Soil incubation study showed biogas digestate to cause higher and more variable short-term N_2O and N_2 fluxes than mineral-N

Caroline Buchen-Tschiskale[1,2]*, Ulrike Hagemann[1], and **Jürgen Augustin[1]**

[1] Leibniz Centre for Agricultural Landscape Research ZALF, Eberswalder Str. 84, 15374 Müncheberg, Germany
[2] Thünen Institute of Climate-Smart Agriculture, Bundesallee 65, 38116 Braunschweig, Germany

Abstract

Today, a large share of mineral fertilizer is substituted by biogas digestates. Biogas digestates are known to promote N_2O production, compared to mineral fertilizer. In particular, the initial phase following fertilizer application is crucial for the N gas release as N_2O and also N_2. However, this period impact has been rarely investigated, especially not across various field sites. Thus, undisturbed soil cores from two fertilizer types (biogas digestate *vs*. mineral fertilizer) at five sites with different site characteristics were investigated in a short-term laboratory experiment under N_2-free helium–oxygen incubation atmosphere. Across sites, biogas digestate soil cores showed significantly higher absolute N_2O fluxes compared to mineral fertilizer soil cores, even though this effect was dominated by samples from one site (Dornburg with the highest biogas digestate fertilization rate). Also relative N_2O fluxes showed a similar tendency. On average, absolute and relative N_2 fluxes differed between the two fertilizer types, while N_2 fluxes were highest at the Dornburg site. A $N_2O/(N_2O+N_2)$ ratio of denitrification below or equal to 0.5 clearly highlighted the importance of N_2O reduction to N_2 for three of five the biogas digestate soil cores. Soil characteristics like bulk density and water-filled pore space as proxies for gas diffusivity in soil, as well as N availability (NO_3^-, NH_4^+), significantly affected the N_2O and N_2 fluxes from the biogas digestate soil cores. While this study presents data on short-term N_2O and N_2 fluxes, there is a need for further studies in order to investigate the dynamics, the duration of the observed effects and their significance at the field scale.

Key words: ammonia / helium–oxygen incubation / $N_2O/(N_2O+N_2)$ ratio of denitrification / N gas exchange

Accepted January 13, 2020

1 Introduction

Gaseous nitrogen (N) emissions from agricultural fields are considered problematic both from an agricultural—in terms of N efficiency—as well as from an environmental perspective (*Cameron* et al., 2013). This is particularly the case for nitrous oxide (N_2O), which is highly relevant due to its global warming potential (*IPCC*, 2013), while it is also expected to be the single most important ozone-depleting substance throughout the 21st century (*Ravishankara* et al., 2009). N_2O mainly originates from agricultural soils, where high N fertilizer inputs (mineral and organic fertilizer) are directly related to N_2O production, resulting in a conversion of 2 to 2.5% of fertilizer N to N_2O (*e.g.*, *Davidson*, 2009) and contributing up to 4.8 Tg N y^{-1} to the anthropogenic N_2O emissions (*IPCC*, 2013).

Apart from N_2O, fertilizer N can be lost in the form of the non-reactive molecular nitrogen gas (N_2), which is non-hazardous from an environmental perspective, but still of great agronomic interest in terms of fertilizer management. In addition to N_2O and N_2, there is a third significant pathway of gaseous N losses as ammonia (NH_3). While for NH_3 there is already a lot of information about the impact of N fertilization available (*e.g.*, *Wolf* et al., 2014; *Seidel* et al., 2017; *Pietzner* et al., 2017), for the other important gas N_2 there is still very limited information on fertilizer-related N_2 losses. This is particularly the case for the application of organic fertilizer in form of fermentation residues, often called biogas digestates (BD), which are increasingly used as organic amendments in agriculture (*Charles* et al., 2017). BD contain large amounts of organic carbon (C), N, and other nutrients, which promote soil microbial activity. In particular, BD may increase ammonium (NH_4^+) concentrations, affect the biological oxygen (O_2) consumption, narrow the C/N ratios, as well as elevate pH values in the soil with repeated additions (*Möller* and *Müller*, 2012; *Möller*, 2015). During BD application under typical spring conditions on often wet soils and by using drag hose or injection technique, BD hotspots can stimulate N_2O production *via* nitrification and denitrification, resulting in a higher potential of gaseous N losses (*Möller* and *Stinner*, 2009; *Köster* et al., 2011; *Senbayram* et al., 2009; 2014). In particular, the modified O_2 availability in combination with high amounts of available organic C provided by BD enhances soil respiration. Moreover, as BD contain mostly water (up to 97% moisture content) (*Möller*, 2015; *Charles* et al., 2017), their addition rapidly saturates micro-pores, which then enhances the

* Correspondence: Dr. C. Buchen-Tschiskale; e-mail: caroline.buchen@thuenen.de

potential for N_2O reduction to N_2 (*Butterbach-Bahl* et al., 2013). However, there is no clear picture about the impact of BD application on N_2 formation up to now (*Köster* et al., 2015; *Fiedler* et al., 2017).

In addition to the effect of fertilizer type (mineral *vs*. organic), N_2O production and consumption processes in agricultural systems are largely influenced by the soil characteristics at the respective location. N_2O and N_2 fluxes are significantly influenced by a range of factors, *e.g*., availability of N, C, and O_2 in the soil (*Weier* et al., 1993; *Conrad*, 1996; *Senbayram* et al., 2012), as well as soil moisture, soil type, soil texture, soil pH, climate, microbial community structure, fertilization, and management (*Butterbach-Bahl* et al., 2013; *Saggar* et al., 2013). In particular, soil moisture might be the most important driving factor during BD application in spring which has not been widely investigated with respect to N_2 fluxes (*Fiedler* et al., 2017).

This is likely due to the fact that the determination of N_2 fluxes from soils is still a delicate matter because of high atmospheric background concentrations complicating the detection of concentration changes in amounts relevant to soil processes (*Groffman* et al., 2006). To overcome this problem, two methods can be used: ^{15}N tracing technique (*i.e.*, analysis of gas fluxes after addition of ^{15}N-labelled substrate) and N_2-free incubation technique (*i.e.*, replacing N_2 by a noble gas like Helium) (*Van Groenigen* et al., 2015). Since the ^{15}N tracing technique requires a homogeneous labeling in order to produce precise results (*Boast* et al., 1988; *Arah*, 1992), it is particularly challenging for heterogeneous substrates such as BD and thus rather expensive. Hence, the N_2-free incubation technique provides an alternative for the investigation of a large number of undisturbed soil cores from different sites, where the actual N_2O and N_2 release can be measured. Generally, the initial phase, *i.e.*, the first days after fertilizer application, is crucial for N_2O emissions (*Senbayram* et al., 2009; *Köster* et al., 2011) and previous BD experiments have indicated a similar immediate reaction also for N_2 (*Köster* et al., 2015; *Fiedler* et al., 2017). Since such measurements on BD are up to now not available under field conditions due to the issues described above and still rare under laboratory conditions, a short-term laboratory incubation experiment would provide reliable data on N_2O and N_2 fluxes with respect to fertilizer type and soil conditions from various field sites, which might be further used to supplement field measurements.

