N dynamics during a 3-year crop rotation fertilized with digestates and cattle effluents

Victor Moinard[1], Antoine Savoie[2], Catherine Pasquier[3], Adeline Besnault[3], Yolaine Goubard-Delaunay[1], Baptiste Esnault[1], Marco Carozzi[1,4,5], Polina Voylokov[1,4], Sophie Génermont[1], Benjamin Loubet[1], Catherine Hénault[3,6], Florent Levavasseur[1], Jean-Marie Paillat[7,8], Sabine Houot[1]

[1]INRAE, AgroParisTech, Université Paris-Saclay, UMR ECOSYS, Palaiseau, 91120, France
[2]INRAE, UE PAO, Nouzilly, 37380, France
[3]INRAE, UR SOLS, Orléans, 45075, France
[4]INRAE, BU EnVisAGES, Palaiseau, 91120, France
[5]INRAE, AgroParisTech, Université Paris-Saclay, UMR SAD-APT, Palaiseau, 91120, France
[6]Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France
[7]CIRAD, UPR Recyclage et risque, Montpellier, 34398, France
[8]ISTOM, UPR ADI-Suds, Angers, 49000, France

Correspondence to: Florent Levavasseur (florent.levavasseur@inrae.fr)

Abstract. On-farm anaerobic digestion is used as a means of producing biogas, with the resulting digestates serving as organic fertilizers. However, such digestates have different fertilizer properties than undigested animal effluents, and are associated with different degrees of N loss. We conducted a field experiment in which cattle slurry and farmyard manure were co-digested with urban and agro-industrial wastes, which represented slightly more than two-thirds of the total. We managed a three-year crop succession (wheat - rapeseed - wheat) with five fertilization systems: no fertilization, mineral fertilizers, cattle manure and slurry, raw digestate, or separated solid and liquid digestates. An exogenous organic matter (EOM) (cattle slurry, liquid or raw digestates) or mineral fertilizer was applied five times in winter and spring. A different type of EOM (cattle manure, solid or raw digestates) was applied twice in summer. After each fertilizer application, we measured ammonia volatilization and N_2O emissions, along with crop N uptake and soil mineral N stocks. Across the three-year rotation, the NH_3 volatilization rate was the lowest in the plot treated with mineral fertilizers (2% of applied total N, TN), followed by cattle effluents (7% TN), liquid and solid digestates (9% TN), and raw digestate (18% TN). Seasonal cumulative N_2O emissions were similar between mineral fertilizers and digestates, and were lower with cattle effluent, mainly because of lower ammoniacal N inputs. Compared to unfertilized crops, the surplus crop N uptake strongly reflected the mineral N content of fertilizers, ammonia volatilization, and the decomposition of EOM in the soil. Liquid digestate and cattle slurry had similar N use efficiencies (37% to 60% depending on the cropping season), while values for raw digestate were lower (25 to 41%), likely due to its larger NH_3 volatilization. Overall, digestates served as an effective N fertilizer but require particular attention to NH3 volatilization.

1 Introduction

Anaerobic digestion (AD) is a process in which organic substrates are degraded by microorganisms under anaerobic conditions to produce biogas, which is then used to produce energy. Various organic substrates can serve as input, such as animal effluents, biowastes, sewage sludge, or energy crops. The residual degraded matter, called digestate, can then be used in agriculture as an organic fertilizer. AD is of particular interest for its potential to produce renewable energy and contribute to mitigate climate change (Cong et al., 2017; Hijazi et al., 2016; Lyng et al., 2018). It can also be used as a biological treatment for household and restaurant biowastes to promote nutrient recycling (Klinglmair and Thomsen, 2020; Moinard et al., 2021). For all these reasons, the use of AD is growing worldwide, particularly in Europe (Scarlat et al., 2018; Stürmer et al., 2020).

Most of the AD plants in Europe are located on farms (European Biogas Association, 2019), where the main inputs are animal effluents, which can be co-digested with other external wastes or agricultural products. The raw digestates produced are often liquid products, similar to classical slurries (Beggio et al., 2019; Guilayn et al., 2019b; Stürmer et al., 2020). During the anaerobic digestion, organic matter is mineralized, with C removed as biogas (carbon dioxide CO_2, methane CH_4), while organic N is mineralized as forms of ammoniacal N ($NH_4^+ + NH_3$) (Möller and Müller, 2012). Therefore, digestates often have a lower ratio of C to N and a higher ratio of total ammoniacal nitrogen to total nitrogen (TAN:TN) than undigested effluents (Möller and Müller, 2012). Digestates also have more stable organic matter than the input effluents (Béghin-Tanneau et al., 2019; de la Fuente et al., 2013; Thomsen et al., 2013). However, the properties of digestate can be modified depending on the identity and concentration of any potential co-substrates (Guilayn et al., 2019b). Following digestion, raw digestate may be treated to separate the solid and liquid phases (Guilayn et al., 2019a). The solid phase, called solid digestate, has high dry matter and volatile solid contents, and is used primarily as a soil amendment to maintain or increase stocks of soil organic matter (SOM) (Houot et al., 2014). The liquid phase, called liquid digestate, has a high TAN content and is used as a substitute for mineral N fertilizers (Houot et al., 2014). At the farm scale, the introduction of an AD system is thus accompanied by a shift from the use of animal effluents as fertilizer to the use of digestates, which have different fertilizer and amendment properties, as well as different environmental impacts.

Multiple studies have demonstrated that liquid or raw digestates can serve as effective substitutes for mineral N fertilizers (Barłóg et al., 2020; Cavalli et al., 2016; Ferdous et al., 2020; Loria et al., 2007; Möller et al., 2008; Verdi et al., 2019). However, their efficiency as N fertilizers may be influenced by their associated rates of ammonia volatilization and mineralization of organic N, which still need to be better characterized (Möller and Müller, 2012; Sharifi et al., 2019). Instead, solid digestates are often applied as a soil amendment only before sowing (Houot et al., 2014; Thomas et al., 2017). As with other types of exogenous organic matter (EOM), such as compost or solid manures, repeated application of solid digestates over the long term could increase the release of mineral N from soil due to increased stocks of organic N (Gómez-Muñoz et al., 2017; Gutser et al., 2005). To date, though, this has been poorly explored with respect to digestates.

Due to their high TAN content and high pH, liquid digestates are more likely to emit ammonia than undigested slurries
(Nkoa, 2014), which decreases their fertilizing potential and contributes to air pollution. This increased volatilization could
be partially offset, however, by their higher rates of infiltration in soils (Möller, 2015; Nicholson et al., 2017). With respect
to solid digestates, ammonia emissions have been poorly explored in the literature. An improved understanding of the impact
of AD on ammonia emissions is critical, as existing life-cycle assessments of the environmental impact of manure digestion
have provided conflicting results: some studies have reported that, through increased ammonia emissions, AD intensifies
environmental acidification (Vaneeckhaute et al., 2018; Zhang et al., 2021), while other analyses have found the opposite
result (Lyng et al., 2015; Styles et al., 2015; Wang et al., 2021). As with all fertilizers, the use of digestate may also generate
N_2O emissions from soils. At the scale of an entire plot, lower N_2O emissions have been reported after the application of
digestate than of undigested slurries (Möller, 2015; Nkoa, 2014), which could be due to the lower content of labile C in the
former. However, the opposite result has also been reported (Verdi et al., 2019). It is likely that N_2O emissions, and the soil
biogeochemical N cycle in general, are strongly influenced by the management practices associated with fertilization,
including spreading periods (e.g., autumn or spring) and the management of crop residues (Möller and Stinner, 2009;
Nicholson et al., 2017). N_2O emissions may also increase as a result of repeated applications (Rosace et al., 2020). Finally,
there is evidence that AD also influences nitrate leaching, but this seems to be due more to its influence on agricultural
systems (e.g., change in crop rotation or cover crop management) than to the physico-chemical properties of the digestate
(Möller, 2015).

Broadly speaking, all of the positive and negative outcomes of the use of digestates are interrelated. For example,
mineralization of the organic N from digestates can increase the soil mineral N content, with possible consequences for crop
growth as well as N_2O emissions. For this reason, it is important to consider the N cycle at the scale of the crop rotation
rather than the plot or season (Möller, 2009; Möller et al., 2008; Sieling et al., 1997; Stinner et al., 2008). To date, most field
studies have been done at the scale of a single year, often focused on the fertilizing and amendment properties of a single
digestate application (Cavalli et al., 2016; Odlare et al., 2014), or have focused only on N losses without considering crop
production (Nicholson et al., 2017). Furthermore, solid and liquid products have typically been applied and analyzed
separately (Cavalli et al., 2016; Möller et al., 2008; Nicholson et al., 2017), whereas both can be applied on the same field by
farmers at different periods.

The objectives of this study were 1) to compare the fertilizing properties of undigested cattle effluents and digestates
originating from diverse livestock, agricultural and industrial wastes, and 2) to evaluate N losses in the field induced by the
use of digestates. More specifically, we asked: What are the factors that explain the N fertilizer efficiencies of different
EOMs (animal effluents or digestates)? Can digestates serve as an efficient substitute for mineral N fertilizer on annual
crops? Do digestates emit more NH_3 and N_2O compared to animal effluents? To answer these questions, we conducted a
three-year field experiment in which we measured N fluxes in a wheat-rapeseed-wheat rotation fertilized using one of five
systems: undigested effluents (solid farmyard manure and liquid slurry), digestates from effluents and other wastes with
phase separation (liquid and solid digestates), digestates without phase separation (raw digestate), mineral fertilizers, and a

control without any fertilization. We evaluated the agricultural performance of the different fertilizers as well as N losses in all treatments. The consistency of all N flows was assessed by analyzing a complete mineral N balance that considered the
100   entire crop succession.

2 Materials and Methods

2.1 Site and anaerobic digester

The field experiment was conducted from September 2016 to July 2019 at the INRAE facility in Nouzilly, Centre-Val de Loire, France (47°32' N, 0°48' E) (Pasquier et al., 2019). The climate is temperate oceanic with a mean annual temperature
105   of 11.9°C and a total annual rainfall of 650 mm. Data on monthly temperature, rainfall, and windspeed during the experiment were collected from a local meteorological station (Supplementary Material 1).

The experimental unit at INRAE (UE PAO, 2024) is a crop-livestock farm with dairy cattle. Until 2014, cattle slurry was typically stored in a covered pit before application, and solid cattle manure was kept on an open platform. In 2014, an anaerobic digester was installed, with a continuous wet mesophilic process, one main digester, and one post-digester. The
110   digester treats 7,500 t of waste per year (fresh weight), consisting of bovine slurry (11% of fresh weight); cattle, sheep, and horse manure (17%); sewage sludge (29%); agroindustrial wastes (18%); grease (8%); and cereal middlings (8%). The remaining inputs consist of site water runoff (9%) and a minor fraction of other wastes (e.g., grass silage, beet pulp). The total retention time is 100 days in the two digesters. At the output of the post-digester, the raw digestate is post-treated with phase separation using a screw press. The liquid phase is stored in an open lagoon, and the solid phase on an open storage
115   platform. All the EOMs are stored approximately six months before their application in the field.

2.2 Field experiment design

Five fertilization systems (Table 1) were tested in five plots of 24 x 75 m each (1800 m^2, one plot per system) (Supplementary Material 2). This design enabled to study the application of EOM using full scale machinery. Each plot is centered on a drain: the purpose of this design was also to measure the N lixiviation from drained water; however, we were
120   not able to value such results. The crop succession was cultivated under conventional management (Supplementary Material 3). Winter wheat (*Triticum aestivum*, var. Syllon) was harvested in 2017, rapeseed (*Brassica napus*, var. Fernando) in 2018, and winter wheat (*Triticum aestivum*, var. Descartes) in 2019. The wheat straws were exported from the field and crop protection products were applied in a similar way in all treatments. The soil was tilled before each sowing, using disc harrows (10 cm depth) and a plow (20 cm depth) before rapeseed sowing and using disc harrows (10 to 15 cm depth) before
125   wheat sowing.

