate. Considering that about 90% of the nitrogen content in the digestate is short acting, the results obtained indicate that the nitrogen rate of mineralization in digestate is very similar to that of urea, confirming that digestate could replace traditional mineral fertilizers.

Key words: digestate / nitrogen / renewable fertilizer / anaerobic digestion / CP MAS ^{13}C NMR

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1 Introduction

Anaerobic digestion (AD) is a biological process that, in the absence of oxygen, allows organic matter degradation to proceed and to produce biogas, a renewable energy source. At the same time, AD leaves a residue called digestate, which is potentially useful as a fertilizer. Digestate fertilizer could offer several benefits, giving greater value to the AD process. Numerous AD plants have been started up in Italy in the last ten years. These plants are concentrated in three regions (Lombardy, Piedmont, and Veneto). Lombardy Region, alone, houses almost half (52.38%) of the total number of pigs bred in Italy (about 4.5 million) (*ERSAF*, 2014). Digestate is characterized by a high content of organic matter (*Tambone* et al., 2009; *Tambone* et al., 2010; *Möller* and *Müller*, 2012) and due to the high content of readily available nitrogen for plants (*Alburquerque* et al., 2012a; *Möller* and *Müller*, 2012), it could be useful to supply nitrogen for open field crops where it is commonly added in the form of mineral fertilizers such as urea (*Šimon* et al., 2015; *Riva* et al., 2016). Currently, in Italy, digestate is not officially recognized as a fertilizer and this poses several problems for its use. This is in spite its fertilizing power and its relative safety, since, in comparison to manure and slurry, digestate is characterized by higher biological stability and lower odour emission (*Orzi* et al., 2010). In recent decades, the application of biomasses to the soil has been encouraged as a positive waste management strategy and as an aid to crop production, in a context where water quality

protection must be considered (*Flavel* and *Murphy*, 2006). There are particular concerns about nitrogen, as shown by the European Nitrate Directive. For this reason, it is important to estimate the real amount of net inorganic nitrogen (N) that may became available for crops from digestate.

The approach to the problem must take into account the different factors, which can control net nitrogen mineralization, such as soil temperature and water content, drying and rewetting events, soil characteristics and, obviously, the actual composition of the organic residues (*Cabrera* et al., 2005). Several studies are available to encourage the use of biomasses as fertilizers (*i.e.*, sewage sludge and compost, for example) (*Adani* and *Tambone*, 2005; *Adani* et al., 2006; *Adani* et al., 2007; *Tambone* et al., 2007), but the results cannot be generalized. This is because the term "biomasses" indicates a variety of organic residues, which can be very different in character (*Tambone* et al., 2010). For example, although the C:N ratio is a typical parameter which is often used to predict organic matter mineralization, some experimenters (*Cabrera* et al., 2005; *Alburquerque* et al., 2012b; *Šimon* et al., 2015) report that organic residues with similar C:N ratios may release different quantities of nitrogen. Probably, this is because the chemical characteristics of both C and N compounds present can also play fundamental roles in the mineralization process.

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The aim of this paper is to evaluate, at laboratory scale, the properties of digestate as a nitrogen fertilizer in comparison to other biomasses: sewage sludge and compost in particular and a typical mineral fertilizer, urea. The study of the different chemical forms of nitrogen evolved was carried out by a laboratory incubation test. During incubation, changes in other parameters related to organic matter mineralization, such as pH and CO_2 evolution, were also measured.

2 Material and methods

2.1 Soil and organic materials: sampling and characterization

The soil used in the experiment (Tab. 1) was a sandy-silty soil (Typic Hapludalf) sampled from the 0 to 20 cm layer of a cultivated field located in Martinengo (Bergamo, North Italy). The soil was air-dried, sieved (2 mm), and stored until needed for the incubation experiment. Soil parameters were determined as follows: soil pH in aqueous solution using a 1:2.5 sample/water ratio, total nitrogen (TKN) by Kjeldahl method, $N-NH_4$ and $N-NO_3$ by following the methods reported by *Faithfull* (2002), and total organic carbon by dichromate methods. For CEC determination, samples were saturated with $BaCl_2$-triethanolamine solution (pH 8.1). Water holding capacity (WHC) was determined by the Stackman box method (*Klute*, 1986). For incubation, the following materials were compared (Tab. 2): a digestate (D), an anaerobically stabilized sewage sludge (SS), a compost (CO) and, as comparison, the fertilizer urea (U), characterized by a high content of nitrogen (460 g kg^{-1}). The digestate sample was obtained from an AD full-scale plant that produces biogas, in which the fed-in biomass was a mix of cow slurry (15,000 Mg y^{-1}) and corn silage (15,000 Mg y^{-1}). The rated power of the plant is 999 kWh with a hydraulic retention time (HRT) of 40 d in one continuously stirred tank reactor (CSTR) operating at mesophilic conditions (37°C). The sewage sludge came from a municipal wastewater treatment plant. Compost, collected from a full-scale plant, was produced by mixing ligno-cellulosic residues *plus* the organic fraction of municipal solid waste (OFMSW) in a 1:2 (v/v) fresh matter ratio. The mixture was composted in a forced aerated pile and, during the process, the pile was watered regularly and turned every 5–6 d for a total of 4 weeks. After that, the compost underwent, in a static pile, a curing phase of 2 months. At the end of the curing phase the compost was ground to pass through a 10-mm mesh. All the plants were located in the Lombardy Region (North Italy).

All samples collected, weighing about 5 kg each, were brought to the laboratory, dried at 40°C for 24 h and then at 105°C for another 24 h (*APHA*, 1992), shredded in a blender, and passed through a 2-mm mesh. The samples were sub-jected to chemical and spectroscopic characterization. Ammonium nitrogen ($N-NH_4$) and total Kjeldahl nitrogen (TKN) were assessed on fresh samples. Dry matter (DM), volatile solids (VS), and total organic carbon (TOC) were determined following procedures reported in the Standard Methods for the Examination of Water and Wastewater (*APHA*, 1992). $N-NH_4$ and TKN were determined by using the analytical method for wastewater sludges (*IRSA CNR*, 1994). Organic N (org-N) was calculated as the difference between TKN and ($N-NH_4 + NO_3$-N). All analyses were performed in triplicate.

