Soil incubation study showed biogas digestate to cause higher and more variable short-term N$_2$O 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$_2$O production, compared to mineral fertilizer. In particular, the initial phase following fertilizer application is crucial for the N gas release as N$_2$O 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$_2$O 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$_2$O 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$_2$/O/(N$_2$O+N$_2$) ratio of denitrification below or equal to 0.5 clearly highlighted the importance of N$_2$O 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$_2$O and N$_2$ fluxes from the biogas digestate soil cores. While this study presents data on short-term N$_2$O 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$_2$O/(N$_2$O+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$_2$O), 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$_2$O mainly originates from agricultural soils, where high N fertilizer inputs (mineral and organic fertilizer) are directly related to N$_2$O production, resulting in a conversion of 2 to 2.5% of fertilizer N to N$_2$O (e.g., Davidson, 2009) and contributing up to 4.8 Tg N y$^{-1}$ to the anthropogenic N$_2$O emissions (IPCC, 2013).

Apart from N$_2$O, 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$_2$O 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; Plettner 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 Möller, 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$_2$O production via nitritification 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

© 2020 The Authors. Journal of Plant Nutrition and Soil Science published by Wiley-VCH Verlag GmbH & Co. KGaA www.plant-soil.com

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
potential for $\text{N}_2\text{O}$ reduction to $\text{N}_2$ (Butterbach-Bahl et al., 2013). However, there is no clear picture about the impact of BD application on $\text{N}_2$ formation up to now (Köster et al., 2015; Fiedler et al., 2017).

In addition to the effect of fertilizer type (mineral vs. organic), $\text{N}_2\text{O}$ production and consumption processes in agricultural systems are largely influenced by the soil characteristics at the respective location. $\text{N}_2\text{O}$ and $\text{N}_2$ 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 $\text{N}_2$ fluxes (Fiedler et al., 2017).

This is likely due to the fact that the determination of $\text{N}_2$ fluxes from soils is still a delicate matter due to 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: $^{15}\text{N}$ tracing technique (i.e., analysis of gas fluxes after addition of $^{15}\text{N}$-labelled substrate) and $\text{N}_2$-free incubation technique (i.e., replacing $\text{N}_2$ by a noble gas like Helium) (Van Groenigen et al., 2015). Since the $^{15}\text{N}$ 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 $\text{N}_2$-free incubation technique provides an alternative for the investigation of a large number of undisturbed soil cores from different sites, where the actual $\text{N}_2\text{O}$ and $\text{N}_2$ release can be measured. Generally, the initial phase, i.e., the first days after fertilizer application, is crucial for $\text{N}_2\text{O}$ emissions (Senbayram et al., 2009; Köster et al., 2011) and previous BD experiments have indicated a similar immediate reaction also for $\text{N}_2$ (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 $\text{N}_2\text{O}$ and $\text{N}_2$ 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 $\text{N}_2\text{O}$ and $\text{N}_2$ 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 $\text{N}_2\text{O}$ and $\text{N}_2$ fluxes and the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio of denitrification. The tested hypotheses were as follows: (1) $\text{N}_2\text{O}$ and $\text{N}_2$ 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 $\text{N}_2\text{O}$ and $\text{N}_2$ 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 $\text{N}_2\text{O}$ emissions at five experimental sites (Ascha, Dedelow, Domburg, 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-

| Site         | UTM coordinates | Elevation (m asl) | Climate   | Soil type | Soil texture |
|--------------|-----------------|-------------------|-----------|-----------|--------------|
|              | Easting         | Northing          | MAT (°C)  | MAP (mm)  |              |
| Ascha        | 33U 328812      | 5429434           | 430       | 7.5       | 807          | Cambisol     | Very loamy sand |
| Dedelow      | 33U 419197      | 5915156           | 38–53     | 8.4       | 485          | Orthic Luvisol | Moderately loamy sand |
| Domburg      | 32U 686442      | 5653450           | 245–255   | 8.8       | 596          | Orthic Luvisol | Very clayey silt |
| Gülzow       | 33U 307700      | 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 |

*FAO Classification (IUSS Working Group, 2007).
over, the Domburg 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 (Thermo-Fisher). NH₄⁺-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 cm³). 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. 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 (Thermo-Fisher). NH₄⁺-N content was analyzed photometrically according to DIN 38406-E5-2 using a Segemented Flow Analysis (Skalar Analytics, CFA-SAN).