By mapping a practical range of parameters, the aim of this laboratory incubation study was to (1) measure short-term N_2O and N_2 fluxes following the application of biogas digestate (BD) and mineral fertilizer (MIN) in undisturbed soil cores from five different field sites, as well as (2) to analyze the effects of soil and fertilization parameters and their interactions on N_2 and N_2O fluxes and the $N_2O/(N_2O+N_2)$ ratio of denitrification. The tested hypotheses were as follows: (1) N_2O and N_2 fluxes shortly after fertilizer application are higher from BD compared to MIN samples, (2) differences between samples from different sites, covering various soil characteristics in Germany, have a greater impact on N_2O and N_2 fluxes than the fertilization type (BD, MIN).

2 Material and methods

2.1 Treatments and fertilization

Soil core samples for the incubation experiment originate from a larger field experiment, established for the "EVA" (Development and Comparison of Optimized Cropping Systems for the Production of Energy Plants under the Variety of Regional Conditions in Germany) project. The project investigated the effects of fertilization with BD on N_2O emissions at five experimental sites (Ascha, Dedelow, Dornburg, Hohenschulen, Gülzow) throughout Germany (Tab. 1) by measuring greenhouse gas emissions from continuous cropping of energy maize (*Zea mays* L.) under different fertilizer regimes from 2011 until 2014 (*Hagemann* et al., 2017). Two fertilizer treatments were selected for sampling: (1) mineral N fertilizer (granular calcium ammonium nitrate; MIN) and (2) organic N fertilizer (biogas digestate; BD). According to common agricultural practice, BD treatments received an application rate equivalent to 70% mineral fertilizer, which explains the generally higher N fertilization rates in this treatment (Tab. 1). More-

Table 1: Characterization of study sites.

Site	UTM coordinates		Elevation (m asl)	Climate		Soil type[a]	Soil texture
	Easting	Northing		MAT (°C)	MAP (mm)		
Ascha	33U 328612	5429434	430	7.5	807	Cambisol	Very loamy sand
Dedelow	33U 419197	5915156	38–53	8.4	485	Orthic Luvisol	Moderately loamy sand
Dornburg	32U 686442	5653450	245–255	8.8	596	Orthic Luvisol	Very clayey silt
Gülzow	33U 307200	5966200	10	8.4	559	Colluvic Stagno-Gleyic Cambisol	Slightly loamy sand
Hohenschulen	32U 564199	6018885	35–40	8.8	760	Stagnic Cambisol	Moderately sandy loam

[a]FAO Classification (*IUSS Working Group*, 2007).

over, the Dornburg site is an exception; here, mineral fertilization was lower due to a planned second MIN application of 100 kg N ha^{-1} (several weeks after soil sampling), which is the common farming practice on that soil.

The BD originates from local biogas plants near the respective study sites, co-fermenting cattle manure and maize silage (all sites), and a small proportion of rye or barley groats (Dornburg and Gülzow). Fertilizers were applied using drag hoses (BD except for the Gülzow site), injection technology (BD in Gülzow), and a common fertilizer spreader (MIN). A representative sample was obtained from the well-mixed BD at all sites and analyzed in duplicate for dry matter content. pH was measured in the fresh sample using a pH meter. Total organic C and N contents were analyzed by dry combustion (elementary analysis). Phosphorus and potassium content was determined by AAS (Atomic Absorption Spectrometry) following DIN ISE 11885 using an ICP-iCAP 6300 DUO (ThermoFisher). NH_4^+-N content was analyzed photometrically according to DIN 38406-E5-2 using a Segemented Flow Analysis (Skalar Analytics, CFA-SAN).

2.2 Soil core sampling and analysis

Soil samples were taken within a few hours following fertilizer application in spring 2012 (second year after the beginning of the experiment), prior to sowing and crop germination. In case of organic fertilization, the applied BD was incorporated using harrows or rotary cultivators within less than 4 h. Five intact soil cores per treatment were taken from a depth of 2 to 8 cm using steel cylinders (volume 250 cm^3). Samples were taken randomly within the plot (MIN treatment) or within the drag hose or injection line (BD treatment). Soil temperature in 2 to 8 cm depth at time of sampling was recorded. Cylinders were immediately sealed, weighed, cooled to 2°C, transported to the laboratory, and kept at that temperature until gas flux measurements were made.

Following soil incubation, soil subsamples (20 g fresh soil) were extracted with 0.0125 M $CaCl_2$ solution [1:4 (w/v)] by shaking for 1 h. The extracts were then filtered and analyzed for NH_4^+-N and NO_3^--N concentrations using spectrophotometry according to *VDLUFA* (2002) with a continuous flow analyzer (Skalar Analytics, CFA-SAN). Soil moisture content was determined gravimetrically after drying a soil subsample at 105°C until constant weight. Bulk density and water-filled pore space (WFPS) were calculated based on sample volume, dry weight and gravimetric water content. Soil pH was determined in 0.01 M $CaCl_2$ solution [1:5 (w/v)] using a glass electrode. An aliquot of each sample was milled and analyzed for soil organic carbon (C_{org}) and total nitrogen (N_t) by dry combustion using an elemental analyzer (Leco Instruments, TruSpec CNS).