The solid-state CP MAS ^{13}C NMR spectra of the samples were acquired at 10 kHz on a Bruker AMX 600 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) using a 4-mm CP-MAS probe. The pulse repetition rate was set at 0.5 s, the contact time at 1 ms, and the number of scans was 3200. The chemical shift scales of CP MAS ^{13}C NMR spectra were referred to tetramethylsilane (δ = 0 ppm). Spectra were elaborated using TOPSPIN 1.3 software (Bruker BioSpin GmbH, Rheinstetten, Germany). This analysis was performed on the three organic materials (CO, SS, D) in order to provide qualitative and quantitative information on their composition. Four types of carbon can be distinguished in the NMR spectrum (Tab. 2): (1) total aliphatic carbon (*Réveillé* et al., 2003; *Pereira* et al., 2005) and proteins (*Dignac* et al., 2000), (2) O-alkyl carbon (*e.g.*, polysaccharides) (*Kögel-Knabner*, 2002) (47–115 ppm), (3) aromatic carbon (*e.g.*, lignin) (*Ussiri* and *Johnson*, 2003) (115–160 ppm), and (4) carbonyl carbon in aliphatic esters, carboxyl groups, and amide carboxyl (160–210 ppm).

2.2 Soil aerobic incubation procedure

Prior to starting the incubation, soil (1000 g dry weight) was weighed into 1000-mL plastic pot containers and deionized water was added to bring moisture to 60% WHC. This value was maintained during the course of the incubation (90 d), on the assumption that these conditions are able to promote organic matter mineralization, minimizing nitrogen losses by the denitrification process (*Drury* et al., 2003; *Šimon* et al., 2015). The experiment was conducted within an incubator at 25°C. To every pot an equivalent of 300 kg ha^{-1} N was added, comparable to a field application for the typical nitrogen fertilization adopted for corn (*Zea mays* L.) in the Lombardy Region (*Riva* et al., 2016) and generally added to the soil at pre-seeding. In particular, considering the organic materials' composition (Tab. 2), the following quantities of DM, expressed as g pot^{-1} (t ha^{-1}), were added to reach the same quantity of nitrogen: 4.49 g pot^{-1} of CO (16.15 t ha^{-1}), 1.71 g pot^{-1} of SS (6.51 t ha^{-1}), 0.53 g pot^{-1} of D (1.91 t ha^{-1}), and 0.18 g pot^{-1} of U (0.65 t ha^{-1}). In the same manner, unamended pots as control (CL) were prepared. All treatments were performed in

Table 1: Characteristics of the soil used in the incubation experiment.

pH	Sand	Silt	Clay	CEC	TOC	TKN	$N-NH_4^+$	$N-NO_3^-$	C/N
		(g kg^{-1} DM)		(cmol$^+$ kg^{-1})		(g kg^{-1} DM)		(mg kg^{-1} DM)	
7.4 ± 0.1	566.7 ± 27.7	362.3 ± 14.1	71.1 ± 8.5	17.1 ± 0.3	14.6 ± 1.2	1.71 ± 0.11	16.4 ± 0.1	19.0 ± 0.2	8.5 ± 0.1

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Table 2: Chemical and spectroscopic (CP MAS ^{13}CNMR) characteristics of the organic matrices used in the experiment.

	DM	VS	TOC	TKN	N-NH$_4^+$	N-NH$_4^+$/TKN	C/N	pH
	(g kg^{-1} FM)		(g kg^{-1} DM)		(g kg^{-1} DM)	(%)		
CO	714.5 ± 2.2	410.1 ± 11.4	237.9 ± 3.6	18.6 ± 0.2	2.4 ± 0.1	13.1	12.8	8.6 ± 0.12
SS	262.5 ± 12.3	604.1 ± 13.2	350.4 ± 14.7	46.1 ± 6.9	7.7 ± 0.2	16.7	7.6	7.6 ± 0.87
D	22.9 ± 1.5	758.6 ± 21.1	440.0 ± 7.9	157.2 ± 9.1	124.0 ± 1.2	78.9	2.8	8.7 ± 1.05
C-type band δ range								
	total aliphatic C (0–47)		O-CH$_3$ or N-alkyl O-alkyl C di-O-alkyl C (47–115)		aromatic C phenol or phenyl ether C (115–160)		carboxyl C keto C (160–210)	
				(ppm)				
CO	19.50		59.44		14.43		6.63	
SS	31.06		45.53		10.57		12.85	
D	45.56		31.98		8.07		14.39	

triplicate to give 15 pots for incubation. Soils' TKN, N-NH$_4$, N-NO$_3$, TOC, and pH were determined for each pot at 0 (immediately after the amending), 3, 7, 10, 16, 23, 30, 37, 48, 62, 76, and 90 d.

Parallel to the aerobic incubation to measure the nitrogen mineralization, a soil respiration test was performed by mixing the soil with the same quantity of CO, SS, D, and U used for the incubation test, as reported above. In this case, the untreated soil (CL) was also tested. The soil respiration was monitored by measuring the CO$_2$ evolution by titrimetric methods (*ISO 16072*, 2002). In brief, the CO$_2$ released by the samples (20 g), closed in a glass pot, was trapped in 0.1 M NaOH and titrated with 0.1 M HCl, using phenolphthalein as indicator after precipitation with 20% BaCl$_2$. The test was conducted in an incubator at 25°C.

2.3 Statistical approach

Biomass chemical analyses were performed in triplicate on samples taken from the 5 kg representative masses. Soil analyses were performed for each replicate pot. Therefore, the standard deviations measured also considered variability due to random differences between the three pots for each treatment. Results obtained were analyzed by ANOVA using Tukey's test to compare mean values and to assess the significance of the differences between mean values. All statistical analyses were carried out using SPSS statistical software (version 16) (SPSS, Chicago, IL).

3 Results

3.1 Soil and biomasses characteristics

The main soil characteristics are shown in Tab. 1. The soil had a pH of 7.4 and the organic carbon and TKN content were 14.6 and 1.71 g kg^{-1} DM, respectively. The soil CEC was 17.1 cmol$^+$ kg^{-1}, a value in agreement with the texture and the organic matter content.