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 cm³). 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 CaCl₂ solution [1:4 (w/v)] by shaking for 1 h. The extracts were then filtered and analyzed for NH₄⁺-N and NO₃⁻-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 CaCl₂ solution [1:5 (w/v)] using a glass electrode. An aliquot of each sample was milled and analyzed for soil organic carbon (Corg) and total nitrogen (Nt) by dry combustion using an elemental analyzer (Leco Instruments, TruSpec CNS).

2.3 Laboratory incubation and gas flux measurements

Simultaneous measurements of N₂O and N₂ fluxes were conducted using the N₂-free helium-oxygen (He-O₂) 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 N₂ 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 N₂ leakage. In order to remove ambient N₂, vessels were evacuated with moderate suction pressure (0.047 bar) and subjected to four subsequent flushing sequences, first with an artificial He–O₂ gas mixture (20.5% O₂, rest He) followed by an artificial He–O₂ gas mixture with traces of CO₂, CH₄, N₂O, and N₂ (20.9% O₂, 340 ppm CO₂, 1,800 ppm CH₄, 0.34 ppm N₂O, 4 ppm N₂, rest He) (Eickenscheidt et al., 2014; Fiedler et al., 2017). An artificial He–O₂ gas mixture was used, because the admixture of ambient concentrations of CO₂, CH₄ and N₂O allows the measurement of possible negative flows of these gases, while the admixture of 4 ppm N₂ improves the precision of measurements of low N₂ concentrations. The approach was based on the assumption that N₂ originally contained in the soil air was completely removed once the N₂ 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–O₂ gas flow rate of 15 mL min⁻¹ to the vessel headspaces was established and continued for 24 h to 48 h until the N₂ concentrations were constant (1 to 2 days). On the following day, the N₂O concentrations in all vessel headspaces and the control were measured once and N₂ concentration three times per day. Concentrations of N₂O were analyzed using a GC (Shimadzu, GC-14B) with an electron capture detector (ECD), while N₂ 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:

\[ F = \frac{M \cdot \rho \cdot P \cdot V \cdot (\Delta c) \cdot V}{A \cdot \Delta t \cdot T} \]

where \( F \) is the flux (µg m⁻² h⁻¹), \( M \) is the molar mass of N₂ or N₂O, respectively (g mol⁻¹), \( \rho \) the atmospheric pressure (Pa), \( V \) is the volume of the vessel, \( \Delta c \) is the difference between in- and outflow gas concentration (mol), \( V \) is the air flow (m³ h⁻¹), \( A \) is the surface area of the soil sample (m²), \( R \) the gas constant (m³ 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–Wilks test. Differences in WFPS, bulk density, N fertilizer rate (Nfert), soil...
NH$_4^+$-N and NO$_3^-$-N concentration, N$_2$ and N$_2$O flux, and the N$_2$O/(N$_2$O+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$_2$O flux, or N$_2$O/(N$_2$O+N$_2$) ratio of denitrification and the soil and fertilization parameters were characterized using the distribution-independent coefficient Spearman’s rho ($ho$). The influence of all variables on the N$_2$ and N$_2$O flux, as well as the N$_2$O/(N$_2$O+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$_{CaCl_2}$ 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$_2$ 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$_2$ 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$_2$, 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$_2$ (%) | NH$_4^+$-N (mg N 100 g$^{-1}$) | NO$_3^-$-N (mg N 100 g$^{-1}$) |
|-----------|--------|-----------------------------|-----------|---------------|---------------|-----------|-------------------------------|-------------------------------|
| Organic N (BD) | Ascha  | 1.28 ± 0.04 A  | 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 B  | 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 Bb  | 17.3 ± 3.2 ab |
|           | Gülzow  | 1.40 ± 0.04 Bc  | 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 Kruskal–Wallis H-Test, post-hoc Student–Newman–Keuls test) of each variable; $n = 5$; $p < 0.05$. 