The five treatments consisted of: (MN) fertilization with only synthetic mineral N solution in late winter and spring, and synthetic mineral P fertilizer when needed; (CSM) fertilization with cattle farmyard manure in summer and cattle slurry in

late winter and spring; (RD) application of raw digestate (without phase separation) in summer, late winter, and spring; (LSD) application of solid digestate in summer and liquid digestate in late winter and spring; and (0N) no fertilization.

130 Fertilizers were applied in two ways. First, mineral fertilizer, cattle slurry, raw digestate, and liquid digestate were applied in late winter and spring, twice on each wheat crop and once on rapeseed (Table 1). The fertilization dates were chosen based on crop development and the ability of the soil to bear the weight of the spreading engines. Then, cattle manure, raw digestate, and solid digestate were applied as soil amendments at the end of summer, once before rapeseed sowing and once before the second wheat sowing (Table 1).

135 The mineral fertilizers mostly comprised a solution of urea ammonium nitrate (UAN) with 30% N (% w/w (weight/weight)) (15% urea-N, 7.5% ammonium-N, 7.5% nitrate-N). We used ammonium nitrate once, in the form of a solid N fertilizer with 33.5% N (w/w) (16.8% nitrate-N, 16.8% ammonium-N). Both were applied with dedicated sprayers, while the liquid EOMs were applied using a trailing hose. Solid digestate and cattle manure were scattered with a manure spreader. In winter and spring, the EOMs were applied to the crops but not buried. In summer, the five plots were tilled 24 h after amendment

140 application to bury the EOMs and prepare the soil for sowing.

In summer, we aimed to apply a similar fresh mass of all organic fertilizers (Table 1). To obtain similar yields in all treatments, then, the doses of fertilizer applied in winter and spring were calculated based on their TAN content. The desired quantities of mineral N were determined for each treatment and each year based on the N demand of the crop, soil mineral nitrogen (SMN) in the whole soil profile in late winter, and, for rapeseed fertilization, crop N uptake in late winter. Due to

145 the difficulty of applying a precise, homogeneous dose with a trailing hose, the actual doses applied varied from these calculations; furthermore, there were small variations among the EOM with respect to composition (Table 1). As a result, the different treatments varied somewhat in their N inputs (Table 1). The input of inorganic N in the MN, RD, and LSD treatments was similar, and was higher than in the CSM treatment. High inputs of organic N were recorded in the CSM, RD, and LSD treatments.

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Table 1: Details of the application of mineral and organic fertilizers in the different treatments. Doses are reported in t ha-1 (fresh weight). Nmin: mineral N. Ntot: total N. Norg: organic N. Values of mineral N also include Urea-N from the UAN solution because of its fast rate of mineralization in the field. MN: mineral fertilizers. CSM: cattle slurry and manure. RD: raw digestate. LSD: liquid and solid digestate.

Crop	Date		MN	CSM	RD	LSD
Wheat 2017	22 March 2017	fertilizer	UAN solution	Cattle slurry	Raw digestate	Liquid digestate
		t ha^{-1}	/	37	38	36
		kg N$_{min}$ ha^{-1}	86	23	86	74
		kg N$_{tot}$ ha^{-1}	86	40	169	137
	19 April 2017	fertilizer	UAN solution	Cattle slurry	Raw digestate	Liquid digestate
		t ha^{-1}	/	60	31	32
		kg N$_{min}$ ha^{-1}	43	46	63	63
		kg N$_{tot}$ ha^{-1}	43	59	129	123
	Total 2017	kg N$_{min}$ ha^{-1}	129	69	149	137
		kg N$_{tot}$ ha^{-1}	129	99	298	260
Rapeseed 2018	2 August 2017	fertilizer	/	Manure	Raw digestate	Solid digestate
		t ha^{-1}	/	35	32	33
		kg N$_{min}$ ha^{-1}	/	20	66	43
		kg N$_{tot}$ ha^{-1}	/	276	133	207
	21 March 2018	fertilizer	UAN solution	Cattle slurry	Raw digestate	Liquid digestate
		t ha^{-1}	/	27	32	36
		kg N$_{min}$ ha^{-1}	99	34	82	98
		kg N$_{tot}$ ha^{-1}	99	88	166	198
	Total 2018	kg N$_{min}$ ha^{-1}	99	54	148	141
		kg N$_{tot}$ ha^{-1}	99	364	299	405
Wheat 2019	19 September 2018	fertilizer	/	Manure	Raw digestate	Solid digestate
		t ha^{-1}	/	12	16	16
		kg N$_{min}$ ha^{-1}	/	10	44	28
		kg N$_{tot}$ ha^{-1}	/	105	86	110
	19 February 2019	fertilizer	UAN solution	Cattle slurry	Raw digestate	Liquid digestate
		t ha^{-1}		24	12	23
		kg N$_{min}$ ha^{-1}	40	47	48	56
		kg N$_{tot}$ ha^{-1}	40	64	57	99
	12 March 2019	fertilizer	UAN solution	Cattle slurry	Raw digestate	Liquid digestate
		t ha^{-1}	/	18	20	18
		kg N$_{min}$ ha^{-1}	80	19	44	39
		kg N$_{tot}$ ha^{-1}	80	40	87	75
	1 May 2019	fertilizer	Ammonium nitrate	Ammonium nitrate	/	/
		t ha^{-1}	/	/	/	/
		kg N$_{min}$ ha^{-1}	41	24	/	/
		kg N$_{tot}$ ha^{-1}	41	24	/	/
	Total 2019	kg N$_{min}$ ha^{-1}	161	100	136	123
		kg N$_{tot}$ ha^{-1}	161	223	230	284
3 years	Total	kg N$_{min}$ ha^{-1}	389	223	433	401
		kg N$_{org}$ ha^{-1}	0	473	394	548
		kg N$_{tot}$ ha^{-1}	389	696	827	949

2.3 Physico-chemical characteristics of exogenous organic matter

All EOMs were sampled for analysis in the field at each application and stored at 4°C; the number of samples was 2 for cattle manure and solid digestate, 5 for liquid digestate and cattle slurry, and 7 for raw digestate.

Dry matter (DM) content was determined after 48 h of drying at 105°C (NF EN 13040). Total volatile solids (VS) and total nitrogen (TN) contents were determined by standard methodologies (mass loss after combustion, NF EN 13039, and total Kjedhal N, NF EN 13654-1, respectively). Mineral nitrogen (Nmin) was extracted with a 1 M KCl solution, then analyzed by colorimetry on a continuous flow analyzer (Skalar, The Netherlands). The nitrate content of EOMs was very low and their Nmin content was approximately equal to the TAN content. The C:N ratio was calculated assuming that the organic C content was half of the VS content. Total P and total K contents were analyzed by inductively coupled plasma optical emission spectrometry (NF EN ISO 11885) after dissolution in aqua regia (NF EN 13346).

Each EOM was mixed with soil and incubated in laboratory conditions for characterization of the kinetics of mineralization or immobilization of N (XP U44-163). For this, fresh soil taken from the experimental field was sampled in the 0-20 cm depth and sieved to 4 mm (see section 2.4). Considering this analysis, raw digestate, solid digestate, and cattle manure were sampled on September 19th, 2018 during field application, and cattle slurry and liquid digestate were sampled on March 12th, 2019 during field application. The fresh EOMs were mixed homogeneously with 500 g of soil in a proportion of 2000 mg C per kg DS (dry soil) and incubated for 175 days in darkness at 28 ± 1°C, in non-limiting conditions for moisture and mineral N. The soil moisture was set to field capacity (25% w/w), as previously measured with a Richard's pressure cell (30,990 Pa). KNO_3 solution was added to the soil–farmyard manure mixture to reach a SMN content of 35 mg Nmin per kg DS; this addition was not necessary for the other EOMs (sufficient amount of mineral N brought by EOM). This mineral N addition avoided any mineral N deficiency, which could have limited the EOM decomposition rate, and highlighted the potential N immobilization (Recous et al., 1995). We used four replicates per treatment and date. Unamended soil was incubated as a control. On days 0, 3, 7, 14, 25, 49, 91, and 175, we extracted and analyzed SMN as described above. For each EOM, net N mineralization or immobilization was expressed as a percentage of the Norg initially present, calculated by subtracting the amount of mineralized N in the unamended control incubation (soil mineralization) from the amount measured in the soil-EOM incubation. We also determined the contribution of available mineral N from the EOM, expressed in kg Nmin in soil per kg FW of EOM added, by taking into account both mineralization/immobilization (as described above) and the initial TAN content of the EOM. Details of the methods and calculations are given in Supplementary Material 4.

Table 2: Physico-chemical characteristics of soil in the upper layer (0–20 cm) of each treatment plot. Results are expressed as mean ± standard deviation (n = 9). SOC: soil organic carbon. CEC: cation exchange capacity. MN: mineral fertilizer. CSM: cattle slurry and manure. RD: raw digestate. LSD: liquid and solid digestate. 0N: unfertilized control.

Treatment	Clay (% w/w)	Silt (% w/w)	Sand (% w/w)	pH	SOC (g kg^{-1})	C:N	CEC (meq 100 g^{-1})
MN	16.1 ± 1.5	59.2 ± 1.3	24.7 ± 0.7	6.7 ± 0.3	14.6 ± 1.3	10.1 ± 0.2	8.9 ± 0.7
CSM	15.4 ± 1.5	57.3 ± 1.3	27.2 ± 1.4	6.4 ± 0.2	14.6 ± 1.3	10.3 ± 0.1	8.5 ± 0.6
RD	15.8 ± 1.7	70.6 ± 1.6	13.6 ± 0.7	6.1 ± 0.2	12.8 ± 1.3	10.4 ± 0.2	6.9 ± 1.1
LSD	17.1 ± 1.4	62.3 ± 1.0	20.6 ± 2.0	6.5 ± 0.4	13.3 ± 1.0	10.1 ± 0.1	8.9 ± 0.7
0N	20.2 ± 3.0	63.5 ± 3.0	16.4 ± 0.9	6.4 ± 0.2	13.4 ± 0.6	10.1 ± 0.2	10.0 ± 1.5

2.4 Soil characteristics

Nine soil samples were taken from each plot at the beginning of the experiment in 2017; the soil was characterized mainly as Stagnic Luvisol, with minor areas described as Haplic Planosol (IUSS Working Group WRB, 2015). Soil depth was approximately 100 cm. From our samples, we analyzed the following properties of the topsoil (0–20 cm depth) (Table 2): particle size distribution, to determine texture (NF X 31-107); total organic C and N contents, using dry combustion, gas chromatography, and a thermal conductivity detector (NF ISO 10694 and NF ISO13878); pH in a water suspension (NF ISO 10390); cation-exchange capacity (CEC), using ammonium acetate (NF X 31-130); $CaCO_3$ content, by acidification and measurement of released CO_2 volume (NF ISO 10693); and SOM, determined by mass loss after combustion (1100°C) and corrected by $CaCO_3$ content. Bulk density was measured in the upper layer in 15 locations using the core method (NF X31-501). Overall, the topsoil was silty loam, with a bulk density of 1.37 g cm^{-3} (standard deviation: 0.05), and low $CaCO_3$ content (1.4 g kg^{-1}, standard deviation: 0.3). We observed a slight gradient in texture between the plots, with an increasing proportion of silt and a decreasing proportion of sand in plots CSM, MN, LSD, 0N, and RD (Supplementary Material 2).

