Article

Organic Matter Composition of Digestates Has a Stronger Influence on N₂O Emissions than the Supply of Ammoniacal Nitrogen

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Abstract: Manures can be treated by solid–liquid separation and more sophisticated, subsequent approaches. These processes generate fertilizers, which may differ in composition and N₂O release potential. The aim of the study was to investigate the influence of processing-related changes in digestate composition on soil-derived N₂O emissions after application to soil. For that purpose, N₂O emissions within the first 7 weeks after fertilization with two raw and eight processed digestates (derived from solid–liquid separation, drying and pelletizing of separated solid, and vacuum evaporation of separated liquid) were measured in the field in 2015 and 2016. Additionally, an incubation experiment was run for 51 days to further investigate the effect of subsequent solid and liquid processing on soil-derived N₂O release. The results showed that, only in 2016, the separation of digestate into solid and liquid fractions led to a decrease in N₂O emissions in the following order: raw digestate > separated liquid > separated solid. N removal during subsequent processing of separated solid and liquid did not significantly influence the N₂O emissions after fertilization. In contrast, the concentrated application of the final products led to contradictory results. Within the solid processing chain, utilization of pellets considerably increased the N₂O emissions by factors of 2.7 (field, 2015), 3.5 (field, 2016), and 7.3 (incubation) compared to separated solid. Fertilization with N-rich ammonium sulfate solution led to the lowest emissions within the liquid processing chain. It can be concluded that the input of less recalcitrant organic C into the soil plays a greater role in N₂O release after fertilization than the input of ammoniacal N. Digestate processing did not generally reduce emissions but apparently has the potential to mitigate N₂O emissions substantially if managed properly.

Keywords: nitrous oxide; treated digestates; techniques; denitrification; separation; pelletization

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1. Introduction

Regional nutrient surpluses caused by a concentration of livestock farming represent a relevant issue worldwide. In Germany, for example, the northwest is the most affected region due to its high livestock density of about 3.5–4.1 livestock units per ha [1,2]. Biogas plants, often based on the digestion of animal manure and dedicated energy crops such as silage maize, further increase the amount of organic manures in such regions [3,4]. Moreover, the timeframe and total amount of organic fertilizer application are limited by legal regulations, such as the German fertilizer ordinance [5,6]. In addition to nitrate leaching, acidification, and eutrophication, the management of organic fertilizers is directly linked to significant gaseous N losses in the form of N₂O [7–10]. Therefore, strategies are needed to either reduce livestock density or to export nutrients out of the affected regions.

Due to their high water content, the transportation of liquid manures over large distances is not feasible.

An approach to facilitate the export of excess nutrients is to reduce the water content by digestate processing. This results in a marketable organic fertilizer which is worth being transported. A common processing technique is solid–liquid separation. This generates a dry-matter-rich solid fraction with a high proportion of the initial phosphorus and a liquid fraction with a high share of nitrogen and potassium [11,12]. In addition to water reduction and improved transportability, processing of digestates might have the potential to reduce climate-relevant N_2O emissions via partial separation of N and organic matter [13–15]. When considering solid and liquid after separation, varying experimental results were reported. Whether the solid or liquid leads to higher emissions after soil application is highly dependent on the digestate feedstock and pretreatment procedure [13,16–20].

Previous studies (e.g., [13,16–24]) mainly focused on the effect of feedstocks in combination with management practices. However, no evaluation of the single steps within an entire processing chain could be found. Therefore, the main objective of the present work was to identify which processing techniques and respective products have the potential to reduce N_2O emissions after soil application in comparison to the untreated digestates. Furthermore, the following aspects were addressed: (i) How does the treatment influence the composition of intermediate and final products? (ii) What is the impact of composition and treatment of digestates on N_2O emissions after application to soil?

For that purpose, solid (raw digestate–separation–drying–pelletizing) and liquid processing chains (raw digestate–separation–vacuum evaporation with partial ammonia stripping) from two fully operating biogas plants were selected, and the composition and emissions after soil application of the respective intermediates and final products were measured. It was generally expected that process-related N removal and the resulting decrease in N content of digestates (solids and liquids) would contribute to lower N_2O production from fertilized soils.

2. Materials and Methods

2.1. Digestate Collection and Composition

The digestates were collected at two biogas plants, which processed liquid and solid fractions after separation, respectively. The feedstocks of the first biogas plant were cattle manure and pig slurry, dedicated energy crops such as silage maize and sunflower ($400 \text{ g}\cdot\text{kg}^{-1}$ total fresh matter, FM), pomace and grape marc ($200 \text{ g}\cdot\text{kg}^{-1}$ FM), and poultry manure ($50 \text{ g}\cdot\text{kg}^{-1}$ FM). In the second plant, pig slurry and cattle manure were digested with silage maize (approximately $220 \text{ g}\cdot\text{kg}^{-1}$ FM), grass silage ($70 \text{ g}\cdot\text{kg}^{-1}$ FM), and cereal silage and grist ($180 \text{ g}\cdot\text{kg}^{-1}$ FM).

In both biogas plants, raw digestate (RD1 and RD2, respectively) was separated into a solid (SS1 and SS2) and a liquid (SL1 and SL2) fraction using a screw press separator (pore size 0.5 mm). After the separation, plant 1 subsequently treated the solid. Biogas plant 2 further processed the solid and liquid, but only the liquid products were considered in this study.

In plant 1, the separated solid (SS1) was dewatered to about 80% dry matter (DM) content (dry solid, DS1) in a solar greenhouse drier and subsequently pelletized (without addition of binding agents). The final pellets (P1) had a diameter of 6 mm and an average length of 14 mm.