Table 2 shows several differences between the matrices. In particular, as expected, CO shows the lowest content of nitrogen (18.6 g kg^{-1} DM) and the biggest C:N ratio (12.8), showing typical characteristics of amendment (*Tambone* et al., 2009; *Tambone* et al., 2010) able to contribute to the soil carbon balance. In contrast, the nitrogen content for D is the highest (157.2 g kg^{-1} DM) and a quantity of about 80% of it is present as the ammonia form, very similar to that of a mineral fertilizer. The C:N ratio for D was 2.8. The sample of SS shows intermediate characteristics between CO and D.

As a consequence of the degradation of the labile organic molecules during AD (*i.e.*, VFA and O-alkyl C), the D spectrum (Tab. 2) is dominated by NMR signals in the 0–47 ppm and 130–160 ppm regions. This means that there had been an accumulation of the more recalcitrant fractions (plant biopolymers, steroid-like molecules, and lignin) contained in the starting organic mixtures subjected to the AD (*Tambone* et al., 2009). Sewage sludge showed a macromolecular composition similar to that of D. The presence of recalcitrant biogenic molecules such as steroids from human faeces (*Réveillé* et al., 2003) and those of non-biogenic origin in the domestic wastewaters (*Schnaak* et al., 1997) were probably responsible for the high alkyl-C content in SS (see 0–47 ppm region) (Tab. 2). The compost showed macromolecular composition different from the other organic materials, *i.e.*, higher O-alkyl-C and aryl-C contents, and lower aliphatic-C content (Tab. 2). Probably, lignocellulosic materials used in the mix as bulking agents led to the higher lignin-like and O-alkyl-Cs contents in the final product (*Ussiri* and *Johnson*, 2003) compared with both D and SS.

3.2 Nitrogen, Total Organic Carbon, C:N ratio and pH evolution

In order to assess the goodness of the analysis, and the possible losses of N-NH$_4$ during the preparation of the pots for incubation and the storage of the samples, its content measured at t = 0 was compared with the N-NH$_4$ calculated, considering the amount added by the biomass and the am-

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monia already present in the soil. The measured values were generally lower (Tab. 4) and this may be due to some volatilization of nitrogen as ammonia during the preparation of the microcosms and their manipulation in order to well homogenize soil and biomass.
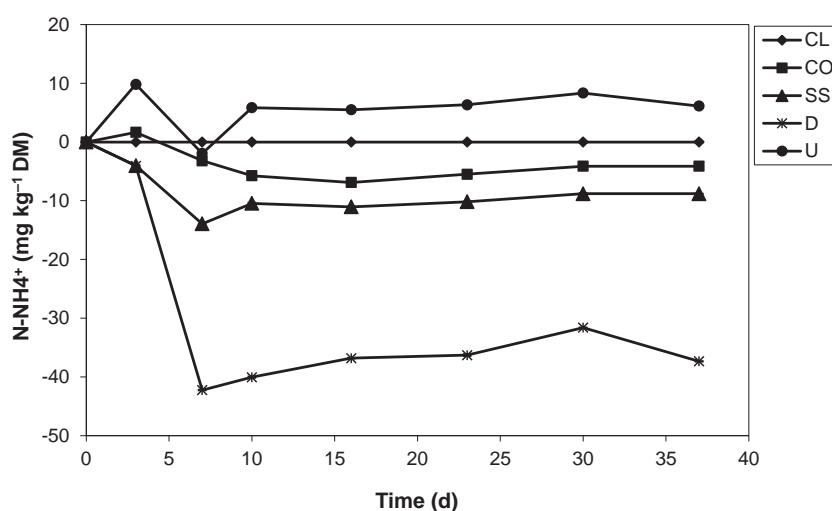
Table 3 reports the soil TKN values detected during incubation for the different treatments. The TKN content was subject to minimal variation caused by ammonia volatilization or denitrification processes (*Šimon* et al., 2015). More interesting was the evolution of the different chemical forms of nitrogen, in particular N-NH$_4$ and N-NO$_3$ during incubation (Tab. 4).

N-NH$_4$ content at time 0 (Tab. 4) reflected the nitrogen contained in this chemical form in the biomasses added and, for this reason, the concentrations followed this trend: D > SS > CO > CL ≅ U. Soil treated only with mineral nitrogen (U) showed a low concentration of N-NH$_4$, similar to that of the control. Subsequently, N-NH$_4$ concentration decreased rapidly in all the treatments (Tab. 4) and, after 30 d, it was detectable only for soils treated with D and U, in which the concentrations were higher from the beginning.

Figure 1 shows the rapid decrease of N-NH$_4$ in soil treated with D in comparison to the other treatments (Tab. 4). A similar trend was shown in the treatment with SS, although with different values. After 7–10 d from the beginning of the test, the N-NH$_4$ evolution trend increased for all the treatments. The only treatment that had a trend greater than of the control (which has values equal to 0, because it is used as a reference in the normalization) is the sample with urea because of the lower content of ammonium at t = 0 (compared to the control), followed immediately by an increase due to the release of N-NH$_4$ by urea. The compost showed a mineralization of organic nitrogen, which was very limited.

Figure 1: Normalized ammonia content derived from fertilizers over time [see Eq. (1) in the text].

Nevertheless, considering N-NO$_3^-$ concentrations (Tab. 4) and soils' pH values (7.4), rapid nitrification represents the most probable fate of N-NH$_4$ contained in the incubated soils, taking into account that the optimum pH ranges for nitrification are 6.6–8 (*Calderon* et al., 2005; *Huang* and *Chen*, 2009), very similar to those of the pH of the soil used for the incubation (Tab. 1).

Table 4 reports the concentration of NO$_3$ during the incubation period. At t = 0 the values were similar for all the treatments, but after only 3 d the concentration went up. In particular, as expected, the NO$_3$ content was lower for the control (maximum value 50.22 mg kg^{-1} at t = 48). Between the amended or fertilized treatments, the soil with compost added showed the slowest trend very similar to that of the control. The maximum concentration of nitrate reached in the microcosm fertilized by urea was about 133 mg kg^{-1} after 62 d of incubation and decreased to around 114 mg kg^{-1} at the end of the test. The treatment with digestate had a development over time which was very similar to that of urea, and the maximum concentration (around 113 mg kg^{-1}) was not statistically different from that of urea (Tab. 4).