2.5 Field trial monitoring

2.5.1 Aboveground biomass, grain yield, and N content

The crops were harvested from the entire plot area (1800 m^2) with classical machinery. The entire grain harvest was weighed and used as a measurement of grain yield, with the uncertainty of the balance serving as the yield uncertainty. Each year, just before harvest, crops were sampled from pooled subplots: three subplots (total 0.2 m^2) for wheat in 2017, three (total 3 m^2) for rapeseed in 2018, and five (total 1.5 m^2) for wheat in 2019. From these, we measured total aboveground and grain biomass. The N content of the grain and straw was analyzed using standard methodology (Dumas N, NF EN ISO 16634-2), and the protein content of the wheat grain was then deduced using a conversion factor (NF EN ISO 16634-2). Using this, we calculated the N export in grain at harvest and the total aboveground N uptake (including straw and grain). Measurement uncertainties were computed using error propagation formulas. Detailed calculations are in Supplementary Material 5.

2.5.2 Ammonia volatilization

210 To analyze ammonia volatilization, we used the inverse dispersion modeling method applied to a multiple source design, as described in Loubet et al. (2018). A dispersion model (FIDES) relates the concentration measured at a given location and height to the emission source and the background concentration by means of a transfer function calculated from measurements of atmospheric turbulence. The model requires the concentration of NH_3 in the air of each plot, as well as background levels, along with measurements of atmospheric turbulence. NH_3 concentrations were measured using ALPHA

215 passive samplers (Adapted Low-cost Passive High Absorption) (Tang et al., 2001).

Each plot was treated as three subplots. Immediately following the application of EOMs or mineral fertilizers, three poles were placed in each plot, one at the center of each subplot, uniformly distributed along the center line. On each pole, sets of two or three passive samplers were installed at two heights (30 cm and 1 m above soil or vegetation). To measure background concentrations of NH_3, three additional poles with three samplers each (3-m high) were installed 300 m from the

220 experimental field in three opposing directions. Samplers were changed regularly, every 12 h for the first 24 to 48 h from the fertilizer application, once a day on days 3 and 4, and once every two days on days 4 to 6. The acid filters were extracted in water and the ammonium concentration of the extracted solution was then determined by conductimetry following separation on a semipermeable membrane (FloRRia, Flow Injection Ammonium Analyzer; EN, the Netherlands). The concentration of NH_3 in the air was calculated based on the exposure time. During the entire measurement period, wind speed and three-

225 dimensional wind direction were measured with a sonic anemometer (20 Hz frequency) installed at a height of 2 m on the field site (Gill Instruments, United Kingdom). A specific modeling procedure was used to account for the temporal delay between the applications of the different fertilizers. Total cumulative NH_3 volatilization (kg N ha^{-1}) after 6 days was estimated by optimization by linear regression and associated uncertainties were computed by a Monte Carlo approach including experimental errors and model uncertainties.

2.5.3 Soil mineral nitrogen and estimation of nitrate leaching

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We assessed the soil mineral nitrogen (SMN) content of the entire soil profile at several points in the experiment: in February, to calculate fertilizer doses; after harvest, to calculate the amount of mineral N left by the crops; and in November (second and third years), to assess potential nitrate leaching in winter. At each date and in each treatment plot, nine soil cores were sampled at four depths (0–20 cm, 20–40 cm, 40–70 cm, 70–100 cm) and pooled in one to three pools depending on the

235 year. SMN content was calculated as the sum of NO_3^--N and NH_4^+-N contents as determined by colorimetry on a continuous flow analyzer (Skalar, The Netherlands) after KCl extraction (Supplementary Material 6). SMN stocks (kg N ha^{-1}) were calculated using soil bulk density (1.37 g cm^{-3} at 0–20 cm, 1.55 g cm^{-3} at 20–40 cm, and 1.60 g cm^{-3} at 40–100 cm).

We estimated nitrate leaching every year as the difference between SMN stocks at the beginning and at the end of winter.

2.5.4 N₂O emissions

240   N₂O emissions were measured using the static chamber method (Gu et al., 2013; Jeuffroy et al., 2013). Each plot was equipped with five manual static chambers (non-steady state chambers). N₂O in air samples was analyzed by gas chromatography with electron capture detector (Trace GC Ultra, Thermo Scientific) and the increase of concentration over the sampling time was modeled using a linear regression, representing the N₂O emission flux; detailed methods are in Supplementary Material 7. Topsoil (0–20 cm) was sampled close to each chamber on each measurement date. Gravimetric

245   soil water content (SWC) (% w/w) was measured by weighing and drying soil at 105°C; these values were then converted to the fraction of water-filled pore space (% WFPS) using the topsoil bulk and assuming a soil particle density of 2.65 g cm^{-3}. SMN content was measured by colorimetry after KCl extraction (see also Supplementary Material 6).

After a winter or spring fertilizer application, N₂O emissions were measured twice a week for two weeks, then once a week for one month, and once a month later (late spring and summer). After a summer fertilizer application, N₂O emissions were

250   measured twice a month, and once a month in late autumn and winter before the next application. In total, N₂O emissions were measured on 40 dates (Supplementary Material 7). We estimated the total emissions during the 50-day period after each fertilizer application (when measurements were most frequent) by integrating N₂O emissions over time, in late summer/autumn (2017, 2018) and late winter/spring (2017, 2018, 2019). To this end, we computed the area under the curve of N₂O emissions as a function of time. The emissions of the five periods (250 days over the course of three years) were then

255   summed. The aim of this was not to accurately estimate the absolute value of N₂O emissions or emission factors, but to enable useful comparisons among fertilization systems with respect to emissions during the periods following applications.

2.6 Crop N uptake and N supply from fertilizers applied in winter and spring

The efficiency of N fertilizer is often assessed through values of crop N uptake. We analyzed two indicators for each crop and each treatment: surplus N uptake by aboveground biomass relative to the 0N treatment (surplus N uptake, kg N ha^{-1}),

260   and N use efficiency (NUE, % TN). We considered the period from winter (February) to harvest. Surplus N uptake and NUE were computed following Eq. 1 and Eq. 2, respectively.

$$\text{surplus N uptake} = (crop\ N_{harvest} - crop\ N_{winter}) - (crop\ N_{harvest}^{0N} - crop\ N_{winter}^{0N}) \qquad (1)$$

$$NUE = \frac{surplus\ N\ uptake}{fertilizer\ TN\ input} \qquad (2)$$

Crop N uptake was the N uptake by the aboveground organs of the crops (kg N ha^{-1}), with the date of analysis indicated by

265   the subscript (winter or harvest). The indication 0N refers to the unfertilized control treatment. N uptake from the time of sowing to winter (crop N$_{winter}$) was considered only for rapeseed; for wheat, crop N$_{winter}$ was considered to be negligible and set as 0 kg N ha^{-1}. The TN input of a fertilizer was the total N applied to the field in late winter and spring (kg N ha^{-1}). Note that the residual effect of former organic fertilizer applications is integrated in the NUE in this experiment.

We also calculated the mineral N supply (kg N ha⁻¹) of each fertilizer to better understand fluctuations in the N supply due to

270   application of fertilizers in each cropping season and treatment. The period considered in this analysis ranged from winter

(February) to harvest.

Mineral N supply was defined as the amount of mineral N available for plants in each plot compared to control, from

February to harvest. It was estimated based on calculations of mineral N input, volatilization, EOM decomposition during

crop growth, and the differences in SMN stock evolution in each treatment compared to the control plot (which also enabled

275   us to assess the differences in SMN stocks in February among the treatments). We assumed that mineralization of SOM was

similar in all treatments and that the effect of the incorporation of former crop residues and organic amendment on SMN

stocks occurred before opening of the N balance in February. Mineral N supply was computed using Eq. 3.

$Mineral\ N\ supply = N_{min}$ fertilizer input – volatilized N +

mineralized or immobilized N due to EOM decomposition + $(SMN_{winter} - SMN_{harvest}) - (SMN^{0N}_{winter} - SMN^{0N}_{harvest})$

280   (3)

N_{min} fertilizer input (kg N ha⁻¹) was computed from the EOM analysis. Volatilized N (kg N ha⁻¹) was measured as described

in section 2.5. The amount of mineralized or immobilized N due to EOM decomposition (kg N ha⁻¹) was estimated from the

percentage of mineralized organic N in a given EOM on day 49 of laboratory incubation (section 2.3); these conditions were

chosen as a proxy for N mineralization between the first fertilizer application and harvest using the temperature function of

285   the STICS soil-crop model (Brisson et al., 2008) and daily values of air temperature. Positive values represent net

mineralization of N (mineral N supply), while negative values indicate net immobilization (mineral N immobilization). SMN

represented the SMN stock in the entire soil profile (kg N ha⁻¹). The time of analysis is indicated by the subscript. 0N refers

to the unfertilized control treatment.

The surplus N uptake was then compared to the mineral N supply to determine whether crop growth could be explained by

290   the supply of N provided by the fertilizers.

2.7 Field N balance

2.7.1 Mineral N balance

To verify the consistency of all N flux measurements, we calculated the balance of mineral N in the soil over the three-year period from February 2017 (the first fertilization of the first wheat crop) until July 2019 (harvest of the second wheat crop).

295 We first computed the sum of initial SMN stocks (measured, section 2.5.3) and mineral N inputs. Mineral N inputs were all processes that produced mineral N in the soil: the mineral N input from fertilizers (measured, Table 1) and the mineralization of organic N from the applied EOMs (see below) and from SOM and crop residues (see below). Atmospheric deposition or biological nitrogen fixation were considered to be negligible.

We then computed the sum of final SMN stocks (measured, section 2.5.3) and mineral N outputs. The mineral N outputs

300 were all processes that remove mineral N from the soil: ammonia volatilization (measured, section 2.5.2), N immobilization in SOM induced by EOM decomposition (see below), nitrate leaching (estimated, section 2.5.3), and N uptake by crops (in aboveground and belowground biomass, see below). Emissions of N_2O and N_2 were not considered in these calculations because estimated N_2O emissions were significantly lower than the other N fluxes, and because we did not measure all emissions of N_2O or N_2. We then compared the mineral N inputs to the mineral N outputs, taking into account the variation

305 in SMN stocks.

The mineralization or immobilization of organic N following EOM applications was deduced from measurements taken from soil incubations conducted under controlled conditions (described in section 2.6), considering the period from the first application to the end of the experiment. Total N uptake in the 0N control treatment was used as a proxy for the net N mineralization in soil due to the decomposition of SOM, crop residues, and roots. We neglected N losses in the 0N treatment,

310 and therefore we may underestimate the N mineralization in soil. N uptake in aboveground plant biomass (grain and crop residues) was measured (section 2.5.1). The N uptake of wheat roots was considered to be 15% of total N uptake (Allard et al., 2013), while the N uptake of rapeseed roots and dead leaves, which was not measured, was considered to be 6% and 11% of the N uptake of aerial rapeseed at harvest, respectively (Malagoli et al., 2005).

2.7.2 Total N balance

315   We also computed the total N balance over the three-year crop rotation to estimate the incorporation of organic N into the SOM. In this calculation, N inputs came from the mineral fertilizers and EOMs, and N outputs were the N in exported crops (measured), ammonia volatilization (measured), and nitrate leaching (estimated, see above). Emissions of N_2O and N_2 were not considered.

2.8 Statistical analyses

320   Statistical analyses were carried out with R software (v4.0.2) (R Core Team, 2020). Because the agriculture production of each plot was measured, the overall degree of uncertainty was low: we knew precisely what the crop yields were. However, variability in crop growth within each plot was not characterized and there was thus no way to statistically compare the relative agricultural performance of the different treatments.

Ammonia volatilization was determined using a reverse modeling approach that provided a mean and standard deviation of

325   the measure for each treatment and fertilization event. Therefore, no statistical tests were used to compare the treatments. Cumulative NH_3 volatilization and N_2O emissions could not be determined for replicates, so no statistical tests were applied to differentiate the treatments.