In plant 2, water was removed from the separated liquid (SL2) in a two-step vacuum evaporator. The evaporation took place at 65°C and 250 hPa. The low pressure induced an evaporation of water, ammonia (NH_3), and other gases. Next, NH_3 was removed from the exhaust steam in a sulfuric-acid-containing washing column to produce ammonium sulfate (ammonium sulfate solution, ASS2). In a second vacuum vessel, the remaining liquid digestate was further dewatered, and a concentrate (CC2) was produced.

The chemical and physical characteristics of raw and treated digestates were analyzed according to VDLUFA [25] and van Soest and Wine [26], and the characteristics are shown

in Tables 1 and 2. In addition, the calculated fractions of soluble materials, hemicellulose, and cellulose can be found in Table S1.

Table 1. Chemical characteristics of the applied raw and processed digestates.

Biogas Plant	Product	Year	DM ⁴	C _t ⁶	C _{org} ⁷	N _t ⁸	NH ₄ -N ⁹	NH ₄ -N:N _t	C:N	pH
			(g·kg ⁻¹ FM ⁵)	(g·kg ⁻¹ DM)						
1	Raw digestate (RD1)	2015	79	348	345	64.1	30.2	0.47	5.4	8.1
	Sep. liquid (SL1) ¹		56	358	354	86.5	43.1	0.50	4.1	8.0
	Sep. solid (SS1) ¹		311	431	429	25.2	6.4	0.25	17.1	8.2
	Dry solid (DS1)		902	416	413	25.6	1.7	0.07	16.2	8.2
	Pellet (P1)		889	402	398	35.1	4.3	0.12	11.5	7.7
	Raw digestate (RD1)	2016	108	435	432	47.6	18.2	0.38	9.1	7.3
	Sep. liquid (SL1) ¹		65	398	394	78.4	36.0	0.46	5.1	7.8
	Sep. solid (SS1) ¹		299	456	454	18.6	3.9	0.21	24.6	8.6
	Dry solid (DS1)		546	461	459	21.4	1.0	0.05	21.5	7.8
	Pellet (P1)		851	447	445	34.9	2.4	0.07	12.8	7.9
2	Raw digestate (RD2)	2015	54	381	376	96.1	62.2	0.65	4.0	7.8
	Sep. liquid (SL2) ¹		50	368	362	103.4	68.6	0.66	3.6	7.8
	Sep. solid (SS2) ¹		225	398	396	28.1	15.0	0.53	14.1	8.3
	Concentrate (CC2) ²		85	375	373	48.8	19.7	0.40	7.7	9.0
	ASS2 ³		322	<2	<2	185.8	181.9	0.98	0.01	1.3
	Raw digestate (RD2)	2016	77	406	403	68.6	41.8	0.61	5.9	7.7
	Sep. liquid (SL2) ¹		47	393	388	106.4	60.8	0.57	3.7	7.9
	Sep. solid (SS2) ¹		174	434	432	32.1	14.4	0.45	13.5	8.9
	Concentrate (CC2) ²		136	399	395	38.7	6.5	0.17	10.3	8.9
	ASS2 ³		278	1	1	202.9	193.7	0.95	0.007	2.4

¹ Sep. liquid/solid: separated liquid/solid; ² CC2: concentrate after vacuum evaporation; ³ ASS2: ammonium sulfate solution after vacuum evaporation; ⁴ DM: dry matter; ⁵ FM: fresh matter; ⁶ C_t: total carbon; ⁷ C_{org}: organic carbon; ⁸ N_t: total nitrogen; ⁹ NH₄-N: ammonium N.

Table 2. Detergent fiber analysis of raw and treated digestates used in this study, according to van Soest and Wine [26].

Biogas Plant	Product	aNDF ⁴ (g·kg ⁻¹ DM)		ADF ⁵ (g·kg ⁻¹ DM)		ADL ⁶ (g·kg ⁻¹ DM)	
		2015	2016	2015	2016	2015	2016
1	Raw digestate (RD1)	411	477	377	438	170	223
	Sep. liquid (SL1) ¹	205	379	204	382	109	244
	Sep. solid (SS1) ¹	683	785	518	649	216	426
	Dry solid (DS1)	533	653	493	607	227	393
	Pellet (P1)	517	583	463	520	225	316
2	Raw digestate (RD2)	96	455	228	411	134	187
	Sep. liquid (SL2) ¹	21	189	254	298	174	193
	Sep. solid (SS2) ¹	727	693	563	524	207	179
	Concentrate (CC2) ²	89	169	260	259	112	141
	ASS2 ³	<10 ^{7,8}	<10 ^{7,8}	<5 ^{7,8}	<5 ^{7,8}	<5 ^{7,8}	<5 ^{7,8}

¹ Sep. liquid/solid: separated liquid/solid; ² CC2: concentrate after vacuum evaporation; ³ ASS2: ammonium sulfate solution after vacuum evaporation; ⁴ aNDF: neutral detergent fiber; ⁵ ADF: acid detergent fiber; ⁶ ADL: acid detergent lignin; ⁷ measured values fall below the detection limits; ⁸ the value is presented as g·kg⁻¹ FM.

Dry matter content was measured by drying at 105 °C until constant weight. The pH of solid digestates was measured in 10⁻² M CaCl₂ solution, while, in the case of the liquids, the original substance was used. The total C content was determined by elemental analysis (vario MAX CN, Elementar Analysensysteme, Hanau, Germany). Total nitrogen (N_t) and NH₄⁺-N contents were measured using the Kjeldahl method and by steam distillation with titration, respectively.