2.3 Laboratory incubation and gas flux measurements

Simultaneous measurements of N_2O and N_2 fluxes were conducted using the N_2-free helium-oxygen (He-O_2) incubation method (*Butterbach-Bahl* et al., 2002), classified as a *flow-through steady-state system* according to *Livingston* and *Hutchinson* (1995). Soil samples were placed in special gas-tight incubation vessels inside a climate box and incubated at 10°C and the respective field moisture, similar to field conditions during fertilization and sampling. The incubation vessels feature double seals back-purged with pure He to inhibit N_2 diffusion from the atmosphere. Each measurement series consisted of five vessels with samples, an empty incubation vessel (blank), and a carrier gas measurement (control), which allows correcting for possible N_2 leakage. In order to remove ambient N_2, vessels were evacuated with moderate suction pressure (0.047 bar) and subjected to four subsequent flushing sequences, first with an artificial He–O_2 gas mixture (20.5% O_2, rest He) followed by an artificial He–O_2 gas mixture with traces of CO_2, CH_4, N_2O, and N_2 (20.9% O_2, 340 ppm CO_2, 1,800 ppm CH_4, 0.34 ppm N_2O, 4 ppm N_2, rest He) (*Eickenscheidt* et al., 2014; *Fiedler* et al., 2017). An artificial He–O_2 gas mixture was used, because the admixture of ambient concentrations of CO_2, CH_4 and N_2O allows the measurement of possible negative flows of these gases, while the admixture of 4 ppm N_2 improves the precision of measurements of low N_2 concentrations. The approach was based on the assumption that N_2 originally contained in the soil air was completely removed once the N_2 concentrations had reached a constant level. Depending on the soil type, this took from one to two days (clay substrates) after the evacuation rinse cycle. Thus, a He–O_2 gas flow rate of 15 mL min^{-1} to the vessel headspaces was established and continued for 24 h to 48 h until the N_2 concentrations were constant (1 to 2 days). On the following day, the N_2O concentrations in all vessel headspaces and the control were measured once and N_2 concentration three times per day. Concentrations of N_2O were analyzed using a GC (Shimadzu, GC-14B) with an electron capture detector (ECD), while N_2 concentrations were analyzed with a micro-GC (Agilent Technologies, 3000 Micro GC) with a thermal conductivity detector (TCD).

After checking for equal gas concentration values for control and blank, the gas flux from the soil samples was calculated using Eq. (1), based on the concentration difference of the respective gas in the incubation vessel headspace over time, taking the control value (concentration in the carrier gas) into account:

$$F = \frac{M \times \rho \times V \times (\Delta c) \times v}{A \times R \times t \times T}, \quad (1)$$

where F is the flux ($\mu g\, m^{-2}\, h^{-1}$), M is the molar mass of N_2 or N_2O, respectively ($\mu g\, mol^{-1}$), ρ the atmospheric pressure (Pa), V is the volume of the vessel, Δc is the difference between in- and outflow gas concentration (mol), v is the air flow ($m^3\, h^{-1}$), A is the surface area of the soil sample (m^2), R the gas constant ($m^3\, Pa\, K^{-1}\, mol^{-1}$), t is the time over which the concentration change was observed, and T the incubation temperature (K).

2.4 Data analysis

Normal distribution was rejected for most variables at the 0.05 significance level using the Shapiro–Wilk test. Differences in WFPS, bulk density, N fertilization rate (N_{fert}) , soil

NH_4^+-N and NO_3^--N concentration, N_2 and N_2O flux, and the $N_2O/(N_2O+N_2)$ ratio of denitrification between treatments within site were analyzed using the Mann–Whitney U-Test; differences among sites within treatments using the Kruskal–Wallis H-Test combined with post-hoc Student–Newman–Keuls test. Pairwise associations between either N_2 flux, N_2O flux, or $N_2O/(N_2O+N_2)$ ratio of denitrification and the soil and fertilization parameters were characterized using the distribution-independent coefficient Spearman's rho (ρ). The influence of all variables on the N_2 and N_2O flux, as well as the $N_2O/(N_2O+N_2)$ ratio of denitrification was determined using generalized linear model (GLM) analysis with stepwise elimination. Initial included variables were treatment (MIN and BD), site, application, WFPS, bulk density, N_{fert}, soil NH_4^+-N and NO_3^--N concentration, as well as bivariate interactions between these factors. All statistical analyses were conducted using SPSS 19.0.1, while figures were created using SigmaPlot 13.0.

3 Results

3.1 Biogas digestate characteristics

BD dry matter and C content varied between 6.6 and 8.4%, and 2.6 and 3.8%, respectively, with Ascha and Gülzow featuring the highest values for both parameters (Tab. 3). All BD were slightly basic (pH range 7.3 to 8.1). Fresh matter N content ranged from 0.4 to 0.5%, with NH_4^+-N being the dominant N compound (50 to 60%).

3.2 Soil characteristics

Site-specific average soil sample bulk density ranged from 1.1 to 1.6 g cm^{-3}, with no significant difference in average bulk density between samples with two fertilization types (Tab. 2). BD samples from Gülzow had the highest bulk density (1.64) and also a particularly high WFPS value of 75 ± 6%. On average, the WFPS of all BD samples (45 ± 4%) significantly exceeded that of MIN samples (27 ± 2%), although this trend was not significant for samples from Ascha. Except for one site, soil pH_{CaCl2} was significantly higher after BD fertilization. Fertilization with BD also significantly increased soil C_{org} concentration for samples from all sites. While average soil N_t concentration was also significantly higher for BD than for MIN samples, this trend was only significant for samples from Dornburg and Gülzow. Regardless of fertilization type, the Gülzow samples featured consistently the lowest soil C_{org} and N_t concentrations.

Across all sites, BD samples exhibited significantly higher average NH_4^+-N and lower NO_3^--N concentrations than MIN samples (Tab. 2), but the latter difference was not significant for all sites. After BD fertilization, NH_4^+-N concentrations were highest in samples from Dornburg and Gülzow, which had the highest N fertilization rate (N_{fert}). In contrast, Dedelow samples featured the highest NH_4^+-N concentrations after MIN fertilization. MIN samples from Dedelow also had the highest overall NO_3^--N concentrations, followed by MIN and BD samples from Dornburg.

Table 2: Average soil bulk density, WFPS, pH_{CaCl_2}, C_{org}, N_t, NH_4^+-N and NO_3^--N concentration by treatment (BD, MIN) and site (± one standard error).[a]