We analyzed the major drivers of NH_3 volatilization following the application of liquid EOMs using a linear model. Solid digestate and cattle manure were excluded from this analysis because the ammonia volatilization of solid EOMs is thought to

330   be driven by different factors than for liquid EOMs. The analyzed dataset contained 17 points, i.e. 5 fertilization dates and 3 EOMs in winter/spring, plus 2 dates with raw digestate in summer. The response variable of the model was the volatilization rate, i.e. the percentage of applied TAN that volatilized. The tested explanatory variables were: mean, maximal, and minimal air temperatures (in °C, over 24 h, 48 h, or 6 days); total rainfall (in mm, over 24 h, 48 h, or 6 days), mean wind speed (in m s^{-1}, over 24 h, 48 h, or 6 days); applied dose of EOM (t FW ha^{-1}); pH of the EOM; DM content of the EOM (g kg^{-1}); and a

335   Boolean variable indicating whether the EOM was buried or not. Different models were built successively using the lm function of R. The best model was selected using the stepAIC function from the *MASS* package (Venables and Ripley, 2002), which uses an automatic "stepwise" approach that iteratively adds or removes one explanatory variable at a time.

Successive models were compared using the Akaike Information Criterion (AIC), and the model with the lowest AIC was selected. The consistency of the model was verified by hand, and the assumptions of the linear model were visually verified.

340 We also analyzed the major determinants of N_2O emissions. The analyzed dataset contained 185 points, i.e. 5 treatments and 37 dates. The response variable of the model was the logarithm of N_2O emissions and the tested explanatory variables were: topsoil water content (% WFPS), topsoil $N-NH_4^+$ content (mg kg DS^{-1}), topsoil $N-NO_3^-$ content (mg kg DS^{-1}), topsoil SMN content (mg kg DS^{-1}), mean daily air temperature (°C), season (last application of fertilizer in either winter/spring or summer), the identity of the last fertilizer applied, the date of measurment (expressed in days from the beginning of the

345 experiment, to evaluate any adaptation effect), and a Boolean variable indicating whether or not the field was fertilized. The best model was determined as described above for NH_3 volatilization.

3. Results

3.1 Characteristics of exogenous organic matter

The two solid products—solid digestate and cattle manure—were similar in many respects. Both had high DM and VS

350 contents, similarly alkaline pH, and similar P content (Table 3). Manure had a slightly higher TN content (8.3 g N kg FW^{-1}) compared to solid digestate (6.6 g N kg FW^{-1}), but a lower TAN:TN ratio (8% vs. 24%, respectively) and a higher K content. The liquid products—cattle slurry, raw digestate, and liquid digestate—all had a higher TAN content compared to solid EOMs and correspondingly high TAN:TN ratios (50% to 60%). Overall, raw and liquid digestates were quite similar, with the main difference being that raw digestate had slightly higher DM and VS contents. Cattle slurry had levels of DM, pH,

355 VS, P, and K that were lower than those in digestates. Slurry also had much lower TN and TAN contents (2.1 g TN kg FW^{-1}) compared to either liquid (4.3 g TN kg FW^{-1}) or raw digestate (4.6 g TN kg FW^{-1}).

The chemical characteristics (DM, VS, TN, TAN, P, K contents) of solid digestates, cattle manure, raw digestates, and liquid digestates varied over the years of the study, with coefficients of variation that reached up to 25%. Values of pH were less variable among the years (coefficients of variation lower than 3%), maybe because it represent the logarithm of H^+ content.

360 In particular, the characteristics of cattle slurry were quite variable, but this was likely due, at least in part, to the fact that the slurry was partly diluted with water in the farm in 2017 and not in 2018 and 2019 (Supplementary Material 8).

Soil incubation with liquid digestate resulted in a net mineralization of organic N (27% of initial organic N (N_{org}) at day 49), while a net immobilization of N was found with cattle slurry (-19%) and solid digestate (-28%). Incubation with raw digestate or cattle manure induced a variable-to-null release of N (Fig. 1a). The availability of mineral N for each EOM,

365   which represented both the mineral N due to TAN inputs and the mineralization of organic N, is displayed in Fig. 1b. Compared to raw digestate, liquid digestate had a slightly lower TAN content but higher levels of N mineralization, and both had higher N availability than the other EOMs. The lower mineral N availability of cattle slurry was mainly due to its lower TAN content and, to a lesser extent, the immobilization of N. Solid digestate had a higher TAN content than manure, but the N immobilization induced by its addition to soil decreased the availability of mineral N. Both solid digestate and cattle

370   manure had equivalent and very low levels of potential mineral N availability.

Table 3: Physico-chemical characteristics of the EOMs, calculated from the analyses performed for each application. Results are depicted as mean ± standard deviation (coefficient of variation).

	Cattle manure	Solid digestate	Cattle slurry	Liquid digestate	Raw digestate
DM (% FW)	32.5 ± 3.4 (11%)	27.2 ± 4.9 (18%)	3.4 ± 2.6 (77%)	4.8 ± 1.1 (23%)	6.6 ± 0.6 (9%)
pH	9.7 ± 0.3 (3%)	9.3 ± 0.1 (1%)	7.4 ± 0.5 (7%)	7.9 ± 0.2 (2%)	8.0 ± 0.1 (2%)
VS (g kg FW^{-1})	253 ± 17 (7%)	232 ± 52 (22%)	24 ± 19.9 (81%)	29 ± 5.7 (20%)	45 ± 3.9 (9%)
TAN (g kg FW^{-1})	0.7 ± 0.2 (22%)	1.6 ± 0.4 (24%)	1.1 ± 0.5 (47%)	2.3 ± 0.3 (13%)	2.6 ± 0.6 (25%)
TN (g kg FW^{-1})	8.3 ± 0.4 (5%)	6.6 ± 0.5 (8%)	2.1 ± 1.0 (49%)	4.3 ± 0.7 (16%)	4.6 ± 0.5 (11%)
TAN:TN (%)	8 ± 1 (17%)	24 ± 4 (16%)	59 ± 16 (28%)	53 ± 3 (5%)	55 ± 13 (24%)
C:N (total N)	15.3 ± 1.8 (11%)	17.8 ± 5.4 (30%)	5.2 ± 2.2 (42%)	3.3 ± 0.2 (6%)	4.9 ± 0.4 (8%)
P (g kg FW^{-1})	2.1 ± 0.1 (5%)	2.5 ± 0.3 (12%)	0.3 ± 0.2 (59%)	0.9 ± 0.3 (34%)	1.0 ± 0.2 (17%)
K (g kg FW^{-1})	11.3 ± 2.4 (21%)	3.6 ± 0.6 (17%)	1.8 ± 1.2 (63%)	3.5 ± 1.0 (29%)	3.6 ± 0.7 (21%)

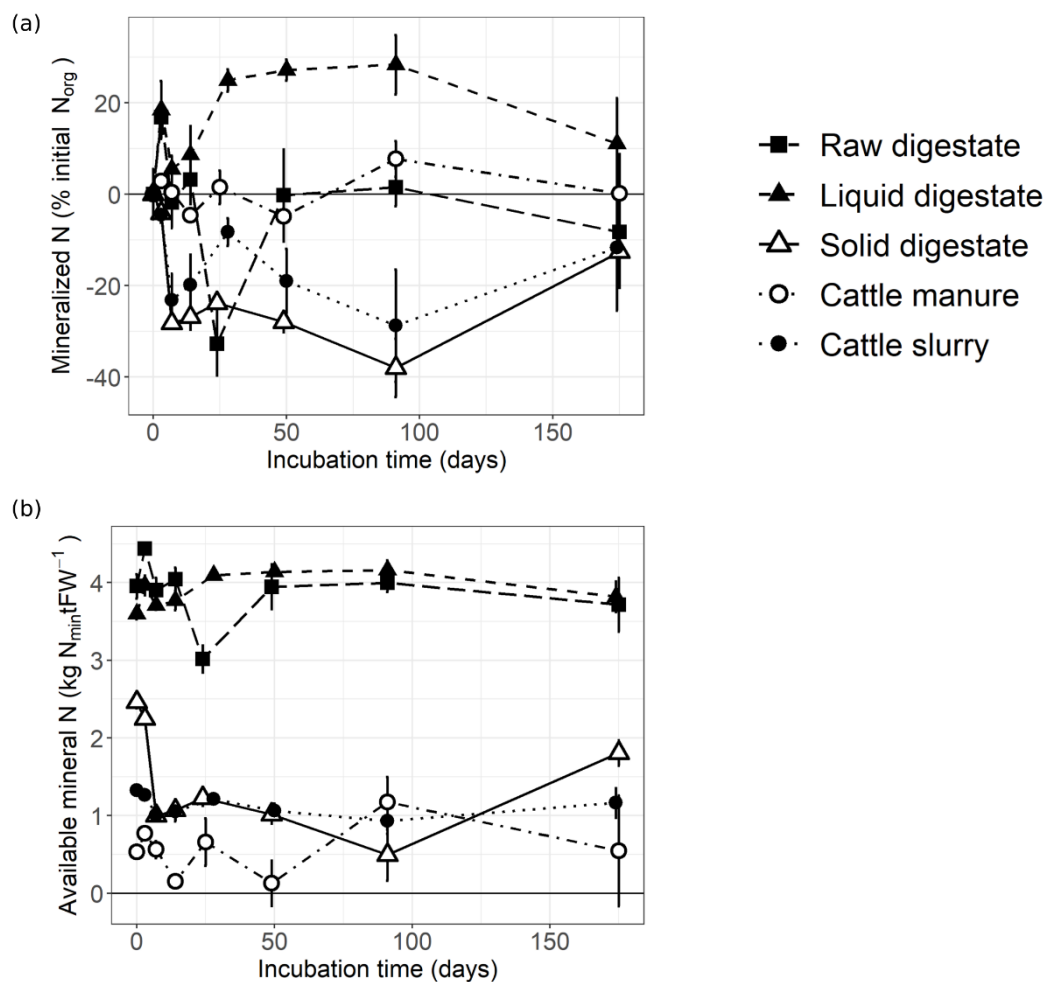



Figure 1: Kinetics of the evolution of mineral N during the incubation of soil-EOM mixtures under laboratory-controlled conditions. In each panel, error bars show one standard error. (a) Mineralized or immobilized N, expressed as a percentage of the initial organic N in the EOM. (b) Available mineral N in the soil that originated from the EOM, which is the sum of mineral N input and organic N mineralized or immobilized.

3.2 Ammonia emissions after application of organic and mineral fertilizers

In summer, the rapid and substantial volatilization associated with all EOMs could be attributed mainly to the high temperatures in that period (Fig. 2a). The very high volatilization rates for cattle manure and solid digestate (up to 100% of TAN) represented 5 to 24 kg of N losses, despite the low TAN content of those EOMs. Volatilization stopped when the EOMs were buried, 1 day after application (detailed data not shown).

In late winter and spring, the EOMs were not incorporated into the soil, and 64% to 100% of the total volatilization occurred within the first 2 days (detailed data not shown). Across the five fertilization events in this period, absolute values and rates

385 of volatilization varied depending on EOM doses, meteorological conditions, and fertilizer characteristics (Fig. 2b). Volatilization varied from 0 to 5 kg N ha^{-1} for UAN solution (0–7% of TAN applied); from 0 to 8 kg N ha^{-1} for cattle slurry (0–40% of TAN applied); from 2 to 16 kg N ha^{-1} for liquid digestate (3–29% of TAN applied); and from 7 to 38 kg N ha^{-1} for raw digestate (13–60% of TAN applied). Over all five applications, the high rates of volatilization associated with EOMs—higher than those of mineral fertilizers (UAN solution)—likely influenced the quantity of N available for crops. Of

390 the liquid EOMs, raw digestate was the most susceptible to volatilization (both the rate and total amount). Liquid digestate had rates of volatilization that were similar or slightly higher than those of cattle slurry, but a higher total volatilization due to the higher doses of applied TAN.