2.2. Experimental Site

A field trial was carried out in spring 2015 and 2016 at the experimental station “Heidfeldhof” (48°42′40″ N, 9°11′45″ E; 389 m a.s.l.). The mean annual air temperature was 10.2 °C and the total annual precipitation was 628 mm for the period 2007–2016. During the measuring period, the air temperature averaged 15.0 °C (2015) and 11.4 °C (2016). Total precipitation was 110 mm in year 1 (27 April–12 June 2015) and 122 mm in year 2 (11 April–30 May 2016).

The experimental fields were located close to each other (approximately 50 m distance), with almost similar soil characteristics (Table 3). Soil type was a Haplic Luvisol from periglacial loess.

Table 3. Main soil characteristics in the A_p-horizon (0–0.3 m depth) of the experimental fields in 2015 and 2016 before fertilization.

Year	Sand (%)	Silt (%)	Clay (%)	Bulk Density (Mg·m ^{−3})	pH (CaCl ₂)	C _{org} (%)	N _t (%)	N _{min} ¹ (kg·ha ^{−1})
2015	2	68	30	1.29	6.5	1.8	0.16	19.9
2016	9	69	22	1.24	7.0	1.1	0.14	7.3

¹ Initial N_{min} (NH₄⁺ and NO₃[−]) at the beginning of each experiment.

2.3. Field Experiment

The field trial was set up on fallow land (between crop rotations) in order to avoid changes in N turnover resulting from plant growth and N uptake. It represented a randomized complete block design with four replicates. We tested two raw and eight processed digestates and an unfertilized control. A total of 44 microplots with a size of 2.25 m² each were established and used for the statistical analyses. The fertilizers were applied once at the beginning of the experiment at a rate of 170 kg N_t·ha^{−1} (the maximum amount of applied N in the form of organic fertilizer as regulated by the German fertilizer ordinance in 2015 [27]; total amounts are presented in Table S2). N₂O emissions after digestate application were measured using the closed chamber method [28]. Each fertilizer was incorporated in a small furrow within the base ring (0.3 m inner diameter) of the chamber. After closing the chamber, four gas samples were taken from the chamber’s atmosphere periodically (15 min intervals) and transferred into 20 mL pre-evacuated glass vials with a syringe.

During the gas measurements, the air temperature inside the chamber was determined. Samples were collected three times a week within the first 2 months after fertilization between 9:00 a.m. and 12:00 p.m., since this time period covers the mean daily soil temperature. Thus, biases during extrapolation of flux rates to daily fluxes due to diurnal soil temperature variations were minimized [29].

After each gas sampling, soil moisture (TDR sensor, FP/mts, Easy Test, Institute of Agrophysics, Polish Academy of Science, Poland), soil temperature (10 cm depth), and air temperature were determined. To verify the volumetric measurement of soil moisture, the gravimetric soil water content was additionally analyzed by collecting 10 random soil samples of the top soil and drying them at 105 °C until constant weight. At the beginning of the experiments (27 April 2015 and 11 April 2016, respectively), stainless-steel cylinders (100 mL) were used to measure the bulk density of the A_p horizon. As a function of the bulk density, the current water-filled pore space (WFPS) was calculated as described by Ruser et al. [30]. The mineral nitrogen (N_{min}) content of the topsoil was determined colorimetrically after extraction of a soil aliquot with 0.5 M K₂SO₄ solution (Continuous Flow Analyzer, AA3 HR; SEAL Analytical, Inc., Norderstedt, Germany). The main physical and chemical characteristics of the soil, such as soil texture and C_{org} and N content, are shown in Table 3.

2.4. Incubation Experiment

For the incubation experiment, the following digestates were compared: solid after separation (SS1), dried (DS1) and pelleted digestates (P1), raw digestate (RD2), liquid after separation (SL2), concentrate (CC2), and untreated control (control). The setup was a completely randomized design with four replicates. For comparison with the field investigation, soil was taken from an area next to the 2016 field trial. It was obtained from the A_p horizon, sieved ≤ 4 mm, and air-dried prior to the experiment. Physical and chemical soil characteristics are shown in Table 3.

A mixture of digestate and soil was incubated in 2.5 L glass jars in a climate chamber at 20 °C for 51 days (in the dark). Each jar contained 700 g air-dried soil, mixed with the respective amount of organic fertilizer equivalent to a total of 170 kg N_t·ha⁻¹ (45.8 mg N_t per glass jar). At the beginning of the incubation, distilled water was added to adjust the soil water content to 60% water-holding capacity (equal to ~50% WFPS, [30]). This ensured the development of aerobic conditions in the soil and was in the range of the field trials, as well as that reported in previous literature [31]. It was chosen in order to simplify the comparison between the results obtained in both experiments. A 20 mL beaker glass was filled with distilled water and set into each glass jar to avoid potential water losses as a result of soil evaporation during the experiment. The glass jars were equipped with a PVC lid and closed airtight. A bulkhead tube fitting was fixed in the middle of the lids and closed with a septum according to the closed chamber method previously outlined. To ensure aerobic conditions during incubation, the jars were opened daily for aeration. Throughout the first 2 weeks, gas samples were taken once a day, whereas, during the rest of the experimental period, the sampling interval was reduced to once a week. For measuring the N₂O flux rates, four samples were collected from the jar's headspace at 15 min intervals. Before taking the first gas sample, the glass jars were opened for aeration. To avoid low pressures during sampling, 150 mL of N₂ was injected into each jar. The collected samples were kept in gas vials until the analysis was performed.