© 2020 The Authors. Journal of Plant Nutrition and Soil Science published by Wiley-VCH Verlag GmbH & Co. KGaA www.plant-soil.com
3.3 N₂O and N₂ fluxes

The measured absolute N₂O 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 N₂O 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 N₂O flux than MIN samples. Between sites, relative N₂O fluxes were different for the BD treatment. However, a trend towards a difference in relative N₂O 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 ≤ 0.05).

The absolute N₂ fluxes across all sites were significantly higher for BD than for MIN samples (Fig. 1b). Relative N₂ fluxes were not significantly different across soil sites, but on average between the two fertilizer types (Tab. 4). The site-specific differences of absolute N₂ 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 N₂O and N₂ flux was considerably higher for BD samples (Fig. 1). BD samples from Dornburg showed the highest N₂O and N₂ flux by far, and—except for N₂O flux in relation to MIN fertilizer input—also the highest proportion of emitted N relative to the amount of applied fertilizer N (Tab. 4).

| Site          | Fertilization date (DD.MM.YYYY) | Organic fertilizer (BD) | N₂O-N flux (µg N m⁻² h⁻¹ kg⁻¹ N_fert) | N₂ flux (µg N m⁻² h⁻¹ kg⁻¹ N_fert) |
|---------------|--------------------------------|-------------------------|--------------------------------------|-----------------------------------|
| Organic N (BD) | Ascha 02.05.2012           | Drag hose               | 0.69 ± 0.21 Aa                      | 6.23 ± 2.74                       |
|               | Dedelow 07.05.2012         | Drag hose               | 0.45 ± 0.17 a                       | 0.09 ± 0.09                       |
|               | Dornburg 12.04.2012        | Drag hose               | 3.68 ± 1.23 Ab                      | 8.35 ± 3.49                       |
|               | Gülzow 26.04.2012          | Injection               | 0.14 ± 0.06 a                       | 1.75 ± 0.85                       |
|               | Hohen- schulen 19.04.2012 | Drag hose               | 0.37 ± 0.15 a                       | 0.42 ± 0.33                       |
|               | Average                     |                         | 1.07 ± 0.35                         | 3.37 ± 1.07 A                       |

| Mineral N (MIN) | Ascha 02.05.2012 | 0.15 ± 0.06 B | 1.23 ± 0.78 |
|                 | Dedelow 07.05.2012| 0.88 ± 0.27 | 0.19 ± 0.19 |
|                 | Dornburg 12.04.2012| 0.61 ± 0.34 B | 1.67 ± 1.30 |
|                 | Gülzow 26.04.2012 | 0.18 ± 0.06 | 0.32 ± 0.32 |
|                 | Hohen- schulen 19.04.2012| 0.21 ± 0.07 | 0.20 ± 0.20 |
|                 | Average                     | 0.40 ± 0.10 | 0.72 ± 0.31 B |

aUppercase 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.
In addition, the proportion of N\textsubscript{2}O relative to the total N\textsubscript{2}O+N\textsubscript{2} fluxes [also known as the N\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{2}) ratio of denitrification] was calculated as an important assessment indicator. The N\textsubscript{2} flux was generally higher than the N\textsubscript{2}O flux, thus resulting in low average values for the N\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{2}) ratio of denitrification. The N\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{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\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{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\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{2}) ratios of denitrification, but particularly BD samples from Gülzow and Ascha had considerably lower N\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{2}) ratios (0.23 to 0.29) than the respective MIN samples (0.63 to 0.81).

3.4 Controls on N\textsubscript{2}O and N\textsubscript{2} fluxes

N\textsubscript{2}O flux from MIN samples was significantly but weakly correlated with N\textsubscript{t} and NH\textsubscript{4} concentrations and (for relative N\textsubscript{2}O flux) with bulk density, while absolute and relative N\textsubscript{2}O flux from BD samples were strongly correlated with C\textsubscript{org}, N\textsubscript{t}, and NO\textsubscript{3} concentrations (Tab. 5). GLM analysis showed NH\textsubscript{4} concentrations, site, WFPS, bulk density, and their interactions to be the most important factors for N\textsubscript{2}O flux (Tab. 6), which reflects the observed significant differences in the N\textsubscript{2}O flux between soil cores from different field sites. Other significant factors influencing the N\textsubscript{2}O flux were application type (i.e., injection vs. drag hose) and fertilization treatment.