Across all seven applications of fertilizers (including summer fertilization), the highest volatilization rates occurred in the RD treatment (33% of volatilized TAN), followed by CSM (25%) and LSD (22%), with very low rates in the MN (mineral

395 fertilizer) treatment (2%) (Fig. 2c), although the short experimental period will tend to underestimate the volatilisation of mineral nitrogen that may last several weeks. Total volatilization rates were similar in the CSM and LSD treatments, but because of the higher TAN input of the latter, total volatilization was 1.8 times higher with LSD than with CSM (Fig. 2c). Phase separation decreased the proportion of volatilized TAN by 33% (LSD vs. RD), and because these two treatments had similar initial TAN inputs, the effect on total volatilization was similar (Fig. 2c). In the CSM, RD, and LSD treatments, there

400 was a substantial amount of volatilization following the summer application of fertilizers, accounting for 51%, 40%, and 44% of total volatilization, respectively (Fig. 2c).

The linear regression model (adjusted r² = 0.84) that best explained the percentage of TAN volatilized after the application of liquid EOMs was:

$$VR = -245.5 + 4.3 \, DM + 11.5 \, T_{max} - 1.1 \, R + 33.3 \, WS - 106.6 \, BURIED. \tag{4}$$

405 where VR was the volatilization rate of ammonia in the 6 days following application (% applied TAN), DM was the dry matter content of the EOM (g kg FW^{-1}), T_{max} was the maximum air temperature in that 6-day period (°C), R was the cumultive rainfall during the 6-day period (mm), WS was the mean wind speed during the 6-day period (m s^{-1}), and BURIED was 1 if the EOM was buried and 0 if not.

The purpose of the model was to identify the main driver of volatilization; it was not intended for use in prediction. The

410   application dose (t FW ha^{-1}) and pH were not selected in the final model, which could be due to the nature of the dataset (low

variation of these variables). All meteorological variables had stronger explanatory power when considered over the 6 days

following fertilizer application than in the 1 or 2 days following application. Maximal temperature was a better explanatory

variable than average or minimal temperature. In the model, volatilization was positively correlated ($p < 0.05$) with DM

content and air temperature, and negatively correlated ($p < 0.05$) with rainfall and burying. Once the optimal model was

415   identified, we used it to interpret the observed differences in volatilization rates among the treatments. In 2017, ammonia

volatilization rates for slurry were low, because the applied slurry had a very low DM content (< 2% FW) that probably

promoted infiltration in the soil. In 2018 and 2019, slurry and liquid digestate had similar DM contents, resulting in similar

rates of volatilization for both products. With respect to the raw digestate, meteorological conditions were more favorable to

volatilization in summer than in winter or spring, but the fact that the raw digestate was incorporated into the soil in summer

420   suppressed volatilization to levels similar to those found in the colder months. In winter and spring, liquid digestate had

similar or lower volatilization rates than raw digestate, probably because of its lower DM content, which enabled faster

infiltration into soil and therefore reduced volatilization. In summer, when volatilization rates were high, more TAN was

applied with raw digestate compared to solid digestate (Table 1); correspondingly, absolute total volatilization was higher in

the RD treatment than in the LSD treatment. It thus appeared that the decrease in volatilization induced by phase separation

425   was a function of two factors: the distribution of TAN inputs throughout the year and the improved infiltration of liquid

digestate in the soil.

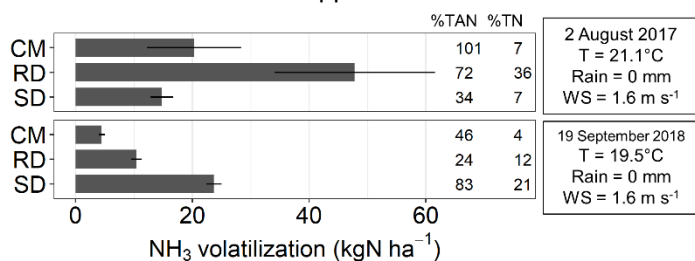
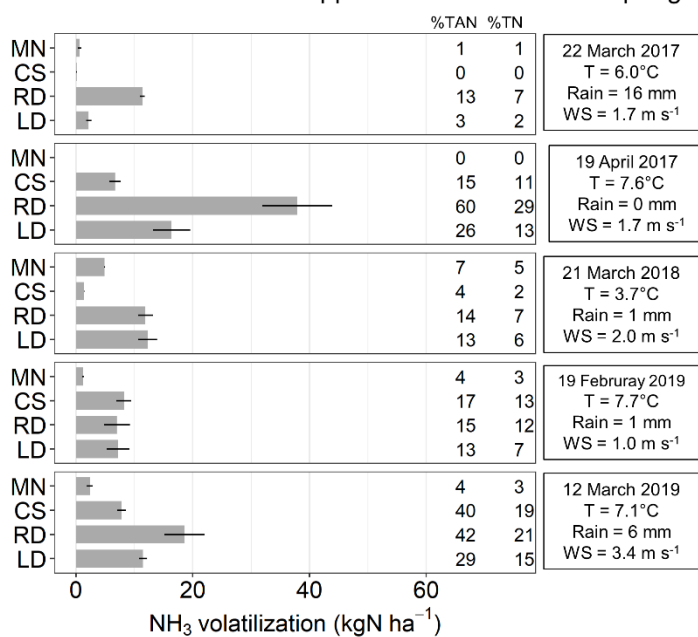
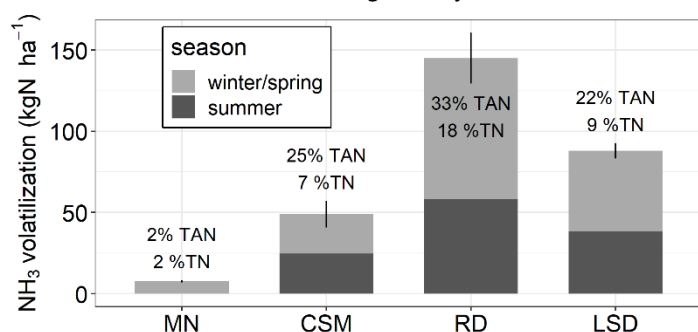



Figure 2: Ammonia emissions 6 days after the application of fertilizers in the field, expressed in kg N ha^{-1}, % of applied TAN, and % of applied TN. In each panel, error bars show one standard error. For each date, mean air temperature (T), total rainfall, and wind speed (WS) during the 6-day period are indicated (A, B). (a) NH$_3$ volatilization following winter and spring applications. MN: Mineral N. CS: Cattle slurry. RD: Raw digestate. LD: Liquid digestate. (b) NH$_3$ volatilization following summer applications.

CM: Cattle manure. RD: Raw digestate. SD: Solid digestate. (c) Total emissions during the three-year period, in each treatment. MN: mineral N. CSM: cattle slurry and manure. RD: raw digestate. LSD: liquid and solid digestate.

3.3 Crop yields and N uptake

435   Excluding the 0N control treatment, the highest yields were always achieved with the MN and LSD treatments, and the

lowest yields with the CSM and RD treatments, with a 15–20% difference between the two groups (Fig. 3a). In each year,

grain yields were strongly correlated with N uptake in the aboveground organs of crops (presented in Fig. 3b; Pearson's r^2 =

0.95 for wheat in 2017 and 2019, r^2 = 0.99 for rapeseed in 2018) as well as with grain N export (r^2 = 0.91 for wheat in 2017

and 2019, r^2 = 0.99 for rapeseed in 2018) (data not shown). The standard quality target for wheat grain was 10% protein,

440   which was only achieved in 2017 in LSD treatment (Fig. 3c). This was likely due to the lack of a third fertilization event

during the upstream stage as is generally recommended in France to enhance grain quality.

Total N inputs differed among the years and treatments (Table 1); generally speaking, mineral N inputs were similar in the

MN, RD, and LSD treatments, and lower in the CSM treatment. It is possible that the summer application of EOMs could

have influenced early rapeseed growth and SMN stocks in February (Supplementary Materials 9 and 10), but our

445   experimental design prevents us from drawing definite conclusions on that point. Instead, we focused on the N efficiency of

EOMs applied in winter and spring (Eq. 2), in order to evaluate the efficiency of the different fertilizers independently of

their N inputs. Values of NUE were the highest for mineral fertilizer (62–66% of total N applied in winter and spring),

followed by cattle slurry (48%–60%), liquid digestate (37%–57%), and raw digestate (22%–41%) (Fig. 4a). Crop

performance was similar in the MN and LSD treatments, due to both high levels of N inputs and high NUE. Instead, the

450   lower crop yields in the CSM treatment appeared to be the result of lower N inputs, since the NUE values of slurry were

similar to those of liquid digestate. The reduced crop performance in the RD treatment was attributed to its low NUE.

The mineral N supply is shown on Fig. 4b. Among the different treatments, these values were strongly correlated with the

surplus uptake of N in crops compared to the non-fertilized control (r^2 = 0.84) (Fig. 4c), although the mineral N supply was

30% higher than the surplus N uptake in aboveground biomass. This difference could be explained by the N uptake in roots

455   as well as N immobilization resulting from the decomposition of former crop residues. The major driver of the mineral N

supply appeared to be TAN input (Fig. 4b), although supply was slightly decreased by both volatilization and N

immobilization during slurry decomposition. For liquid digestate, the decrease mineral N supply due to volatilization was

compensated by the mineralization of organic N. For raw digestate, instead, volatilization significantly reduced the mineral N supply, which explained its lower NUE compared to liquid digestate. Overall, the efficiency of N use and the high total N input obtained with liquid digestate enabled the achievement of the desired crop yields and N uptake. Instead, both of these outcomes were less than optimal in the plots treated with cattle slurry or raw digestate, in the former case because of the lower doses of applied N and in the latter case due to lower N use mostly caused by NH_3 volatilization.