2.5. Trace Gas Analysis and Flux Rate Calculation

The analysis of the gas samples was performed using a gas chromatograph (GC 450, Bruker Daltonik, Bremen, Germany) coupled with an autosampler (GX-281, Gilson, Limburg, Germany). Further details on the GC measurements can be found in Petrova et al. [32].

The calculation of flux rates took into account N₂O concentration of the four gas samples (ppb), air temperature (°C), covered soil area (m²), and volume (L) of the chamber headspace. While, for the field experiment, the volume of the headspace was equivalent to the internal volume of the closed chamber, for the incubation jars, it was calculated differently. The sum of actual headspace (above soil surface), 150 mL N₂ injection, and air-filled pore space in the soil formed the total air volume. The N₂O fluxes were calculated using the R-Package “gasfluxes” [33,34].

2.6. Statistical Analysis

The datasets were statistically analyzed using the software SAS (v 9.4). Residuals were tested for normality and homogeneity of variance. If required, data were logarithmically transformed prior to analysis to fulfil the pre-requirements. For estimating the effect of digestate treatment on cumulative N₂O emissions, a one-way ANOVA was performed for each experimental year and for both experiments separately. This model used data of 44 experimental units. Here, the digestate treatment acted as a main independent variable. Additionally, the significant differences among the treatments were calculated using a Tukey HSD test ($p \leq 0.05$). Differences were presented using a letter display [35]. If transformed data were analyzed, means were back-transformed for presentation purpose only. In this case, standard errors were back-transformed using the data methods. Furthermore, two linear regression models were run to quantify the effect of C:N or NH₄⁺-N:N_t of digestates on the cumulative N₂O emissions. Biogas plant was used as a dummy variable.

To test the effect of environmental variables (air and soil temperature, rainfall, and WFPS) on the N_2O fluxes, a stepwise multiple regression (forward procedure, F to enter: 4.000, F to remove: 3.900) was conducted for each combination of year and digestate.

3. Results

3.1. Field Experiment

3.1.1. Weather Conditions in 2015

Within the first measuring period, the main rainfall events were in weeks 1, 3, 5, and 7 with total amounts of precipitation ranging between 16 and 36 mm per week (Figure 1a). Air temperature (2 m height) varied between 8 and 25 °C, whereas soil temperature (10 cm depth) ranged between 10 and 32 °C (Figure 1a). The highest soil moisture contents were associated with rainfall events and accounted for 55% and 56% WFPS in the first and third weeks of the measurements (Figure 1a).

Figure 1. Air temperature (2 m height), soil temperature (10 cm depth), daily precipitation, and mean water-filled pore space during the field trial (a: year 2015, d: year 2016); mean N_2O flux rates ($n = 4$) as affected by digestate from biogas plant 1 (b: year 2015, e: year 2016) and plant 2 (c: 2015, f: year 2016); sep.: separated, concentrate: concentrate after vacuum evaporation, ASS2: ammonium sulfate solution of biogas plant 2.

3.1.2. Daily N_2O Fluxes in 2015

In 2015, the N_2O flux rates ranged between 4 (DS1, 27th April) and 930 $\mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ (P1, 4th May) (Figure 1b,c). The highest flux was determined 1 week after

fertilizer application within the first period of steady rainfall (36 mm in total, 27 April–3 May 2015) (Figure 1b). An increase in flux rates was also observed in the other treatments, including the control.

At the beginning of week 3, air temperature rose by 5 °C. The separated liquid of both biogas plants showed increasing flux rates, which averaged 537 (SL1) and 216 $\mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ (SL2). A similar pattern was also found in plots treated with RD1 and SS2. At the end of week 3, the next rain event occurred, followed by a second N_2O peak in P1. Furthermore, air and soil temperature dropped and WFPS increased to 56%. Despite additional rainfall events during the following 2 weeks, N_2O fluxes remained low. During the sixth experimental week, flux rates increased again in all treatments before dropping to the baseline level in week 7.

The stepwise multiple regression indicated a significant effect of the environmental conditions on the mean N_2O fluxes (air temperature: $r^2 = 0.36$, $p < 0.05$ (independent variable entered in step 1) and WFPS: $r^2 = 0.66$, $p < 0.05$ (independent variable entered in step 2)). It can be noted that 27–65% of the N_2O variability was predicted by differences in air temperature among the sampling dates (Table 4). With the exception of RD1, SL1, P1, and SL2, N_2O fluxes were also affected by soil temperature. In these three treatments, an additional effect of the WFPS on N_2O release was found.

Table 4. N_2O release as affected by air temperature (x_1), water-filled pore space (x_2), soil temperature (x_3), and rainfall (x_4) during the first experimental year (2015). Coefficient of determination and function of stepwise multiple regression (Forward procedure, F to enter: 4.000, F to remove: 3.900).