Treatment	Site	Bulk density (g cm^{-3})	WFPS (%)	pH_{CaCl_2}	C_{org} (%)	N_t (%)	NH_4^+-N (mg N 100 g^{-1})	NO_3^--N (mg N 100 g^{-1})
Organic N (BD)	Ascha	1.28 ± 0.04 Aa	40 ± 6 a	7.0 ± 0.3 A	1.45 ± 0.16 Aa	0.16 ± 0.02 ab	17.3 ± 8.2 Aab	2.4 ± 0.8 ab
	Dedelow	1.23 ± 0.04 ab	31 ± 3 Aa	6.8 ± 0.2 A	1.19 ± 0.06 Aab	0.14 ± 0.01 ab	4.1 ± 3.4 Aa	4.8 ± 1.0 Aa
	Dornburg	1.09 ± 0.03 b	46 ± 3 Aa	7.4 ± 0.1 A	1.56 ± 0.07 Aa	0.21 ± 0.01 Aa	23.8 ± 3.6 Ab	12.4 ± 2.5 a
	Gülzow	1.64 ± 0.04 Ac	75 ± 6 Ab	7.3 ± 0.1 A	1.02 ± 0.03 Ab	0.12 ± 0.00 Ab	22.0 ± 3.0 Ab	0.4 ± 0.2 Ab
	Hohenschulen	1.14 ± 0.02 ab	34 ± 4 Aa	6.9 ± 0.2	1.22 ± 0.05 Aab	0.14 ± 0.01 ab	11.9 ± 5.9 Aab	3.6 ± 1.6 ab
	Average	1.28 ± 0.04	45 ± 4 A	7.1 ± 0.1 A	1.29 ± 0.05 A	0.15 ± 0.01 A	15.8 ± 2.6 A	4.7 ± 1.0 A
Mineral N (MIN)	Ascha	1.44 ± 0.03 Ba	35 ± 3 a	6.3 ± 0.1 Bab	1.13 ± 0.02 Ba	0.12 ± 0.00 ab	2.0 ± 0.3 Ba	5.9 ± 0.8 a
	Dedelow	1.11 ± 0.04 b	17 ± 1 Bb	7.5 ± 0.0 Ba	0.99 ± 0.02 Bab	0.16 ± 0.01 a	12.7 ± 1.9 Bb	24.3 ± 2.5 Bb
	Dornburg	1.14 ± 0.01 b	24 ± 0 Ba	6.2 ± 0.1 Bab	1.05 ± 0.02 Ba	0.16 ± 0.01 Ba	7.0 ± 1.9 Bab	17.3 ± 3.2 ab
	Gülzow	1.40 ± 0.04 Bac	33 ± 3 Ba	5.9 ± 0.1 Bb	0.86 ± 0.01 Bb	0.10 ± 0.00 Bb	1.8 ± 0.9 Ba	4.8 ± 1.9 Ba
	Hohenschulen	1.22 ± 0.04 bc	24 ± 1 Ba	6.5 ± 0.0 a	0.97 ± 0.02 Bab	0.12 ± 0.01 ab	4.6 ± 1.2 Bab	9.3 ± 2.2 a
	Average	1.26 ± 0.04	27 ± 2 B	6.5 ± 0.1 B	1.00 ± 0.02 B	0.13 ± 0.01 B	5.6 ± 1.0 B	12.3 ± 1.8 B

[a]Uppercase letters denote significant differences between treatments within site or across-site averages (non-parametric Mann–Whitney U-Test); lowercase letters denote significant differences among sites within a treatment (non-parametric Kruskall–Wallis H-Test, post-hoc Student–Newman–Keuls test) of each variable; $n = 5$; $p < 0.05$.

Table 3: Characterization and amount of organic and mineral fertilizer by study site.

Site	Fertilization date (DD.MM.YYYY)	Organic fertilizer (BD)											Mineral fertilizer (MIN)	
		Application type	Volume (m^3 ha^{-1})	N_{fert} (kg N ha^{-1})	Dry matter (%DM)	pH	C_t (%FM)	N_t (%FM)	C_t:N_t ratio	NH_4^+-N (%FM)	P (%FM)	K (%FM)	Fertilizer type	N_{fert} (kg N ha^{-1})
Ascha	02.05.2012	Drag hose	46	202	8.43	7.3	3.35	0.44	7.6	0.24	0.08	0.43	CAN[b]	150
Dedelow	07.05.2012	Drag hose	57	251	6.90	7.9	2.60	0.44	5.9	n.a.[a]	0.06	0.25	CAN[b]	160
Dornburg	12.04.2012	Drag hose	79	332	6.60	7.9	2.77	0.42	6.6	0.21	0.08	0.32	CAN[b]	74[c]
Gülzow	26.04.2012	Injection	52	262	7.93	8.1	3.84	0.50	7.7	0.30	0.14	0.37	CAN[b]	160
Hohenschulen	19.04.2012	Drag hose	51	254	6.90	7.4	n.a.[a]	0.50	n.a.[a]	0.28	0.08	0.37	CAN[b]	160

[a]Missing data;
[b]granular calcium ammonium nitrate;
[c]only first application, second application of 100 kg N ha^{-1} MIN fertilizer took place after soil core sampling.

3.3 N_2O and N_2 fluxes

The measured absolute N_2O flux ranged from 37 to 1,221 μg N m^{-2} h^{-1} and from 23 to 140 μg N m^{-2} h^{-1} for BD and MIN samples, respectively (Fig. 1a). Across all sites, BD samples showed a significantly higher absolute N_2O flux than MIN samples. However this effect was caused by samples from only two sites, particularly from Dornburg where BD samples exhibited a 27-fold higher N_2O flux than MIN samples. Between sites, relative N_2O fluxes were different for the BD treatment. However, a trend towards a difference in relative N_2O fluxes between the BD and MIN treatment can be assumed from Tab. 4, but could not be confirmed with non-parametric statistics setting a significance level ($p \leq 0.05$).

The absolute N_2 fluxes across all sites were significantly higher for BD than for MIN samples (Fig. 1b). Relative N_2 fluxes were not significantly different across soil sites, but on average between the two fertilizer types (Tab. 4). The site-specific differences of absolute N_2 flux between fertilization types were not significant, due to high between-sample variability (coefficient of variation 42 to 100%). Although differences among sites were generally less pronounced than differences between fertilization types, between-site variability of the N_2O and N_2 flux was considerably higher for BD samples (Fig. 1). BD samples from Dornburg showed the highest N_2O and N_2 flux by far, and—except for N_2O flux in relation to MIN fertilizer input—also the highest proportion of emitted N relative to the amount of applied fertilizer N (Tab. 4).

Table 4: Average relative (per kg^{-1} applied N_{fert}) N_2O and N_2 flux by treatment (BD, MIN) and site (± one standard error).[a]

Treatment	Site	N_2O-N flux (μg N m^{-2} h^{-1} kg^{-1} N_{fert})	N_2 flux (μg N m^{-2} h^{-1} kg^{-1} N_{fert})
Organic N (BD)	Ascha	0.69 ± 0.21 Aa	6.23 ± 2.74
	Dedelow	0.45 ± 0.17 a	0.09 ± 0.09
	Dornburg	3.68 ± 1.23 Ab	8.35 ± 3.49
	Gülzow	0.14 ± 0.06 a	1.75 ± 0.85
	Hohenschulen	0.37 ± 0.15 a	0.42 ± 0.33
	Average	1.07 ± 0.35	3.37 ± 1.07 A
Mineral N (MIN)	Ascha	0.15 ± 0.06 B	1.23 ± 0.78
	Dedelow	0.88 ± 0.27	0.19 ± 0.19
	Dornburg	0.61 ± 0.34 B	1.67 ± 1.30
	Gülzow	0.18 ± 0.06	0.32 ± 0.32
	Hohenschulen	0.21 ± 0.07	0.20 ± 0.20
	Average	0.40 ± 0.10	0.72 ± 0.31 B

[a]Uppercase letters denote significant differences between treatments within site or across-site averages (non-parametric Mann–Whitney U-Test); lowercase letters denote significant differences among sites within treatment (non-parametric Kruskall–Wallis H-Test. post-hoc Student–Newman–Keuls test); $n = 5$; $p < 0.05$.

