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Field Application of Organic Fertilizers Triggers N_2O Emissions From the Soil N Pool as Indicated by ^{15}N-Labeled Digestates

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Anaerobic digestion (AD) can generate biogas while simultaneously producing digestate which can be used as fertilizer. Feedstocks used for AD influence digestate composition, which in turn may affect carbon (C) and nitrogen (N) turn-over in soils and subsequently influence nitrous oxide (N_2O) emissions after soil application. Assessment of greenhouse gas emissions from digestates can help to evaluate the overall sustainability of an agricultural production system. The objective of this study was therefore to evaluate and understand the effect of differences in digestate composition on *in situ* N_2O emissions within the 1st weeks after application of seven digestates. The digestates were derived from different feedstocks and ^{15}N-labeled, either in total N or only in ammonium-N. Therefore, the experimental design enabled us to differentiate between potential N_2O-N sources (i.e., digestate N or soil N). Furthermore, it allowed to distinguish to some extent between organic-N and ammonium-N as potential N sources for denitrification. Digestates were homogeneously incorporated into the upper 5 cm of microplots in an arable Haplic Luvisol in South Germany at a rate of 170 kg N ha^{-1}. After application, N_2O fluxes were measured for ~60 days (May-July) using the closed chamber method in 2 experimental years. Mainly due to higher precipitations in the 1st year, cumulative N_2O emissions were higher (312–1,580 g N_2O-N ha^{-1}) compared to the emissions (133–690 g N_2O-N ha^{-1}) in the 2nd year. Between 16–33% (1st year) and 17–38% (2nd year) of N_2O emissions originated from digestate N, indicating that digestate application triggered N_2O production and release mainly from soil N. This effect was strongest immediately after digestate application. It was concluded that the first (short term) peak in N_2O emissions after digestate application is largely related to denitrification of soil-N. However, the experimental setup does not allow to differentiate between the different denitrification pathways. Weather conditions showed a substantial effect on N_2O emissions, where the correlation between N_2O and CO_2 flux rates hinted on denitrification as main N_2O source. The effect of digestate composition, particularly organic N from the digestate, on soil N_2O emissions seems to be of minor relevance.

Keywords: anaerobic digestion, biogas slurry, organic fertilizer, greenhouse gas emissions, stable isotope, field experiment

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INTRODUCTION

In the EU, about 180 million tons of anaerobic digestate are estimated to be produced per year, most of which is used as organic fertilizer (Corden et al., 2019). Digestates have been shown to have the potential to substitute mineral fertilizers and contribute to a sustainable soil management (Gutser et al., 2005; Cavalli et al., 2016; Verdi et al., 2019). However, application of organic as well as mineral nitrogen (N) fertilizers is also known to increase greenhouse gas (GHG) emissions from soils. Globally, agriculture contributes up to 20% to carbon dioxide equivalents (CO_2-eq.) from all human activities (2010–2017), with nitrous oxide (N_2O) and methane (CH_4) as main GHGs (FAO, 2020). About 60% of anthropogenic N_2O emissions are emitted by agricultural soils (Ciais et al., 2013), thus it is of high relevance to assess N_2O in relation to fertilizer application.

Studies have shown that digestates might lead to a higher risk of N_2O formation than manures, which is related to the higher share of ammonium (NH_4^+-N) after AD (Möller and Stinner, 2009). Ammonium is quickly nitrified to nitrate (NO_3^-), which can further be denitrified to dinitrogen gas (N_2). Both processes, as well as nitrifier denitrification, bear the risk of producing N_2O and are considered as main N_2O source from soils (Granli and Bøckman, 1994; Bremner, 1997; Koola et al., 2010). Application of liquid manures like slurry or digestates provides available N and carbon (C), which in turn promotes heterotrophic activity (oxidation of C, N, S, etc.), depleting oxygen (O_2) availability in soil, and thus favors creation of anaerobic microsites that ultimately trigger N_2O production and release via denitrification (Chadwick et al., 2000; Petersen et al., 2003). Hence, N_2O emissions largely depend on the availability of labile organic C (C_{org}), mineral N, O_2 and water in the soil and their subsequent effect on soil microbial processes (Flessa and Beese, 2000; Ruser et al., 2001). However, AD has also been reported to reduce the N_2O potential compared to the initial feedstock e.g., by decreasing slurry viscosity or increasing the recalcitrance of organic matter (OM) (Petersen, 1999; Möller, 2015).

The different organic substrates that are used as feedstock for anaerobic digestion (AD) affect the physico-chemical characteristics of the digestate (Fouda et al., 2013; Zirkler et al., 2014). For example, comparing food wastes and maize silage, food wastes are already processed goods with a high degradability and high protein content. Thereby, food waste-based digestates tend to have a higher OM degradability and a higher share of NH_4^+-N than maize silage, that could enhance soil microbial activity (Möller and Müller, 2012; Guilayn et al., 2020).

Based on compositional differences, such as N content, C/N ratio and OM degradability [C_{org}/organic N (N_{org})], it can be assumed that digestates from different feedstocks will show differences in N_2O emissions after field application. However, a differentiated consideration of the GHG emission potential for digestates from different feedstocks is currently scarce, and therefore will be the main research focus of this study.

The largest share of N_2O release during the growing season usually occurs shortly after field application, with further peaks correlated to rainfall-events (Guzman-Bustamante et al., 2019; Herr et al., 2019) or freeze-thaw periods (Flessa et al., 1995; Rochette et al., 2008). For this reason, the following experiment was conducted to evaluate digestates regarding short-term N_2O emissions on fallow land. To calculate the amount of N_2O derived from the digestate, ^{15}N-stable isotope labeling was used. The following hypotheses were tested:

(1) Digestates with varying physical and chemical properties will show different temporal N_2O and ^{15}N-N_2O flux patterns.
(2) Application of these digestates will also result in different cumulative N_2O emissions and N_2O emission factors.
(3) The amount of N_2O-N directly derived from the digestate will differ among the digestate types.

MATERIALS AND METHODS

^{15}N Labeling and Digestate Production

Labeled anaerobic digestates were prepared by cultivation of ^{15}N-enriched plants in a comparable approach as applied by Schouten et al. (2012). Maize (*Zea mays* L. cv. Ronaldinio), ryegrass (*Lolium perenne* L. cv. Kentaur), and sugar beet (*Beta vulgaris* subsp. vulgaris, Altissima Group) were ^{15}N labeled, by addition of ^{15}N ammonium sulfate ($(NH_4)_2SO_4$) as fertilizing solution. Ryegrass was cultivated in sand culture in 12 kg boxes of 10 cm height. For fertilization, 96.2 mg N kg $^{-1}$ as $(NH_4)_2SO_4$ (30 atom% ^{15}N) solution was applied before sowing ryegrass. We cut the ryegrass three times in 30-days intervals. Sugar beet was grown in Mitscherlich pots with 12 kg sand. After pre-growing sugar beet seedlings in peat, two plants were set for each pot. Four rates of $(NH_4)_2SO_4$ (50 atom% ^{15}N) solution were applied during growth (in total 1.5 g N per pot). Maize was grown in a hydroponic system with two plants per 10-liter pot. Nutrient solution adapted after Engels (1999) with modified N concentration was exchanged twice a week. Within the first 5 weeks of growth, NH_4^+-N concentration was gradually increased, while NO_3^--N supply was decreased to acclimate maize plants to primary NH_4^+-N nutrition. After this adaption phase for the plants, the N concentration was kept stable at 0.5 mM NO_3^--N and 3 mM NH_4^+-N, in the form of calcium nitrate and ammonium sulfate. For ^{15}N labeling, four additions of NH_4^+-N were substituted by 50 atom% ^{15}N- NH_4^+ and applied at BBCH stages: 16–19, 30–33, 51–55, and 71. As commonly done for maize, as energy crop, it was harvested at the dough-ripe stage. After harvest, ryegrass, maize sugar beet, as well as sugar beet leaves were immediately cut and homogenized by short blending (Thermomix TM31, Wuppertal, Gemany). The ^{15}N enrichment of crops and harvest residues was determined by IRMS with previous freeze-drying, leading to 19.3 atom% ^{15}N in maize, 26.1 atom% in ryegrass, 43.8 atom% in sugar beet, and 45.3 atom% in sugar beet leaves. After weighing the ^{15}N-plant biomass into small portions, they were frozen at −20°C until anaerobic digestion in a batch reactor as previously described by Brulé (2014), Mönch-Tegeder et al. (2014).

Anaerobic digestion of the ^{15}N-labeled plants and plant residues was carried out at the State Institute of Agricultural Engineering and Bioenergy, at the University of Hohenheim. Before, digestates from maize, grass silage and sugar beet were

TABLE 1 | Physico-chemical digestate properties, ^{15}N-labeling and ^{15}N abundance.

