SOIL, 3, 161–176, 2017
https://doi.org/10.5194/soil-3-161-2017

SOIL Open Access EGU

Potential short-term losses of N_2O and N_2 from high concentrations of biogas digestate in arable soils

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Received: 9 February 2017 – Discussion started: 28 February 2017
Revised: 14 August 2017 – Accepted: 27 August 2017 – Published: 28 September 2017

Abstract. Biogas digestate (BD) is increasingly used as organic fertilizer, but has a high potential for NH_3 losses. Its proposed injection into soils as a countermeasure has been suggested to promote the generation of N_2O, leading to a potential trade-off. Furthermore, the effect of high nutrient concentrations on N_2 losses as they may appear after injection of BD into soil has not yet been evaluated. Hence, we performed an incubation experiment with soil cores in a helium–oxygen atmosphere to examine the influence of soil substrate (loamy sand, clayey silt), water-filled pore space (WFPS; 35, 55, 75 %) and application rate (0, 17.6 and 35.2 mL BD per soil core, $250\,cm^3$) on the emission of N_2O, N_2 and CO_2 after the usage of high loads of BD. To determine the potential capacity for gaseous losses, we applied anaerobic conditions by purging with helium for the last 24 h of incubation. Immediate N_2O and N_2 emissions as well as the $N_2 / (N_2O+N_2)$ product ratio depended on soil type and increased with WFPS, indicating a crucial role of soil gas diffusivity for the formation and emission of nitrogenous gases in agricultural soils. However, emissions did not increase with the application rate of BD. This is probably due to an inhibitory effect of the high NH_4^+ content of BD on nitrification. Our results suggest a larger potential for N_2O formation immediately following BD injection in the fine-textured clayey silt compared to the coarse loamy sand. By contrast, the loamy sand showed a higher potential for N_2 production under anaerobic conditions. Our results suggest that short-term N losses of N_2O and N_2 after injection may be higher than probable losses of NH_3 following surface application of BD.

1 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (Myhre et al., 2013), with agriculture being its single largest anthropogenic source, contributing about $4.1\,Tg\,N_2O\text{-}N\,yr^{-1}$ or 66 % of total gross anthropogenic emissions, mainly as a result of mineral nitrogen (N) fertilizer and manure application (Davidson and Kanter, 2014). The generation of nitrogen gas (N_2) is of agronomic interest in terms of nutrient management since such gaseous losses may imply a significant loss of N from the soil–plant system (Cameron et al., 2013; Friedl et al., 2016). However, from an environmental stance, N_2 is innocuous and, thus, the preferred type of gaseous N loss from soil (Davidson et al., 2015). In general, the improvement of N use efficiency and thus the decrease in N losses in crop production are paramount in the presence of challenges like food security, environmental degradation and climate change (Zhang et al., 2015).

Digestion residues (biogas digestate, BD) from biogas plants are used as organic amendment in agriculture. However, compared to undigested amendments, digestion results in an increased pH, a higher proportion of ammonium (NH_4^+) and a narrowed C / N ratio of BD (Möller and Müller, 2012). These altered chemical properties may promote biochemical reactions in the soil that are responsible for the formation of gaseous N species like N_2O, nitric oxide (NO), N_2 and ammonia (NH_3) (Nkoa, 2013).

Significant losses of N as NH_3 may occur within the first hours after manure application (Quakernack et al., 2012). To reduce NH_3 losses, the application of BD by injection is recommended, but this measure can simultaneously increase the potential for N_2O losses compared to surface application (Velthof and Mosquera, 2011; Wulf et al., 2002). On the one hand, high NH_4^+ concentrations in the injection band promote nitrification, consuming O_2 and releasing N_2O (Christensen and Rowe, 1984). On the other hand, increased amounts of C in the injection band also promote respiration, additionally depleting O_2 supply (Dell et al., 2011). Altogether, the conditions during the initial phase after injection of BD foster microsites favourable for microbial denitrification, which also promote the formation of N_2 due to anaerobic conditions (Köster et al., 2015; Webb et al., 2010).

There is a wealth of biotic and abiotic processes in soils that produce N_2O and N_2, depending on mineral N content, C availability and temperature, most of which are enhanced by anoxic or at least suboxic conditions (Butterbach-Bahl et al., 2013). The amounts and the relative share of N_2 and N_2O in the overall gaseous N emissions depend – among other factors – on the degree of O_2 restriction (Firestone and Davidson, 1989). Soil physical and biotic factors (i.e. diffusion permitted by soil porosity in conjunction with water-filled pore space (WFPS) as well as consumption of O_2 by heterotrophic respiration and nitrification) control the aerobic status of a soil (Ball, 2013; Maag and Vinther, 1999; Uchida et al., 2008). In general, clayey soils exhibit a lower gas diffusivity compared to coarse-textured soils. This regularly results in higher denitrification in the former with higher N_2O emission rates, but also a higher probability for the consecutive reduction to N_2 (Ball, 2013; Gu et al., 2013; Senbayram et al., 2014).

There is a general lack of knowledge about the effects of high BD concentration on gaseous N losses as they might appear after injection into soils and their interactions with O_2 limiting factors like soil texture and WFPS, as well as temperature and heterotrophic respiration. Thus, we applied the helium–oxygen (He–O_2) incubation technique (Butterbach-Bahl et al., 2002) in a laboratory experiment to evaluate the effect of the factors suggested above on the emission of N_2O and N_2 from different soils. Simultaneously, CO_2 flux was determined as an indicator for microbial O_2 consumption, O_2 diffusion and also for the degradability of organic C applied with BD (Blagodatsky and Smith, 2012). We hypothesized that (1) N_2O and N_2 emissions will increase with WFPS, (2) gaseous N losses will also be affected by BD application rate, i.e. the hypothetical concentration of C and N resulting from injection, and (3) the clayey silt will induce higher gaseous N losses than the coarse loamy sand.

Table 1. The examined factors soil texture, water-filled pore space (WFPS) and amount (i.e. concentration) of nitrogen (N) applied with biogas digestate (BD) with their respective levels applied in the present study, resulting in 18 treatments with three replicates each. The temperature was manipulated consecutively during the incubation.

Factor (*n*)	Levels		
Soil texture (2)	loamy sand	clayey silt	
WFPS (%) (3)	35	55	75
BD-N (kg ha^{-1}) (3)	0	160	320
Temperature (°C) (2)	2	15	

2 Material and methods

2.1 Selected soils, sampling of soil cores and biogas digestate

Two soils were selected and both were adjusted to three levels of WFPS and three quantities of BD (Table 1), resulting in 18 factor combinations with three replicates. Temperature was increased from 2 °C during the first 2 days to 15 °C for the last 3 days of the incubation. Intact soil cores (diameter 7.2 cm, height 6.1 cm, volume 250 cm^3) were taken with sample rings in the range from 0 to 0.10 m depth from two sites with different textures, i.e. sandy loam and clayey silt. The sandy loam samples were collected from a Stagnic Luvisol (IUSS Working Group WRB, 2006) located in Gülzow (northeast Germany) in the ground moraine of the Weichselian glacial period at 53°48′35″ N and 12°4′20″ E. The clayey silt samples were collected from a Haplic Luvisol located in Dornburg between the foothills and the lowlands of central Germany at 51°0′8″ N and 11°39′25″ E (see Table 2 for more details on soil characteristics). After field sampling, the soil cores were dried for 48 h at 40 °C to facilitate adjustment of WFPS.

Both sites have been cultivated with similar crop rotations used as feedstock for biogas production and have been amended with BD for the past 9 years. The crop rotation on the sandy loam consisted of maize (*Zea mays* L.), rye (*Secale cereale* L.), sorghum (*Sorghum bicolor* (L.) Moench), winter triticale (× *Triticosecale* Wittmack), ryegrass (*Lolium perenne* L.) and winter wheat (*Triticum aestivum* L.). The only difference in the crop rotation on the clayey silt was the cultivation of sudan grass (*Sorghum* × *drummondii*) instead of sorghum.