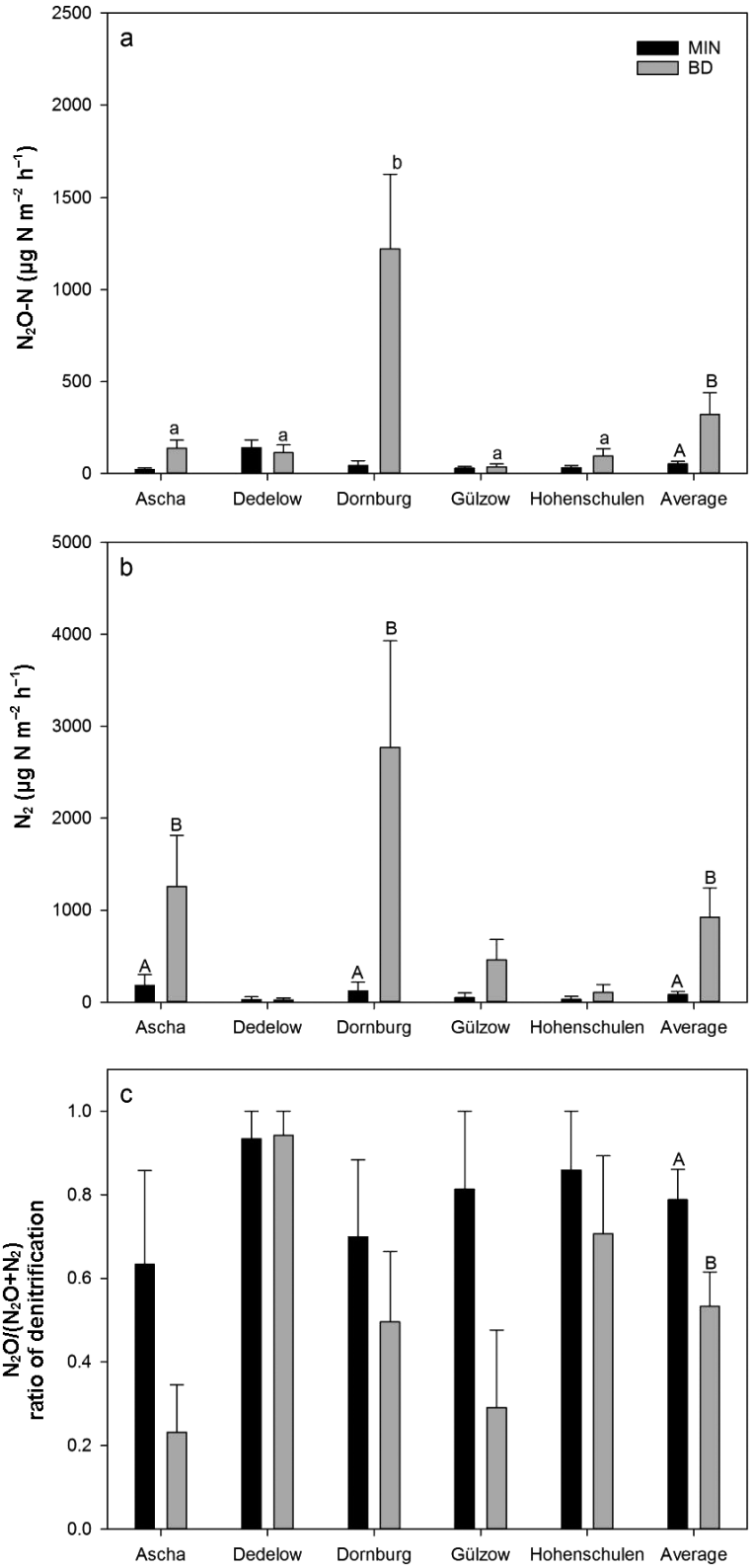

Figure 1: N_2O (a) and N_2 (b) flux as well as $N_2O/(N_2O+N_2)$ ratio of denitrification (c) by treatment (MIN, BD) and site. Error bars show ± one standard deviation. Note: Uppercase letters denote significant differences between treatments within site or across-site averages (non-parametric Mann-Whitney U-Test); lowercase letters denote significant differences among sites within treatment (non-parametric Kruskall–Wallis H-Test. post-hoc Student–Newman–Keuls test).

In addition, the proportion of N_2O relative to the total N_2O+N_2 fluxes [also known as the $N_2O/(N_2O+N_2)$ ratio of denitrification] was calculated as an important assessment indicator. The N_2 flux was generally higher than the N_2O flux, thus resulting in low average values for the $N_2O/(N_2O+N_2)$ ratio of denitrification. The $N_2O/(N_2O+N_2)$ ratio of denitrification was lower for BD than for MIN samples (Fig. 1c). Samples from Dedelow were an exception to this trend and showed similar high $N_2O/(N_2O+N_2)$ ratios of denitrification (> 0.93) for both fertilizer types. However, due to the high variability, differences between the sites were not significant. There was no significant effect of fertilization on site-specific $N_2O/(N_2O+N_2)$ ratios of denitrification, but particularly BD samples from Gülzow and Ascha had considerably lower $N_2O/(N_2O+N_2)$ ratios (0.23 to 0.29) than the respective MIN samples (0.63 to 0.81).

3.4 Controls on N_2O and N_2 fluxes

N_2O flux from MIN samples was significantly but weakly correlated with N_t and NH_4^+-N concentrations and (for relative N_2O flux) with bulk density, while absolute and relative N_2O flux from BD samples were strongly correlated with C_{org}, N_t, and NO_3^--N concentrations (Tab. 5). GLM analysis showed NH_4^+-N concentrations, site, WFPS, bulk density, and their interactions to be the most important factors for N_2O flux (Tab. 6), which reflects the observed significant differences in the N_2O flux between soil cores from different field sites. Other significant factors influencing the N_2O flux were application type (*i.e.*, injection *vs*. drag hose) and fertilization treatment.

While no correlations between absolute or relative N_2 flux and the control parameters were observed for MIN samples, the N_2 flux from BD samples was significantly correlated with NH_4^+-N, pH, C_{org}, N_t (only the absolute N_2 flux), and WFPS. Overall, the main factor complexes controlling the N_2 flux were (1) fertilization treatment and its interactions with NH_4^+-N concentration, followed by (2) WFPS, application type, and their interaction, while the relative importance of site was considerably smaller than for N_2O (Tab. 6).