	DM (%)	pH (water)	EC ($\mu S\ cm^{-1}$)	C_t (% DM)	C_{org} (% DM)	^{15}N labeling	^{15}N (atom%)	N_t (g kg^{-1} FM)	NH_4^+-N/N_t (%)	C/N	C_{org}/N_{org}
Maize (M)	6.7	8.3	4,370	37.8	37.1	N_t	7.4	6.77	67.9	3.7	11.3
Grass (G)	8.2	9.1	4,250	28.0	27.3	N_t	11.7	4.41	58.8	5.2	12.4
Sugar beet (SB)	3.9	8.5	1,380	16.9	15.9	N_t	6.3	2.03	63.6	3.3	8.5
Sugar beet leaves (SBL)	4.8	8.8	1,640	18.5	17.2	N_t	12.2	1.97	65.8	4.5	12.2
Organic waste (OW)	13.7	7.7	3,100	31.2	30.0	NH_4^+-N	5.36	6.33	56.9	6.8	15.1
Food waste (FW)	4.3	8.1	3,650	36.7	36.3	NH_4^+-N	5.36	6.79	67.6	2.3	7.1
Cattle slurry (CS)	9.3	7.9	3,380	38.3	37.9	NH_4^+-N	5.36	4.06	56.7	8.8	20.1

TABLE 2 | Soil characteristic at the beginning of the experiment spring 2016 (1st year) and 2017 (2nd year), mean mineral N (N_{min}) (± standard deviation ($n = 2$).

	pH ($CaCl_2$)	Total C (%)	Total N (%)	C/N	NH_4^+-N (kg N ha^{-1})	NO_3^--N (kg N ha^{-1})	N_{min} (kg N ha^{-1})
1st year	7.0	1.25	0.14	8.9	3.52 ± 1.70	27.5 ± 3.4	31.0 ± 5.1
2nd year	6.8	1.13	0.13	9.0	1.84 ± 0.41	30.2 ± 1.4	32.1 ± 1.8

collected from biogas plants in southern Germany to be used as inoculum for AD of the ^{15}N-feedstocks (maize, ryegrass, sugar beet, and leaves). Digestates were "starved" for 10 days according to the German standard VDI 4630 guideline (2016) to minimize residual gas production. During this starvation phase, the vessels were kept open and stirred to volatilize ammonia (NH_3) from the inoculum. By decreasing NH_4^+-N in the digestate, hence total N concentration, a high ^{15}N-signature could be assured, with only marginal N dilution of the added ^{15}N-feedstock. Prior to AD, the inoculum was sieved to produce a homogeneous slurry. ^{15}N-labeled ryegrass, maize, sugar beet, and sugar beet leaves were separately added to the substrate-specific inoculum in a ratio of 1:2.5 organic total solids (oTS) (VDI 4630, 2016). Anaerobic digestion was carried out in 2 liter fed-batch systems under mesophilic temperature at 37.5 ± 1°C for 60 days. During digestion, three feeding portions of ^{15}N enriched plant substrates were added: at the start of the experiment, after 20, and after 40 days, respectively. Due to the amount of oTS added by the digestates, the ^{15}N amount of the feedstocks was diluted by N contained in the inoculum, leading to a lower labeling of the ^{15}N-digestates compared to the initial plant feedstock (**Table 1**).

Additionally, digestates from existing biogas plants were included and the mineral NH_4^+-N fraction was labeled: organic waste digestate, food waste digestate, and cattle slurry digestate. The digestates were analyzed for total N and NH_4^+-N. Each digestate was filled into a glass beaker and put into a rotating water bath for 12 h at 37°C, to volatilize a small amount of NH_3. Afterwards the digestates were analyzed again for total N and NH_4^+-N to assess the amount of N that was emitted. The lost N was substituted by addition of ^{15}N-enriched $(NH_4)_2SO_4$ solution to 5 atom% ^{15}N excess. If more N was lost than resupplied by ^{15}N-NH_4^+, ammonium chloride solution was added.

Experimental Design

The experiment was performed at the research station "Heidfeldhof" at the University of Hohenheim, 13 km south of Stuttgart, in South-Germany. The research station has a mean annual precipitation of 686 mm and a mean annual temperature of 8.8°C, monitored by a local meteorological station. The soil type of the arable field was a Haplic Luvisol (IUSS Working Group, 2015) with a silty loam soil texture (2% sand, 68% silt, and 30% clay), a bulk density of 1.24 g cm^{-3} in the upper 30 cm. Soil analytical results are presented in **Table 2**. The micro-plot field experiment (1 × 1 m plot size) was conducted as randomized block design with four replicates per treatment in 2 years 2016 (1st) and 2017 (2nd year) from May to July. The treatments consisted of one unfertilized control and seven ^{15}N-labeled digestates based on maize (M), grass (G), sugar beet (SB), sugar beet leaves (SBL), organic waste (OW), food waste (FW), and cattle slurry (CS) (**Table 1**).

Gas Measurements and Analysis

The closed chamber system was used to monitor N_2O, CO_2, and methane (CH_4) soil fluxes (Hutchinson and Mosier, 1981). The system consisted of a polyvinyl chloride (PVC) base ring (30 cm inner diameter) and a corresponding chamber (Pfab et al., 2011). Within the center of the 1 m^2 micro plot, the PVC base ring was embedded 10 cm deep in the soil. The ^{15}N-labeled digestates (**Table 1**) were applied at a rate of 170 kg N ha^{-1}, meaning 1.2 g N per base ring and quickly incorporated into the upper 5–10 cm of the soil. In order to do so, a 10 cm deep furrow was dug across the ring, using a spade. Digestate was filled into the furrow, covered by soil and mixed. The same procedure was done for the unfertilized control using water. The amount of water (290 ml) corresponded to the average volume of digestate application. Directly after application, the first gas measurement was performed. For gas sampling between 8.00 and 12.00 am, the base ring was covered with the dark, vented PVC chamber, sealed by a rubber ring to collect the trace gas. The chambers were closed for 45–60 min. The first gas sample was directly taken after closure, followed by additional sampling every 15–20 min using a syringe and transferred into evacuated 20 ml gas vials. At the same time, two additional gas samples were collected into 100 ml vials at the start and end of each measurement for

^{15}N-N_2O determination. Soil and chamber temperature were recorded within each block from two random plots at beginning and end of sampling. Within the 1st month, gas samples were taken 3–4 times a week. Afterwards the sampling frequency was reduced to once or twice per week, with additional samplings after strong rainfall events. In both years 20 gas samplings were performed and measured for N_2O, CO_2, and CH_4, whereas 13–15 out of 20 ^{15}N-N_2O gas samples could be measured due to cost and time reasons in the 1st and 2nd year, respectively.

Gas samples were measured with a gas chromatograph (GC 450 Greenhouse Gas Analyzer, Bruker Daltonic, Bremen, Germany) equipped with electron capture detector (ECD) and flame ionization detector (FID) and an automatic sampler (GX-281, Gilson, Limburg, Germany). During GC measurements, concentrations of N_2O and CO_2 were analyzed with a ^{63}Ni ECD and CH_4 concentrations were determined with the FID. Fluxes of N_2O, CO_2, and CH_4 were calculated by an extended version of the R (R Core Team, 2016) package "gasfluxes" (Fuß and Asger, 2014).

Analysis of Digestate and Soil

Digestates were dried at 105°C for dry matter (DM) analysis. Total C (C_t) was measured by Dumas combustion via elemental analysis (Elementar vario MAX CN, Analysensysteme GmbH, Hanau, Germany). Carbonate content was determined volumetrically using the Scheibler method according to DIN 10693 (2014). Thus, organic C can be calculated as the difference between C_t and Carbonate-C. Total N (N_t) and NH_4^+ of fresh matter (FM) digestate sample was determined by Kjeldahl method. Organic N was derived by the difference of NH_4^+ from N_t. The pH value was measured in FM digestate using 0.01 mol L^{-1} calcium chloride solution (1:10 w/w).

Soil mineral N (N_{min})was determined by extraction with 0.5 M potassium sulfate solution (1:4) and measured colorimetrically with a photometer (Flow-injection-analyzer 3 QUAAtro, SEAL Analytical, UK). Bulk density of the top soil was determined using 100 ml stainless steel cylinders in the field.

Total N and C, and ^{15}N-signature of ^{15}N-labeled plant substrates, soil and digestate was measured with a CN-elemental analyzer (EuroVector, HEKAtech, Wegberg, Germany) with Isotope Ratio Mass Spectrometer (IRMS) (Delta plus Advantage, Thermo Finnigan, Bremen, Germany). For the determination of the ^{15}N abundance in N_2O we used an IRMS delta plus (Finnigan MAT, Bremen, Germany) coupled with an automated PreCon-Interface (Brand, 1995).