N ₂ O Release ¹	Model R ²	Air Temp		Rainfall	Soil Temp		WFPS ²		Final Equation of Stepwise Forward Regression
(g N ₂ O-N ha ⁻¹ d ⁻¹)		(°C)	(mm)	(°C)	(%)				
		Partial R ²	p-Value		Partial R ²	p-Value	Partial R ²	p-Value	
RD1	0.55	0.29	0.002	n.s.		n.s.	0.26	0.008	$y = -250.1 + 10.6x_1 + 4.4x_2$
SL1	0.27	0.27	0.020	n.s.		n.s.		n.s.	$y = -78.7 + 12.4x_1$
SS1	0.61	0.33	<0.001	n.s.	0.28	0.004		n.s.	$y = -11.7 + 8.6x_1 + 3.7x_3$
DS1	0.68	0.31	<0.001	n.s.	0.15	<0.001	0.22	n.s.	$y = 8.3 + 11.2x_1 - 6.1x_3$
P1	0.65	0.28	0.003	n.s.		n.s.	0.37	<0.001	$y = -553.9 + 16x_1 + 10.7x_2$
RD2	0.67	0.34	<0.001	n.s.	0.33	0.001		n.s.	$y = -26.2 + 15.5x_1 - 6.9x_3$
SL2	0.60	0.36	<0.001	n.s.		n.s.	0.24	0.007	$y = -126.3 + 6.1x_1 + 2.2x_2$
SS2	0.65	0.32	<0.001	n.s.	0.33	0.001		n.s.	$y = -23.9 + 17.3x_1 - 7.8x_3$
CC2	0.68	0.32	<0.001	n.s.	0.36	<0.001		n.s.	$y = -5.5 + 12.1x_1 - 5.6x_3$
ASS2	0.64	0.37	<0.001	n.s.	0.27	0.003		n.s.	$y = -43.2 + 15.7x_1 - 6.5x_3$
Control	0.75	0.35	<0.001	n.s.	0.40	<0.001		n.s.	$y = -24.4 + 12.5x_1 - 5.9x_3$

¹ RD1/2: raw digestate; SL1/2: liquid fraction; SS1/2: solid fraction; DS1: dry solid; P1: pellet; CC2: concentrate; ASS2: ammonium sulfate solution; number behind treatment abbreviations: number of respective biogas plant; ² WFPS: water-filled pore space.

3.1.3. Weather Conditions in 2016

Compared to year 1, the second observation period was characterized by a higher precipitation (122 mm) and lower mean air temperature, which ranged between 3 and 18 °C (Figure 1d). The soil temperature fluctuated between 7 and 33 °C (Figure 1d). The water-filled pore space reached two main peaks in the beginning of the experiment (58% WFPS, 18 April) and on the last measurement day (57% WFPS, 30 May).

3.1.4. Daily N_2O Fluxes in 2016

In 2016, all treatments showed lower N_2O fluxes than in 2015 (Figure 1e,f). One week after fertilization, the highest flux rate (294 $\mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) was measured in P1 (Figure 1e). It was observed in combination with the highest WFPS peak, which occurred after total rainfall of 36 mm and a temperature drop to 7 °C within the first experimental week.

During week 4 (2–8 May), a second significant increase in flux rates was observed in the treatments RD1, SL1, P1 and RD2, SL2, CC2 (Figure 1e,f). In addition, a continuous temperature rise and a decrease in WFPS were measured (Figure 1d). After the next heavy rain (at the end of week 7), N₂O fluxes in all treatments increased once again with exception of RD2.

In contrast to the results in year 1, the stepwise multiple regression indicated that weather conditions had a lower effect on the mean N₂O fluxes during 2016 (temperature: $r^2 = 0.19$, $p < 0.05$ (independent variable entered in step 1 of stepwise regression) and WFPS: $r^2 = 0.46$, $p < 0.05$ (independent variable entered in step 2)). The N₂O fluxes measured in untreated control, SS1, DS1, and ASS2 were partly predicted by daily rainfall (Table 5). In plots treated with SL2, air temperature showed an effect on N₂O production in the soil. Furthermore, a significant relationship between soil temperature and N₂O flux rates, as well as between WFPS and N₂O flux rates, was observed in the treatments RD1 and CC2. In the treatments SL1, P1, and SS2, weather conditions did not contribute to the variability of the flux rates.

Table 5. N₂O release as affected by air temperature (x_1), rainfall (x_2), water-filled pore space (x_3), and soil temperature (x_4) during the second experimental year (2016). Coefficient of determination and function of stepwise multiple regression (Forward procedure, F to enter: 4.000, F to remove: 3.900).

N ₂ O Release ¹	Model R ²	Air Temp		Rainfall		Soil Temp		WFPS ²		Final Equation of Stepwise Forward Regression
(g N ₂ O-N ha ⁻¹ d ⁻¹)		(°C)		(mm)		(°C)		(%)		
		Partial R ²	p-Value	Partial R ²	p-Value	Partial R ²	p-Value	Partial R ²	p-Value	
RD1	0.54		n.s.	0.40	<0.001	0.14	0.037		n.s.	$y = -22.1 + 14x_2 + 2.2x_4$
SL1			n.s.		n.s.		n.s.		n.s.	$y = \text{Intercept}$
SS1	0.50		n.s.	0.50	<0.001		n.s.		n.s.	$y = 7.4 + 3.1x_2$
DS1	0.31		n.s.	0.31	0.010		n.s.		n.s.	$y = 10.6 + 3.5x_2$
P1			n.s.		n.s.		n.s.		n.s.	$y = \text{Intercept}$
RD2	0.49	0.20	0.018	0.29	0.016		n.s.		n.s.	$y = -35.8 + 6.2x_1 + 11.1x_2$
SL2	0.36	0.36	0.005		n.s.		n.s.		n.s.	$y = -11.6 + 3.7x_1$
SS2			n.s.		n.s.		n.s.		n.s.	$y = \text{Intercept}$
CC2	0.77		n.s.	0.57	<0.001	0.13	0.002	0.06	0.051	$y = -215.8 + 18.6x_2 + 3.2x_3 + 4.3x_4$
ASS2	0.45		n.s.	0.45	0.001		n.s.		n.s.	$y = 10.2 + 4.8x_2$
Control	0.70		n.s.	0.70	<0.001		n.s.		n.s.	$y = 4.9 + 4x_2$

¹ RD1/2: raw digestate; SL1/2: liquid fraction; SS1/2: solid fraction; DS1: dry solid; P1: pellet; CC2: concentrate; ASS2: ammonium sulfate solution; number behind treatment abbreviations: number of respective biogas plant; ² WFPS: water-filled pore space.