Like for N_2, the $N_2O/(N_2O+N_2)$ ratio of denitrification from BD samples was significantly correlated with soil NH_4^+-N concentration, pH, and WFPS (Tab. 5). GLM analysis revealed that the $N_2O/(N_2O+N_2)$ ratio of denitrification is mainly controlled by site, bulk density and NH_4^+-N concentration (Tab. 6), and the interaction of NH_4^+ and WFPS, whereas the influence of treatment, WFPS, N_t content and their interactions with soil parameters was of far lesser relevance than for N_2O and N_2 fluxes.

Table 5: Bivariate non-parametric correlations (Spearman's ρ) between absolute or relative N_2O and N_2 flux, $N_2O/(N_2O+N_2)$ ratio of denitrification and bulk density, WFPS, N_{fert}, soil NH_4^+-N and NO_3^--N concentration.[a]

Treatment	Variable	Bulk density (g cm^{-3})	WFPS (%)	pH_{CaCl_2}	C_{org} (%)	N_t (%)	N_{fert} (kg N ha^{-1})	NH_4^+-N (mg N 100 g^{-1})	NO_3^--N (mg N 100 g^{-1})
Organic N (BD)	absolute N_2O flux	–0.376	–0.066	0.080			**0.643*****	**0.497***	0.243
								0.157	**0.721*****
	absolute N_2 flux	–0.061	**0.423***	**0.643*****	**0.460***	**0.433***	0.153	**0.676*****	0.000
	relative N_2O flux	–0.356	–0.036	0.115			**0.661*****	**0.521****	0.220
								0.195	**0.699*****
	relative N_2 flux	–0.004	**0.402***	**0.613*****	**0.456***	0.394	0.100	**0.649*****	–0.015
	$N_2O/(N_2O+N_2)$ ratio	–0.264	**–0.556****	**–0.661*****	–0.202	–0.185	0.052	**–0.692*****	0.362
Mineral N (MIN)	absolute N_2O flux	–0.343	–0.332	0.381	–0.102	**0.416***	0.261	**0.456***	0.364
	absolute N_2 flux	0.085	0.212	–0.157	0.185	0.169	–0.235	0.047	–0.086
	relative N_2O flux	**–0.404***	–0.330	0.306	–0.041	**0.476***	0.081	**0.416***	0.366
	relative N_2 flux	0.044	0.194	–0.147	0.208	0.199	–0.291	0.059	–0.057
	$N_2O/(N_2O+N_2)$ ratio	–0.098	–0.234	0.181	–0.144	–0.117	0.224	–0.001	0.119

[a]Correlations in bold are significant at: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

4 Discussion

A single application of mineral or organic fertilizer during sowing in spring is common practice in maize cultivation. Fertilization thus usually takes place under conditions favorable for N_2O and N_2 emissions, *i.e.*, high soil moisture levels in combination with increasing soil temperatures (*Butterbach-Bahl* et al., 2013). As a consequence, a major part of the annual N_2O emissions is directly related to fertilization and often emitted within hours to days after fertilizer application (*Heintze* et al., 2017), together with a less known amount of N_2, which in our experiment was found to be up to 2,773 μg N m^{-2} h^{-1} (average) within the first two to four days. The present experimental set-up allowed studying short-term N_2O and N_2 fluxes shortly after fertilizer application with respect to different soil characteristics in Germany, but not aimed to study the dynamics after BD and MIN application on different field sites. In order to study N_2O and N_2 dynamics, a longer incubation period (up to several weeks) with high-frequency measurements would have been necessary.

4.1 Impact of fertilizer type and application (BD *vs.* MIN)

In agreement with our first hypotheses, average N_2O and N_2 fluxes were significantly higher from BD than from MIN treatments in soil cores across five sites (Fig. 1). Moreover lower average $N_2O/(N_2O+N_2)$ ratios of denitrification were found in the BD soil cores across sites, except those from the Dedelow site. These findings are in line with a previous laboratory experiment by *Senbayram* et al. (2009), who reported that soils treated with BD derived from maize emitted more N_2O than soils treated with MIN fertilizer. The average value of N_2O fluxes following BD application was strongly influenced by the sample from the Dornburg site (Fig. 1a), where BD samples exhibited a 27-fold higher N_2O flux than MIN samples, mainly due to the four times higher N application rate of BD compared to MIN fertilization. Despite slightly different boundary conditions (different biogas digstate, slightly higher temperature with 15°C), a subsequent laboratory study by *Fiedler* et al. (2017) showed higher N_2O fluxes overall but compared to Gülzow significantly higher N_2O fluxes again for the Dornburg soil cores. On the other hand, *Heintze* et al. (2017) investigated N_2O fluxes following BD application (160 kg N ha^{-1}) for the Ascha site within a 21-day incubation experiment and found maximum mean N_2O fluxes of 7.8 $\pm$ 5.6 μg N m^{-2} h^{-1}. The measured N_2O fluxes were thus up to ten times lower than in our study (*i.e.*, 139 $\pm$ 43 μg N m^{-2} h^{-1}). Differences in N_2O fluxes between the three related studies might be due to the well-known spatial and temporal variability of N_2O, particularly because samples were taken in different years and soil cores were treated differently [*i.e.*, drying and rewetting for WFPS adjustment in *Fiedler* et al. (2017)]. However, N_2 fluxes from the BD treat-

Table 6: Summary statistics of generalized linear model (GLM) analysis describing the influence of treatment (BD, MIN), application type, site, WFPS, bulk density, soil pH, and soil N_t, NH_4^+-N and NO_3^--N concentration, as well as N_{fert} (and their interactions) on the absolute N_2, N_2O flux and $N_2O/(N_2O+N2)$ ratio of denitrification.[a]

	N_2 (mg N m^{-2} h^{-1})		N_2O (µg N m^{-2} h^{-1})		$N_2O/(N_2O+N_2)$ ratio of denitrification	
	Wald Z	***p***	**Wald Z**	***p***	**Wald Z**	***p***
Intercept	0.001	*0.977*	6.355	***0.012***[a]	1.544	*0.214*
Treatment (BD, MIN)	6.131	***0.013****	15.259	≤ ***0.001****	†[b]	
Application type	6.359	***0.012****	23.680	≤ ***0.001****	†	
Site	11.284	***0.024****	25.453	≤ ***0.001****	17.749	***0.001****
WFPS	16.355	≤ ***0.001****	14.231	≤ ***0.001****	1.830	*0.176*
Bulk density	5.174	***0.023****	5.480	***0.019****	4.175	***0.041****
Soil pH_{CaCl2}	†		†			
Soil N_t	†		6.543	***0.011****	3.702	*0.054*
Soil NH_4^+-N	16.375	≤ ***0.001****	29.256	≤ ***0.001****	4.938	***0.026****
N_{fert}	†		†		6.398	***0.011****
Treatment × WFPS	2.866	*0.090*	†		†	
Treatment × NH_4^+-N	16.120	≤ ***0.001****	†		†	
Application type × WFPS	5.133	***0.023****	†		†	
Site × Bulk density	9.443	*0.051*	28.162	≤ ***0.001****	†	
Site × WFPS	†		28.480	≤ ***0.001****	†	
WFPS × NH_4^+-N	†		29.224	≤ ***0.001****	3.964	***0.046****