The BD used for the incubation was obtained from a biogas plant at "Gut Dalwitz", an organic farm in northeast Germany. The feedstock for the anaerobic fermentation in the plant consisted of 60 % maize, 20 % solid cattle manure, 10 % dry chicken manure and 10 % rye. The digestate was analysed by LUFA Rostock, Germany, and had a pH of 8.3, 2.91 % organic C, 0.16 % dissolved organic C (DOC), 0.54 %

Table 2. Characteristics of both soils. Texture and mean values with standard deviations (in parentheses) for carbon (C, $n = 9$), nitrogen (N, $n = 9$), pH ($n = 3$), bulk density (BD, $n = 3$) and mineral N (NO_3^- and NH_4^+, $n = 3$) of both soils in 0–10 cm depth after field sampling.

Texture	C ($mg\,g^{-1}$)[a]	N ($mg\,g^{-1}$)[a]	pH[b]	Bulk density ($g\,cm^{-3}$)[c]	NO_3^- ($mg\,kg^{-1}$)[d]	NH_4^+ ($mg\,kg^{-1}$)[d]
Loamy sand	6.99 (0.29)	0.67 (0.05)	7.2 (0.1)	1.4 (0.0)	1.0 (0.2)	0.6 (0.3)
Clayey silt	10.77 (0.28)	1.19 (0.06)	7.2 (0.0)	1.5 (0.0)	1.8 (0.2)	0.3 (0.2)

[a] Measured with the analyser TruSpec CNS, Leco Instruments GmbH, Germany; performed according to ISO 10694 (elemental analysis) for C and according to ISO 13878 (elemental analysis) for N. [b] Measured in H_2O with TitraMaster 85, Radiometer Analytical SAS, France; performed according to *VDLUFA Methodenbuch*, Vol. 1, chap. 5.1.1. [c] Measured on 250 cm^3 soil cores. [d] Measured with the analyser CFA-SAN, Skalar Analytical B.V., the Netherlands; performed according to ISO 14256.

Table 3. Chronological order of the incubated factor combinations. Two different factor combinations with their respective repetitions ($n = 3$) were placed together for each weekly incubation course (see Fig. 1). The factors were combined by (1) soil (loamy sand: LS, clayey silt: CS), (2) amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and (3) WFPS (%).

Week	Factor combination 1	Factor combination 2
1	LS – No BD – 35 %	LS – No BD – 55 %
2	LS – No BD – 75 %	LS – Low BD – 35 %
3	LS – Low BD – 55 %	LS – Low BD – 75 %
4	LS – High BD – 35 %	LS – High BD – 55 %
5	LS – High BD – 75 %	CS – No BD – 35 %
6	CS – No BD – 55 %	CS – No BD – 75 %
7	CS – Low BD – 35 %	CS – Low BD – 55 %
8	CS – Low BD – 75 %	CS – High BD – 35 %
9	CS – High BD – 55 %	CS – High BD – 75 %

N and 0.27 % NH_4-N in undried material with a dry matter content of 9.4 %.

2.2 Adjustment of WFPS and addition of N

For adjustment of WFPS, the dry and undisturbed soil cores were moistened dropwise. The respective quantities of water were calculated based on the bulk density and an assumed particle density of 2.65 $g\,cm^{-1}$ and they were reduced by the expected moisture input from subsequent addition of BD. The soil cores were then mixed with BD and finally repacked to reach nutrient concentrations comparable to those in injection bands. The amounts of added BD were calculated with an assumed injection of 160 $kg\,N\,ha^{-1}$ into the soil, with row spaces of 0.15 m (narrow injection bands with low BD concentration, LOBD) and 0.30 m (wide injection bands with high BD concentration, HIBD). These are common ranges used for injection machinery and correspond to 17.6 and 25.3 mL BD per sample ring. After this procedure, the soil cores were sealed with plastic lids and stored immediately at 2 °C until the beginning of the incubation within a week.

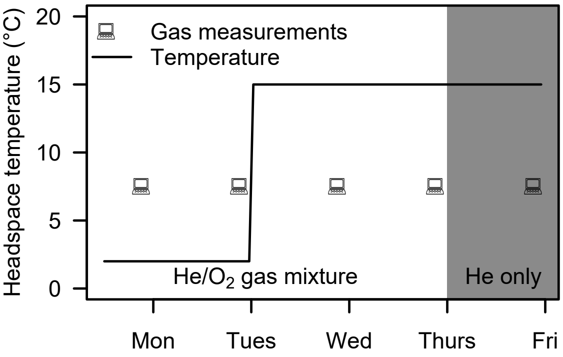

Figure 1. Course of incubation and gas measurements with respect to atmosphere and temperature of the headspace after 2 days of pre-incubation at 2 °C in the He / O_2 gas mixture. Gas concentrations of the headspace were determined on 5 consecutive days, i.e. Monday to Friday in the morning. After the first 2 measurement days, the headspace temperature was increased from 2 to 15 °C. Additionally, after the fourth measurement day, the aerobic helium / oxygen gas mixture in the headspace was replaced by a pure helium atmosphere.

2.3 Determination of gas fluxes

The measurements of N_2, N_2O and CO_2 fluxes were applied following the He–O_2 method (Butterbach-Bahl et al., 2002; Scholefield et al., 1997). Six soil cores (i.e. the repetitions of two factor combinations at a time, Table 3) were placed simultaneously in special gas-tight incubation vessels inside a climate chamber. Analyses were conducted in the laboratory of the Institute for Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany. Before flux measurements, the vessels were evacuated to 0.047 bar and flushed with an artificial He / O_2 gas mixture (20.49 % O_2, 345.5 ppm CO_2, 359 ppb N_2O, 1863 ppb CH_4, 2.46 ppm N_2, rest He) four times consecutively to remove ambient N_2. Subsequently, the air temperature of the climate chamber was set to 2 °C and a continuous He / O_2 gas flow rate of 15 $mL\,min^{-1}$ was applied to the vessel headspace for 72 h to remove residues of N_2 from soil cores by diffusion, including a restricted N_2 production by decreased microbial activity. After this pre-incubation, on the following 2 days the headspace concentration of N_2O and

CO_2 was measured once daily in the morning. To compensate for the lower precision of the detector for N_2 in relation to the detector for N_2O and CO_2 (Eickenscheidt et al., 2014), N_2 concentrations were measured consecutively three times daily in the morning. Immediately after the last measurement on the second day, the temperature was set to 15 °C and the measurements were continued for another 2 days. Finally, the He / O_2 gas mixture was substituted by pure He and, following 24 h of acclimatization, gas measurements were carried out once again (Fig. 1) to determine the generation of N_2O and N_2 in a completely anaerobic soil matrix. The latter step is important to get a clue about the actual potential for gaseous N losses after highly concentrated BD application. The settings of the chromatographs for gas analyses are described in Eickenscheidt et al. (2014). Gas fluxes were calculated according to Eq. (1):

$$f = \frac{M \times p \times v \times dc}{R \times T \times A}, \tag{1}$$

where f is the flux (N_2 and CO_2: $\mathrm{mg\,m^{-2}\,h^{-1}}$, N_2O: $\mathrm{\mu g\,m^{-2}\,h^{-1}}$), M the molar mass in $\mathrm{g\,mol^{-1}}$ (N_2: 28, CO_2: 44, N_2O: 44), p the air pressure (Pa), v the air flow ($\mathrm{L\,h^{-1}}$), R the gas constant ($8.31\ \mathrm{J\,mol^{-1}\,K^{-1}}$), T the temperature inside the chamber (K), A the area of the incubation vessel (m^2) and dc the difference of gas concentrations (N_2 and CO_2: ppm, N_2O: ppb) between inlet and outlet of a vessel.

To enhance the tightness against atmospheric N_2 contamination, the lids of the incubation vessels were permanently purged with helium. We obtained blank values by inserting aluminium blocks into the vessels before each measurement cycle. Since these blank values were usually steady with means of 1.9 ($1\sigma = 0.9$) ppm N_2, 349.6 ($1\sigma = 11.4$) ppb N_2O and 353.9 ($1\sigma = 13.5$) ppm CO_2, we suggest that the vessels were tight. Derived from the blank values, the lowest detectable fluxes were on average 0.427 ($1\sigma = 0.271$) $\mathrm{mg\,N_2\text{-}N\,m^{-2}\,h^{-1}}$, 3.6 (3.1) $\mathrm{\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}}$ and 0.918 (0.693) $\mathrm{mg\,CO_2\text{-}C\,m^{-2}\,h^{-1}}$. For flux estimation, the blank values were subtracted from the values measured at the respective outlet. Estimated fluxes from the soil cores smaller than the respective blank fluxes of each day were set to zero.