Statistics and Calculations

Trapezoidal linear interpolation of daily gas fluxes (N_2O and CH_4) was used to calculate total cumulative emissions for the 55–58 days of the experiment in the 1st and 2nd year, respectively. The percentage of N_2O-N originating from digestate N (Nd) was calculated by equation (1), with digestate *i* at sampling time *t*. Atom%^{15}N excess was calculated by subtraction of the natural abundance of N_2O in the atmosphere (0.369 atom%) from the measured ^{15}N. The daily N_2O flux rate ($\mu g\ N_2O$–$N\ m^{-2}\ h^{-1}$) was multiplied with *Nd* in equation (2) to determine the amount of N_2O derived from digestate (^{15}N-N_2O) as reported by Senbayram et al. (2009). We calculated the recovery (%) of ^{15}N applied by summing up ^{15}N content of the soil at the end of the experiment and cumulative ^{15}N-N_2O loss. This sum was then divided by the amount of ^{15}N applied by the digestate as described by Pfab (2011).

$$Nd_{i,t}\ (\%) = \frac{atom\%^{15}N\ excess\ digestate_i}{atom\%^{15}N\ excess\ N_2O_{i,t}} \quad (1)$$

$$^{15}N - N_2\ O_{i,t}\ \ \left(\mu g\ N_2\ O - N\ \ m^{-2}\ h^{-1}\right) = Nd_{i,t}\ (\%) * N_2O\ flux_{i,t} \quad (2)$$

Digestate derived fluxes (^{15}N-N_2O) were also linearly interpolated to calculate cumulative (cum) ^{15}N-N_2O emissions. The total share of N derived from digestate (total Nd) in cumulative N_2O was calculated by Equation (3). As suggested by Schleusner et al. (2018), the amount of primed N_2O-N lost by fertilizer application was calculated by a simplified approach accounting for cumulative N_2O-N emissions of the unfertilized control treatment (Equation 4), without considering other gaseous losses *via* NH_3 or N_2.

$$total\ Nd_i\ (\%) = \frac{cum\ ^{15}N - N\ _2O_i\ (g\ N_2O\ ha^{-1})}{cum\ N_2O_i\ (g\ N_2O\ ha^{-1})} * 100 \quad (3)$$

$$Primed\ N_2O_i\ (g\ N_2O\ ha^{-1}) = \left(cum\ N_2O_i - cum\ ^{15}N_2O_i\right) - cum\ N_2O\ control \quad (4)$$

$$EF_i\ (\%) = \frac{\left(cum\ N_2O_i - cum\ N_2O\ control\right)\ g\ N_2O\ ha^{-1}}{170\ kg\ ha^{-1} * 1000} * 100 \quad (5)$$

N_2O emission factors (EFs) were calculated according to the IPCC guidelines for direct emissions (Equation 5), meaning total cumulative N_2O-N emissions accounted for the control, per applied N (IPCC, 2019). The disaggregated IPCC N_2O EF for "other N inputs in wet climates" with the default value of 0.6% was applied for comparison, where other N inputs refer to organic amendments such as digestates. Field conditions of the experimental site showed a positive water balance and fit with IPCC conditions for wet climates (IPCC, 2019). Greenhouse gas emissions of N_2O and CH_4 were transformed to CO_2-eq. to assess the total global warming potential (GWP) of each digestate. The default values of 296 g g^{-1} CO_2 for N_2O and 24 g g^{-1} CO_2 for CH_4 were applied to the measured emissions. Ammonia (NH_3) volatilization within the first 72 h was derived from the ALFAM2 model to calculate potential indirect N_2O emissions (Hafner et al., 2019). The model is used to predict NH_3-N losses within the first 72 h from animal slurry and therefore holds a higher uncertainty for digestates. Digestate NH_3-N losses mainly served as an indicator for the amount of indirect N_2O emissions. Indirect emissions from NO_3^- leaching were not

accounted for. According to IPCC (2019) 1% of NH_3-N losses was assumed to be re-deposited as N_2O-N. Soil organic C stocks were presumed to be stable over the experimental period, thus, CO_2 fluxes were not considered for the calculation of total GWP (Herr et al., 2020).

Water filled pore space (WFPS) was calculated by Equation (6) using the measured volumetric water content (WC_{vol}) and porosity (P),

$$WFPS\ (\%) = \frac{WC_{vol}}{P} * 100 \quad (6)$$

where P is depicted as soil bulk density (ρd) and solid particle density (ρs) (Equation 7). For ρs the density of quartz (2.65 g cm^{-3}) was assumed.

$$P\ (\%) = \left(1 - \frac{\rho d}{\rho s}\right) * 100 \quad (7)$$

For each year, a regression analysis of N_2O fluxes was calculated, using a stepwise forward selection in a multiple linear regression approach. Air temperature (2 m height), WFPS and CO_2 fluxes were included as independent variables within the model (8). Only significant variables remained in the model ($\alpha = 0.05$) and the square root of the partial R^2 was determined. Same regression procedure was applied for cumulative N_2O and ^{15}N-N_2O emissions within each year separately. For this approach the effect of digestate composition was determined, using NH_4^+-N share, and the ratios C/N and C_{org}/N_{org} in model (9).

$$y_{it} = \mu + \beta_1 temp_t + \beta_2 WFPS_{it} + \beta_3 CO_{2it} + b_{it} + e_{it} \quad (8)$$

$$y_i = \mu + \beta_1 CN_i + \beta_2 C_{org} N_{org_i} + \beta_3 NH_{4i} + b_i + e_i \quad (9)$$

where y_i is the observation of the i^{th} digestate treatment, μ represents the average response, β_n are the parameters of fixed effects, b_i is the complete block effect and e_i is the error of y_i.

Significant differences among treatments for cumulative N_2O, CH_4, CO_2, as well as ^{15}N-N_2O and total Nd (%) were determined by the Proc MIXED procedure and the Tukey test ($\alpha = 0.05$). The MIXED procedure can fit various mixed linear models to data and produces the appropriate statistics (SAS Institute Inc, 2015). All statistical analyses were performed with SAS 9.4 (SAS Institute, Cary NC, USA). Graphics were produced with SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany).

RESULTS

Meteorological Conditions

Weather conditions showed distinct differences in precipitation between the 2 experimental years. Over the 1st and 2nd year, 183 and 178 mm of precipitation were measured during the 55 and 58 days when the experiment lasted, respectively (**Figure 1**). Within

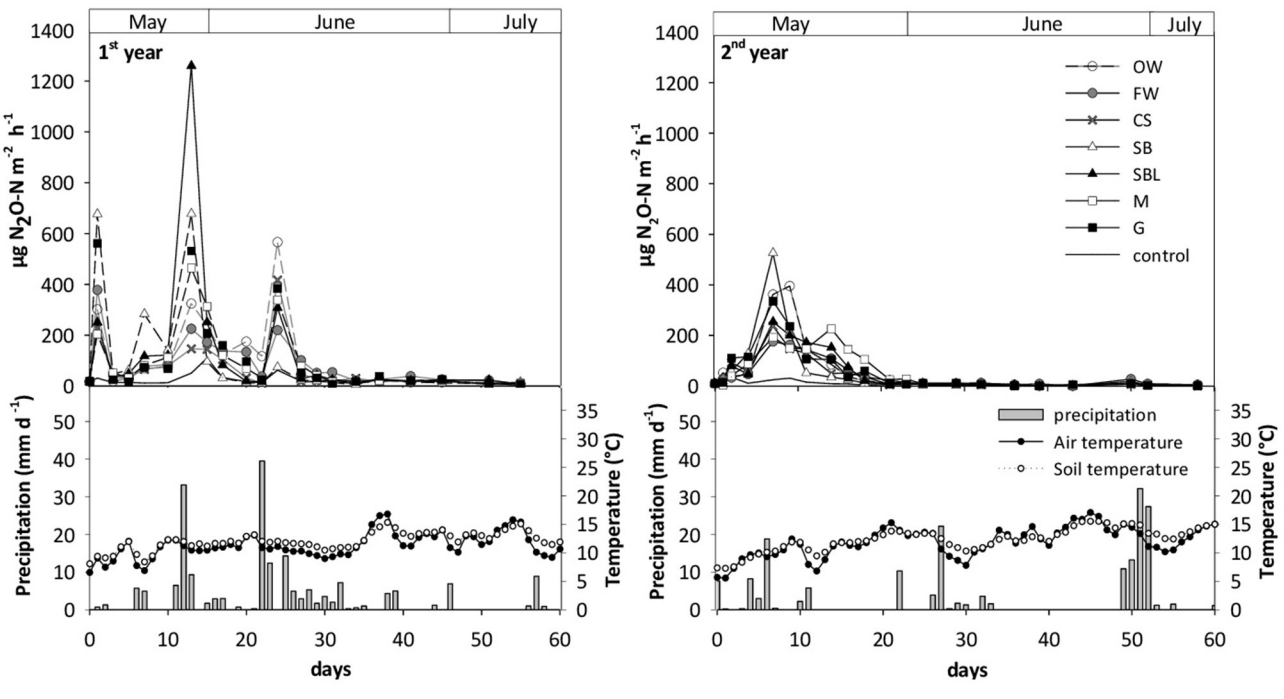

FIGURE 1 | Mean daily N_2O fluxes after application of different digestates based on organic waste (OW), food waste (FW), cattle slurry (CS), sugar beet (SB), sugar beet leaves (SBL), maize (M), grass (G), and unfertilized soil (control) measured from May to July 2016 and 2017 (1st and 2nd year) at Heidfeldhof. Digestate application of 170 kg N ha^{-1} on day 1. Standard error of measurements was excluded due to improved clarity, but shown in ^{15}N fluxes (**Figures 2**, **3**). Climate data with mean daily precipitation on the left y-axis and mean daily temperature on the right y-axis.