[a]Asterisks denote significant factors ($\alpha = 0.05$);
[b]† Redundant parameter or parameter interaction.

ment between the two subsequent studies were found to be similar for the two sites (Dornburg and Gülzow). As no MIN fertilizer treatment was investigated by *Fiedler* et al. (2017), a direct comparison between the two studies was not possible. N_2 fluxes were also higher from BD samples than MIN samples; hence, the calculated average $N_2O/(N_2O+N_2)$ ratio of denitrification was lower for BD compared to MIN samples, even though N_2 fluxes were higher than N_2O fluxes in both fertilizer treatments. Compared to another incubation study using BD samples with an N_2 release of up to 13 mg N m^{-2} h^{-1} within five days of incubation (*Köster* et al., 2015), present mean N_2 fluxes were considerably smaller. This might be explained by a slightly different set-up with BD application after two days of helium incubation, a higher fertilization rate of 250 kg N ha^{-1} and a significantly higher WFPS of 90%, which could favor N_2O reduction to N_2 in the study by *Köster* et al. (2015). In the present case, N_2 fluxes were up to ten-fold higher than N_2O fluxes, which underlined the assumption that a substantial share of N_2O formed by denitrification was further reduced to N_2 [see also low $N_2O/(N_2O+N_2)$ ratios of denitrification], although NO_3^- availability was limited (Tab. 2).

4.2 Effect of soil conditions from the different field sites

Soil biochemical processes in agricultural systems are influenced by physical and chemical soil conditions at the respective field sites. For fertilized agricultural sites, the impacts of fertilization rate, substrate composition and fertilizer application type have to be added as important influencing factors.

4.2.1 N level and substrate composition

As this study was not designed to test a large gradient of fertilizer input, the choice of N fertilization levels was based on typical local management preferences for maize cultivation. Local fertilization rates were based on the assumption that 70% of the N of the biogas digestate will be available for the plant in the year of application. Correspondingly, increased N doses *via* biogas digestate application were planned from the outset in this case to compensate for this factor, which thus increased the BD compared to the MIN fertilization rate. This resulted in a range of fertilizer N inputs from 202 to 332 kg N ha^{-1} in the BD treatments (Tab. 2) and likely the lack of any statistical effect of the N fertilization rate (N_{fert}) on short-term N_2 and N_2O fluxes (Fig. 1). In particular, the Dornburg site exceeded the

compensation by a large margin, which has led to significant differences between the two N fertilizer treatments (232 kg N ha^{-1} were applied in the BD treatment compared to 74 kg N ha^{-1} MIN treatment). These differences were reflected in the absolute and relative N_2O fluxes at this site (Fig. 1, Tab. 4). Some recent results have shown that N_2O and N_2 fluxes did not increase with increasing the BD application rate (*Fiedler* et al., 2017), whereas *Sänger* et al. (2014) found an increase in net nitrification rates with increasing application rates, which was linked to the greater availability of NH_4^+. Moreover, the application of local BD instead of a standardized BD might have masked any site or treatment effects *via* introduction of uncertainty due to differences in BD quality, but all applied BD originated from co-fermentation of cattle slurry and maize silage featuring similar physical and chemical characteristics. While the dry matter content of the applied BD (6.6 to 8.4%) was slightly higher than reported for mono-fermented maize BD (4.9 to 5.9%; *Senbayram* et al., 2009; *Svoboda* et al., 2013), it was representative of co-fermented slurry-maize BD (5.0 to 8.5%; *Kluge* et al., 2008). The observed range of pH and C:N ratios were also typical for mono- and co-fermented maize BD (pH of 7.6 to 8.6 and C:N ratio of 5.2 to 7.6; *Senbayram* et al., 2009; *Chen* et al., 2012; *Svoboda* et al., 2013). Regardless of the small differences in the applied BD fertilizers, digestate properties and therefore also the C and N dynamics are always influenced by the type of substrate input for anaerobic digestion (*Sänger* et al., 2014). Due to the usage of BD fertilizers from local farmers, the quantities of BD fertilizers applied were to some extent different for the respective sites, which must be considered when interpreting results. When relative N_2 fluxes were calculated, no site differences within the BD treatment were found (Tab. 4). Nevertheless, the much higher variability of the N_2 fluxes of the BD treatment compared to N_2 fluxes of the MIN is an indication that the quantity and composition of the BD may to have a strong impact on the N_2 fluxes (*i.e.*, the share of N_2O reduction to N_2).

Higher N_2O fluxes after BD application were likely related to stimulated microbial activity caused by the addition of some easily degradable C and N and the resulting consumption of soil O_2, which in turn may enhance N losses due to denitrification, in particular also the reduction of N_2O to N_2 (*Petersen* and *Sommer*, 2011; *Köster* et al., 2015; *Heintze* et al., 2017). Moreover, it can be assumed that the increased amount of NH_4^+ due to BD application, which was also found here, probably enhanced the process of nitrifier-denitrification, *i.e.*, the oxidation of NH_4^+ to NO_2^- and its subsequent reduction to NO and N_2O by autotrophic ammonia oxidizing microorganisms. Since nitrifier-denitrification particularly occurs in soils with high N availability but low C_{org}, BD application has been found to stimulate this process under low O_2 availability (*Eickenscheidt* et al., 2014). However, a multitude of additional sources of N_2O production has to be considered, due to the known wealth of N_2O production processes and their simultaneous occurrence in soils (*Butterbach-Bahl* et al., 2013).

In addition to the discussed N_2O, NO, and N_2 losses, a significant N loss *via* ammonia (NH_3) can also occur within the first hours after organic fertilizer application (*Webb* et al., 2010; *Wolf* et al., 2014; *Ni* et al., 2015), which should be mentioned for the sake of completeness. It is supposed that up to 15% of NH_4^+ is probably volatized as NH_3 within the first 10 h after surface application of BD (*Quakernack* et al., 2012), which might have also affected the share of N_2O and N_2 losses.