While no correlations between absolute or relative N\textsubscript{2} flux and the control parameters were observed for MIN samples, the N\textsubscript{2} flux from BD samples was significantly correlated with NH\textsubscript{4} concentrations, pH, C\textsubscript{org}, N\textsubscript{t}, and WFPS. Overall, the main factor complexes controlling the N\textsubscript{2} flux were (1) fertilization treatment and its interactions with NH\textsubscript{4} concentration, followed by (2) WFPS, application type, and their interaction, while the relative importance of site was considerably smaller than for N\textsubscript{2}O (Tab. 6).

Like for N\textsubscript{2}, the N\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{2}) ratio of denitrification from BD samples was significantly correlated with soil NH\textsubscript{4} concentration, pH, and WFPS (Tab. 5). GLM analysis revealed that the N\textsubscript{2}O/(N\textsubscript{2}O+N\textsubscript{2}) ratio of denitrification is mainly controlled by site, bulk density and NH\textsubscript{4} concentration (Tab. 6), and the interaction of NH\textsubscript{4} and WFPS, whereas the influence of treatment, WFPS, N\textsubscript{t} content and their interactions with soil parameters was of far lesser relevance than for N\textsubscript{2}O and N\textsubscript{2} fluxes.
tion and bulk density, WFPS, Nfert, soil NH3-N and NO3-N concentration. Absolute N2O and N2 fluxes shortly after fertilizer application with the present experimental set-up allowed studying short-term emissions in soil cores across five sites (Fig. 1). Moreover lower absolute N2O/(N2O+N2) ratios of denitrification were found in the BD soil cores across sites, except those from the Dedorow 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 N2O than soils treated with MIN fertilizer. The average value of N2O fluxes following BD application was strongly influenced by the sample from the Dornburg site (Fig. 1a), where BD samples exhibited a 27-fold higher N2O 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 digestate, slightly higher BD compared to MIN fertilization. Despite slightly different boundary conditions (different biogas digestate, slightly higher temperatures), the measured N2O fluxes were thus up to ten times lower than in our study (i.e., 139 ± 43 μg N m⁻² h⁻¹). Differences in N2O fluxes between the three related studies might be due to the well-known spatial and temporal variability of N2O, 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, N2 fluxes from the BD treat-

| Treatment | Variable | Bulk density (g cm⁻³) | WFPS (%) | pH_CaCl2 | Corg (%) | N2 (%) | Nfert (kg N ha⁻¹) | NH3-N (mg N 100 g⁻¹) | NO3-N (mg N 100 g⁻¹) |
|-----------|----------|----------------------|----------|----------|----------|--------|-------------------|----------------------|----------------------|
| Organic N (BD) | absolute N2O flux | −0.376 | −0.066 | 0.080 | 0.643*** | 0.497* | 0.243 |
| | absolute N2 flux | −0.061 | 0.423* | 0.643*** | 0.460* | 0.433* | 0.153 | 0.676*** | 0.000 |
| | relative N2O flux | −0.356 | −0.036 | 0.115 | 0.661*** | 0.521** | 0.220 |
| | relative N2 flux | −0.004 | 0.402* | 0.613*** | 0.456* | 0.394 | 0.100 | 0.649*** | −0.015 |
| | N2O/(N2O+N2) ratio | −0.264 | −0.556** | −0.661*** | −0.202 | −0.185 | 0.052 | −0.692*** | 0.362 |
| Mineral N (MIN) | absolute N2O flux | −0.343 | −0.332 | 0.381 | −0.102 | 0.416* | 0.261 | 0.456* | 0.364 |
| | absolute N2 flux | 0.085 | 0.212 | −0.157 | 0.185 | 0.169 | −0.235 | 0.047 | −0.086 |
| | relative N2O flux | −0.404* | −0.330 | 0.306 | −0.041 | 0.476* | 0.081 | 0.416* | 0.366 |
| | relative N2 flux | 0.044 | 0.194 | −0.147 | 0.208 | 0.199 | −0.291 | 0.059 | −0.057 |
| | N2O/(N2O+N2) ratio | −0.098 | −0.234 | 0.181 | −0.144 | −0.117 | 0.224 | −0.001 | 0.119 |