As expected, higher values of organic carbon characterized the treatments with D, CO, and SS, compared to the control and the treatment fertilized with urea (Tab. 5). In particular, the samples with compost showed the highest level of organic carbon, considering that they had received a larger amount of biomass, due to the low nitrogen content in the compost, in order to reach the adequate fertilizer value (300 kg ha^{-1} of N).

Table 3: TKN concentration in the soil during the incubation.

days	CL	CO	SS	D	U
			(mg kg^{-1} DM)		
t = 0	1650 ± 22	1850 ± 85	2010 ± 71	1740 ± 57	1930 ± 71
t = 3	1650 ± 31	1760 ± 65	1520 ± 41	1610 ± 68	1610 ± 63
t = 10	1710 ± 58	1720 ± 14	1690 ± 33	1800 ± 47	1890 ± 13
t = 16	1610 ± 101	1710 ± 19	1750 ± 97	1750 ± 19	1780 ± 41
t = 30	1690 ± 77	1680 ± 39	1830 ± 48	1760 ± 27	1680 ± 92
t = 48	1700 ± 31	1710 ± 27	1740 ± 94	1780 ± 62	1760 ± 101
t = 90	1700 ± 23	1750 ± 88	1710 ± 11	1740 ± 57	1820 ± 31
Δ (%)	+3.0	−5.4	−14.9	0.0	−5.7

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Table 4: N-NO$_3^-$ and N-NH$_4^+$ concentration in the soil during the incubation.

days		CL	CO	SS	D	U
				(mg kg^{-1} DM)		
t = 0	N-NO$_3^-$	19.02 ± 2.50a[a]	22.16 ± 2.92a	22.02 ± 1.62a	16.16 ± 0.88a	14.64 ± 3.23a
	N-NH$_4^+$	16.45 ± 0.93ab[a] (16.4)[b]	20.72 ± 2.39ab (27.18)	25.40 ± 3.15b (29.56)	53.94 ± 7.34c (82.12)	14.46 ± 0.53a (16.40)
t = 3	N-NO$_3^-$	22.88 ± 1.37a	38.08 ± 6.73b	32.19 ± 3.82ab	36.59 ± 4.42b	22.84 ± 6.43ab
	N-NH$_4^+$	13.79 ± 1.93a	19.56 ± 3.20a	18.58 ± 4.48a	47.08 ± 10.87b	13.45 ± 4.11a
t = 7	N-NO$_3^-$	31.35 ± 5.53a	39.61 ± 8.25a	30.09 ± 1.57a	68.49 ± 6.28b	24.20 ± 3.72a
	N-NH$_4^+$	8.95 ± 0.90c	9.89 ± 0.94c	3.82 ± 1.63b	4.03 ± 0.23b	0.89 ± 0.16a
t = 10	N-NO$_3^-$	32.18 ± 4.28a	39.75 ± 2.99a	38.69 ± 2.09a	77.75 ± 10.55b	90.46 ± 7.07c
	N-NH$_4^+$	4.28 ± 0.08b	2.66 ± 0.28a	2.63 ± 0.38a	1.57 ± 0.87a	4.01 ± 0.43b
t = 16	N-NO$_3^-$	32.29 ± 12.46a	46.51 ± 3.73a	44.81 ± 10.21a	93.96 ± 17.56b	102.36 ± 5.72c
	N-NH$_4^+$	2.77 ± 0.08a	n.d.[c]	0.52 ± 0.73a	3.30 ± 1.25a	2.14 ± 0.42a
t = 23	N-NO$_3^-$	44.76 ± 6.23a	40.16 ± 2.69a	46.82 ± 15.92bc	93.35 ± 12.20b	106.63 ± 28.33c
	N-NH$_4^+$	1.37 ± 1.21ab	n.d.	n.d.	2.42 ± 0.24b	1.59 ± 0.28ab
t = 30	N-NO$_3^-$	38.61 ± 4.05a	46.21 ± 15.65a	61.35 ± 7.67a	90.17 ± 3.32ab	97.75 ± 5.19b
	N-NH$_4^+$	n.d.	n.d.	n.d.	5.72 ± 2.42b	2.22 ± 0.17a
t = 37	N-NO$_3^-$	42.02 ± 18.98ab	51.81 ± 3.79a	60.34 ± 14.31ab	107.21 ± 25.99b	122.51 ± 12.87c
	N-NH$_4^+$	n.d.	n.d.	n.d.	n.d.	n.d.
t = 48	N-NO$_3^-$	50.22 ± 3.82a	53.23 ± 16.24a	73.24 ± 5.86b	99.37 ± 10.30b	120.15 ± 16.48c
	N-NH$_4^+$	n.d.	n.d.	n.d.	n.d.	n.d.
t = 62	N-NO$_3^-$	47.77 ± 6.51ab	60.98 ± 6.94a	65.90 ± 2.66a	99.05 ± 5.79b	132.80 ± 10.42c
	N-NH$_4^+$	n.d.	n.d.	n.d.	n.d.	n.d.
t = 76	N-NO$_3^-$	44.41 ± 7.68a	57.45 ± 7.09a	73.27 ± 6.07a	113.28 ± 7.53b	129.93 ± 30.72c
	N-NH$_4^+$	n.d.	n.d.	n.d.	n.d.	n.d.
t = 90	N-NO$_3^-$	35.92 ± 10.24a	64.51 ± 3.05b	76.02 ± 12.09b	114.71 ± 13.26c	113.81 ± 7.60d
	N-NH$_4^+$	n.d.	n.d.	n.d.	n.d.	n.d.
Δ (%)	N-NO$_3^-$	47.05	65.65	71.03	85.91	87.14

[a]In the same column, values for the same parameter followed by the same letter are not statistically different (*p* < 0.05) according to Tukey's test.
[b]Theoretical value data calculated by considering biomasses N-NH$_4^+$ content (Tab. 1 for CL, Tab. 2 for fertilizers) and total biomass dosed (DM) (see section 2).
[c]n.d.: not detectable.

Figure 3 reports the evolution of pH during the test. Data show that the biomass added to the soil influenced soil pH (7.45). In particular, the treatment with digestate was statistically higher (7.84) than the others at t = 0 due to the high pH value of the digestate (8.71). During the test, a decrease in pH occurred for all soils fertilized.