4.2.2 Soil physical properties

Since the study sites were selected to represent a wide range of typical agricultural soil conditions, this study allowed the evaluation of the study results in light of common agricultural practice throughout Germany while lacking systematic gradients of potential impact factors. It is thus reasonable that the soil cores taken from five study sites represent a variety of soil physical properties regarding differences in soil moisture, soil type and texture, which also had a certain impact on the N gas release (see impact of site in the GLM). In particular, the parameter interaction of study site and bulk density, as well as soil moisture (*i.e.*, WFPS) had a significant impact on the N_2O fluxes. In the case of bulk density, the greater variability in the BD treatment might be due to the local, strip-like soil loosening during the incorporation of the applied BD using harrows or rotary cultivators and the possibility of not always exact removal of steel cylinders in the drag hose line. The on average higher proportion of WFPS in the BD treatment is due to localized application of high amounts of water in the form of liquid digestate, which will have influenced the N_2O fluxes more than the N_2 fluxes. It might also be possible that MIN fertilizer granulates were not entirely dissolved at the time of soil core sampling, which might also lead to a certain bias. However, when looking at single impact factors, bulk density and WFPS were important factors affecting the N_2, N_2O release as well as the $N_2O/(N_2O+N_2)$ ratio of denitrification (Tab. 6). For example, for the study site Dedelow, only a limited denitrification activity was previously assumed due to the sandy soil texture and low WFPS contents, which are positive for soil aeration (*Del Grosso* et al., 2000; *Ball*, 2013). However, $N_2O/(N_2O+N_2)$ ratios of denitrification < 0.4 were found for the Dedelow samples, which indicates a great contribution of N_2O reduction to N_2. By contrast, high WFPS values (up to 81%) were found for BD in the Gülzow samples, probably also resulting from the different fertilizer application technique *via* injection. Although soil moisture conditions were optimal for complete denitrification, N_2 fluxes ranged on a moderate level (460 ± 224 µg N m^{-2} h^{-1}). By using stable isotope labeling, *Senbayram* et al. (2009) also found denitrification to be the dominant process in soils having 58% and 76% WFPS values. However, for a WFPS value of 58% (*Senbayram* et al., 2009), a significant contribution of nitrification was revealed, which could be also the case for the other sites in this study (WFPS < 49%; Tab. 2).

Overall, these observations were more pronounced in the BD treatment than in the MIN treatment, which might be additionally promoted by the high water content of the digestate (BD treatment) compared to a dry granulated MIN fertilizer. BD contain mostly water (up to 97% moisture content), which can lead to the short-term saturation of micro-pores (*Charles* et al., 2017), thus limiting O_2 diffusivity and favoring denitrification (*Tiedje* et al., 1984). This may apply in particular for the Dornburg site, where in addition to the highest BD fertilization rates, which led to high NO_3^--N and NH_4^+-N (Tab. 2), also a

great amount of water was added during BD application. Moreover, the Dornburg site has a very clayey silt soil texture, which often corresponds to limiting O_2 diffusivity and the greater potential for the occurrence of anaerobic microsites, which in combination with the effects of BD application could have favored conditions for denitrification and might explain the high N_2 fluxes for the Dornburg samples (Fig. 1).

4.2.3 Soil chemical properties

Regarding the chemical soil properties, large differences between the BD and MIN treatment were present (Tab. 2). BD application increased differences and variability among sites due to higher pH values, higher C_{org} contents, higher NH_4^+-N and lower NO_3^--N concentrations in BD samples compared to MIN samples. However, it is not possible to distinguish whether the differences result from the application of the BD in this year or already resulted from the generally good supply of the soils with BD from the previous experimental year. Apart from soil physical properties, N availability (*i.e.*, NH_4^+ and NO_3^-) is known as an important driver for N_2O and N_2 processes. Here, it seems that the higher N_2O and in particular N_2 gas release from the BD compared to the MIN treatment was driven by the interaction of physical (*i.e.*, high soil moisture) and chemical properties (*i.e.*, high NH_4^+-N concentrations and C contents) (see Tab. 5). Following BD application, the NH_4^+-N concentration was enhanced, which probably stimulated the N_2O release *via* nitrification (*Köster* et al., 2011). However, along with increased amounts of C_{org} following BD application, which promote soil respiration and thus interact with O_2 availability, the potential for denitrification losses (*i.e.*, N_2O and N_2 fluxes) due to anaerobic soil conditions increased (*Köster* et al., 2015). This might be also the case here, although NO_3^- availability (*i.e.*, substrate for denitrifiers) was limited. However, limited NO_3^- availability at the beginning can also promote the reduction of N_2O ongoing nitrification in anaerobic microsites, because C_{org} availability for denitrification becomes more and more depleted (*i.e.*, decreasing CO_2 rates) and lower O_2 consumption allow nitrification (*Weier* et al., 1993; *Köster* et al., 2011). In turn, with increasing NO_3^- availability again, this would then have caused the shift back from N_2 to N_2O as the main denitrification end product (*Senbayram* et al., 2009). Due to the focus on short-term results from different sites within the present N_2-free incubation experiment, it was not possible to investigate any interactions of NO_3^- and NH_4^+ dynamics.

5 Conclusions

Despite the limited scope of this experiment, this study provides valuable insights into short-term N_2O and N_2 fluxes as well as the $N_2O/(N_2O+N_2)$ ratio of denitrification following the initial phase of fertilizer application (biogas digestate *vs*. mineral fertilizer) from different field sites. On average, results demonstrated higher absolute N_2O and N_2 fluxes from biogas digestate samples compared to mineral fertilizer samples. Relative (to fertilizer input) N_2O fluxes were different between sites, but not across sites, while relative N_2 fluxes were only different across sites. Due to the higher N_2 than N_2O fluxes, the low $N_2O/(N_2O+N_2)$ ratios of denitrification highlighted the importance of N_2O reduction to N_2, in particular for the biogas digestate compared to the mineral fertilizer soil cores. While variability in N gas release between the investigated soil cores were lower for mineral fertilizer samples, soil conditions like bulk density and WFPS as proxies for gas diffusivity in the soil particularly affected the N_2O production and reduction rates in the biogas digestate treatment. Nevertheless, the presented results also emphasize the need for further investigations of the dynamics and the duration of the observed effects and their significance under field conditions. However, a first estimate of the short-term N_2 gas release in the field during the initial phase might be possible, when applying the $N_2O/(N_2O+N_2)$ ratio of denitrification to field measurements.

Acknowledgments

The study was funded by the *German Federal Ministry of Food and Agriculture* and managed by the *Agency for Renewable Resources* under grants *22021008* (*08NR210*). We thank all our partners from the Christian-Albrechts-University Kiel (CAU), University of Rostock (URO), University of Applied Sciences Weihenstephan-Triesdorf (HSWT), Thünen Institute and the Leibniz Centre for Agricultural Landscape Research (ZALF), particularly the persons involved in field sampling [*Madlen Pohl, Monique Andres, Nicole Jurisch* (ZALF), *Marcus Rohwer, Achim Seidel* (CAU), *Sebastian Fiedler* (URO), *Gawan Heintze* (HSWT)] and gas measurements (*Bertram Gusovius*, ZALF). We would also like to acknowledge the anonymous reviewers for their constructive comments.

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