2.4 Soil analyses after incubation

After incubation, the soil cores were stored at 2 °C until they were extracted with 0.1 M KCl solution (the soil-to-extract ratio was 1 : 4; standardized extraction method of the commissioned laboratory at Leibniz Centre for Agricultural Landscape Research e.V.) and analysed for NH_4^+ and nitrate (NO_3^-) using spectrophotometry according to DIN ISO 14256 with the continuous flow analyser CFA-SAN, Skalar Analytical B.V., the Netherlands, and for DOC by combustion according to DIN ISO 10694 with the analyser RC 612, Leco Instruments GmbH, Germany.

2.5 Statistical analysis

All statistical analyses were done using R statistical software version 3.2.3 (R Core Team, 2016) with the data of the measuring days under a He–O_2 atmosphere. Data from the vessels with the factor combination of 35 % WFPS and LOBD with clayey silt were omitted due to technical reasons during sample preparation. For the final period of pure He headspace, some gas concentration data could not be documented. For loamy sand, this affects all WFPS levels with LOBD (N_2 and N_2O), the treatment 75 % WFPS with 320 kg N h^{-1} (N_2O and CO_2) and for the clayey silt the treatment 35 % WFPS without amendment (N_2O and CO_2).

To account for repeated measurement of vessels, linear mixed effect models were applied with the R package "lmerTest" version 2.0-33 (Kuznetsova et al., 2016) for fluxes of each gas type. The three pseudo-replicated fluxes from the N_2 measurements of each vessel were averaged for each day to obtain the same number of observations as for N_2O and CO_2 fluxes. The fixed structure of models included soil type, WFPS, amount of digestate, temperature, NO_3^- and DOC contents after incubation as well as the fluxes of N_2O (in the model for N_2) and CO_2 (in the models for N_2, N_2O and N_2 / [N_2+N_2O] product ratio). Soil NH_4^+ was omitted since it showed high autocorrelation with the amount of BD applied. The individual soil cores in the vessels were set as random effect (nested within the week of incubation and with allowance for a variable slope of the effect each day) with regard to lack of independence of consecutive measurements. The model responses for N_2, N_2O and CO_2 were log transformed (ln[value + 1]) since gas fluxes from soils usually show lognormal distributions (Kaiser et al., 1998). The function "step" was used for automatic backward selection of models based on AIC (Akaike's information criterion). The skewness (γ) was calculated with the R package "moments" version 0.14 (Komsta and Novomestky, 2015) to check residuals for normal distribution and $|\gamma| \leq 2$ was assumed as appropriate (West et al., 1995). For mixed-effect models, p values of the ANOVA (type 2) were calculated based on Satterthwaite's approximation.

Cumulated gas fluxes were estimated with a bootstrap method using function "auc.mc" of the R package "flux" version 0.3-0 (Jurasinski et al., 2014). In short, the fluxes for the period of aerobic headspace were cumulated in 100 iterations, while for each run two fluxes were omitted randomly. Then, the resulting data were used to calculate means and standard deviations.

3 Results

3.1 Soil NH_4^+, NO_3^- and DOC contents

The calculated application of NH_4^+-N from BD per kilogram of soil approximated for the sandy loam was 135.8 mg (LOBD) and 271.5 mg (HIBD), and for the clayey silt it was

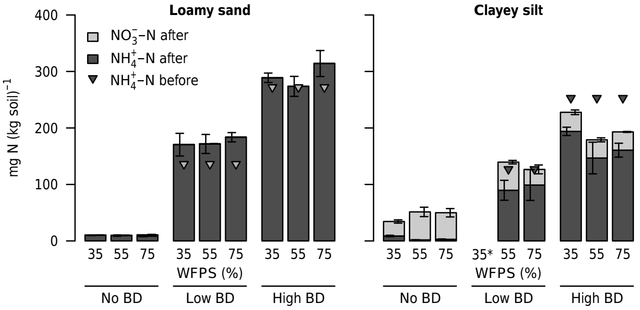

Figure 2. Ammonium and nitrate contents from loamy sand and clayey silt after incubation with different water-filled pore spaces (WFPSs, %) and amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). Error bars denote standard deviations. In general, the ammonium content increased with digestate application, with lower amounts detected in the clayey silt. Nitrate was found almost exclusively in the latter soil. For comparison, inverted triangles show calculated amounts of applied ammonium, which may differ from actual rates due to heterogeneity of biogas digestate. One treatment (*) was omitted from all analyses due to technical reasons.

126.7 mg (LOBD) and 253.4 mg (HIBD). The NO_3^- content of BD was negligible. In general, the NH_4^+ content of the soils after incubation increased with digestate application, with lower amounts detected in the clayey silt. Nitrate was found almost exclusively in the latter soil (Fig. 2).

The amounts of measured DOC increased with the application rate of BD, but with higher magnitudes for the loamy sand than for the clayey silt (Table 4).

3.2 CO_2 fluxes

CO_2 fluxes showed clear differences between the soils: under all combinations of temperature and oxygen, the fluxes were always larger from loamy sand compared to clayey silt (Table A1). In general, mean fluxes from loamy sand increased with the amount of digestate during each of the different periods regarding temperature and headspace aerobicity, but showed no obvious pattern with WFPS. There was no clear trend of fluxes with the amount of amendment, but a slight trend of decreasing fluxes with increasing WFPS could be seen for the clayey silt. However, the predictive power of WFPS on CO_2-C fluxes was minor since it was eliminated during stepwise regression fitting. By contrast, soil type, amount of digestate, temperature and the DOC content after the incubation had significant ($p < 0.01$) effects (Table 5).

3.3 N_2O fluxes

The mean N_2O fluxes from the loamy sand in the He–O_2 headspace were virtually zero, independent of temperature and WFPS as well as the amount of BD application (Fig. 3, Table A2). In contrast, the emissions of the clayey silt increased with temperature and were highest at 15 °C with intermediate WFPS and amount of BD, i.e. 6.2 mg N_2O-N m^{-2} h^{-1} at 55 % with LOBD, respectively. Surprisingly, at 15 °C, increasing the amount of BD up to HIBD did not increase the observed N_2O efflux; rather, it decreased the efflux significantly ($p < 0.05$, Tukey's HSD) at 55 % and also, but not significantly, at 75 % WFPS (Fig. 3, Table A2). According to the linear mixed model for N_2O fluxes in aerobic conditions, WFPS, amount of digestate, temperature, DOC content of soil after incubation and CO_2 fluxes had significant ($p < 0.001$) effects on N_2O flux (Table 5).

Under anaerobic headspace conditions, the overall highest mean N_2O flux was observed from the clayey silt at 35 % WFPS with HIBD (11.7 mg N_2O-N m^{-2} h^{-1}). The same soil showed a tendency of decreasing N_2O fluxes with increasing WFPS and amendment. In the loamy sand, the pure He atmosphere induced increasing mean N_2O fluxes (up to 1.3 mg N_2O-N m^{-2} h^{-1}) with increasing WFPS (Fig. 3, Table A2). Thus, the anaerobic headspace only induced a change in the loamy sand by increasing emissions.

3.4 N_2 fluxes

From the loamy sand, no or only small rates of N_2 were detected at both temperatures under a He–O_2 atmosphere (Fig. 4, Table A3). The clayey silt showed mean fluxes of up to 1.4 mg N_2 m^{-2} h^{-1} at 2 °C (all incubations with 75 % WFPS) and up to 3.8 mg N_2 m^{-2} h^{-1} at 15 °C (75 % WFPS with LOBD), but no fluxes in all BD treatments with 35 % WFPS. Put simply, temperature had a small effect on N_2 emissions from the sandy loam, with no consistent influence of WFPS and the amount of BD. In contrast, the clayey silt emitted increasing fluxes with increasing temperature and WFPS. However, the application rise from LOBD up to HIBD at 15 °C resulted in slightly, but not significantly ($p >$ 0.05, Tukey's HSD) decreased fluxes (Fig. 4, Table A3). The summary of the linear mixed model for N_2 fluxes under aerobic conditions revealed significant effects ($p < 0.05$) of soil type, WFPS, the amount of digestate, temperature, DOC content after incubation and N_2O flux (Table 5).