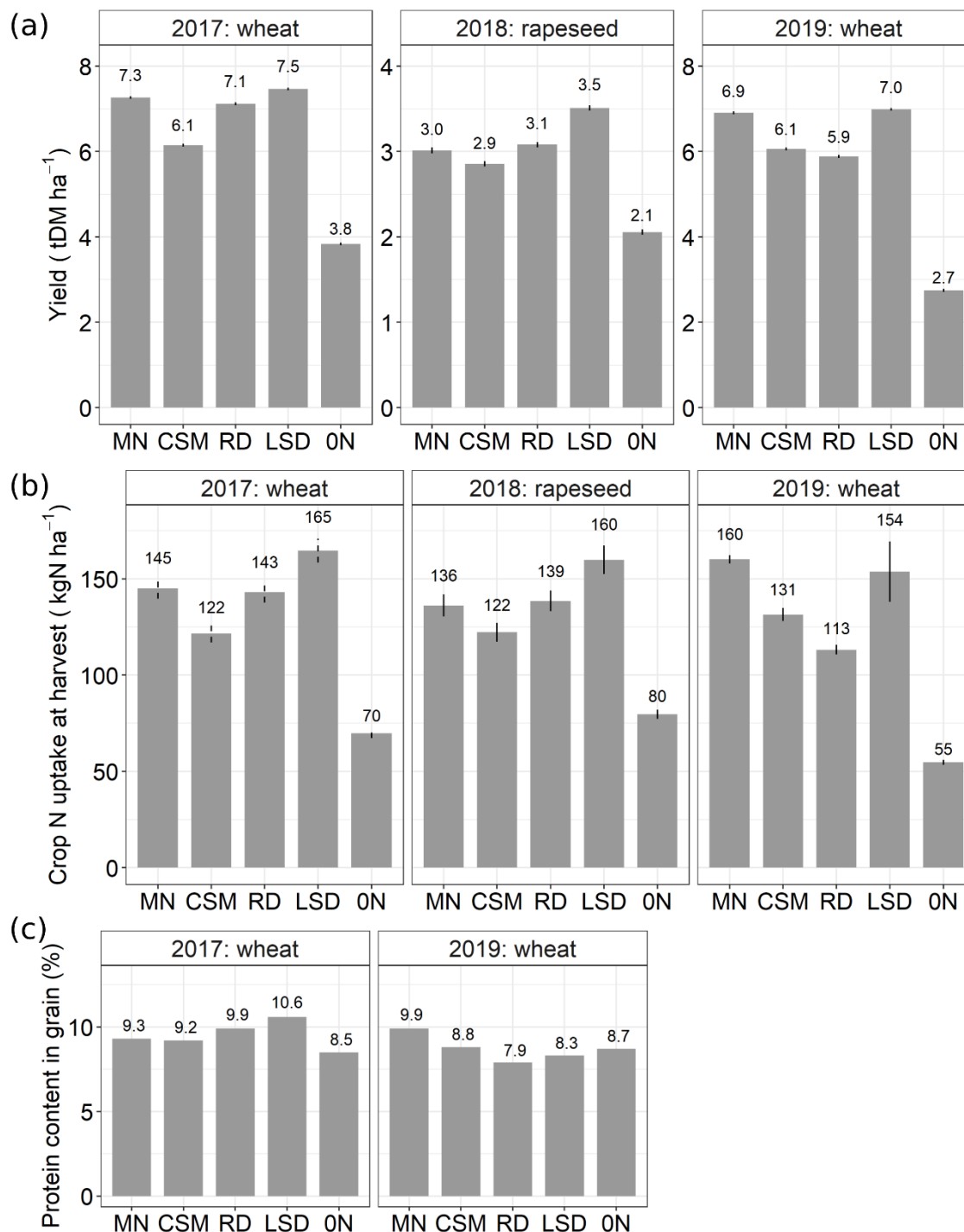



Figure 3: Crop performance during the three-year crop succession. Error bars show measurement uncertainty. **MN: mineral N. CSM: cattle slurry and manure. RD: raw digestate. LSD: liquid and solid digestate. 0N: unfertilized control. (a) Grain yield. (b) N uptake by crop (aboveground organs) at harvest date. In 2017,** uncertainty could not be calculated **because there was no**

replication of the measurement of N content; the dotted error bars shown represent an estimated uncertainty with the same relative uncertainty in N content as obtained for wheat in 2019. (c) Protein content in wheat grain (2017 and 2019). The measures were not repeated and no uncertainty could be estimated

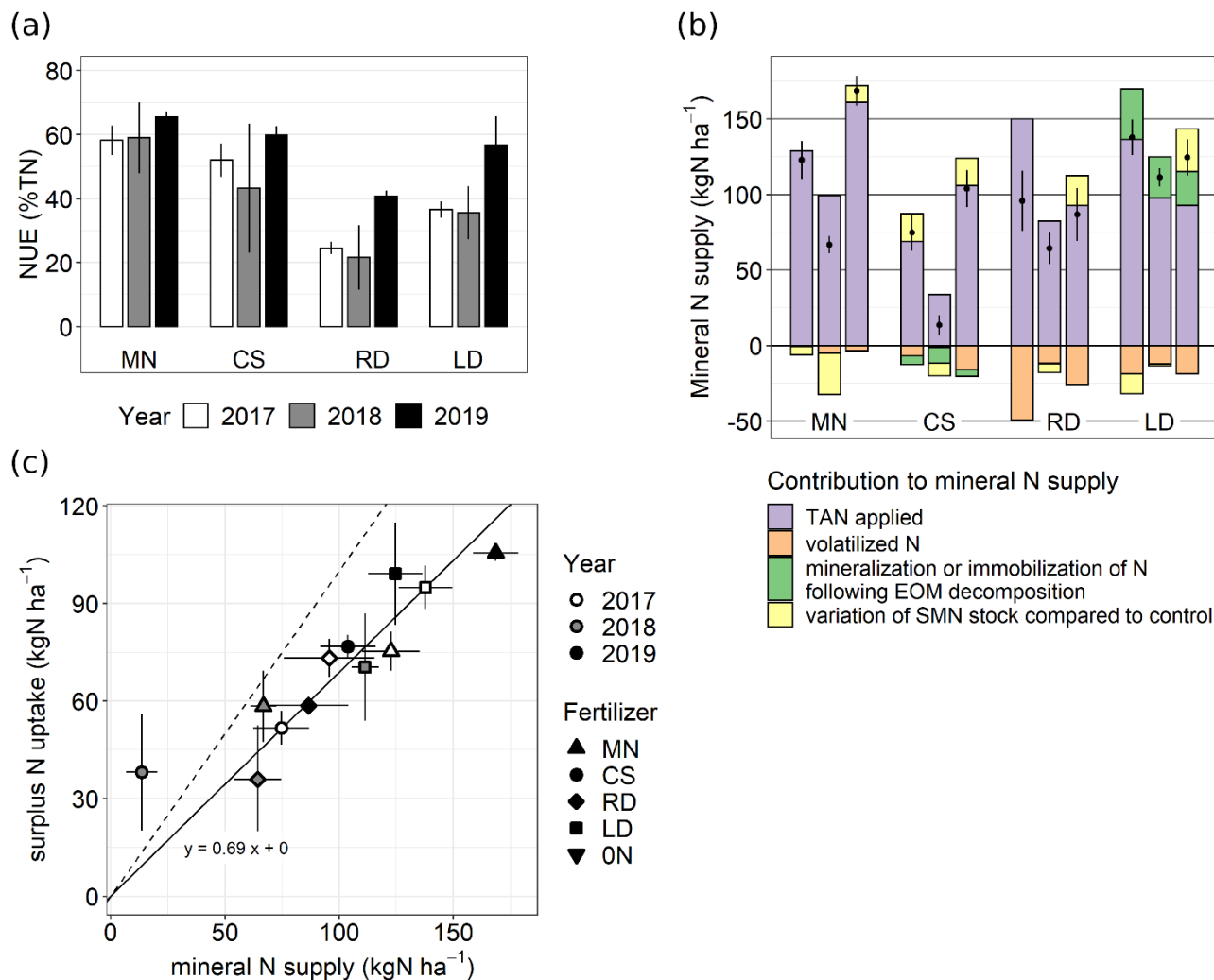



Figure 4: (a) Nitrogen use efficiency (NUE) of fertilizers in the different treatments. (b) Mineral N supply calculated between February and harvest. Black dots indicate mineral N supply, error bars represent one standard error. Histograms depict the decomposition of the mineral N supply into its four components: TAN, volatilization, mineralization or immobilization of N due to EOM decomposition, and variations in soil mineral N stocks compared to control. Positive values indicate processes that supply mineral N; negative values indicate processes that remove mineral N. For each treatment, the bars represent the mineral N supply in 2017, 2018, and 2019, from left to right. (c) Correlation between surplus N uptake and the mineral N supply calculated between February and harvest. The solid line represents the best regression line passing through the origin. The dotted line represents a 1:1 relationship. Error bars represent measurement uncertainty. Pearson's r^2 was 0.84. MN: Mineral N fertilizer. CS: Cattle slurry. RD: Raw digestate. LD: Liquid digestate.

3.4 N₂O emissions

Every year, in all treatments, we observed a peak in N₂O emissions in spring in the month following fertilizer application, up to 75 g N_2O-N ha^{-1} day^{-1} (Supplementary Material 11). Emissions in both digestate treatments (LSD and RD) were similar, while those following the application of cattle slurry (CSM treatment) were slightly lower. In spring 2018, the use of mineral fertilizers induced N₂O emissions that were much higher than those associated with the use of digestates, but in the other two years of the study, similar levels were obtained with the MN, LSD, and RD treatments (spring of 2017 and 2019). In autumn, N₂O emissions were low in all treatments, often below 10 g N_2O-N ha^{-1} day^{-1} (Supplementary Material 11).

When we summed the emissions associated with each treatment over the five periods following fertilizer applications (50 days each, 250 days total), we found that the emission rates of surplus N₂O emissions (compared to the 0N control) were highest in the MN treatment, followed by RD, LSD, and CSM, corresponding to $0.67\% \pm 0.07\%$, $0.22\% \pm 0.03\%$, $0.21\% \pm 0.02\%$, and $0.11\% \pm 0.03\%$ of total N applied, respectively (Fig. 5). The same order was observed when emissions were calculated based on the amount of mineral N applied: $0.67\% \pm 0.06\%$, $0.49\% \pm 0.06\%$, $0.41 \pm 0.05\%$, and $0.38\% \pm 0.11\%$ of mineral N applied for the MN, LSD, RD, and CSM treatments, respectively. However, because the emissions were not measured over the course of the whole year, these percentages cannot be considered emission factors, and can only be used for the purpose of comparing treatments. All EOMs (CSM, RD, and LSD) had similar ratios of measured N₂O emissions to mineral N input, and these were all lower than that obtained with mineral fertilizer (MN). Therefore, cumulative N₂O emissions were the highest in the MN treatment (Fig. 5), followed closely by both RD and LSD. Cumulative N₂O emissions were about 50% lower in the CSM treatment probably because of the lower dose of mineral N applied. The emissions in autumn, following summer application, represented 7%, 16%, 19%, and 34% of total emissions for the MN, LSD, RD, and CSM treatments, respectively, even though this period represented 40% of the total measurement duration.

We investigated the drivers of daily N₂O emissions with a linear regression model. The model that best explained (adjusted $r^2 = 0.42$) N₂O emissions was:

$$log(N_2O) = -5.379 + 0.026\ NH_4^+ + 0.154\ NO_3^- + 0.048\ SWC + 0.048\ T + 1.441\ FERTILIZED + 1.017\ SEASON \qquad (5)$$

where $log(N_2O)$ was the logarithm of N₂O emissions expressed in g N_2O-N ha^{-1} day^{-1}, NH_4^+-N was the content of NH_4^+ in the topsoil (mg NH_4^+-N kg DS^{-1}), NO_3^--N was the content of NO_3^- in the topsoil (mg NO_3^--N kg DS^{-1}), SWC was the soil

505 water content (%WFPS) in the topsoil, T was the air temperature (°C), FERTILIZED was equal to 0 if the treatment was

(0N) and 1 if not, and SEASON was equal to 1 if the last application of fertilizer was in winter or spring, and 0 if not.