3.3 Soil respiration

The dynamics of soil organic matter degradation in the incubated containers over time was studied by measuring the amount of carbon dioxide evolved during a respiration test. Tab. 6 reports the data expressed as mg CO_2 g^{-1} referred to

Table 5: Organic carbonic concentration in the soil during the incubation.

days	CL	CO	SS	D	U
			(mg kg^{-1} DM) or (g C pot^{-1})		
t = 0	14.58 ± 0.68a*	15.70 ± 1.11b	15.04 ± 0.03b	15.08 ± 0.00b	14.44 ± 0.45b
t = 3	14.31 ± 0.45a	15.77 ± 0.37c	15.07 ± 0.08bc	14.76 ± 0.25ab	14.89 ± 0.20ab
t = 10	15.12 ± 1.39a	16.38 ± 0.24a	15.55 ± 0.22a	15.27 ± 0.55a	15.27 ± 0.86a
t = 16	14.95 ± 0.08a	15.99 ± 0.66a	15.02 ± 0.98a	16.10 ± 0.13a	14.69 ± 0.14a
t = 30	13.63 ± 0.01a	15.56 ± 0.12c	14.32 ± 0.30b	14.25 ± 0.41b	14.04 ± 0.05ab
t = 48	14.90 ± 0.26a	15.06 ± 0.40a	14.36 ± 0.31a	14.45 ± 0.39a	14.78 ± 0.29a
t = 90	13.56 ± 1.36a	15.43 ± 0.31a	14.44 ± 0.58a	14.21 ± 0.39a	14.64 ± 0.37a

[a]In the same column, values followed by the same letter are not statistically different ($p < 0.05$) according to Tukey's test.
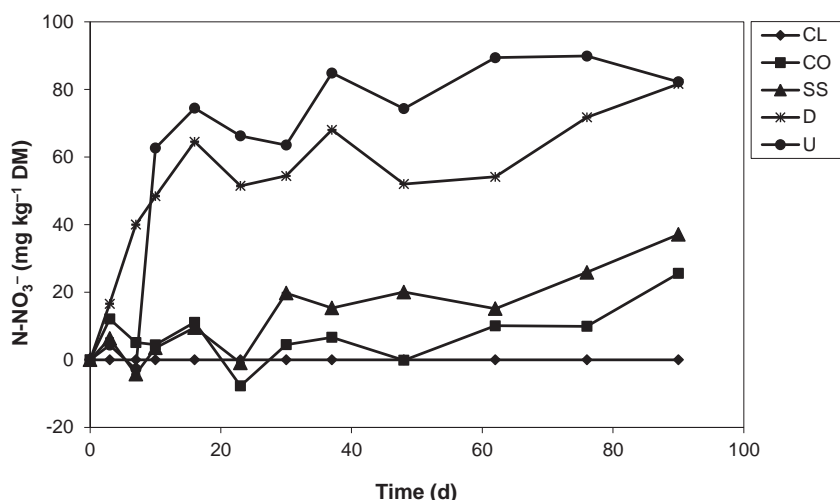
Figure 2: Normalized nitrate content derived from fertilizers over time [see Eq. (2) in the text].
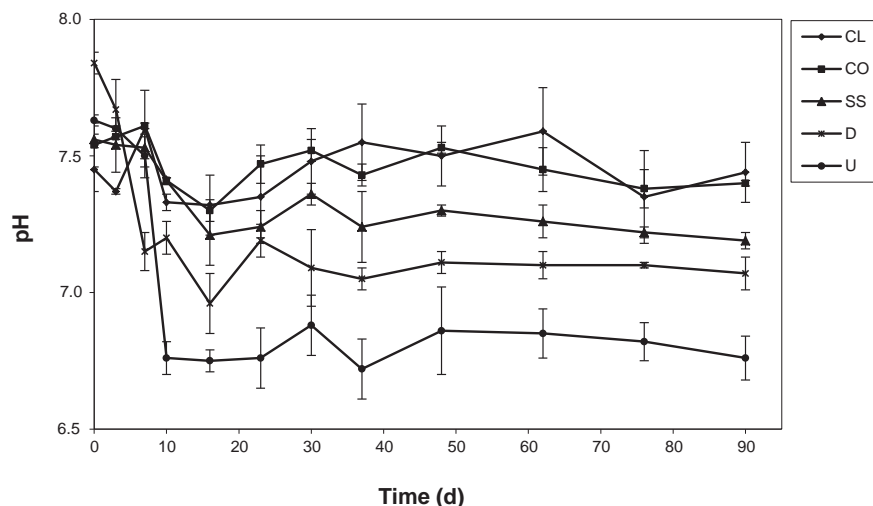
Figure 3: pH variation during the incubation.

the organic carbon present, representing the sum of the carbon in the soil control and the quantity added by the different biomasses. As expected, the lower concentration of CO_2 evolved from the control, representing the basal soil respiration. The treatment with urea showed a rapid increase in the early days but later settled on values lower than those of the other fertilized treatments, although higher than that of the control (Tab. 6).

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Table 6: Soil carbon respiration.

	CL	CO	SS	D	U
			(mg CO_2 g^{-1} TOC)		
t = 3	$15.09 \pm 2,10a$[a]	$17.52 \pm 4.05ab$	$24.39 \pm 3.66ab$	$30.38 \pm 2.11b$	$27.94 \pm 9.59ab$
t = 7	$25.14 \pm 8.71a$	$30.36 \pm 4.05ab$	$39.02 \pm 4.22ab$	$44.97 \pm 3.65b$	$35.55 \pm 10.08ab$
t = 10	$54.69 \pm 10.89a$	$62.48 \pm 3.50ab$	$79.86 \pm 3.66b$	$79.60 \pm 6.32b$	$64.12 \pm 13.73ab$
t = 16	$78.57 \pm 10.89a$	$89.34 \pm 4.05ab$	$107.91 \pm 5.59b$	$111.20 \pm 7.59b$	$86.98 \pm 15.40ab$
t = 23	$99.95 \pm 6.53a$	$106.85 \pm 3.50a$	$131.07 \pm 4.22b$	$135.51 \pm 5.57b$	$107.30 \pm 15.40a$
t = 30	$121.95 \pm 8.71a$	$127.29 \pm 5.35a$	$154.85 \pm 7.61b$	$154.35 \pm 5.57b$	$121.90 \pm 15.24a$
t = 37	$148.35 \pm 7.85a$	$149.48 \pm 6.07a$	$180.46 \pm 5.59b$	$179.87 \pm 5.57b$	$142.22 \pm 19.17a$
t = 48	$181.03 \pm 3.77a$	$178.67 \pm 3.50a$	$208.50 \pm 3.66b$	$207.82 \pm 9.65b$	$172.69 \pm 20.98a$
t = 76	$213.09 \pm 4.35ab$	$209.62 \pm 4.05ab$	$239.59 \pm 4.22b$	$240.03 \pm 12.80b$	$203.80 \pm 20.98a$
t = 90	$247.66 \pm 5.76ab$	$241.74 \pm 6.07ab$	$270.68 \pm 6.34b$	$267.37 \pm 11.14b$	$234.91 \pm 20.98a$