After switching the atmosphere to pure He, the N_2 fluxes from the sandy loam increased more than 60-fold. In contrast to aerobic conditions, all measured factor combinations showed mean fluxes of up to 35.1 mg N_2 m^{-2} h^{-1} (55 % with 320 kg N ha^{-1}) (Fig. 2, day 5 in Table A3). Mean fluxes from clayey silt increased only up to 9.3 mg N_2 m^{-2} h^{-1} in amended treatments. Thus, the loamy sand exhibited a much more intense reaction under anaerobic headspace conditions.

3.5 N_2 / (N_2+ N_2O-N) product ratio

No clear trend of the product ratio of N_2 / (N_2+ N_2O-N) was found for incubations of the loamy sand. However, there was a clear distinction between the ratios for this soil under aero-

Table 4. Mean DOC values from soils, measured after incubation, with standard deviations in parentheses for the respective treatments differing in amount of applied biogas digestate (BD) and water-filled pore space (WFPS).

kg digestate-N ha^{-1}	WFPS (%)	mg DOC (kg soil)$^{-1}$	
		Loamy sand	Clayey silt
	35	41.4 (2.7)	18.9 (1.1)
0	55	38.6 (3.1)	19.8 (1.4)
	75	43.7 (1.4)	19.0 (1.8)
	35	197.4 (20.7)	NA
160	55	190.5 (19.3)	68.3 (12.7)
	75	362.2 (40.0)	63.2 (9.6)
	35	316.8 (25.3)	358.1 (26.3)
320	55	312.5 (14.3)	94.8 (13.6)
	75	500.1 (33.4)	105.9 (14.8)

NA: data not available.

Table 5. ANOVA table (type 2, p values calculated based on Satterthwaite's approximation) of the linear mixed-effect models for estimated fluxes of N_2, N_2O, N_2 / (N_2+N_2O) product ratio and CO_2 in an aerobic He–O_2 atmosphere. Soil type, water-filled pore space (WFPS), amount of digestate, temperature, NO_3^- and DOC content of soil after incubation, and fluxes of N_2O and CO_2 were set as possible independent variables. The individual soil rings were set as the random effect (nested within the respective week and with the allowance for varying slopes for each day of measurements). The random effect was always significant.

Response	Fixed effects							
	Soil type	WFPS	Digestate amount	Temperature	NO_3^- post	DOC post	N_2O flux	CO_2 flux
N_2	0.026	< 0.001	0.008	0.037	[a]	0.001	< 0.001	[a]
N_2O	[a]	< 0.001	< 0.001	< 0.001	[a]	< 0.001	[b]	< 0.001
N_2 / (N_2+N_2O)	0.005	0.004	[a]	[a]	[a]	[a]	[b]	[a]
CO_2	< 0.001	[a]	< 0.001	< 0.001	[a]	0.007	[a]	[b]

[a] Variable eliminated during stepwise regression selection. [b] Variable was not included in the original regression.

bic and anaerobic atmospheres: while the ratios were close to zero in the former, they were close to 1 in the latter (Fig. 5). In contrast, in the clayey silt the ratios increased with WFPS and were affected by digestate amendment under both the aerobic and the anaerobic atmospheres, in which the highest ratios (up to 0.8) were found in treatments without digestate and at least 55 % WFPS. The digestate-amended treatments showed ratios around or above 0.5, with the exception of the 35 % WFPS treatments, which had ratios close to zero. According to the linear mixed model, the product ratio under aerobic conditions was affected significantly ($p < 0.01$) by soil type and the amount of digestate (Table 5).

4 Discussion

4.1 Increased BD application rate did not increase N_2O and N_2 losses, probably due to inhibitory effect of high NH_4^+ concentrations

In the loamy sand, the higher NH_4^+ content measured after the incubation cycle compared to the calculated NH_4^+ application rates may result from heterogeneity of BD itself (Andruschkewitsch et al., 2013). By contrast, the considerable lower values after incubation in the clayey silt could be attributed to a higher fixation of NH_4^+ as NH_3 by clay minerals, enhanced by the increased pH of BD (Kissel et al., 2008).

The overall N_2O fluxes corresponded well with those from other studies with similar incubation conditions and application rates of BD in terms of nitrogen per hectare (Köster et al., 2015; Senbayram et al., 2012; Severin et al., 2015). However, the latter studies assumed a distribution of BD into soil using a cultivator, which implies a smaller concentration of BD than we actually applied. Although we observed differences in N_2O emissions between soils, soil type was not confirmed as a significant effect. Nevertheless, WFPS and temperature, which are well-known controls of N_2O generation (Maag and Vinther, 1999), showed significant influences. Both are physical (by gas diffusion) and biological (by increased metabolic activity and consequently increased O_2 consumption by respiration) drivers for O_2 availability (Ball, 2013; Maag and Vinther, 1999). Accordingly, CO_2 flux (resulting from respiration of O_2) generally increased with tem-

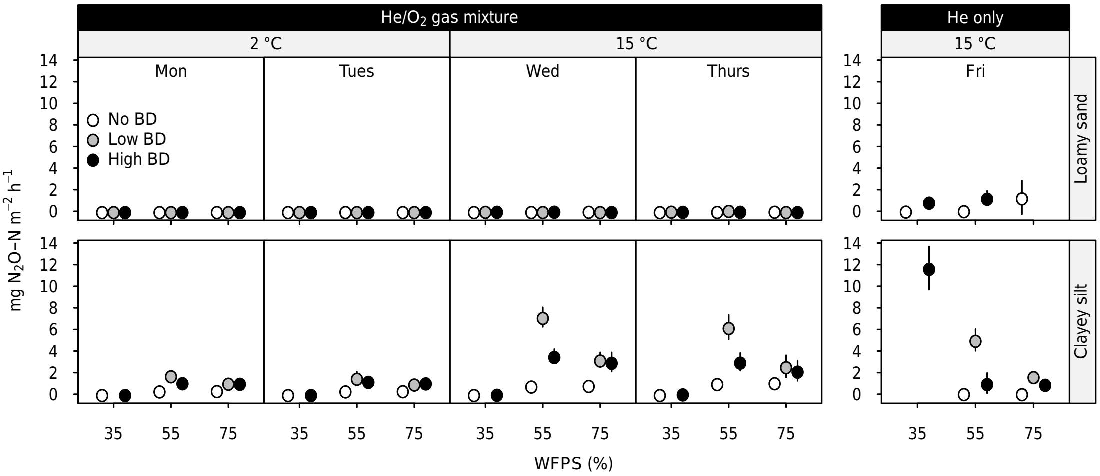

Figure 3. Mean N_2O fluxes ($mg\,N\,m^{-2}\,h^{-1}$) from loamy sand and clayey silt incubated under different water-filled pore spaces (WFPSs, %) with different amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). The first to the fourth days of the incubation were measured in an aerobic He–O_2 headspace (with 2 days at 2 °C followed by another 2 days at 15 °C) while on the fifth day measurements were conducted in an anaerobic headspace with pure He (at 15 °C). Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. Under an aerobic atmosphere, N_2O fluxes from loamy sand were negligible, while fluxes from clayey silt showed an increase with temperature, especially with higher WFPS and intermediate amounts of digestate. Under an anaerobic atmosphere, mean fluxes from loamy sand increased slightly, but significantly (Tukey's HSD, $p < 0.05$). The fluxes from clayey silt showed no significant differences (Tukey's HSD, $p < 0.05$) compared to the day before, with the exception of 35 % WFPS, at which mean flux increased strongly in the treatment with 320 kg digestate N ha^{-1}.