TABLE 3 | Output of (stepwise forward) regression analysis for N_2O fluxes in the 1st and 2nd year, testing for the inclusion of parameters CO_2 flux, water filled pore space (WFPS), and soil temperature into the model (model 8).

Year	Partial R^2			R^2	Model R^2	*F*-value	*p*-value
	WFPS	Soil temp	CO_2	$\sum$			
1st year	0.2064			0.281	0.207	160	<0.001
			0.0693		0.277	58.7	<0.001
		0.0048			0.282	4.08	0.0438
2nd year	0.1574			0.209	0.166	117	<0.001
		0.0459			0.213	36.1	<0.001
			0.0053		0.218	4.22	0.0404

the first 30 days of measurements the rainfall pattern differed, showing 155 mm in the 1st compared to 86.2 mm precipitation in the 2nd year. In the 1st year, two strong rainfall events occurred on day 12 (33.2 mm) and day 22 (39.5 mm). In contrast, the 2nd year showed lower rainfall events on day 6 and 27 with 18.8–22.2 mm, and two stronger events at the end of the experiment on day 51 and 52 (32.2–27.4 mm). The mean air temperature over the experimental period was 17.1°C in the 1st and 18.0°C in the 2nd year.

Temporal N_2O Fluxes

Nitrous oxide fluxes measured in the 2 experimental years showed distinct differences in peak number and flux magnitude. In both years N_2O pulses occurred directly after digestate fertilization and after strong rainfall events (**Figure 1**). Three major peaks were detected in the 1st year: one directly after digestate application, the second and third peak after 13 and 24 days, and a minor peak after 1 week, following strong rainfall events on day 12 and 23. Highest N_2O flux rate in the 1st year was measured with the SBL treatment on day 13 (1,260 μg N_2O-N m^{-2} h^{-1}). In the 2nd year, the N_2O pulse developing directly after N fertilization did not reach the same magnitude as in the 1st year and appeared 1 day later. Highest N_2O flux in the 2nd year followed a rainfall event 1 week after digestate application reaching up to 424 μg N_2O-N m^{-2} h^{-1} with SB (**Figure 1**). The peak decreased sharply in case of SB, and gradually until day 14 for the other digestates. After another strong rainfall event on day 22 (2nd year), only FW showed a slight N_2O rise (38 μg N_2O-N m^{-2} h^{-1}). Approaching the end of the experiment, 50 days after digestate application, a small peak (5.22–21.9 μg N_2O N m^{-2} h^{-1}) was noted within 4 days of continuous rainfall (**Figure 1**).

In both years, WFPS showed a significant positive linear correlation with N_2O flux rates, $r = 0.400$ ($p < 0.001$) and $r = 0.454$ ($p < 0.001$) in the 1st and 2nd year, respectively. Similarly, CO_2 fluxes correlated with N_2O fluxes, exhibiting $r = 0.233$ ($p < 0.001$) in the 1st year, and a weaker coefficient of correlation in the 2nd year ($r = 0.144$, $p < 0.001$). Soil temperature showed a negative correlation with N_2O fluxes ($r = -0.340$; $p < 0.001$) in the 2nd year, but no significant correlation in the 1st year. All parameters (WFPS, soil temperature and CO_2) combined in a linear regression model (model 8) could account for 28.1 to 20.9 % of the prediction of N_2O fluxes in the 1st and 2nd year, respectively (**Table 3**).

Temporal ^{15}N-N_2O Fluxes

Total N_2O and digestate derived ^{15}N-N_2O fluxes in the 1st year are shown in **Figure 2** and the 2nd year data are shown in **Figure 3**. A comparable trend was observed for both $^{15}N_t$- and $^{15}NH_4^+$-N-labeled digestates in each year, with variations in flux magnitude of ^{15}N-N_2O among digestates (**Supplementary Tables 2, 3**).

In the 1st year, the emerging peak directly after digestate application showed a low ^{15}N signature, indicating that 92.4–96.5% of N_2O was derived from soil internal N sources (**Supplementary Table 2**). Within the first 10 days after digestate application, ^{15}N-N_2O fluxes showed no significant differences among treatments (**Supplementary Table 2**). Only on day 7, a small peak in ^{15}N-N_2O (13–87 μg ^{15}N-N_2O-N m^{-2} h^{-1}) appeared, and SB showed significantly higher emissions than M, OW, FW, and CS. On that day, ∼18–30% of N_2O-N was derived from digestates (**Figure 2** and **Supplementary Table 2**). During the highest peak on day 13, a significant proportion of digestate-based N_2O-N (31–59%) was emitted. Highest ^{15}N-N_2O among digestates was measured with SBL (539 μg ^{15}N-N_2O-N m^{-2} h^{-1}), not significantly different from G (338 μg ^{15}N-N_2O-N m^{-2} h^{-1}). Following the two peaks on day 7 as well as on day 13, lower total N_2O and ^{15}N-N_2O fluxes were measured, but the share of digestate-derived N was still relatively high (**Supplementary Table 2**). The last major peak appeared on day 24 (10–114 μg ^{15}N-N_2O-N m^{-2} h^{-1}), with 10–27% N_2O-N originating from digestates. Flux rates peaking on day 24 were comparable among most treatments, and only OW exhibited significantly higher flux rates than SB. Prior to peaks of day 24, OW already indicated a rising flux rate on day 20, being significantly higher than all digestates, except G and FW. The flux rate further increased on day 22, where OW significantly exceeded all other treatments (**Figure 2** and **Supplementary Table 2**). Within the first 3 weeks of measurements, flux pattern of CS digestate significantly differed from the other treatments, where ^{15}N-N_2O gradually increased after 7 days and reached its maximum on 13 (**Figure 2**). At both peaks, on day 13 and 24, ^{15}N-N_2O flux rates of CS were in a comparable range (48–40 μg ^{15}N-N_2O-N m^{-2} h^{-1}). The 2nd year showed a similar temporal pattern in ^{15}N-N_2O abundance over the duration of the experiment. The first pulse after digestate application was observed 2 days after application with more than 80% soil-borne N_2O-N. Only CS showed lower soil-borne N_2O-N, thus highest digestate derived N_2O-N (45%) among digestates (**Supplementary Table 3**). The major peak in total N_2O and ^{15}N-N_2O appeared after 1 week (**Figure 3**), with highest ^{15}N-N_2O flux measured in G (189 μg ^{15}N-N_2O-N m^{-2} h^{-1}) showing 67% digestate-derived N. In contrast, 28–36% of N_2O-N was emitted from the other digestate treatments on that day (**Supplementary Table 3**). From day 7 to day 18, ^{15}N-N_2O gradually decreased for all digestates to 0.3–20 μg ^{15}N-N_2O-N m^{-2} h^{-1}, except for M being significantly higher (66.1 μg ^{15}N-N_2O-N m^{-2} h^{-1} on day 18). From day 18 through 29, the M