Correlations in bold are significant at: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 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 N2O and N2 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 N2O 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 N2, 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 N2O and N2 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 N2O and N2 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 N2O and N2 fluxes were significantly higher from BD than from MIN treatments in soil cores across five sites (Fig. 1). Moreover lower average N2O/(N2O+N2) ratios of denitrification were found in the BD soil cores across sites, except those from the Dedorow 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 N2O than soils treated with MIN fertilizer. The average value of N2O fluxes following BD application was strongly influenced by the sample from the Dornburg site (Fig. 1a), where BD samples exhibited a 27-fold higher N2O 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 digestate, slightly higher temperatures), the measured N2O fluxes were thus up to ten times lower than in our study (i.e., 139 ± 43 μg N m⁻² h⁻¹). Differences in N2O fluxes between the three related studies might be due to the well-known spatial and temporal variability of N2O, 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, N2 fluxes from the BD treat-

© 2020 The Authors. Journal of Plant Nutrition and Soil Science published by Wiley-VCH Verlag GmbH & Co. KGaA www.plant-soil.com
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. N2 fluxes were also higher from BD samples than MIN samples; hence, the calculated average N2O/(N2O+N2) ratio of denitrification was lower for BD compared to MIN samples, even though N2 fluxes were higher than N2O fluxes in both fertilizer treatments. Compared to another incubation study using BD samples with an N2 release of up to 13 mg N m⁻² h⁻¹ within five days of incubation (Köster et al., 2015), present mean N2 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 N2O reduction to N2 in the study by Köster et al. (2015). In the present case, N2 fluxes were up to ten-fold higher than N2O fluxes, which underlined the assumption that a substantial share of N2O formed by denitrification was further reduced to N2 [see also low N2O/(N2O+N2) ratios of denitrification], although NO₃ 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 (Nfert) on short-term N₂ and N₂O fluxes (Fig. 1). In particular, the Dornburg site exceeded the

| N₂ (mg N m⁻² h⁻¹) | N₂O (µg N m⁻² h⁻¹) | N₂O/(N₂O+N₂) ratio of denitrification |
|-------------------|---------------------|-------------------------------------|
| Wald Z            | p                   | Wald Z                              | p                             |
| Intercept         | 0.001               | 0.977                               | 6.355                         | 0.012*                          |
| Treatment (BD, MIN) | 6.131               | 0.013*                              | 15.259                        | ≤ 0.001*                        |
| Application type  | 6.359               | 0.012*                              | 23.680                        | ≤ 0.001*                        |
| Site              | 11.284              | 0.024*                              | 25.453                        | ≤ 0.001*                        |
| WFPS              | 16.355              | ≤ 0.001*                            | 14.231                        | ≤ 0.001*                        |
| Bulk density      | 5.174               | 0.023*                              | 5.480                         | 0.019*                          |
| Soil pH CaCl₂     | †                   | †                                   | †                             | †                               |
| Soil N_t          | †                   | 6.543                               | 0.011*                        | 3.702                           |
| Soil NH₄₋N        | 16.375              | ≤ 0.001*                            | 29.256                        | ≤ 0.001*                        |
| N_fert            | †                   | †                                   | †                             | †                               |
| Treatment × WFPS  | 2.866               | 0.090                               | †                             | †                               |
| Treatment × NH₄₋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 × NH₄₋N      | †                   | 29.224                              | ≤ 0.001*                      | 3.964                           |

| a Asterisks denote significant factors (α = 0.05); b Redundant parameter or parameter interaction. |
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 N₂O fluxes at this site (Fig. 1, Tab. 4). Some recent results have shown that N₂O and N₂ 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 NH₃-N. 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 N₂ fluxes were calculated, no site differences within the BD treatment were found (Tab. 4). Nevertheless, the much higher variability of the N₂ fluxes of the BD treatment compared to N₂ fluxes of the MIN is an indication that the quantity and composition of the BD may have a strong impact on the N₂ fluxes (i.e., the share of N₂O reduction to N₂).