3.1.5. Cumulative N₂O Emissions

The average cumulative N₂O emissions in year 1 were significantly ($p < 0.05$) higher than in year 2 (Figure 2a,b). This effect was also observed in the untreated control, which emitted almost 70% less in 2016 compared to 2015.

Concerning biogas plant 1 (solid processing chain) in both experimental years, the treatments raw digestate (RD1), separated liquid (SL1), and pellet (P1) showed higher cumulative emissions than the solids SS1 and DS1. In 2015, the separation of raw digestate (RD1) led to a slight increase in N₂O emissions after application of the subsequent liquid fraction (SL1). This was not the case in the second experimental year. After utilization of the separated liquid, 0.2–0.7% of applied N_t was released as N₂O. In terms of the further processing of separated solid, increasing cumulative emissions were determined especially after pellet (P1) application. On average, they were equivalent to 0.05 (dry solid, DS1) and 0.5% of applied N_t (P1), respectively.

Within biogas plant 2 (liquid processing chain), the differences in digestate composition did not significantly influence the cumulative N₂O emissions in 2015 (Figure 2a). In 2016, SL2 and SS2 resulted in lower emissions (0.2–0.09% of applied N_t) when compared to RD2 (Figure 2b). Regarding the subsequent processing of SL2, the cumulative emissions increased after applying the N-depleted concentrate (0.4% of applied N_t) and decreased after ASS2 utilization (0.07% of applied N_t).

Figure 2. Mean cumulative N₂O emission in the field trial ($n = 4 \pm$ standard error) as affected by digestate and biogas plant in the two experimental years (a: 2015; b: 2016); concentrate: concentrate after vacuum evaporation, ASS: ammonium sulfate solution; at least one identical letter indicates nonsignificant differences among treatments, according to a Tukey HSD test ($p \leq 0.05$).

Within biogas plant 1, N₂O emissions were negatively correlated to the C:N ratio of the digestates (2015: $r^2 = 0.71$, $p < 0.05$; 2016: $r^2 = 0.64$, n.s.), while, in plant 2, this effect could not be verified (data not shown). Concerning the influence of the fertilizer NH₄⁺-N:N_t ratio on the N₂O emissions, no correlation was found during both observation periods.

3.2. Incubation Experiment

3.2.1. Daily N₂O Fluxes

During the first week of incubation, the N₂O release increased in all fertilizer treatments (Figure 3). The highest N₂O flux rate ($679 \mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) was determined 3 days after fertilization in the pellet treatment of biogas plant 1 (P1, Figure 3a). Concerning the liquids of plant 2, SL2 and CC2 showed the first significant N₂O peaks on day 4 followed by a second small increase in SL2 on day 8 (Figure 3b). During the next 6 weeks, the flux rates remained on a low level in all treatments of both biogas plants. Additional N₂O peaks were measured in SS1 ($75 \mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, day 16), DS1 ($89 \mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, day 25) and SL2 ($58 \mu\text{g N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, day 44).

3.2.2. Cumulative N₂O Emissions

The subsequent processing of solid significantly affected N₂O emissions. The highest cumulative N₂O emission during the whole incubation period ($1.5 \text{ kg N}_2\text{O-N}\cdot\text{ha}^{-1}$ on average) was determined in the pellet treatment (P1) and corresponded to 0.8% of applied N_t (Figure 4). Lower cumulative emissions (0.03 and 0.2% of applied N_t) were observed when separated and dry solid (SS1 and DS1) were utilized. Concerning the liquid processing (biogas plant 2), treatments showed no significant effect on the N₂O release. The application of raw digestate (RD2) caused the lowest cumulative emissions within the plant ($0.18 \text{ kg N}_2\text{O-N}\cdot\text{ha}^{-1}$ or 0.02% of applied N_t), which did not differ significantly from those of the untreated control ($0.15 \text{ kg N}_2\text{O-N}\cdot\text{ha}^{-1}$). The post-treatments SL2 and CC2 showed slightly higher cumulative emissions than RD2 and released 0.2% (SL2) and 0.14% of applied N_t (CC2), respectively.

Figure 3. Mean N_2O flux rates ($n = 4$) as affected by digestate and biogas plant (a: biogas plant 1; b: biogas plant 2) measured during the incubation; sep.: separated, concentrate: concentrate after vacuum evaporation.

Figure 4. Mean cumulative N_2O emissions ($n = 4 \pm$ standard error) as affected by digestate and biogas plant measured during the incubation; sep.: separated, concentrate: concentrate after vacuum evaporation; at least one identical letter indicates nonsignificant differences between the treatments, according to a Tukey HSD test ($p \leq 0.05$).

A strong negative correlation ($r^2 = 0.96$, $p < 0.05$) between the cumulative emissions and the C:N ratio of the digestates was found within biogas plant 1. For plant 2, the C:N ratio did not show any effect on the N_2O release. Furthermore, the N_2O emissions were not affected by the $NH_4^+ - N:N_t$ ratio of digestates (data not shown).