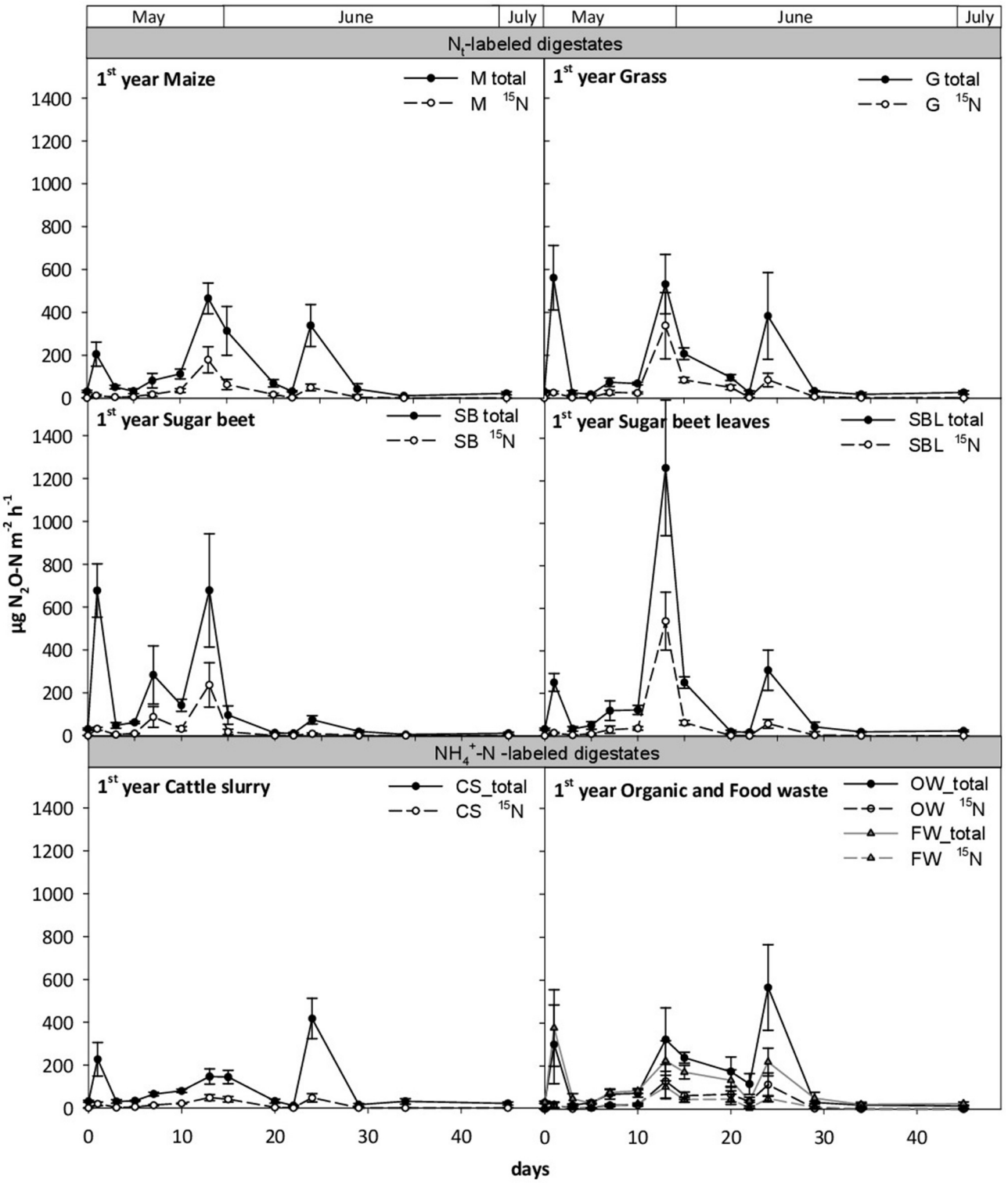

FIGURE 2 | Mean daily N_2O fluxes (total) and digestate-derived ^{15}N-N_2O fluxes (^{15}N) within the 1st year Digestates from maize (M), grass (G) sugar beet (SB), and sugar beet leaves (SBL) were $^{15}N_t$-labeled (mineral and organic N) and digestates based on cattle slurry (CS), organic waste (OW), and food waste (FW), were ^{15}N-labeled only in the NH_4^+-N pool. Error bars indicate the standard error ($n = 4$).

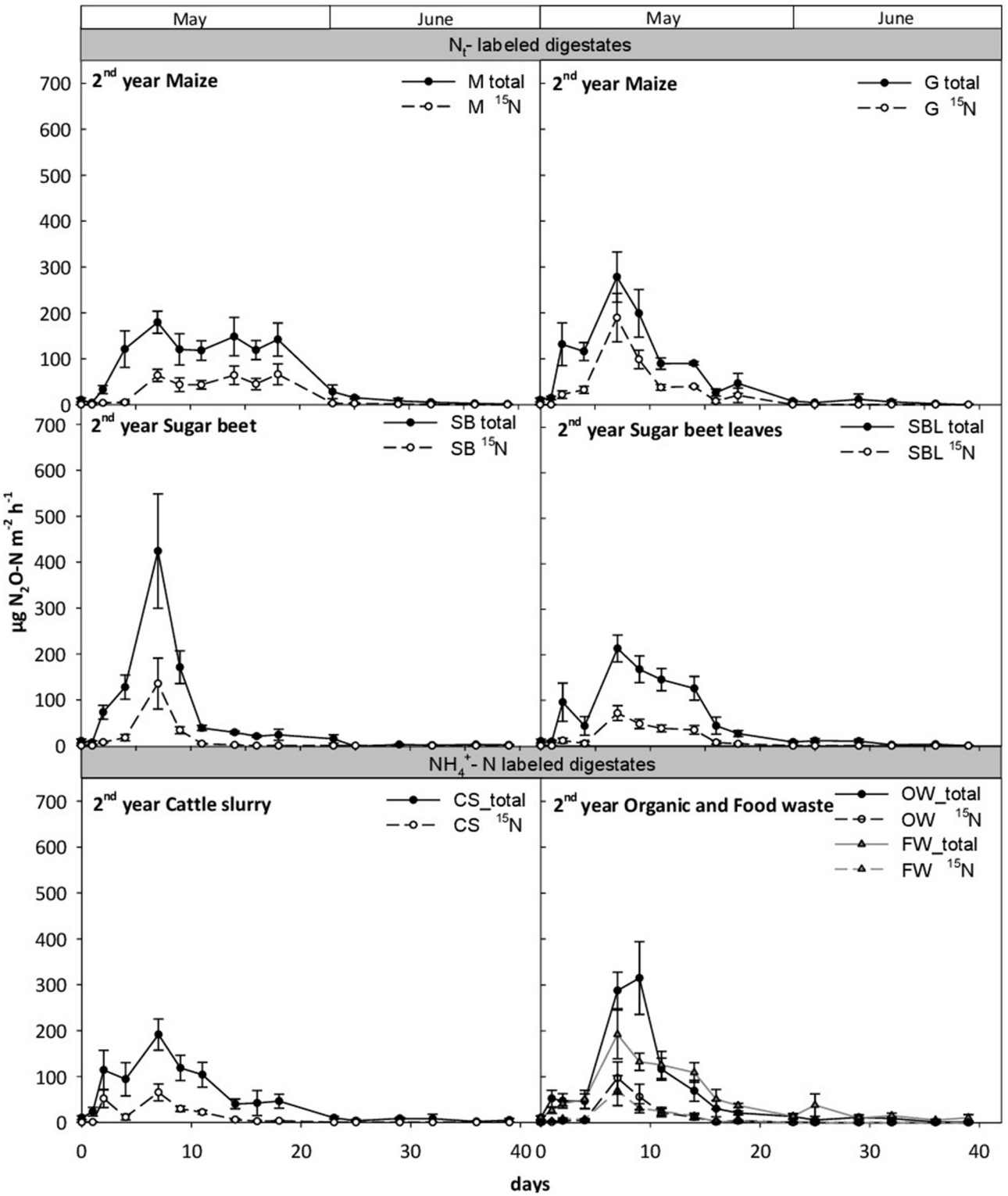

FIGURE 3 | Mean daily N_2O fluxes (total) and digestate-derived ^{15}N-N_2O fluxes (^{15}N) within the 2nd year Digestates from maize (M), grass (G) sugar beet (SB), and sugar beet leaves (SBL) were $^{15}N_t$-labeled (mineral and organic N) and digestates based on cattle slurry (CS), organic waste (OW), and food waste (FW), were ^{15}N-labeled only in the NH_4^+-N pool. Error bars indicate the standard error ($n = 4$).

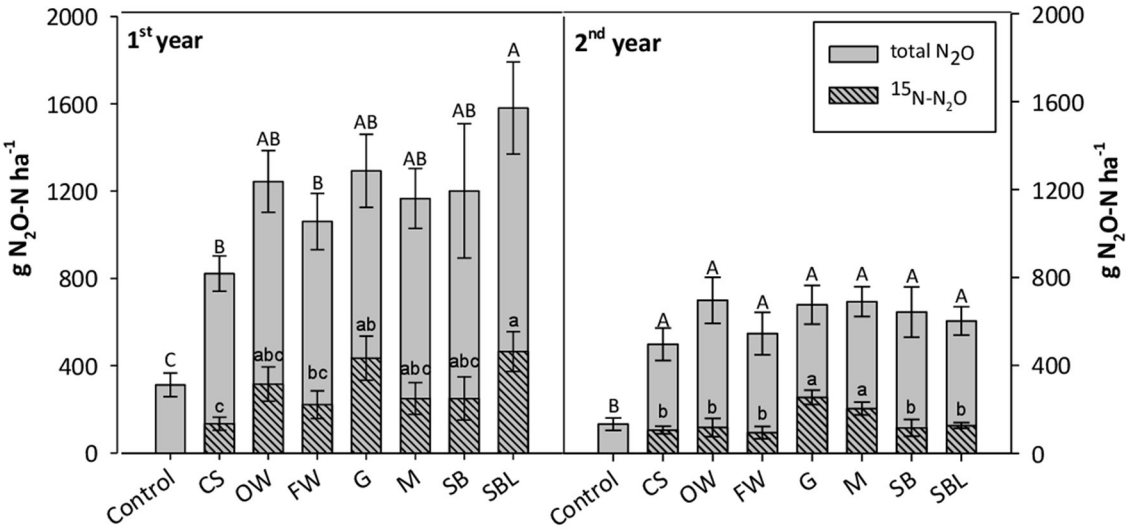

FIGURE 4 | Total cumulative N_2O-N emissions derived from digestate (^{15}N-N_2O) 55 (1st year) and 58 days (2nd year) after digestate application. In both years, measurements were conducted from May to July. Unfertilized soil served as control. Digestates from cattle slurry (CS), organic waste (OW), food waste (FW) were ^{15}N-labeled only in NH_4^+-N; digestates based on grass (G), maize (M), grass (G), sugar beet (SB), and sugar beet leaves (SBL) were $^{15}N_t$-labeled (mineral and organic N). Error bars show standard error ($n = 4$). Different letters indicate significant differences at $p < 0.05$ (Tukey Test); Capital letters refer to total N_2O and small letters to ^{15}N-N_2O.