Higher N₂O 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 O₂, which in turn may enhance N losses due to denitrification, in particular also the reduction of N₂O to N₂ (Petersen and Sommer, 2011; Köster et al., 2015; Heintze et al., 2017). Moreover, it can be assumed that the increased amount of NH₃-N due to BD application, which was also found here, probably enhanced the process of nitrifier-denitrification, i.e., the oxidation of NH₃ to NO₂ and its subsequent reduction to NO and N₂O by autotrophic ammonia oxidizing microorganisms. Since nitrifier-denitrification particularly occurs in soils with high N availability but low Corg, BD application has been found to stimulate this process under low O₂ availability (Eickenscheidt et al., 2014). However, a multitude of additional sources of N₂O production has to be considered, due to the known wealth of N₂O production processes and their simultaneous occurrence in soils (Butterbach-Bahl et al., 2013).

In addition to the discussed N₂O, NO, and N₂ losses, a significant N loss via ammonia (NH₃-N) 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 NH₃-N is probably volatilized as NH₃ within the first 10 h after surface application of BD (Quakernack et al., 2012), which might have also affected the share of N₂O and N₂ 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 N₂O 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 N₂O fluxes more than the N₂ 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 N₂, N₂O release as well as the N₂O/(N₂O+N₂) 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, N₂O/(N₂O+N₂) ratios of denitrification < 0.4 were found for the Dedelow samples, which indicates a great contribution of N₂O reduction to N₂. 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, N₂ fluxes ranged on a moderate level (460 ± 224 x 10⁻³ 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 O₂ 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 NO₃-N and NH₃-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 $N_2$ and $N_2O$ fluxes, the low $N_2O/(N_2+N_2)$ ratios of denitrification highlighted the importance of $N_2O$ reduction to $N_2$, in particular for the biogas digestate treatment 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 Weißenstephan-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 Heinitz (HSWT)] and gas measurements [Bertram Gusovius, ZALF]. We would also like to acknowledge the anonymous reviewers for their constructive comments.

References

Arah, J. R. M. (1992): New formulae for mass spectrometric analysis of nitrous oxide and dinitrogen emissions. Soil Sci. Soc. Am. J. 56, 795–800.

Ball, B. C. (2013): Soil structure and greenhouse gas emissions: a synthesis of 20 years of experimentation. Eur. J. Soil Sci. 64, 367–373.

Boast, C. W., Mulvaney, R. L., Baveye, P. (1988): Evaluation of nitrogen-15 tracer techniques for direct measurement of denitrification in soil: I. Theory. Soil Sci. Soc. Am. J. 52, 1317–1322.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Ries, R., Zechmeister-Boltenstern, S. (2013): Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Phil. Trans. R. Soc. B Biol. Sci. 368. DOI: https://doi.org/10.1098/rstb.2013.0122.

Buchent-Tschiskale, Hagemann, Augustin J. Plant Nutr. Soil Sci. 2020, 000, 1–12
Short-term N$_2$O and N$_2$ fluxes from biogas digestate

Livingston, G. P., Hutchinson, G. L. (1995): Enclosure-Based Measurement of Trace Gas Exchange: Applications and Sources of Error, in Matson, P., Harris, R. (eds.): Biogenic Trace Gases: Measuring Emissions from Soil and Water. Blackwell Science Inc., Oxford, UK, pp. 14–51.

Möller, K. (2015): Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review. Agron. Sustain. Dev. 35, 1021–1041.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12, 242–257.

Möller, K., Stinner, W. (2009): Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides). Eur. J. Agron. 30, 1–16.

Ni, K., Köster, J. R., Seidel, A., Pacholski, A. (2015): Field measurement of ammonia emissions after nitrogen fertilization—A comparison between micrometeorological and chamber methods. Eur. J. Agron. 71, 115–122.