4. Discussion

4.1. General Factors Influencing the N_2O Release

In the current study, the field application of raw or treated digestate caused a temporary increase in soil N_2O emissions. Although different feedstocks and processing approaches were used in both biogas plants, the resulting emissions of corresponding products did not differ significantly in most cases. It is assumed that, in our work, the environmental conditions, especially soil moisture and temperature, were of major importance and mostly overlaid digestate composition-related effects. During the first experimental year, warmer conditions with relatively high precipitation favored N_2O production and release. Increasing fluxes after fertilizer application and in connection with soil wetting (i.e., rainfall) (Figure 1) have already been reported in literature [22,36–39]. In accordance with Hayakawa et al. [22] and Häfner et al. [40], the highest N_2O peaks were observed within the first rainfall event (week 1). Hayakawa et al. [22] assumed denitrifying activity as the main driver, since they measured a simultaneous increase in CO_2 release. The simultaneous CO_2 release was also noted by Häfner et al. [40], who stated that at least part of the emitted CO_2 originates from digestate carbonate C, indicating that such a CO_2 release cannot only be assigned to the decomposition of organic matter. In the present study, the N_2O release directly after fertilization might have been a result of nitrification followed by denitrification, which was triggered by the high initial NH_4^+ content of digestate. On the other hand, the supply of easily degradable organic C and ammoniacal N with the digestates might have further promoted denitrification by (i) providing electrons as reduction equivalents [41], and (ii) stimulating O_2 consumption and consequently leading to more anoxic conditions [21,42–44].

The subsequent peaks in N_2O fluxes determined during the following weeks can be partly associated with further rainfall events, which was also described in previous studies [40,45–47]. During rather dry periods organic decomposition was reduced [48]. Subsequent rainfall induced soil moisture content which favored mineralization of residual organic molecules derived from digestates [48,49]. It provided labile N and C for denitrifying bacteria. Thus, denitrification was promoted and is assumed to be the main source for the N_2O production. Measurements of WFPS during the relevant N_2O peaks (WFPS of 55–58%, Figure 1a,d) also indicate that nitrification and denitrification occurred simultaneously [50]. It can be concluded that weather conditions affected the emissions mainly immediately after digestate application and their influence decreased with time.

4.2. N_2O Emissions Affected by Changes in Digestate Composition Due to Processing

4.2.1. Solid–Liquid Separation

Generally, the application of the same amount of N_t as separated solid or liquid led to similar or lower emissions than the utilization of raw digestate. This can be explained by the partial separation of two important factors: organic C (solid) which supplies electrons during nitrate reduction and ammoniacal N (liquid) which serves as substrate for combined nitrification–denitrification [13]. If the actual N partition due to the solid–liquid separation into single fractions (12% separated solid, 88% separated liquid) [51] is taken into account, the cumulative N_2O emissions from both fractions added up to 70–95% of the value of raw digestates in most cases. These results are slightly higher than those of Askri et al. [13] who reported cumulative N_2O emissions from both separated products of up to 50% of raw digestate. Hence, it can be concluded that solid–liquid separation potentially reduces the overall N_2O emissions after field application due to a partial separation of mineral N and organic C. Therefore, in untreated digestates, the interaction of these two compounds seems to boost the emissions.

Concerning the comparison between the separated products (liquid and solid) within each plant, the lower cumulative emissions after application of the solid fraction were in line with the previous literature [16,17]. The solid was characterized by a higher total C content, higher amounts of recalcitrant fractions, and a lower content of mineral N than raw digestate (Tables 1 and 2). The mentioned characteristics mainly result from processing and subsequent gaseous N losses during storage of the solid fraction [10,52]. During anaerobic digestion, a shift in particle size distribution toward larger and more recalcitrant particles takes place [53]. The solid–liquid separation of digestate removes further solids (>0.5 mm) and allocates most of the NH_4^+ to the liquid fraction (Table 1). The following storage of the separated solid further increases the proportion of large to small particles due to preferred biological decomposition of the smaller ones [54] and reduces the NH_4^+ content due to N immobilization and gaseous N losses (NH_3 volatilization, denitrification) [52]. As a result, the solid contains less soluble compounds, especially mineral N (Table 1 and Table S1). Additionally, the larger particles are more resistant to degradation and, consequently, the O_2 consumption is probably reduced [12,55] due to the lower C mineralization. Both lower substrate availability (N as NH_4^+) and higher aeration likely reduced the N_2O release from denitrification after field application. Within 7 weeks, the cumulative N_2O emissions in the solid treatment were 30–100% of the raw digestate (Figure 2a,b). This indicates the potential of separated solid to reduce the emissions after application compared to the precursor, as also reported in previous studies [15,56]. However, a concluding statement about the overall greenhouse gas reduction potential should also consider N losses, which might occur during processing and storage [10], as well as methane and carbon dioxide emissions.

4.2.2. Subsequent Processing of Separated Liquid

In contrast to our initial assumption, the N removal during subsequent processing of separated liquid did not clearly affect the N_2O emissions after application of the respective products.

When comparing separated liquid with its partially dewatered concentrate post treatment, no significant effect was found (Figures 2 and 4), although separated liquid had at least a threefold higher NH_4^+ content (based on total FM) and a lower C:N ratio. Since the proportion of recalcitrant fractions, such as lignin, was lower in concentrate (Table 2), it can be assumed that the presence of degradable C influenced bacterial growth and activity and, thus, triggered the N_2O emissions. The addition of labile C can also induce N_2O emissions derived from soil mineral N [47,57,58], which might have enhanced this effect in the case of concentrate (Figure 2b). Furthermore, a part of initial labile N applied with separated liquid was probably immobilized by soil microflora immediately after fertilization [59], which mitigated the total amount of N_2O emitted from this treatment. These results generally suggest that the supply of ammoniacal N via liquid digestates is less relevant for the emissions after field application. This effect was also shown in the case of ammonium sulfate treatment (ASS2).