TABLE 4 | Total cumulative N_2O-N, ^{15}N-N_2O-N emissions, and primed N_2O-N emissions after 55 (1st) and 58 days (2nd year).

Year	Total cumulative N_2O-N		Cumulative ^{15}N-N_2O-N		Primed N_2O-N	
	1st	2nd	1st	2nd	1st	2nd
			g N ha^{-1}			
Control	312 ± 54 c	133 ± 28 b				
N_t -labeled digestates						
Maize	1,166 ± 137 ab	690 ± 68 a	250 ± 73 abc§	203 ± 29 a AB	604 ± 82 ab	354 ± 48 ns
Grass	1,293 ± 167 ab	676 ± 88 a	434 ± 102 ab§	255 ± 33 a A	547 ± 119 ab	289 ± 56 ns
Sugar beet	1,201 ± 308 ab	643± 114 a	251 ± 99 abc§	116 ± 38 b B	638 ± 209 ab	394 ± 78 ns
Sb-leaves	1,580 ± 211 a	602 ± 65 a	465 ± 92 a§	127 ± 13 b B	804 ± 121 a	343 ± 55 ns
NH_4^+-N -labeled digestates						
Organic waste	1,244 ± 142 ab	697 ± 105 a	315 ± 79 abc§§	118 ± 42 b§§	617 ± 77 ab	446 ± 84 ns
Food waste	1,060 ± 129 b	545 ± 97 a	221 ± 63 bc§§	94.2 ± 27.8 b§§	528 ± 95 ab	318 ± 121 ns
Cattle slurry	822 ± 81 b	496 ± 74 a	133 ± 30 c§§	106 ± 17 b§§	376 ± 57 b	257 ± 60 ns

Unfertilized soil as control and application of Nt -labeled or NH_4^+-N -labeled digestates. Mean values ± standard error (n = 4). Different letters indicate significant differences at $p < 0.05$ (Tukey Test), ns = no significant differences. Small letters represent statistical differences among all treatments. For cumulative ^{15}N-N_2O-N, large letters refer to significant differences only among N_t-labeled or NH_4^+-N-labeled digestates.

§no significant differences among N_t -labeled labeled digestates, when excluding NH_4^+-N-labeled digestates.

§§no significant differences among NH_4^+-N-labeled digestates, when excluding N_t -labeled digestates.

treatment continued to show higher ^{15}N fluxes compared with the other digestates, even though these emission rates were quite low (from 1.1 to 2.7 μg ^{15}N-N_2O-N m^{-2} h^{-1}).

Cumulative N_2O and ^{15}N-N_2O Evolution and Emission Factors

Total cumulative N_2O emissions in the 1st year (302–1,345 g N_2O-N ha^{-1}) were more than twice as high as in the 2nd year (124–613 g N_2O ha^{-1}) (**Figure 4** and **Table 4**). In both years, digestates lead to significantly higher N_2O emissions than the unfertilized control. Differences among digestates were observed only in the 1st year, with significantly higher N_2O emissions for SBL compared to CS and FW (**Figure 4**). Compared to total N_2O emissions, digestate-based ^{15}N-N_2O emissions indicated larger differences between the different treatments in both years (**Figure 4**). In the 1st year, G and SBL emitted significantly more ^{15}N-N_2O than CS, while all other treatments did not differ significantly. In the 2nd year, highest ^{15}N-N_2O emission was

TABLE 5 | Share of digestate derived N_2O-N on total N_2O emissions (Total Nd) and N_2O-N emission factors after 55 (1st) and 58 days (2nd year).

Year	Total Nd		N_2O emission factor	
	1st	2nd	1st	2nd
		%		
N_t -labeled digestates				
Maize	20.3 ± 4.2 bc B	29.3 ± 2.6 ab B	0.50 ± 0.08 ab	0.33 ± 0.05 ns
Grass	32.9 ± 5.5 a A	37.8 ± 1.1 a A	0.58 ± 0.10 ab	0.32 ± 0.05 ns
Sugar beet	18.2 ± 3.8 bc B	16.8 ± 2.8 bc C	0.52 ± 0.18 ab	0.30 ± 0.07 ns
Sb-leaves	28.8 ± 1.8 ab B	21.2 ± 1.3 c C	0.75 ± 0.12 a	0.29 ± 0.04 ns
NH_4^+-N -labeled digestates				
Organic waste	24.2 ± 4.1 abc[§]	16.5 ± 4.8 c[§]	0.55 ± 0.08 ab	0.33 ± 0.06 ns
Food waste	20.1 ± 4.1 bc[§]	20.7 ± 7.0 bc[§]	0.44 ± 0.08 ab	0.24 ± 0.06 ns
Cattle slurry	15.7 ± 2.4 c[§]	21.6 ± 1.9 bc[§]	0.30 ± 0.05 b	0.21 ± 0.04 ns

Mean values ± standard error (n = 4). Different letters indicate significant differences at $p < 0.05$ (Tukey Test), ns = no significant differences. Small letters represent statistical differences among all treatments For total Nd, large letters refer to significant differences only among N_t-labeled or NH_4^+-N-labeled digestates.
[§]no significant differences among NH_4^+-N-labeled digestates, when excluding N_t -labeled digestates.

measured with G and M, while all NH_4^+-N-labeled digestates OW, FW and CS, as well as SB and SBL were comparable. Calculated amounts of primed N_2O-N showed that significant higher N_2O-N losses were induced by SBL compared with CS in the 1st year (**Table 4**). In the 2nd year, there were no significant differences among digestates. Total Nd emitted by the digestates was 16–33% in the 1st, and 17–38% in the 2nd year (**Table 5**). Grass digestate tended to show the highest share of digestate derived Nd in both years.

There was no correlation of digestate properties (C/N, C_{org}/N_{org}, NH_4^+-N/N_t) with N_2O emissions. The respective digestate characteristics did not help to predict cumulative N_2O or ^{15}N-N_2O emissions in a multiple (stepwise forward) linear regression model (model 9).

According to IPCC guidelines, ∼0.6% of the annual amount of total N of organic amendments applied as fertilizer is lost as N_2O-N in wet climates (IPCC, 2019). In the 1st year, most digestates approached this IPCC EF within only 55 days and SBL even exceeded it with 0.75% (**Table 5**). Only FW and CS indicated lower EFs than the IPCC default value in the 1st year, with 0.44 and 0.30%, respectively. Related to the overall lower cumulative N_2O emissions of the 2nd year, mean N_2O EFs were below 0.33% and in a comparable range for all digestates.

Total ^{15}N recovery within cumulative N_2O and soil N at the end of the experiment was 10–57% and 27–64% in the 1st and 2nd year, respectively (**Supplementary Table 4**). The largest share of digestate ^{15}N remained in the soil.

Total Global Warming Potential

Cumulative CH_4-C emissions were significantly higher in the 1st year compared to the 2nd. In both years, unfertilized soil served as CH_4 sink (−147 to −184 g CH_4-C ha^{-1}) (**Table 5**). Within the 1st year, emissions among digestates ranged between 0.26 and 1.82 kg CH_4-C ha^{-1} and decreased in the following order SBL ≥ CS ≥ FW, OW, SB ≥ M, G ≥ control. In the 2nd year, digestates as well as unfertilized soil were comparable and digestates indicated CH_4-C emissions close to zero (**Table 6**).

TABLE 6 | Modeled NH_3-N losses over the first 72 h after application (ALFAM2 model) and total cumulative CH_4 fluxes of digestates and unfertilized soil (control) after 55 (1st) and 58 days (2nd year).

	NH_3-N		Total cumulative CH_4	
	1st year	2nd year	1st year	2nd year
	kg NH_3-N ha^{-1}		kg CH_4-C ha^{-1}	
Control			−0.184 ± 0.202 d	−0.147 ± 0.106[ns]
Maize	6.2	0.5	0.360 ± 0.231 cd	−0.0369 ± 0.044[ns]
Grass	5.3	0.4	0.257 ± 0.133 cd	0.0171 ± 0.122[ns]
Sugar beet	2.8	0.5	0.848 ± 0.068 bc	−0.0608 ± 0.067[ns]
Sugar beet leaves	3.2	0.5	1.82 ± 0.394 a	0.0778 ± 0.129[ns]
Organic waste	3.9	0.4	0.767 ± 0.142 bc	−0.0518 ± 0.075[ns]
Food waste	4.8	0.5	0.671 ± 0.106 bc	−0.0876 ± 0.057[ns]
Cattle slurry	5.1	0.4	1.29 ± 0.369 ab	0.0550 ± 0.079[ns]

For CH_4, mean values ± standard error (n = 4). Different letters indicate significant differences in CH_4 emissions at $p < 0.05$ (Tukey Test); ns = no significant differences.