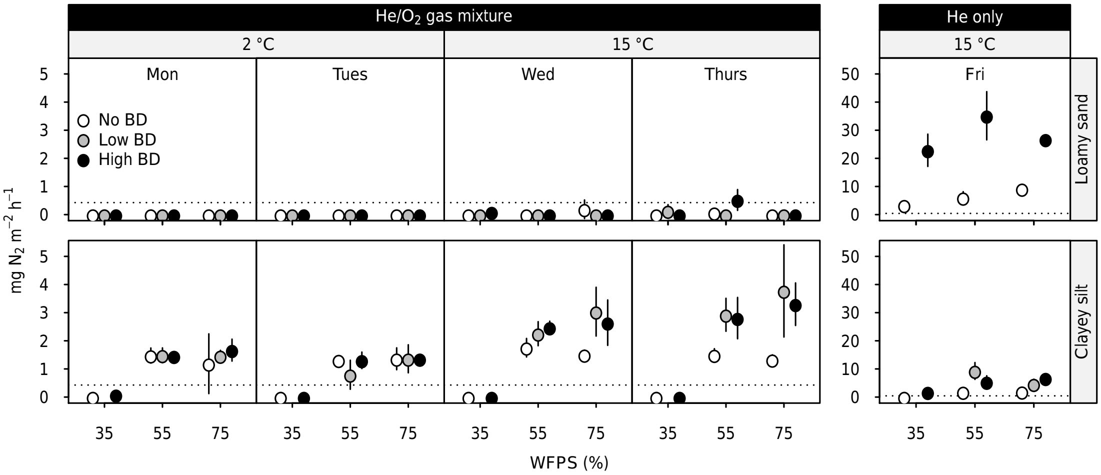

Figure 4. Mean N_2 fluxes ($mg\,m^{-2}\,h^{-1}$) from loamy sand and clayey silt incubated under different water-filled pore spaces (WFPSs, %) with different amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). The first to the fourth days of the incubation were measured in an aerobic He–O_2 headspace (with 2 days at 2 °C followed by another 2 days at 15 °C) while on the fifth day measurements were conducted in an anaerobic headspace with pure He (at 15 °C). Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. The dotted horizontal lines depict the average blank value; single flux rates lower than the respective lank value were set zero. Under an aerobic atmosphere, N_2 fluxes from loamy sand were zero or rather negligible, while fluxes from clayey silt show a distinct increase with WFPS and higher fluxes at 15 °C. Under an anaerobic atmosphere, mean fluxes from loamy sand increased by orders of magnitude, while the fluxes from clayey silt increased as well, but more gently compared to the sand.

perature and was also identified as significant by regression selection.

The mean N_2 fluxes of up to 0.5 (loamy sand) and 3.8 $mg\,N\,m^{-2}\,h^{-1}$ (clayey silt) at 15 °C (Fig. 5, Table A3) were considerably smaller than the mean fluxes of up to 13.0 $mg\,m^{-2}\,h^{-1}$ observed by Köster et al. (2015) during the first 5 days of their incubation. Although the amount of BD in terms of applied N (250 kg ha^{-1}) was comparable, Köster

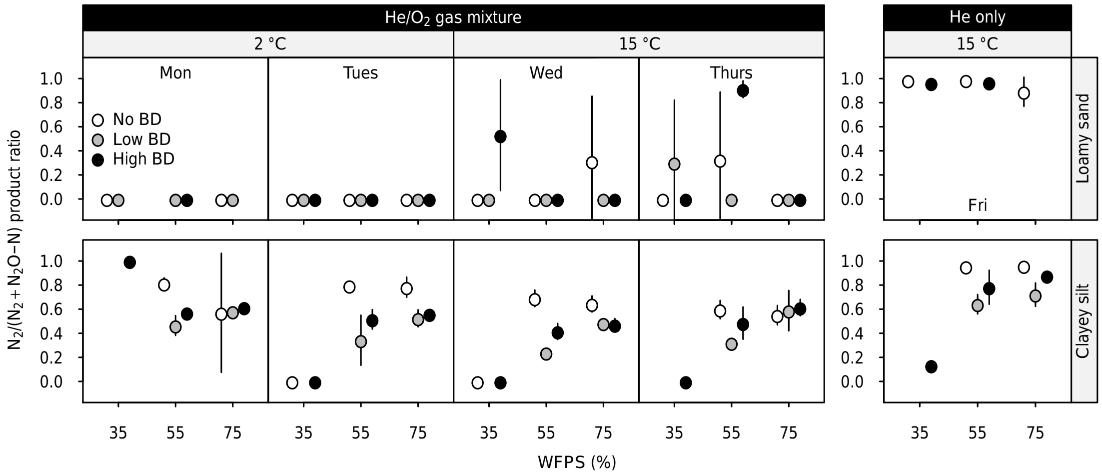

Figure 5. Mean N_2 / (N_2+ N_2O-N) product ratio from loamy sand and clayey silt incubated under different water-filled pore spaces (WFPSs, %) with different amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). The first to the fourth days of the incubation were measured in an aerobic He–O_2 headspace (with 2 days at 2 °C followed by another 2 days at 15 °C) while on the fifth day measurements were conducted in an anaerobic headspace with pure He (at 15 °C). Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. For the loamy sand, there was a clear distinction of the ratios between aerobic and anaerobic atmospheres: while the ratios tended to 0 in the former, they tended to 1 in the latter, regardless of temperature or amount of digestate. For the clayey silt, ratios increased with WFPS and were highest from the unamended treatments under both the aerobic and the anaerobic atmospheres.

et al. (2015) used a higher WFPS of 90 %, which may have increased the generation of N_2. In contrast to N_2O emission rates, the observed N_2 fluxes depended not only on WFPS but also on soil type (Table 5). This is most likely due to the direct influence of soil structure on diffusivity and the resulting supply with O_2 (Balaine et al., 2016; Butterbach-Bahl et al., 2013). N_2O flux also showed a significant effect during regression selection for N_2. N_2O is the direct precursor of N_2 in denitrification; thus, the flux of the latter depends on the availability of the former. However, temperature showed no significant effect.

N_2 / (N_2+ N_2O) product ratios were significantly determined only by soil type and WFPS: while no clear trend was observable for the loamy sand, there was a pronounced effect in the clayey silt (Fig. 4). We attribute the lack of a trend in loamy sand to generally adverse conditions for the formation of N_2O and N_2, i.e. a sufficient supply of O_2 (see Sect. 4.2). Conversely, the influence of WFPS apparently mirrored favourable conditions in the clayey silt (Table 5). Simultaneously, with increasing WFPS, the reduction of N_2O as an alternative electron acceptor under reduced O_2 supply accelerates (Tiedje, 1988). Accordingly, no or rather small fluxes of the investigated gaseous N species were generally found in our presumably well-aerated treatments with 35 % WFPS.

In our study, one treatment (clayey silt, 55 % WFPS, LOBD) showed exceptionally large mean N_2O fluxes of up to 7.1 mg N m^{-2} h^{-1} (Fig. 3, Table A2). This could be evidence that the injection of such commonly applied amounts of BD-N (i.e. 160 kg N ha^{-1}) may favour much larger losses of N_2O compared to an even distribution of BD in a soil surface due to larger substrate concentration in injection slits. However, with higher amendments (i.e. HIBD), we observed partially significant ($p < 0.05$, Tukey's HSD) reductions of N_2O and a decreasing tendency of N_2 emissions (Tables A2, A3). In line with this, the amount of BD showed a significant effect during the regression selection on N_2O, but not on N_2 fluxes (Table 5). A coherent reasoning for the rather smaller emissions of highly amended HIBD treatments might lie in an inhibitory effect of NH_3 on nitrification. Accordingly, Kim et al. (2006) found a selective inhibition of NO_2^- oxidation in the presence of 14 to 17 mg NH_3-N L^{-1}. Our calculated application rates in the treatments with HIBD amounted to at least 253.4 mg NH_4^+-N (kg soil)$^{-1}$ for the clayey silt (Fig. 3), which corresponds to 13.0 mg NH_3-N (kg soil)$^{-1}$ at 15 °C when applying the pH of the BD and assuming all extractable NH_4^+-N to be in solution (Emerson et al., 1975). Hence, we consider this inhibitory effect as the reason for the missing increase in N_2O and N_2. Nevertheless, because we mixed the BD with the soil, one would expect a lower in situ NH_3 fixation by clay minerals in tubular injection slits (Kissel et al., 2008), resulting in probably lower N_2O and N_2 fluxes from clayey soils due to a more marked inhibitory effect.