[a]In the same column, values followed by the same letter are not statistically different ($p < 0.05$) according to Tukey's test.

4 Discussion

4.1 Nitrogen evolution

Data reported in Tab. 4 show that N-NH$_4$ concentration decreased rapidly in all the treatments and, only for soil treated with D and U, already reached at time zero of N-NH$_4$, it was detectable after 30 d. This trend agrees with those of other experiments and it is independent of soil characteristics and biomasses added (*Huang* and *Chen*, 2009; *Alburquerque* et al., 2012a). N-NH$_4$ evolution can be explained by considering that different mechanisms contribute to a rapid transformation of ammonia into the nitrate form (nitrification) by soil microorganisms or to a direct volatilization as NH$_3$ (*Soaud* et al., 2011).

As proposed by *Griffin* et al. (2004), to better understand N-NH$_4$ evolution from biomasses or fertilizer added, the values reported in Tab. 4 were elaborated to calculate the quantity of ammonia (N-NH$_{4\,fert}^{+}$), originating during the time of incubation, by using the following formula:

$$N - NH_{4\,fert}^{+}(mg\ kg^{-1}soil) = \left\{ [NH_4^+]_t - [NH_4^+]_{t=0} \right\}_{fert}$$
$$- \left\{ [NH_4^+]_t - [NH_4^+]_{t=0} \right\}_{test}. \quad (1)$$

Figure 1 reports the trend of N-NH$_{4fert}$ during incubation. These values consider both the variation in the concentration at the beginning (t = 0) and at the different times, all normalized for N-NH$_4$ produced in the control (CL). In this way, the initial content of ammonia is not considered and it is possible to focus attention on the variation in N-NH$_4$ which originated from the biomasses or the fertilizer added. The rapid decrease of N-NH$_4$ in soil treated with D (Fig. 1) is probably due to the high content of soluble N-NH$_4$ at time zero (Tab. 4) and the successive N-NH$_4$ evolution trend for all the treatments is

probably a consequence of organic matter degradation. For the compost, a mineralization of organic nitrogen, which is very limited, can be assumed as the consequence of its high biological stability.

Nevertheless, considering N-NO$_3^-$ concentrations (Tab. 4) and soils' pH values (7.4), the rapid nitrification represents the most probable fate of N-NH$_4$ contained in the incubated soils, taking into account that the optimum pH ranges for nitrification are 6.6–8 (*Calderon* et al., 2005; *Huang* and *Chen*, 2009), very similar to the pH of the soil used for the incubation (Tab. 1).

At time zero, the concentration of NO$_3^-$ was similar for all the treatments (Tab. 4), but, after only 3 d, the concentrations went up and the soil amended with compost showed a trend very similar to that of the control. In this respect, we need to emphasize that the nitrogen contained in the compost, because it is in a stable form and fixed in the organic component, is characterized by slow rates of mineralization with the result that the release will be inadequate to supply crop needs, but also, since it is uncontrolled, makes the element available over a time period that will be very difficult to predict.

The ammonium decrease could be caused both by nitrification and by mechanisms of immobilization by microorganisms (*Bar-Tal* et al., 2004). This fact is nevertheless correlated to the C:N ratio that, during the incubation period, assumes values that promote the nitrification mechanism. The soil sample enriched with urea showed the greatest levels of concentration of nitrate (Tab. 4) after an initial lag phase of 7 d, probably due to the activation of the process of nitrogen transformation at first from urea to ammonia and afterwards from ammonia into nitrate. In the same way, it is interesting to note that the presence of digestate led to a high value of nitrate after only 7 d from the beginning, an index of a rapid nitrification pro-

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cess (Tab. 4). This result, similar for digestate and urea, suggests that nitrogen in digestate, both because of the chemical form in which it occurs as well as because of the mechanism by which it is released, was comparable to that of a nitrogenous mineral fertilizer.

For both the ammonia and nitric nitrogen, the concentration values reported in Tab. 4 were elaborated in accordance with *Griffin* et al. (2004), to reach values of nitrified net accumulated nitrogen, according to the formula:

$$N - NO_3^-{}_{cum}(mg\ kg^{-1}soil) = \left\{ [NO_3^-]_t - [NO_3^-]_{t=0} \right\}_{fert}$$
$$- \left\{ [NO_3^-]_t - [NO_3^-]_{t=0} \right\}_{test}.$$
(2)

Figure 2 shows the trend of the new variable N-NO$_3^-{}_{cum}$ during the test.

The new variable takes into account the changes in concentration between the initial time (t = 0) and any subsequent time t_n, all normalized to N-NO$_3^-{}_{cum}$ evolved in the control. In this way, it does not consider either the initial content of nitrate in the soil or the content of nitrate that originates in the control during the test, in this way focusing attention only on the changes in concentration of N-NO$_3^-{}_{cum}$ due to biomass or fertilizers added (*Griffin* et al., 2004).

The trend of the nitrification process in the treatment with digestate was faster than the other ones, probably because of the greater presence of soluble N-NH$_4$ present in the biomass added and, consequently, into the soil. The microcosms with urea had a longer initial lag phase (7 d), but later, they showed a greater amount of nitrate. Figure 2 shows a limited nitrification in microcosms with compost, confirming what was said before.