The lower emissions observed in ASS2 (year 2, field experiment) might have been a result of the negligible C supply compared to the concentrate. Moreover, a short-term NH_3 volatilization probably occurred directly after soil application of ASS2 [60–64], which reduced the amount of NH_4^+ added to soil and available for N_2O production. A reduced availability of introduced mineral N and consequently low N_2O release can also be attributed to partial N immobilization during the initial experimental phase, as reported by Fanguiero et al. [65]. Although NH_3 volatilization and N immobilization were not measured in the present work, it is assumed that the absence of C in ASS2 was the main reason for the low N_2O emissions observed in the second year. During year 1, weather conditions (higher temperature and relatively high precipitation) probably overlaid this effect.

4.2.3. Further Processing of Separated Solid

Concerning the solid processing chain, the significant reductions in N_2O emissions after application of the intermediate products (separated solid and dried solid, both low

in ammoniacal N) compared to raw digestate underlined, once again, the importance of simultaneous substrate availability (N as NH_4^+) in the presence of degradable organic C. Even though considerable amounts of solids (and organic matter) were applied to the soil in the separated solid and dried solid treatments (Table S2), N_2O emissions were still limited by the availability of NH_4^+ and did not differ significantly from the control. Between these two solids, emissions were also not significantly different. This result was not in line with the finding of Askri et al. [13] who reported a fourfold increase in N_2O emissions after fertilization with dried solid. However, since they assumed that the mineralization of N already present in the soil was of higher importance for the N_2O production than the introduction of additional mineral N via fertilizer, the discrepancies between both studies could be explained by soil-related effects, such as soil type, characteristics, and nutrient level. Another reason for their findings could be an elevated substrate biodegradability, which would favor microbial growth and activity.

In general, by removing water from the separated solid, a dried fertilizer with similar total C content, lower NH_4^+ concentration, and higher soluble material load is produced (Table 1 and Table S1). In the present study, these changes led to a slightly higher but not significant N_2O release after application compared to the separated solid (Figures 2 and 4). The lower share of recalcitrant fractions in the dried solid (Tables 1 and 2) provides some indications for consecutive changes in the composition of the organic matter during the drying process [66–70]. Consequently, it can be concluded that, in the case of dried solid, the addition of easily degradable C in combination with soil-derived NO_3^- triggered the N_2O production and release. This notion is consistent with previous findings, based on experiments with ^{15}N -labeled manures [40,47,57,58]. It highlights the complexity of the presented issue and the importance of additional determination of the responsible N source. Additionally, the interaction among soil parameters, added organic matter fractions, and weather conditions should be taken into consideration.

The high N_2O emissions observed after pellet application (Figures 2 and 4) are in accordance with the results of Hayakawa et al. [22], who compared poultry manure with its pelleted post-treatment. In general, the strong increase in temperature during pelletizing affects the properties of organic compounds and creates constant inner microsites [32,71–74]. According to Petrova et al. [32], the water addition to the pellet activated the decomposition of its organic matter by indigenous microflora. Consequently, an enhanced microbial O_2 consumption favored the development of anaerobic microsites inside the pellet and, thus, stimulated denitrification. In addition, the increase in soil NO_3^- content after pellet application measured by Hayakawa et al. [22] indicated a probable nitrification at the soil–pellet interface. Since the temporal changes in soil NO_3^- content were not observed in the present study, denitrification inside the pellets was probably the main emission driver. It seems that concentrated application of organic manures, either in the form of pellets or via fertilizer injection [42,75,76], results in a strong increase in N_2O emissions from the soil. It was shown that both the composition of organic manure and the interaction between applied manures and soil strongly affect N_2O emissions.

5. Conclusions

The present study showed that, aside from environmental conditions, it is not possible to draw general conclusions regarding how digestate composition influences the processes driving the N_2O emissions after fertilization. For example, the N removal during further processing of separated solid and liquid does not affect the emissions after application of the respective products. More relevant for N_2O production and release are the strong interactions between digestate composition, O_2 consumption triggered by decomposition of applied C, nitrification of fertilizer mineral N, and denitrification induced by added C and soil mineral N.

When considering the different processing techniques, it can be concluded that separation of raw digestate has the potential to reduce N_2O emissions after soil application. This reduction potential is also true for the dried separated solid. Additionally, separated

solid and dried solid can outperform a mineral fertilizer, such as ammonium sulfate, with respect to soil-derived N_2O emissions. However, pelletizing of the dried product stimulates water-induced denitrifying activity inside the pellet body and results in a dilemma. Much higher N_2O emissions after soil application counteract positive aspects such as excellent storability and transportability properties. Regarding the utilization of different liquid fractions (concentrate and ammonium sulfate), further field and laboratory experiments are recommended to better understand the interactions between the processes driving N_2O release after fertilization. Last but not least, future assessments of digestate post-treatments should consider covering the whole digestate value chain, including N_2O and other emissions during processing, storage, and field application.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112215/s1>: Table S1. Organic carbon composition of tested digestates. Contents of soluble materials, hemicellulose, and cellulose were calculated from detergent fiber analysis; Table S2. Amount of fresh matter (FM), organic carbon (C_{org}), and ammonium nitrogen ($\text{NH}_4\text{-N}$) equivalent to $170 \text{ kg N}_t \cdot \text{ha}^{-1}$ applied as digestates.

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