In both years, the release of CO_2-eq after digestate application was significantly higher than in the control (**Table 7**). Significant differences among digestates were only noted in the 1st year, where SBL caused significantly higher total CO_2-eq. than M, FW, and CS. In both years, N_2O emissions made up the largest share in total GHG emissions, based on CO_2-eq, above 85.6% in the 1st and almost 100% in the 2nd year.

DISCUSSION

Temporal N_2O and ^{15}N-N_2O Fluxes

The high temporal variability of N_2O fluxes in this study, with increased flux rates after application of crop residues or organic fertilizers and after rainfall events, was similarly documented in

TABLE 7 | Carbon dioxide equivalents (CO_2-eq.) of unfertilized soil (control) and soil after application of different digestates, originating from maize, grass, sugar beet, sugar beet leaves, organic waste, food waste, and cattle slurry, based on cumulative N_2O and CH_4 emissions (kg ha^{-1}) after 55 (1st) and 58 days (2nd year); and indirect N_2O emission as NH_3-N volatilization over 72 h after application.

Year	Treatment	Share of total CO_2_eq.			Total CO_2-eq.
		N_2O direct	N_2O indirect[§]	CH_4	
		%			kg ha^{-1}
1st	Control	103	-	−3.45	139 c
	Maize	92.9	5.17	1.91	583 b
	Grass	94.7	4.13	1.16	634 ab
	Sugar beet	91.9	2.60	5.48	599 ab
	Sugar beet leaves	90.7	1.92	7.34	808 a
	Organic waste	93.5	3.06	3.42	618 ab
	Food waste	91.1	4.32	4.62	540 b
	Cattle slurry	85.6	5.51	8.87	447 b
2nd	Control	104	-	−3.88	62.3 b
	Maize	100	0.746	−0.55	322 a
	Grass	101	0.632	−1.44	314 a
	Sugar beet	98.6	0.831	0.56	303 a
	Sugar beet leaves	101	0.867	−1.59	278 a
	Organic waste	100	0.606	−0.14	325 a
	Food waste	98.3	0.981	0.76	258 a
	Cattle slurry	101	0.877	−1.49	231 a

Different letters indicate significant differences at $p < 0.05$ (Tukey Test).
[§]*indirect emissions only based on NH_3 loss, nitrate leaching was not accounted for.*

other experiments (Pfab et al., 2012; Herr et al., 2019). Ultimately, N_2O fluxes leveled off 30 days after digestate application in both years with drying of the soil during warm periods with low rainfall. Dry conditions with low WFPS have often been reported to result in low N_2O emissions from arable soils even if these soils were well-provided with microbial easily degradable C and available N (Möller and Stinner, 2009; Pezzolla et al., 2012). However, in the 2nd year strong rainfall events were recorded 50 days after digestate application and only caused a minor increase in N_2O fluxes (**Figure 1**). Hence, digestate-related effects were short-term and had the highest impact on N_2O release within the first 30 days after application.

First Peak After Digestate Application

Peaks evolving shortly after organic N fertilizer application, such as digestates or manures, have been reported by several studies (Wulf et al., 2002; Johansen et al., 2013; Holly et al., 2017). As indicated by ^{15}N measurements in both years, the first N_2O peak after digestate application showed a low ^{15}N abundance, demonstrating that more than 90% of N_2O-N was derived from soil N (**Figures 2**, **3**). However, the experimental setup does not allow for a differentiation between nitrification and denitrification. Therefore, we can only conclude that the first N_2O peak was mainly derived from soil N. The high share of soil-borne N suggests that the addition of OM positively affected microbial activity which further enhanced the turnover of native soil-N, as also stated by Schleusner et al. (2018). Furthermore, digestate or slurry application moistened the soil close to the applied fertilizer, another factor that has been shown to promote denitrification of NO_3^--N (Comfort et al., 1990). Moreover, CO_2 flux rates were elevated directly after digestate fertilization (**Supplementary Figure 1**), supporting the assumption of increased microbial activity which further stimulated denitrification of NO_3^- by O_2 depletion (Buchen-Tschiskale et al., 2020). However, these are only speculations, as soil N_{min} and its ^{15}N-signature was not measured during the experiment. It should also be considered that digestates contain carbonate-C (HCO_3^- and CO_3^{2-}): the higher the total N content, the higher the carbonate-C content (Georgacakis et al., 1982). Carbonate-C in the digestates can also contribute to soil CO_2 release within the 1st days after application (Chen et al., 2011). For example, carbonate-C release from digestates can occur after application to acidic soils (Chen et al., 2011), which is not the case in present study, or due to microbial turnover processes (Tamir et al., 2013). Therefore, the immediate effects of digestate application on soil microbial activity and the related CO_2 release might be masked by decomposition of carbonate-C to CO_2. In order to elucidate the driving processes related to the N turnover processes in the soil shortly after digestate application, a more detailed measurement of the pathways of the different fractions of soil and digestate N (NH_4^+, N_{org}), as well as digestate C (C_{org}, carbonate-C), is necessary.

Rainfall-Induced Peaks

The emission pattern found in present study strongly coincided with the precipitation pattern, providing a major indication that the environmental conditions are the main driving factor for soil N_2O fluxes. Also the unfertilized control showed a significant increase in N_2O flux rates after rainfall, whereas almost no fluxes were observed in dry periods. The occurrence of increased N_2O fluxes in conjunction with heavy rainfall events, hence a high soil WFPS, is typical for arable fields and has extensively been described in the literature (Pfab et al., 2011; Senbayram et al., 2014; Ruser et al., 2017).

Contribution of Digestate N and Soil N Pool to N_2O Emissions

The largest rainfall-induced N_2O peaks in both years, had also the highest ^{15}N abundance, with up to 56–66% of N_2O-N derived from the digestate (**Figures 3**, **4** and **Supplementary Tables 2, 3**). Although it was shown that even at a high soil moisture of 70% WFPS nitrification may also contribute to the N_2O-release from soils (Ruser et al., 2006), the positive correlations between N_2O flux rates and CO_2 flux rates as well as between N_2O fluxes and soil moisture (**Table 3**) indicate that denitrification is the driving process releasing N_2O after rainfall. The contribution of denitrification to the N_2O release generally increases with increasing soil moisture (Davidson, 1991). When compared to soil air, the $\sim 10^{-4}$ lower diffusion coefficient for atmospheric O_2 in soil water (Heincke and Kaupenjohann, 1999) restricts O_2 delivery, the creation of anaerobic conditions is favored. Similarly, the turn-over of fresh OM, as indicated by the increased CO_2 fluxes, further depletes O_2 availability and thus fuels anaerobiosis (Flessa and Beese, 1995).

The largest peaks evolved 13 (1st year) and 7 days (2nd year) after digestate application, where presumably digestate NH_4^+-N was already nitrified (Johansen et al., 2013) and available for denitrification, thus, explaining the high share of digestate-based N_2O-N. Senbayram et al. (2009) observed that nitrification of ^{15}N-labeled digestate rapidly increased 1 week after application in a pot experiment initiating a rise in N_2O flux rates, as also noted in our study. It cannot be excluded that beside denitrification also nitrification contributed a share of the measured N_2O. Yet, Köster et al. (2011) measured the intramolecular ^{15}N distribution in N_2O within a 43-days incubation experiment, showing that bacterial denitrification was the main process emitting N_2O after application of food waste digestate, driven by C availability. This is in line with other studies, reporting that the largest N_2O-N contribution of digestates was caused by denitrification, even at 65% WHC (Senbayram et al., 2009). Later N_2O peaks (1st year, day 24) showed lower ^{15}N-N_2O fluxes, hence indicating an increasing share of N_2O from soil-internal N. This shift in ^{15}N-N_2O abundance over the measuring period indicates increased effects of the soil microbial processes, affecting N availability and N_2O emissions, and might result from mineralization of digestate $^{15}N_{org}$-N and subsequent processes. A comparable shift was observed by Senbayram et al. (2014).

For both, NH_4^+-N or N_t-labeled digestates, the low shares of fertilizer-derived N_2O-N supported the notion that the largest source of N_2O was native soil N (> 62%, **Table 5**). The open hypothesis of an "enhanced soil-derived N_2O" stated by Senbayram et al. (2014), regarding the low share of emitted digestate-N, can therefore be confirmed. This triggering effect on N_2O emissions due to digestate application was accounted for by a simplified calculation via equation 4. The amount of triggered N_2O-N reflects the high share of soil-derived N_2O, and was approximately half of total cumulative N_2O emissions (**Table 4**). Significant differences in primed N_2O-N among digestates followed the same trend as N_2O emissions. For NH_4^+-N-labeled digestates, N_2O-N losses which might originate from digestate N_{org}, were not accounted for. Therefore, the amount of primed N_2O-N might be overestimated.