4.2 Total Organic Carbon and C:N ratio evolution

To reach the same quantity of nitrogen in the pots, corresponding to 300 kg ha^{-1}, the samples of soil necessarily received different quantities of carbon, and, in particular, the one treated with compost received the largest amount of biomass. Organic matter added to the soil undergoes the attack of microorganisms that degrade the less recalcitrant compounds producing CO$_2$. The values of TOC should therefore remain constant if the degradation is inhibited or organic matter is highly stable. Net variations of TOC do not occur, although there is a general tendency to decrease during the test incubation (Tab. 5). There may also be random errors or lack of homogeneity in the collection of soil from the microcosms to consider.

The C:N ratio is significant when describing the dynamics of mineralization of organic matter in soil (*Sikora* and *Szmidt*, 2001). Generally, low values of the C:N ratio promote microorganism activity, and observation of the C:N ratio can be useful in assessing any loss of carbon or nitrogen from the system. Generally, if the C:N ratio is less than 8, release of carbon and nitrogen are favored (*Cabrera* et al., 2005). Values be-

tween 8 and 12 lead to a substantial balance between the reactions of mineralization and conservation. In our study, at t = 0, as expected, there was evidently a higher value of C:N for the treatment with compost: this was due to the larger amount of organic carbon brought in with this biomass (1.067 g kg^{-1}), greater than for the other treatments. However, the value of C:N detected for compost (12.82) was not very high, making it possible to speculate that there was very slow mineralization. The consequence is a very slow nitrogen release (Fig. 2) from compost. For the other biomasses at t = 0, the value of the C:N ratio was quite low (8–8.5) and it was therefore more favorable to organic matter mineralization. A significantly negative correlation was detected between cumulative nitrogen nitrified at the end of the trial and the values of the C:N ratio of the biomass used (compost, sludge and digestate) (mg kg^{-1} N-NO$_3^-$ *vs.* C:N, $r = -0.94$, $n = 3$, $p < 0.05$). In general, during the test no C:N ratio variation occurred for the different treatments. Considering the N mineralization from the three biomasses and according to the literature (*Rowell* et al., 2001), another interesting negative correlation was that between the alkyl-C content (Tab. 2) and the N-NO$_3^-$ concentration (Tab. 4) at the end of the experiment (mg kg^{-1} N-NO$_3^-$ *vs.* alkyl-C, $r = -0.95$, $n = 3$, $p < 0.05$).

4.3 pH evolution

Decreases in the pH values during the incubation period (Fig. 3) are probably due to the accumulation of nitrate and the simultaneous loss of ammonium (*Huang* and *Chen*, 2009). This was shown by the fact that the values of pH followed the content of N-NO$_3^-$ evolved (U > D > SS > CO $\cong$ CL). The trends of pH values were closely linked to the forms of nitrogen present, especially for the treatments with a higher concentration of this (digestate and urea). This result is demonstrated by a high correlation between values for pH and content of nitrate (pH *vs.* N-NO$_3^-$, $r = -0.81$, $n = 12$; $p < 0.05$, for SS; $r = -0.92$, $n = 12$, $p < 0.05$, for D; $r = -0.94$, $n = 12$, $p < 0.05$, for U).

Nitrate content was correlated in part with the amount of the element already present at t = 0, but a decisive contribution came from the ammonia which was transformed into nitrate and, finally, we can also assume that a portion of organic nitrogen was mineralized and transformed into nitrate. In order to quantify the contribution of the organic nitrogen, the soils' respiration was considered to estimate the rate of mineralization.

4.4 Soil respiration

Data previously discussed suggest that the dynamic of soil organic matter, as expected, plays a key role in the nitrogen release from the different biomasses tested. In this context, the measure of the basal soil respiration (data reported in Tab. 6 for soil CL), was compared, in terms of mg CO$_2$ g^{-1} referred to the organic carbon present, between the different microcosms over time. In the treatment with urea, the nitrogen added probably promoted the mineralization of the native organic matter present in the soil (priming effect). SS and D had very similar trends, but it was slightly higher for the digestate.

Most likely, the organic carbon added by SS (0.633 g kg⁻¹ DM) and D (0.233 g kg⁻¹ DM), and especially its composition, led to maintaining higher biological activity (*Alburquerque* et al., 2012b). The compost, despite representing the treatment with the greatest amount of carbon added (1.067 g kg⁻¹ DM), showed a respiration rate which was lower and similar to those of control and urea. In this regard, as amply demonstrated in the literature (*Swift* et al., 1979; *Parnaudeau* et al., 2004), the mineralization processes depend more than the amount of organic carbon on its macromolecular composition. As is well known, the composting process leads to the degradation of the more easily fermentable organic fractions with the consequent concentration of slowly degradable compounds (*i.e.*, cellulose and lignin). Characterization of biomasses through CPMAS NMR spectroscopy (Tab. 2) can better clarify these dynamics.

Data reported in Tab. 6 show that the respiration rate was greater for the treatments with digestate and sewage sludge, while it was lower for control, urea, and compost, and these differences became more pronounced during the test and allowed us to distinguish two groups of samples: CL, U, and CO on one side, SS and D on the other. This means that the respiration rate could represent the differences assessed by CPMAS NMR analysis in the macromolecular composition. Although with the compost a greater amount of carbon was added, this was characterized by complex carbohydrates, typically cellulose, and ligno-humic compounds (Tab. 2), while, in contrast, more easily degradable organic compounds characterized digestate and sewage sludge, in agreement, for digestate, with *Alburquerque* et al. (2012a), for a similar incubation and method used. The respiration of the carbon contained in the control was similar to that of the compost because, probably, the soil had not received recent additions and stable organic compounds characterized it. This means that the different characteristics in the quality of the organic matter added affected, more than the quantity, the degradation rate.

In particular, NMR spectroscopy (Tab. 2) showed the different composition of the carbon compounds present: digestate and sewage sludge had a higher content of aliphatic-C (45.56 and 31.06%, respectively) and aromatic-C content (8.07 and 10.57%, respectively) was less than that of compost (Tab. 2). This resulted in a more rapid degradation by microorganisms of aliphatic molecules, such as short chain fatty acids, sterols and proteins, and then a greater rate of degradability than for the most recalcitrant compounds (lignin, phenols, and tannins).