High NH_4^+ loads in conjunction with an increased pH favour NO_2^- accumulation because NO_2^--oxidizing bacteria are less resilient against high concentrations of NH_3 than NH_3-oxidizing bacteria (Anthonisen et al., 1976). This NO_2^- should have protonated then partly to toxic and unstable HNO_2, which drives biological and chemical production of

NO and N_2O for detoxification (Venterea et al., 2015). Although we did not determine NO_2^-, we suggest a dominant role of nitrifier denitrification, i.e. NO_2^- reduction, in the generation of N_2O during our experiment, especially during the anaerobic headspace conditions at the end of the incubation, resulting in the relatively small NO_3^- recovery in both soils. Accordingly, coupled nitrification–denitrification and bacterial denitrification have been found to dominate the production of N_2O directly after application of BD (Köster et al., 2011; Senbayram et al., 2009). However, N_2O-N losses were clearly larger than N_2 losses under aerobic headspace in the clayey silt. This indicates that much of the N gas loss was driven by processes other than canonical denitrification. Under the above-mentioned conditions, NO-N losses may exceed N_2O losses (Venterea et al., 2015), making it important to take NO measurements into account in future studies.

Supposing that 15 % of NH_4^+-N is volatilized as NH_3 within the first 10 h after surface application of BD (Quakernack et al., 2012), the losses from the NH_4^+ amounts we applied would average to 80 mg NH_3-N m^{-2} h^{-1} (LOBD) and 160 mg H_3-N m^{-2} h^{-1} (HIBD). The actual losses of up to 11.7 mg N_2O-N m^{-2} h^{-1} at 30 % WFPS in the clayey soil (Table A2) or of up to 35.1 mg N_2 m^{-2} h^{-1} at 55 % WFPS in the sandy loam (Table A3) from our HIBD treatments add up to 117 mg N_2O-N and 351 mg N_2, respectively, for the same period. Hence, increased N_2O and N_2 emissions following injection of BD might effectively cause higher N losses compared to a surface application and deserve closer attention in future.

4.2 Different effects of soil diffusivity on N_2O and N_2 fluxes

Apparently, the tested factors affected the N_2O and N_2 fluxes from both soils in a different way. A specific soil characteristic that exhibits such a fundamental control on biogeochemical processes such as denitrification is the diffusivity for O_2 (Ball, 2013; Letey et al., 1980; Parkin and Tiedje, 1984), which is a main soil characteristic responsible for the appearance of anaerobic microsites. In general, diffusivity integrates the soil porosity, i.e. pore continuity and size as well as WFPS, which control both soil N_2O and N_2 emissions (Balaine et al., 2016; Ball, 2013; Letey et al., 1980). Soils with a coarser texture like the loamy sand have a higher proportion of macropores and thus a higher gas diffusion compared with fine-textured soils like the clayey silt we used (Groffman and Tiedje, 1991). This lets us expect conditions that are more favourable for N_2O and N_2 generation in the latter due to relatively poor diffusion characteristics and, thus, a smaller O_2 supply. Actually, although we incubated the soils at comparable levels of WFPS and BD amendments, the apparent lower diffusivity led to larger N_2O and N_2 production in the treatments with the clayey silt in relation to the loamy sand.

The role of the distinct diffusivities of both soils is corroborated by our observations of the gas fluxes in anaerobic headspace. With switching the He–O_2 atmosphere in the headspace to pure He, the denitrification potential can be tested because anaerobicity eliminates respiration processes that use O_2 as an electron acceptor (Parkin and Tiedje, 1984). We acknowledge DNRA and anammox, for example, as possible additional sources of N_2O and N_2 under such conditions but we were not able to quantify their contribution. The anaerobic headspace induced a considerable increase in N_2O fluxes in the loamy sand, but not in the clayey silt. Concurrently, the N_2 fluxes increased in both soils, but pronouncedly, i.e. more than 60-fold, in the sandy loam. These observed changes resulting from oxygen deprivation imply that, during the previous aerobic conditions, the diffusivity of the sandy loam was too high to allow for a sufficient establishment of anaerobic microsites, while the clayey silt ensured a moderate diffusional constraint to maintain suboxic conditions. In general, only N_2O fluxes from treatments with negligible fluxes during the previous aerobic period increased under anaerobic conditions, including all treatments with loamy sand (Fig. 3, Table A2). At the same time, there was a reduction of N_2O fluxes in most clayey silt treatments. However, a closer look reveals that virtually all of the latter treatments showed increased N_2 flux rates. Hence, there was an enhanced reduction of N_2O to N_2, which is reflected in the increased N_2 / (N_2 + N_2O) product ratio (Fig. 5) and points to intensified reduction of N_2O due to the lack of oxygen (Parkin and Tiedje, 1984). The much larger N_2 fluxes from the loamy sand compared to the clayey silt might have been caused as well by poor NO_3^- availability (Fig. 2) and a high availability of C (Table 4), which promoted the reduction of N_2O to N_2 (Senbayram et al., 2012). Further, we found no evidence for any shortage of substrate in the clayey silt during the subsequent anaerobic headspace conditions. However, the cumulated fluxes of both N_2 and N_2O amounted to a maximum absolute loss of 9.4 ($1\sigma = 0.3$) mg N per kg soil in the clayey silt with LOBD and 55 % WFPS, which was 7.4 %

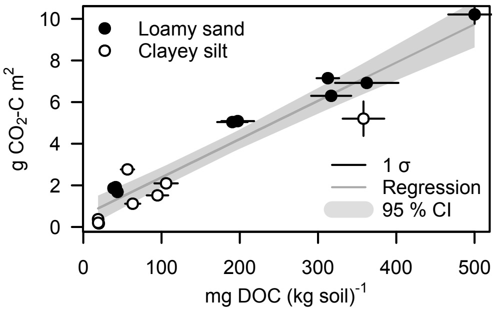

Figure 6. Regression between DOC (mg per 100 g soil) measured after the incubation and the respective cumulated CO_2 emissions (g C m^{-2}) during the period of aerobic headspace with their standard deviations and confidence interval (95 %). If error bars are not visible, they are smaller than the symbols of the means. Both soils showed increasing emissions with increasing soil DOC contents as well as a good regression fit ($R^2 = 0.91$, $p < 0.001$).

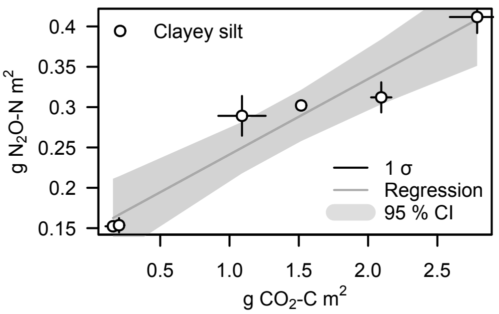

Figure 7. Regression between cumulated CO_2 emissions (g C m^{-2}) and the respective cumulated $N_2O + N_2$ emissions (g N m^{-2}) from the clayey silt with WFPS > 35 % during the period of aerobic headspace with their standard deviations and confidence interval (95 %). If error bars are not visible, they are smaller than the symbols of the means. The proportional increase in CO_2 and the N gas species shows a good regression fit of $R^2 = 0.93$ ($p = 0.001$).

of the calculated NH_4^+-N applied with BD (Fig. 2). Conversely, the N_2 / (N_2+N_2O) product ratios increased only slightly (Fig. 5) and, in contrast to the loamy sand, there were still significant N_2O fluxes in the clayey silt (Fig. 3). This points to still-sufficient stocks of NO_3^- in the latter (Senbayram et al., 2012). In fact, the NO_3^- stock was greater in the clayey silt than in loamy sand after incubation (Fig. 2). Thus, we suggest that the gas fluxes were unaffected by the change to anaerobic headspace in the clayey silt due to already low O_2 concentrations as a result of poor diffusivity. In conclusion, distinct gas diffusivities of both soils can be proposed as the main reason for the differing N_2O and N_2 fluxes.