The purpose of the model was to identify the main driver of N_2O emissions—not to be used for prediction—and was better

when it included both soil NO_3^- and NH_4^+ content instead of total mineral N content. All explanatory variables had a slope

significantly different than 0 ($p < 0.05$). The variables "date" and "last fertilizer used" were not selected in the model. The

510 different treatments influenced N_2O emissions mainly through their NO_3^- and NH_4^+ contents, independently of the type of

fertilizer applied. Therefore, the low level of N_2O emissions in the CSM treatment was induced by the low mineral N content

in winter and spring (Supplementary Material 11). The contribution of autumn emissions to cumulative emissions was low

because of the lower SWC in autumn than in spring, but also because of the slightly lower mineral N content in soil

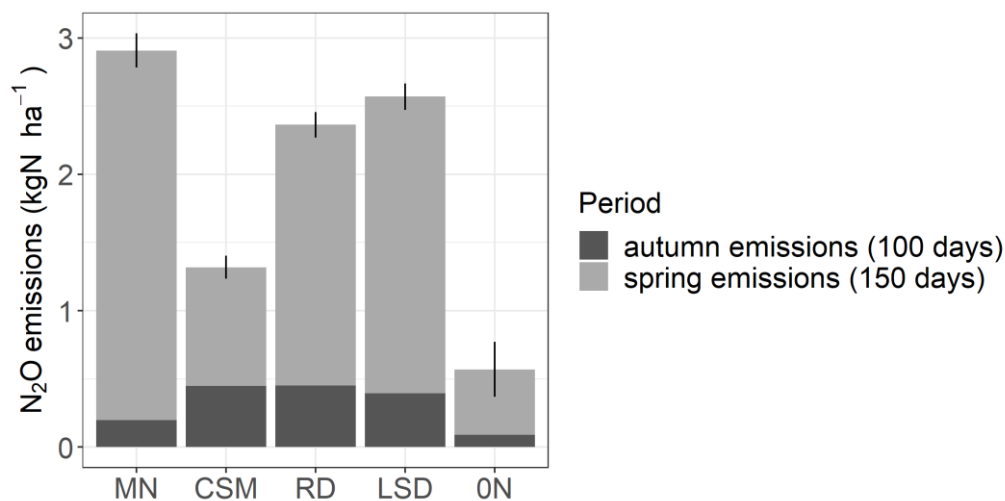
(Supplementary Material 11).

515

Figure 5: Total measured N_2O emissions during 250 days of measurement over three years. Values do not represent the real absolute value of N_2O emissions. Error bars show one standard error.

3.5 Soil mineral N stocks and estimated nitrate leaching

During the drainage period in winter 2017/2018 (rapeseed cultivation), SMN stocks were low in all treatments (around 25 kg

520 N ha^{-1} in the whole soil profile) because of substantial N uptake by rapeseed in the autumn (Supplementary Material 9). High

amounts of nitrate leaching were unlikely during this period (Supplementary Material 10).

In the autumn and early winter of 2018/2019, N uptake by wheat was low, resulting in high levels of SMN in the soil profile

in late autumn: around 50 kg N ha^{-1} in the MN and 0N treatments, 75 kg N ha^{-1} in the CSM and RD treatments, and 125 kg N

ha^{-1} in the LSD treatment (Supplementary Material 10). SMN stocks decreased during the drainage period in 2018/2019

525   (Supplementary Material 10), with nitrate leaching estimated at 6 kg N ha^{-1}, 15 kg N ha^{-1}, 15 kg N ha^{-1}, 21 kg N ha^{-1}, and 82

kg N ha^{-1} in the MN, 0N, RD, CSM, and LSD treatments, respectively. In the RD and CSM treatments, the summer

application of raw digestate and manure added relatively low amounts of mineral N (Table 1). Moreover, the mineralization

of organic N was low, as observed during our incubation experiments (Fig. 1a). Therefore, the slightly higher SMN stocks in

the RD and CSM treatments (with respect to MN) were consistent with the relatively low surplus of leaching observed.

530   However, at the end of autumn, the SMN stock in the LSD treatment was higher than in the other treatments, and

consequently nitrate leaching was also larger. Compared to the 0N control, the surplus leaching that occurred in the LSD

treatment corresponded to 60% of the N applied in summer 2018 as solid digestate. This could only be explained by a high

degree of mineralization of organic N, which was inconsistent with the kinetics of N immobilization observed in our

laboratory incubations (Fig. 1a). We concluded that the application of organic fertilizers before wheat sowing may carry a

535   significant risk of nitrate leaching.

3.6 Field N balance

3.6.1 Mineral N balance

The balance of mineral N in the soil at the scale of the entire three-year crop rotation is displayed in Fig. 6. The flux of

mineral N in the soil was the highest in the RD and LSD treatments, followed by MN, and was lowest in the CSM treatment.

540   The main sources of mineral N inputs were the mineralization of N from SOM (soil supply) and TAN from fertilizers, with

the mineralization of organic N from EOMs playing a much smaller role. The main output of mineral N was plant N uptake,

which represented 95%, 84%, 85%, and 70% of mineral N inputs from fertilizers and soil in the CSM, LSD, MN, and RD

treatments, respectively. N losses (leaching and volatilization) represented 2%, 15%, 24%, and 25% of mineral N inputs

from fertilizers and soil in the MN, CSM, RD, and LSD treatments, respectively. N losses were equivalent to 3% of plant N

545   uptake in the MN treatment, and were higher in the CSM (16%), LSD (30%), and RD (34%) treatments.

When viewed across the three cropping seasons, our calculations of the mineral N balance in the soil appeared to be quite accurate. The difference between outputs and inputs of soil mineral N were -89 kg N ha^{-1} (15% of mineral N inputs), 50 kg N ha^{-1} (11%), -5 kg N ha^{-1} (1%) and 105 kg N ha^{-1} (16%) in the MN, CSM, RD, and LSD treatments, respectively (Fig. 6). Considering the uncertainties associated with measurement (N uptake, volatilization, leaching, mineralization from EOMs,

550 SMN stocks, soil N furniture), and the processes that were not accounted for here (e.g., mineralization of different quantities of crop residue between the plots, N$_2$ and N$_2$O losses, atmospheric deposition), these balances were fairly consistent, which improves our confidence in the measured and estimated N fluxes.

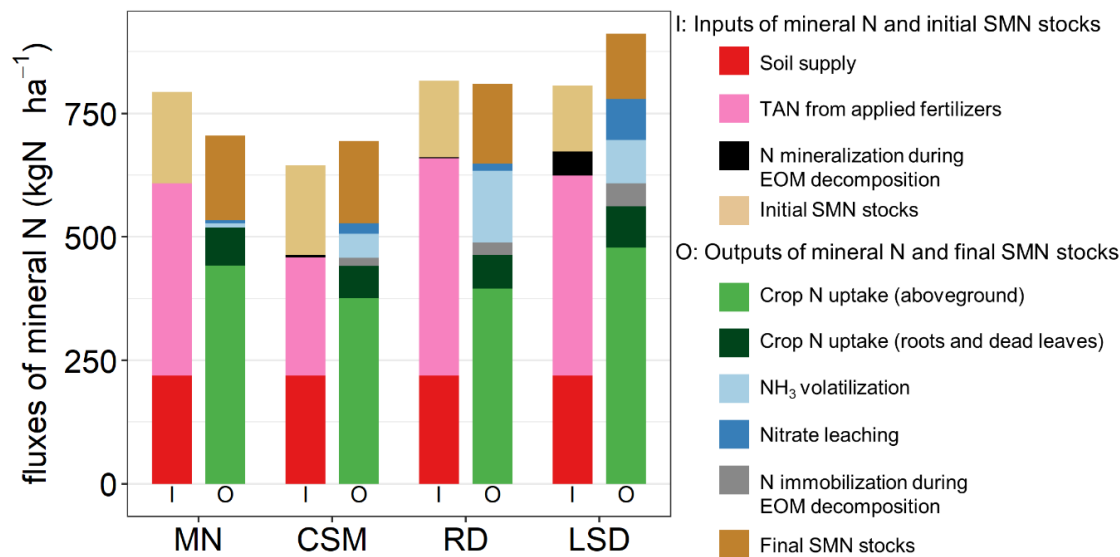



Figure 6: **Mineral N balance in the field at the scale of the three-year crop rotation. (I): Inputs, i.e. processes that produce mineral**
555 **N in the soil. Initial SMN stocks are also considered. (O): Outputs, i.e. possible processes explaining the decrease in mineral N**
stocks in soil. Final SMN stocks are also considered.

3.6.2 Total N balance

In the MN treatment, the outgoing and incoming fluxes of N were equivalent to each other (Table 4), indicating no change in soil organic nitrogen (SON) storage. In the treatments using organic fertilizers, the N inputs were larger than the outputs

560 (Table 4). The positive N balance in the organic treatments represented 44%, 43%, and 41% of total N inputs in the CSM, RD, and LSD treatments. It also represented ca. 10% of the initial soil organic N stock (3.7 t ha^{-1} on average). This reflected the potential contribution to soil organic matter from organic fertilization, i.e. the so-called amendment properties of the EOM.

Table 4: Total N balance in the field at the scale of the three-year crop rotation in the different treatments. MN: mineral N fertilizers. CSM: cattle slurry and manure. RD: raw digestate. LSD: liquid and solid digestates. N inputs consisted of total N applied by fertilizers. N outputs consisted of crop N export, leaching, and volatilization.

N fluxes (kg N ha^{-1})	MN	CSM	RD	LSD
Organic N input	0	423	423	537
Inorganic N input	389	239	440	405
Total N input	389	662	863	942
Total N output	380	373	491	558
Balance of N fluxes	9	290	372	384

4. Discussion

4.1 Digestates have different characteristics than animal effluents

Due to import of nutrients with co-substrates, digestates had different characteristics than undigested animal effluents. The raw digestate examined here was similar in many respects to previously described digestates created from sewage sludge, biowastes, and food/agroindustry residues (Guilayn et al., 2019b). The characteristics of the liquid and solid digestates in this study fit well with the expected results associated with low-performance phase separation (Guilayn et al., 2019a). Based on these analytical characteristics, we calculated that phase separation removed only 27% of the DM content from the raw digestate. In other respects, the liquid digestate was largely similar to the raw digestate, as was previously observed for low-efficiency phase separation (Guilayn et al., 2019a).

We observed positive mineralization of organic N after the application of liquid, but not raw, digestate to soil under controlled conditions; such variability in mineralization has been reported in multiple studies (Alburquerque et al., 2012; Cavalli et al., 2016; de la Fuente et al., 2013; Tampio et al., 2016). Likewise, N immobilization after the application of solid digestate has often been observed (Cavalli et al., 2017; Chiyoka et al., 2014; de la Fuente et al., 2013). Using a more synthetic approach, Levavasseur et al. (2022) recently highlighted the variability in N mineralization kinetics after the addition to soil of various types of EOMs, including digestates, cattle slurries, and cattle farmyard manures. Our results were all in the range of the N kinetics reported in their study.

As expected, the slurry and the raw and liquid digestates had high TAN content and high nitrogen use efficiencies; they could thus serve as adequate substitutes for mineral N fertilizers. Instead, due to their lower TAN content, and because the mineralization of their organic N was low or negative, solid digestates and manure were poor fertilizers, but likely contributed to organic matter storage.

4.2 The use of different exogenous organic matters influences N losses in the field

Ammonia and N_2O emissions, together with nitrate leaching, were observed after the application of EOMs. In most respects, our results were consistent with those in the published literature: meteorological conditions and the method of application had a strong influence on NH_3 volatilization for liquid EOMs (Hafner et al., 2019), but there was also a positive correlation between DM content and volatilization, which was expected given the decreased infiltration rates associated with higher DM (Hafner et al., 2019; Pedersen et al., 2021). However, previous research had highlighted an increase in NH_3 volatilization with pH or application dose (Hafner et al., 2019), which was not detected in our results. This could possibly be explained by the limitations of our dataset or the confounding effects of meteorological conditions. Indeed, due to the diversity in meteorological conditions over the course of this study, measured rates of ammonia volatilization ranged from 2% to 60% of TAN for liquid and raw digestates, which was consistent with previous measurements (Nicholson et al., 2017; Räbiger et al., 2020; Riva et al., 2016). The volatilization rates for cattle manure and cattle slurry were also consistent with those reported in the ALFAM2 database (Hafner et al., 2018). In general, mineral fertilizers had lower volatilization rates than the organic fertilizers. Anaerobic digestion increased the risks of NH_3 volatilization, though these rates could be brought down by post-treatment phase separation. Compared to the original raw digestate, the liquid phase had lower ammonia emissions, which were attributed to its higher rate of infiltration. A similar effect of phase separation on ammonia emission had previously been reported, but for undigested slurry rather than digestate (Amon et al., 2006; Nyord et al., 2012). Overall, the use of phase-separated digestates resulted in similar rates of ammonia volatilization as found with undigested effluents, but higher absolute amounts of volatilization due to the higher TAN inputs in the LSD treatment versus CSM. To our knowledge, the current study is the first to present results on rates of ammonia volatilization for solid digestate. Despite the lower TAN content of solid digestate, the high volatilization rate observed here demonstrates that these emissions can be significant and should not be ignored, as has occurred in the past (Amon et al., 2006; Holly et al., 2017).