This was confirmed by the positive correlation ($r = 0.89$, $n = 3$, $p < 0.05$) between the values of CO_2 accumulated at the end of the test (t = 90) (Tab. 6) and the relative concentrations of the portion of the spectrum between 0 and 47 ppm, representing the aliphatic compounds. There was also a positive correlation with the region of the carbon of the carboxyl groups ($r = 0.99$, $n = 3$; $p < 0.05$) that were present in low molecular weight molecules such as fatty acids and nitrogen compounds which were rapidly degradable. Otherwise, there was a negative correlation with the concentration of aromatic carbon and carbohydrates, in particular cellulose (respectively, $r = -0.96$, $n = 3$, $p < 0.05$; $r = -0.92$, $n = 3$, $p < 0.05$), due to the greater resistance to microbial degradation of these compounds given by their more complex macromolecular structure.

4.5 Nitrogen balance

Based on the concentrations of nitrate detected (Tab. 4) and on the basis of previous observations about the degradability of the carbon, the rate of mineralization of organic matter was calculated. The aim was to assess the relationship between the concentrations of nitrate measured, distinguish the fraction already present, and which resulted from the processes of ammonification and nitrification. Table 7 shows the values of the nitrogen balance for the treatments fertilized. In order to verify the accuracy of the measures, a comparison was made between the data of N-NO$_3$ measured and data obtained through the balance between the different forms of nitrogen (Tab. 7). Assuming that the concentrations of N-NO$_3$ follow an asymptotic curve and then, after a certain time from the start of the test, reach a constant value, a single value of N-NO$_3$ was considered for each treatment obtained from the average of the values of N-NO$_3$ measured from t = 10 to t = 90 (end of the test). Furthermore, it is assumed that the theoretical content of N-NO$_3$ is the result of the sum of three compo-

Table 7: Nitrogen balance.

	N-NO$_3^-$ detected	N org added	Mineralization rate	N-NO$_3^-$ produced			N-NO$_3^-$ Σ	Δ
				N-NO$_3^-$ t$_0$	*N-NH$_4^+$* t$_0$	N org		
	(mg kg⁻¹)		(%)	(mg kg⁻¹ DM)				
CO	51.18	75.59	5.93	22.16	20.72	4.48	47.37	+3.81
SS	60.05	71.05	19.09	22.02	25.40	13.57	60.99	−0.94
D	98.76	48.37	55.04	16.16	53.94	26.62	96.72	2.04
U	112.93	93.35	100[a]	14.64	10.48	93.35	118.47	−5.54

[a]It is assumed that all the nitrogen introduced with the urea is mineralized.

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nents: nitrate content at t = 0 (no losses due to denitrification), ammonium content at t = 0 (entirely evolved in nitrate) and organic nitrogen transformed, first as N-NH$_4$, and then as nitrate. To calculate the coefficient of mineralization, we used the data of CO$_2$ evolved (Tab. 6) compared to the organic carbon added with the different fertilizers. The coefficient of mineralization thus obtained allowed us to calculate the proportion of organic N mineralized and then nitrified. As reported in Tab. 7, there is a good correspondence between the measured and calculated values, highlighting that there were no losses of nitrogen from the microcosms.

Data reported in Tab. 7 show that the higher quantity of organic nitrogen was added by compost and sewage sludge, while the lower quantity occurred with digestate (78.8% of N-NH$_4^+$). For the treatment with urea, nitrogen was all added in inorganic form, therefore all transformable into N-NH$_4$ (mineralization coefficient assumed equal to 100%). For the other treatments the values of this coefficient were, in ascending order, CL < CO < SS < D. In particular, for the digestate the high coefficient of mineralization (> 50% in 90 d) and the low content of organic N led to this treatment giving a ready fertilizing effect, reducing the long term release and the potential loss of nitrate. When the nitrogen contained in a biomass is present, above all, in organic form, it is available to plants only after mineralization over a period which is not easily predictable. If we correlate the values of soil respiration (CO$_2$ evolution accumulated at t = 90) with the content of N-NO$_3^-$ measured in different treatments the results are very interesting: mg CO$_2$ g^{-1} dry soil *vs.* mg kg^{-1} N-NO$_3^-$, r = 0.94, 0.93, 0.93, 0.98, 0.93; n = 11; p < 0.05 for CL, CO, SS, D, and U, respectively. As shown by *Calderon* et al. (2005) and also in our work, the evolution of CO$_2$ is positively correlated (mg CO$_2$ g^{-1} dry soil *vs.* mg kg-N- NH$_4^+$, r = 0.80, n = 5, p < 0.05) to the initial content of N-NH$_4$, stimulating, at the same time, soil microbial activity for nitrification and respiration.

5 Conclusions

The experiment using incubation, which was conducted, showed that the mineralization of nitrogen in digestate is very similar in terms of both quantity and quality to that of urea. For this reason we can assume similar suitability for digestate to be used in open fields for crops to replace traditional mineral fertilizers. The high content of nitrogen in ammonia form in digestate and the high rate of mineralization of organic matter lead to the conclusion that about 90% of the total nitrogen content in the digestate is short acting. The quota of organic nitrogen that remains in the soil, susceptible to mineralization which is not controlled, and therefore potentially leached in the absence of uptake by plants, can be assumed to have a limited impact on the leaching of nitrates into groundwater. In this regard further studies are necessary to assess the dynamics of mineralization and the evolution of nitrogen forms on time scales greater than those considered in this work, even in most agricultural years, and in real environmental conditions (open fields). Given the heterogeneity that characterizes this type of biomass, it is still essential to carry out a characterization of digestate in order to meet the needs of crops in relation to different climatic conditions. Additional processes of separation by centrifugation or sedimentation could

help to improve the fertilizing properties of digestate and facilitate management. In addition, an improvement of the distribution system is necessary to limit the losses by volatilization of NH$_3$ by immediate injection. Identifying digestate as a "real" fertilizer (*i.e.*, a renewable fertilizer) could allow this material to exit from the current limited horizon for its use, promoting actions leading to the adoption of this resource on a larger territorial scale, and recognizing its potential contribution to major environmental protection, thus, meeting the concept of sustainable agriculture.

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