Petersen, S. O., Sommer, S. G. (2011): Ammonia and nitrous oxide interactions: Roles of manure organic matter management. Anim. Feed Sci. Technol. 166, 503–513.

Pietzner, B., Ganski, B., Klobenz, B., Bednorz, D., Tauchnitz, N., Bischoff, J., Köbbke, S., Meißner, R., Christen, O. (2017): Impact of slurry strip-till and surface slurry incorporation on NH$_4$ and N$_2$O emissions on different plot trials in Central Germany. Soil Till. Res. 169, 54–64.

Quakermack, R., Pacholski, A., Techo, A., Herrmann, A., Taube, F., Kage, H. (2012): Ammonia volatilization and yield response of energy crops after fertilization with biogas residues in a coastal marsh of Northern Germany. Agric. Ecosyst. Environ. 160, 66–74.

Ravishankara, A. R., Daniel, J. S., Portmann, R. W. (2009): Nitrous oxide (N$_2$O): the dominant ozone-depleting substance emitted in the 21st century. Science 326, 123–125.

Saggar, S., Jha, N., Deslippe, J., Bolan, N. S., Luo, J., Giltrap, D. L., Kim, D.-G., Zaman, M., Tillman, R. W. (2013): Denitrification and N$_2$O:N$_2$ production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. Sci. Total Environ. 465, 173–195.

Sänger, A., Geisseler, D., Ludwig, B. (2014): C and N dynamics of a range of biogas slurries as a function of application rate and soil texture: a laboratory experiment. Arch. Agron. Soil Sci. 60, 1779–1794.

Seidel, A., Pacholski, A., Nyord, T., Vestergaard, A., Paalman, I., Herrmann, A., Kage, H. (2017): Effects of acidification and injection of pasture applied cattle slurry on ammonia losses, N$_2$O emissions and crop N uptake. Agric. Ecosyst. Environ. 247, 23–52.

Senbayram, M., Chen, R., Budai, A., Bakken, L., Dittert, K. (2012): N$_2$O emission and the N$_2$O/(N$_2$O:N$_2$) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agric. Ecosyst. Environ. 147, 4–12.

Senbayram, M., Chen, R., Mühlung, K.-H., Dittert, K. (2009): Contribution of nitrification and denitrification to nitrous oxide emissions from soils after application of biogas waste and other fertilizers. Rapid Commun. Mass Spectrom. 23, 2489–2498.

Senbayram, M., Chen, R., Wienforth, B., Herrmann, A., Kage, H., Mühlung, K.-H., Dittert, K. (2014): Emission of N$_2$O from Biogas Crop Production Systems in Northern Germany. BioEnergy Res. 7, 1223–1236.

Svoboda, N., Taube, F., Wienforth, B., Klüß, C., Kage, H., Herrmann, A. (2013): Nitrogen leaching losses after biogas residue application to maize. Soil Till. Res. 130, 69–80.
Tiedje, J. M., Sexstone, A. J., Parkin, T. B., Revsbech, N. P. (1984): Anaerobic processes in soil. Plant Soil 76, 197–212.

Van Groenigen, J., Huygens, D., Boeckx, P., Kuyper, T. W., Lubbers, I. M., Rütting, T. P., Groffman, P. M. (2015): The soil N cycle: new insights and key challenges. Soil 1, 235–256.

VDLUFA (2002): Methodenbuch I. Die Untersuchung von Böden. VDLUFA-Verlag, Darmstadt, Germany.

Webb, J., Pain, B., Bittman, S., Morgan, J. (2010): The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—a review. Agric. Ecosyst. Environ. 137, 39–46.

Weier, K. L., Doran, J. W., Power, J. F., Walters, D. T. (1993): Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. Soil Sci. Soc. Am. J. 57, 66–72.

Wolf, U., Fuß, R., Höppner, F., Flessa, H. (2014): Contribution of N₂O and NH₃ to total greenhouse gas emission from fertilization: results from a sandy soil fertilized with nitrate and biogas digestate with and without nitrification inhibitor. Nutr. Cycl. Agroecosys. 100, 121–134.