In interaction with soil diffusivity, respiration affects the aerobicity of a soil matrix by concurrent consumption and formation of O_2 and CO_2 as well. Depending on microbial C availability, respiration could be indicated by DOC, though not all DOC may be readily degradable (Cook and Allan, 1992). Generally, DOC content after our incubation increased with application rate of BD (Table 4), but DOC content was always smaller in clayey silt. This might reflect a stronger sorption of C and thus a lower availability for respiration in the clayey silt compared to loamy sand (Kaiser and Guggenberger, 2000). If we compare DOC concentrations with cumulated flux rates of CO_2 over the period of aerobic headspace, we find a good regression fit ($R^2 = 0.91$, $p < 0.001$) for both soils (Fig. 6), indicating a sufficient availability of C from BD for respiration and, thus, implicitly also for denitrification (Reddy et al., 1982). Moreover, as increased DOC enhanced respiration (Table A1), it consequently affected O_2 consumption and, thus, also the emergence of anaerobic microsites (Azam et al., 2002). Accordingly, there is also a good correlation between cumulated CO_2 and $N_2O + N_2$ fluxes for the same period from the clayey silt ($R^2 = 0.93$, $p = 0.001$), when the treatments with 35 % WFPS (which showed virtually no N emissions) are omitted (Fig. 7). However, there was no such a correlation for the loamy sand. This confirms the interactive effect of diffusivity (induced by both the soils and WFPS) and C availability on the emissions of N_2O and N_2, which, nevertheless, interacted with the inhibitory effect of high NH_4^+ loads on nitrification (see Sect. 4.1).

5 Relevance and implications

Our aim was to estimate the effect of differing soil environmental conditions on gaseous N losses – and not to draw conclusions about the long-term dynamics of N_2 and N_2O emissions after BD application in concentrations similar to injection. In another laboratory study at a WFPS of 65 %, Senbayram et al. (2009) measured only one peak within 2 days without a repeated increase later, regardless of the amount of applied BD. Thus, we assume that a single peak shortly after application holds also true for our incubation. We also assume the measurements after only 24 h of anaerobicity in the headspace to be representative for the emission potential since in similar studies Wang et al. (2011, 2013) showed that the emission of N_2 and N_2O peaked within less than 24 h after switching their headspace from aerobic to anaerobic conditions.

In summary, as hypothesized, N_2O and N_2 emissions as well as the N_2 / (N_2O+N_2) product ratio increased with WFPS, most probably due to restricted supply of O_2. Contrary to our second hypothesis, the gaseous losses of N_2O and N_2 did not increase with the application rate of BD. This indicates an inhibitory effect of high NH_3 and NH_4^+ concentrations on nitrification, which are typically found in BD. At the same time, the N_2 / (N_2O+N_2) product ratio tended to decrease with application rate as supposed, probably due to a copious supply with NO_2^- and NO_3^- from oxidized BD-NH_4^+. Confirming our third hypothesis, the fine textured clayey silt induced larger gaseous N losses and a higher N_2 / (N_2O+N_2) ratio than the coarse loamy sand by the apparent distinct diffusivities of both soils. Overall, there was a larger potential for formation of N_2O in the fine-textured clayey silt compared to the coarse loamy sand after the application of high concentrations of BD as they may appear after injection. However, the loamy sand showed a large potential for N_2 formation under anaerobic headspace conditions.

Since coupled nitrification–denitrification N losses from injected BD seem to be massive in this study, the short-term emissions of N_2O and N_2 after injection appear to offset the reduced NH_3-N losses that would have hypothetically arisen from surface application. Further investigations are needed regarding the dynamics and the duration of the observed effects and their reliability for field conditions.

Data availability. The data underlying the figures are accessible publicly via the Supplement.

Appendix A

Table A1. Mean CO_2-C fluxes with standard deviations in mg m^{-2} h^{-1} from the loamy sand and the clayey silt, treated with different water-filled pore spaces (WFPSs, %), amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and different temperature regimes (°C) under aerobic (He–O_2) and anaerobic (He) atmospheres. The column "Day" denotes the consecutive measuring days of the respective incubation cycle. Different letters after fluxes indicate significant differences (Tukey's HSD, $p < 0.05$) within each soil and measuring day. Zeros as last digits were omitted.

Day	Atmosphere	Temperature (°C)	WFPS (%)		mg CO_2-C m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He–O_2	2	35	No BD	6.8 ± 2.4 cd	0 ± 0 c
1	He–O_2	2	35	Low BD	22 ± 3.5 bcd	NA
1	He–O_2	2	35	High BD	23.3 ± 9.3 bc	22.8 ± 2.8 ab
1	He–O_2	2	55	No BD	6 ± 0.7 d	4.6 ± 7.9 bc
1	He–O_2	2	55	Low BD	34.4 ± 3.1 b	34.5 ± 11.6 a
1	He–O_2	2	55	High BD	28 ± 3.2 b	15.9 ± 3.4 abc
1	He–O_2	2	75	No BD	9.4 ± 1.4 cd	0 ± 0 c
1	He–O_2	2	75	Low BD	37.5 ± 6 b	15.5 ± 12.1 abc
1	He–O_2	2	75	High BD	68.3 ± 12.1 a	24.5 ± 2.7 a
2	He–O_2	2	35	No BD	9.8 ± 3.5 c	1.3 ± 1.4 b
2	He–O_2	2	35	Low BD	23 ± 3.9 bc	NA
2	He–O_2	2	35	High BD	30.9 ± 2.2 b	22.2 ± 2.4 a
2	He–O_2	2	55	No BD	8.7 ± 1.5 c	0.6 ± 1 b
2	He–O_2	2	55	Low BD	33.4 ± 0.9 b	27.6 ± 12.3 a
2	He–O_2	2	55	High BD	35.9 ± 2.7 b	14.4 ± 1.9 ab
2	He–O_2	2	75	No BD	8.3 ± 1.5 c	0 ± 0 b
2	He–O_2	2	75	Low BD	31.9 ± 3 b	13 ± 9.3 ab
2	He–O_2	2	75	High BD	57.6 ± 14.8 a	18.3 ± 4 a
3	He–O_2	15	35	No BD	42.5 ± 4.5 c	6.7 ± 0.7 b
3	He–O_2	15	35	Low BD	114.3 ± 12.2 b	NA
3	He–O_2	15	35	High BD	149.5 ± 9.4 b	130.9 ± 105 a
3	He–O_2	15	55	No BD	41.3 ± 3.5 c	3.2 ± 0.4 b
3	He–O_2	15	55	Low BD	108.7 ± 10.1 b	57.8 ± 12.2 bc
3	He–O_2	15	55	High BD	162.1 ± 9.6 b	26.8 ± 0.7 bc
3	He–O_2	15	75	No BD	44.1 ± 9.8 c	3.2 ± 0.7 b
3	He–O_2	15	75	Low BD	150.4 ± 19 b	26.4 ± 11.8 bc
3	He–O_2	15	75	High BD	249.7 ± 53.5 a	35.3 ± 6 bc
4	He–O_2	15	35	0	48.7 ± 6 c	15.1 ± 4.9 cd
4	He–O_2	15	35	Low BD	114.3 ± 6.4 b	NA
4	He–O_2	15	35	High BD	156.9 ± 15.4 a	65.7 ± 2.2 a
4	He–O_2	15	55	No BD	48 ± 3.4 c	4.2 ± 0.2 d
4	He–O_2	15	55	Low BD	109 ± 14.4 b	51.2 ± 15.1 ab
4	He–O_2	15	55	High BD	177.7 ± 7.5 a	26.6 ± 2.3 cd
4	He–O_2	15	75	No BD	34 ± 7.8 c	6.7 ± 4 d
4	He–O_2	15	75	Low BD	168.7 ± 0.4 a	22.1 ± 14.8 cd
4	He–O_2	15	75	High BD	166.3 ± 23.1 a	34.1 ± 5.7 bc
5	He	15	35	No BD	11.2 ± 0.6 d	NA
5	He	15	35	Low BD	54.8 ± 9.3 c	NA
5	He	15	35	High BD	149.3 ± 3.9 a	45.8 ± 2.1 a
5	He	15	55	No BD	13.6 ± 1.9 d	3.4 ± 0.6 c
5	He	15	55	Low BD	55.2 ± 4.4 bc	32 ± 11.4 ab
5	He	15	55	High BD	164.5 ± 3.5 a	15.2 ± 10.7 bc
5	He	15	75	No BD	20.9 ± 2.3 d	3.6 ± 0.1 c
5	He	15	75	Low BD	75 ± 7.3 b	20.6 ± 8.5 bc
5	He	15	75	High BD	NA	26.1 ± 2.6 ab

Table A2. Mean N_2O-N fluxes with standard deviations in mg m^{-2} h^{-1} from the loamy sand and the clayey silt, treated with different water-filled pore spaces (WFPSs, %), amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and different temperature regimes (°C) under aerobic (He–O_2) and anaerobic (He) atmospheres. The column "Day" denotes the consecutive measuring days of the respective incubation cycle. Different letters after fluxes indicate significant differences (Tukey's HSD, $p < 0.05$) within each soil and measuring day. Zeros as last digits were omitted.