The peak in N_2O emissions in late winter and spring, although rather low, was consistent with levels previously reported following the application of either digestate (Möller and Stinner, 2009; Nicholson et al., 2017; Verdi et al., 2019) or undigested effluents (Bell et al., 2015). We observed a positive relationship between N_2O emissions and soil moisture, soil mineral N content, and temperature, consistent with previous reports (Butterbach-Bahl et al., 2013). Here, emissions were

lower in summer and autumn, which we attributed to the dryness of the soil, as has been observed previously for digestates (Pezzolla et al., 2012) and other types of EOMs (Parnaudeau et al., 2009). Other studies examining the effect of animal effluents measured higher emissions in autumn or summer compared to spring (Bell et al., 2015, 2016), but these appeared to

615   be the result of high rainfall and levels of soil moisture. Overall, the rates of N_2O emission relative to applied mineral N were similar in all EOM treatments, and slightly lower than with mineral fertilizers. On the contrary, previous studies had detected higher N_2O emission rates from digestate compared to mineral fertilizers (Buchen-Tschiskale et al., 2020; Köster et al., 2011). Furthermore, we did not observe any effect of the quality of the different EOMs as reported by Charles et al. (2017), lower N_2O emission rates with digestate than with undigested slurry as found by Köster et al. (2015), Möller (2015) and

620   Nkoa (2014), or any decrease in N_2O emissions as a result of phase separation as published by Askri et al. (2016) and Möller (2015). We hypothesize that the effect of soil mineral N content, soil moisture levels, and air temperature may have masked some or all of these effects. In general, our results were most consistent with those of Häfner et al. (2021), who reported that the identity or type of digestate used played only a minor role in explaining N_2O emissions. Here, lower N_2O emissions were found with the CSM treatment than with other treatments, but this was mainly due to its lower N input. Therefore, our main

625   conclusions are that the type of fertilizer used is less important than the quantity of mineral N inputs and that there appears to be no supplementary risk of N_2O emissions from the use of organic rather than mineral fertilizers.

Unlike NH_3 volatilization or N_2O emissions, we estimated nitrate leaching only indirectly. Here, the amount of surplus nitrate leaching associated with rapeseed (in respect to MN treatment) seemed to be low. Räbiger et al. (2020) estimated a N leaching ranging from 5 to 18% of total N input with a summer application of digestate on rapeseed using a modeling

630   approach. These authors confirmed the dependency of N leaching phenomenon on climate and soil type, supporting our results of N leaching under these environmental conditions. Similarly, the low amount of nitrate leaching we estimated following summer applications of raw digestate or cattle manure on wheat were consistent with the results of Nicholson et al. (2017), who estimated that leaching represented 15% of total N following summer applications of slurry or digestate on winter wheat. The extent of leaching following the application of solid digestate was surprisingly high, as discussed above.

635   Although we did not detect high amounts of leaching in all treatments, this result suggests that the application of EOMs in autumn before wheat sowing may promote nitrate leaching, as reported by Sieling et al. (1997).

The values of NUE estimated in this study were likely shaped by the successive EOM applications in spring and in summer. In the literature, we could not find any references to the NUE of digestates applied to rapeseed, but our values for raw

640   digestate (Fig. 4) were consistent with those obtained for raw digestates on barley by Thomas et al. (2017) and Baral et al. (2017) (19% and 37%, respectively). The main factor driving fertilizer efficiency was TAN content; indeed, this is often used as a proxy for fertilizer efficiency (de França et al., 2021; Iocoli et al., 2019; Tampio et al., 2016). However, our results suggest that this is an oversimplification, as argued by Möller and Müller (2012). NUE may be decreased by volatilization (Frost et al., 1990; Quakernack et al., 2012) as well as influenced by the decomposition of EOM in the soil (mineralization of

645   organic N or N immobilization) (Abubaker et al., 2012; Cavalli et al., 2017). Therefore, reducing volatilization could have both environmental and agronomical benefits. Indeed, the lower volatilization and higher mineralization associated with the LSD treatment compared to RD explained the former's higher NUE. Overall, the substitution of mineral fertilizers with digestate did not affect crop performance. In our case, slightly higher NUE was observed for cattle slurry than for liquid digestate, but because the liquid and raw digestates had higher N contents than cattle slurry, it is possible to apply less

650   material to achieve the same required N dose. For this reason, our digestates, enriched by the addition of agricultural and urban wastes were more convenient to use as fertilizers than cattle slurry.

One disadvantage of EOM application is that it could only occur when the soil was dry enough to support the heavy spreading engine, which placed a constraint on fertilization dates. Compared to mineral fertilizers, EOM doses could not be fractionated as easily, and for technical reasons, it was not possible to apply a third dose of EOM to improve the protein

655   content of wheat grain. One potential solution could be the use of a combination of digestate and mineral fertilizers, as previously proposed (de França et al., 2021; Odlare et al., 2014). The use of EOMs instead of mineral fertilizers induced higher inputs of total N and organic matter in the field, and we would therefore expect that their use could increase the stocks of SOM, as the N balance showed. However, such an effect must be studied over a longer term than was possible in our experiment (Bhogal et al., 2018).

660   Generally speaking, the kinetics of N mineralization observed in our laboratory incubations were consistent with field observations (consistent evolution of SMN stocks in autumn, good correlation between the estimated mineral N supply and

N uptake). Likewise, Cavalli et al. (2017) also reported a strong correlation between the N availability calculated from incubations and that measured in field experiments. However, the N immobilization that was detected during the incubation of solid digestate was not observed in the field. This result was unexpected because the two experiments used the same soil, and fresh digestate was used in the incubation. Levels of SMN were high, and did not inhibit the decomposition of solid digestate. One explanation could be that the soil was drier in the field in autumn than during the incubation, which could possibly have limited decomposition; however this hypothesis is not completely satisfactory.

4.4 Assessing the N balance in a field experiment

This study presents an original field experiment aimed at comparing different fertilization systems using full-scale machinery close to a real farm management, from which it was possible to measure numerous N fluxes. Because we used a single large plot for each treatment, rather than a classical block design with smaller plots, we were limited in the types of statistical analyses we could perform; we addressed this problem for each measured N flux, in particular in focusing on measurement uncertainties. In the experiment, the N doses that were applied differed among the treatments. We first compared the N fluxes relative to the applied N in each treatment (NH_3 and N_2O emission rates, NUE). We then studied the total N fluxes among the different treatments, taking into account that it was easier to apply large N dose with digestates than with animal effluents.

Despite these limitations, we were able to obtain numerous measurements of N fluxes that enabled us to propose a consistent soil mineral N balance in the field at the scale of the entire three-year rotation. One of our main findings was a strong correlation between the surplus crop N uptake and the N use efficiency of the fertilizers, which we identified through the measurement of multiple N fluxes within a single experiment. This confirmed our solid understanding of the N cycle in the field, and highlighted the link between the N losses (NH_3 volatilization) and the N use efficiencies of the different EOMs. With this information, it becomes possible to more thoroughly comprehend the effects of fertilization with digestates, and to consider in an integrative way both the fertilization of crops and the N losses. This work is essential in measuring the trade-offs associated with the use of EOMs. In this experiment, the use of digestate in the end of winter enabled strong crop fertilization, but this was offset by N losses, and in particular, NH_3 volatilization. These results confirmed the importance of reducing N losses to increase the efficiency of fertilization with digestate (Möller, 2009). Here, our application dates did not

allow us to consistently bury the EOMs, which could have limited the volatilization risk. In the future, the application and burying of digestates on bare soil should be studied to ensure that the N input can be exploited by later crops without an increase in nitrate leaching or N_2O emissions (Emmerling et al., 2020).

690 This experiment compared the behavior of N at the scale of an entire field following the application of cattle effluents and digestates. One important area for further study is the co-digestion of cattle effluents with additional inputs, which not only influence the characteristics of EOMs, but also—via the import of external wastes—the absolute quantity of available nutrients at the scale of the whole farm. There is thus much that remains to be understood about the effect of anaerobic digestion on N fluxes and balance at the scale of the whole farm (Möller, 2009).

Conclusions

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Here we present the results of a three-year field experiment examining different fertilization strategies based on cattle effluents, digestates, or mineral N fertilizers. The main N flows in the field were investigated, and, over the entire three-year crop rotation, we were able to estimate a consistent soil mineral N balance, suggesting that our analysis addressed the majority of the processes that affect mineral N fluxes at the scale of the field. Undigested cattle slurry and raw and liquid

700 digestates were used as N fertilizers in winter, without incorporation into the soil. Overall, NUE values were well explained by TAN content, ammonia emissions, and N kinetics resulting from the decomposition of organic N in soil. In the summer, cattle manure and raw and solid digestates were applied and buried to add organic matter to the soil. Due to the high N content and NUE (37% to 57%) of liquid digestate, the system based on liquid and solid digestates resulted in similar crop performance as was achieved with mineral fertilizers, but also led to higher NH_3 volatilization (9% of applied N). With

705 respect to N_2O emissions, we identified no additional risks of using digestates instead of mineral fertilizers. Because it was less able to infiltrate deeply in the soil, raw digestate was more prone to NH_3 volatilization (18% of applied N), which also reduced its NUE (22% to 41%) compared to that of liquid digestate. Cattle effluents had similar NUE values (48 to 60%) and similar NH_3 volatilization rates (7% of applied N) as liquid and solid digestates, but cattle slurry was less convenient to use as a fertilizer than liquid digestate because of the larger doses required (lower N content). The use of digestates also likely

710 contributed to an increase in SOM storage compared to mineral fertilizers. We conclude that digestates have strong potential

for use as fertilizers, but care must be taken to limit NH_3 volatilization. The introduction of anaerobic digestion on a dairy farm transforms the quality of EOMs, but can also introduce additional nutrients to the system via the inclusion of external co-substrates. Therefore, it is necessary to assess the real on-farm impacts of such a system in order to understand the changes in both the characteristics and the quantities of EOMs produced.

715 **Data availability**

Data from this study are publicly available at https://doi.org/10.15454/5MOZKJ (Pasquier et al., 2019)

Author contribution

Author contribution according to the CRediT Taxonomy. Victor Moinard (VM), Antoine Savoie (AS), Catherine Pasquier (CP), Adeline Besnault (AB), Yolaine Goubard-Delaunay (YGD), Baptiste Esnault (BE), Marco Carozzi (MC), Polina Voylokov (PV),
720 **Sophie Génermont (SG), Benjamin Loubet (BL), Catherine Hénault (CH), Florent Levavasseur (FL), Jean-Marie Paillat (JMP), Sabine Houot (SH)**

Contributor role	VM	AS	CP	AB	YGD	BE	MC	PV	SG	BL	CH	FL	JMP	SH
Conceptualization		X								X				X
Data curation	X	X	X	X	X	X	X	X						
Formal analysis	X	X	X				X	X	X	X				
Funding acquisition		X									X			X
Investigation	X	X	X	X	X	X	X	X						
Methodology	X	X							X	X	X	X		X
Project administration		X	X											X
Supervision													X	X
Validation	X	X	X				X	X	X	X	X	X	X	X
Visualization	X													
Writing – original draft preparation	X													
Writing – review & editing	X	X	X				X		X	X	X	X	X	X

Competing interests

The authors declare that they have no conflict of interest.

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