In general, the rather comparable share of total digestate-derived N_2O-N losses among the digestates with different labeling approaches indicates that digestate-N_{org} plays only a minor role in short-term N_2O formation. Senbayram et al. (2014) labeled only the mineral N fraction of a digestate and found 31% of N_2O-N was derived from the digestate mineral fraction. The share of digestate-derived N_2O-N losses among NH_4^+-N-labeled digestates FW, OW and CS ranged from 15.7 to 24.2% over the 2 years. For these digestates as well as for the digestates in the study of Senbayram et al. (2014) it cannot be excluded that non-labeled organic N was mineralized and emitted as N_2O. However, the N_t-labeled digestates, M, G, SB, and SBL showed a rather comparable range with 18.2–37.8% digestate-N being emitted as N_2O over the 2 years. Similar to our findings, also other studies reported a higher share of N_2O-N originating from the soil N pool than from fertilizer N. For instance, only 22% of N_2O-N was derived from ^{15}N-labeled manure after 22 days (Ingold et al., 2018) or 40.4% from ^{15}N-urea after 35 days (Roman-Perez and Hernandez-Ramirez, 2020) in incubation experiments. In a field study, NH_4^+-N-labeled cattle slurry was applied, which produced higher fertilizer-derived N_2O emissions within the first 10 days, but higher soil-derived N_2O 11–22 days after application (Dittert et al., 2001). However, the study was carried out on grassland and using the injection technique (Dittert et al., 2001), which has been reported to increase N_2O emissions compared to trail hose application with immediate incorporation (Herr et al., 2019).

N_2O Emissions Affected by Fertilizer Type

As previously described, N_2O fluxes were shaped and influenced by weather conditions and soil microbial processes. Environmental conditions and soil type may play a more important role than the fertilizer type, as previously suggested by Senbayram et al. (2014): the authors noted no significant differences in N_2O emissions between mineral and organic N fertilization. However, in both years, significant differences among digestates were noted on several sampling dates, for N_2O as well as ^{15}N-N_2O fluxes (e.g., flux rates from M digestate vs. fluxes from SB digestate in **Figure 2** and **Supplementary Tables 1–3**), indicating that digestate composition affects N_2O emissions. This supports hypothesis (1), that digestates from different feedstocks will differ in N_2O flux rates.

However, regarding cumulative effects, there was no clear indication that the digestate type influenced total N_2O emissions. This was supported by the lack of a significant correlation between digestate composition (NH_4^+/N, C/N, C_{org}/N_{org}) and cumulative N_2O or $^{15}N_2O$. Only measurements of the 1st year showed significant differences among digestates. Therefore, hypothesis (2) had to be rejected for the 2nd year and could be partly accepted for the 1st year. Yet, when separating cumulative ^{15}N-N_2O data into N_t-labeled and NH_4^+-N-labeled digestates, there was a significant effect of C/N ratio in the 1st year, predicting 22.1% of ^{15}N-N_2O emissions of $^{15}N_t$-labeled digestates ($R^2 = 0.36$, F-value $= 3.81$, p-value $= 0.077$). For the 2nd year, C_{org}/N_{org} accounted for 39.2% ($R^2 = 0.56$, F-value $= 9.85$, p-value $= 0.0094$) of ^{15}N-N_2O emissions among $^{15}N_t$-digestates (M, G, SB, and SBL). Also Abubaker et al. (2013) noted significantly different cumulative N_2O emissions after 24 days between two types of urban waste digestates, which were low or high organic C. For NH_4^+-N-labeled digestates, there was no significant relation of digestate properties to ^{15}N-N_2O emissions. Hence, the correlation between digestate properties and N_2O emissions seems more strongly related to the total N and N_{org} content of digestates, than NH_4^+-N. Regarding the total share of digestate-derived N_2O-N (Nd), significant differences among digestates (**Table 5**) could support hypothesis (3).

Ultimately, the results of the present study suggest that the different digestate types influenced cumulative N_2O, flux rates and digestate derived N_2O-N only marginally. Hence, N_2O emissions were more strongly affected by environmental conditions (**Table 3**). The effect of digestate properties on total N_2O emissions was overlaid to some extent by the high amount of N_2O from the native soil N pool. Abubaker et al. (2013) incubated two digestates in three soil textures and noted considerable

differences regarding emission peaks and cumulative N_2O-N emissions among digestates, particularly in the sandy soil. In loam, digestates showed comparable total N_2O emissions (Abubaker et al., 2013). Therefore, N_2O emissions discussed in this study might differ on soils with different soil textures or amendment history (Rosace et al., 2020). In this context, soil texture, soil amendment history and fertility status, especially OM content, plays a crucial role, exceeding the effect of digestate properties.

Digestate Emission Factors and Practical Consequences

Digestate EFs determined in this study (0.21–0.75%) were all within the range of the IPCC default value, except for SBL in the 1st year (**Table 5**). However, these EFs will not cover the whole year and might underestimate the total EF of the digestates. Shang et al. (2020) determined 10–30% lower EFs when only the growing season and not the whole year N_2O emissions were considered. Moreover, the authors found that the differences between EFs of the whole year and growing season were higher with higher precipitation (Shang et al., 2020). The experimental design of the present study used bare soil, hence there were no crops removing the applied digestate N. Crop N uptake could have decreased available N from the soil as well as soil moisture, which could have lowered digestate-derived N_2O emissions and EFs. Thorman et al. (2020) determined annual N_2O EFs from different organic amendments, top-dressed to a cereal crop (0.15–0.73% in 2011 and 0.27–0.51% in 2012), which were in a comparable range with our EFs. Most digestate EFs did not show significant differences, except SBL compared with CS in the 1st year, thus hypothesis (2) cannot be fully confirmed.

Soil derived N_2O-N contributed to a large extent to digestate EFs. As a consequence of the high share of N_2O-N from the native soil pool within the first 30 days after digestate application, crop cultivation should be synchronized with available soil N. In particular, mineral N from the soil pool should be taken up by the crops, before digestates are applied. Thereby, the triggering effect of short-term soil-enhanced N_2O emissions by digestates could be decreased. For example, N_{min} supply in the present study would be sufficient for maize cultivation in the early growth stage. Digestates could then be top-dressed ∼1 month after emergence when most of soil M was already taken up by the crop. Also de Neve (2017) emphasized that in ideal cropping systems fertilizer availability and soil mineral N should be synchronized with crop demand, which could mitigate potential N losses.

Experimental Limitations

Determination of N_2O isotopomers in the present study, including the $\delta^{18}O$ and site preference of ^{15}N in the N_2O molecule, could have helped to understand the underlying soil microbial processes, differentiating between denitrification and nitrification (Köster et al., 2015). Yet, distinguishing nitrifier denitrification from nitrification is not possible using site preference (Köster et al., 2011). A dual isotope labeling approach of ^{15}N and ^{18}O-labeled water would be required (Koola et al., 2010), which is not feasible in field studies (Baggs, 2008). Also the $N_2O/N_2O+ N_2$ product ratio could have provided a better indication of denitrification in the study (Buchen-Tschiskale et al., 2020). However, measuring N_2 in the field is rather difficult due to the high N_2 background level in the atmosphere, as well as its spatial and temporal heterogeneity (Groffman et al., 2009). Instead N_2 is often studied in incubation experiments using an artificial helium–oxygen atmosphere (Scholefield et al., 1997). Regular soil N_{min} and $^{15}N_{min}$ analysis at the sampling dates could have given a hint for respective microbial processes, but would not have completely identified them. Thus, allocating the specific N_2O pathways after digestate application in the field is still challenging and needs further research and suitable methods to provide accurate measurements (Well et al., 2019).

CONCLUSION

The major finding of this study was the large share of N_2O-N from the soil pool, showing that digestate application triggers "enhanced soil-derived N_2O." The major driving forces of the emission pattern are the weather conditions, the specific chemical composition of digestates do have only minor effects on the denitrification. The different ^{15}N-labeling approaches of the digestates indicate that contribution of the organic fraction seems to be of very low significance for short-term N_2O emissions. The ^{15}N labeling approach helped to determine the source of N_2O emissions, but not the underlying processes (nitrifier denitrification or heterotrophic denitrification). Analysis of isotopomers and N_2 is needed to further identify the N_2O-releasing microbial processes in the soil. Emission factors were comparable for most digestates, but reached and even exceeded the default IPPC EF (0.6%) within only 60 days in the 1st year.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

FH prepared original draft, including statistical analysis, and graph production. RR supported calculation of global warming potential, provided guidance, and new input for determination of indirect N_2O emissions. The basis for the experimental idea was based on KM, with further contribution by RR regarding design of the field experiment, sampling frequency, and measurement technique. IC-M supported calculation of ^{15}N abundance in N_2O from IRMS data. FH and IC-M conducted the experiment. All authors reviewed and proofread the whole manuscript and gave critical feed-back to all sections.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.614349/full#supplementary-material

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