Day	Atmosphere	Temperature (°C)	WFPS (%)		mg N_2O-N m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He–O_2	2	35	No BD	0 ± 0	0 ± 0 c
1	He–O_2	2	35	Low BD	0 ± 0	NA
1	He–O_2	2	35	High BD	0 ± 0	0 ± 0 c
1	He–O_2	2	55	No BD	0 ± 0	0.3 ± 0.1 c
1	He–O_2	2	55	Low BD	0 ± 0	1.7 ± 0.4 a
1	He–O_2	2	55	High BD	0 ± 0	1.1 ± 0.1 b
1	He–O_2	2	75	No BD	0 ± 0	0.4 ± 0.1 c
1	He–O_2	2	75	Low BD	0 ± 0	1 ± 0.1 b
1	He–O_2	2	75	High BD	0 ± 0	1 ± 0.2 b
2	He–O_2	2	35	No BD	0 ± 0	0 ± 0 d
2	He–O_2	2	35	Low BD	0 ± 0	NA
2	He–O_2	2	35	High BD	0 ± 0	0 ± 0 cd
2	He–O_2	2	55	No BD	0 ± 0	0.3 ± 0.1 bc
2	He–O_2	2	55	Low BD	0 ± 0	1.5 ± 0.6 a
2	He–O_2	2	55	High BD	0 ± 0	1.2 ± 0.2 a
2	He–O_2	2	75	No BD	0 ± 0	0.4 ± 0.1 bc
2	He–O_2	2	75	Low BD	0 ± 0	1 ± 0.1 ab
2	He–O_2	2	75	High BD	0 ± 0	1.1 ± 0.2 a
3	He–O_2	15	35	No BD	0 ± 0 cd	0 ± 0 c
3	He–O_2	15	35	Low BD	0 ± 0 abc	NA
3	He–O_2	15	35	High BD	0 ± 0 ab	0 ± 0 c
3	He–O_2	15	55	No BD	0 ± 0 bcd	0.8 ± 0.2 c
3	He–O_2	15	55	Low BD	0 ± 0 bcd	7.1 ± 0.9 a
3	He–O_2	15	55	High BD	0 ± 0 a	3.5 ± 0.7 b
3	He–O_2	15	75	No BD	0 ± 0 ab	0.8 ± 0.2 c
3	He–O_2	15	75	Low BD	0 ± 0 d	3.2 ± 0.7 b
3	He–O_2	15	75	High BD	0 ± 0 cd	3 ± 0.9 b
4	He–O_2	15	35	No BD	0 ± 0 b	0 ± 0 c
4	He–O_2	15	35	Low BD	0 ± 0 ab	NA
4	He–O_2	15	35	High BD	0 ± 0 ab	0.1 ± 0.1 c
4	He–O_2	15	55	No BD	0 ± 0 b	1 ± 0.2 bc
4	He–O_2	15	55	Low BD	0.1 ± 0.1 a	6.2 ± 1.1 a
4	He–O_2	15	55	High BD	0 ± 0 ab	3 ± 0.8 b
4	He–O_2	15	75	No BD	0 ± 0 ab	1.1 ± 0.3 bc
4	He–O_2	15	75	Low BD	0 ± 0 b	2.6 ± 1 b
4	He–O_2	15	75	High BD	0 ± 0 b	2.2 ± 0.9 b
5	He	15	35	No BD	0.1 ± 0	NA
5	He	15	35	Low BD	NA	NA
5	He	15	35	High BD	0.9 ± 0.1	11.7 ± 2 a
5	He	15	55	No BD	0.1 ± 0	0.1 ± 0 c
5	He	15	55	Low BD	NA	5 ± 1 b
5	He	15	55	High BD	1.2 ± 0.7	1.4 ± 0.8 c
5	He	15	75	No BD	1.3 ± 1.6	0.1 ± 0 c
5	He	15	75	Low BD	NA	1.7 ± 0.3 c
5	He	15	75	High BD	NA	1 ± 0.3 c

Table A3. Mean N_2 fluxes with standard deviations in $\mathrm{mg\,m^{-2}\,h^{-1}}$ from the loamy sand and the clayey silt, treated with different water-filled pore spaces (WFPSs, %), amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and different temperature regimes (°C) under aerobic (He–O_2) and anaerobic (He) atmospheres. The column "Day" denotes the consecutive measuring days of the respective incubation cycle. Different letters after fluxes indicate significant differences (Tukey's HSD, $p < 0.05$) within each soil and measuring day. Zeros as last digits were omitted.

	Atmosphere	Temperature (°C)	WFPS (%)		$\mathrm{mg\,N_2\,m^{-2},h^{-1}}$	
					Loamy sand	Clayey silt
1	He–O_2	2	35	No BD	0 ± 0	0 ± 0 bc
1	He–O_2	2	35	Low BD	0 ± 0	NA
1	He–O_2	2	35	High BD	0 ± 0	0.1 ± 0.1 bc
1	He–O_2	2	55	No BD	0 ± 0	1.5 ± 0.3 a
1	He–O_2	2	55	Low BD	0 ± 0	1.5 ± 0.3 a
1	He–O_2	2	55	High BD	0 ± 0	1.5 ± 0 a
1	He–O_2	2	75	No BD	0 ± 0	1.2 ± 1.1 a
1	He–O_2	2	75	Low BD	0 ± 0	1.5 ± 0.2 a
1	He–O_2	2	75	High BD	0 ± 0	1.7 ± 0.4 a
2	He–O_2	2	35	No BD	0 ± 0	0 ± 0 c
2	He–O_2	2	35	Low BD	0 ± 0	NA
2	He–O_2	2	35	High BD	0 ± 0	0 ± 0 c
2	He–O_2	2	55	No BD	0 ± 0	1.3 ± 0.1 a
2	He–O_2	2	55	Low BD	0 ± 0	0.8 ± 0.5 b
2	He–O_2	2	55	High BD	0 ± 0	1.3 ± 0.3 a
2	He–O_2	2	75	No BD	0 ± 0	1.4 ± 0.4 a
2	He–O_2	2	75	Low BD	0 ± 0	1.4 ± 0.5 a
2	He–O_2	2	75	High BD	0 ± 0	1.4 ± 0.1 a
3	He–O_2	15	35	No BD	0 ± 0 b	0 ± 0 e
3	He–O_2	15	35	Low BD	0 ± 0 b	NA
3	He–O_2	15	35	High BD	0.1 ± 0.1 ab	0 ± 0 e
3	He–O_2	15	55	No BD	0 ± 0 b	1.8 ± 0.3 cd
3	He–O_2	15	55	Low BD	0 ± 0 b	2.3 ± 0.4 bc
3	He–O_2	15	55	High BD	0 ± 0 b	2.5 ± 0.2 ab
3	He–O_2	15	75	No BD	0.2 ± 0.3 a	1.5 ± 0.2 d
3	He–O_2	15	75	Low BD	0 ± 0 b	3 ± 0.9 a
3	He–O_2	15	75	High BD	0 ± 0 b	2.6 ± 0.8 ab
4	He–O_2	15	35	No BD	0 ± 0 b	0 ± 0 c
4	He–O_2	15	35	Low BD	0.1 ± 0.2 b	NA
4	He–O_2	15	35	High BD	0 ± 0 b	0 ± 0 c
4	He–O_2	15	55	No BD	0.1 ± 0.1 b	1.5 ± 0.2 b
4	He–O_2	15	55	Low BD	0 ± 0 b	2.9 ± 0.6 a
4	He–O_2	15	55	High BD	0.5 ± 0.4 a	2.8 ± 0.7 a
4	He–O_2	15	75	No BD	0 ± 0 b	1.3 ± 0.2 bc
4	He–O_2	15	75	Low BD	0 ± 0 b	3.8 ± 1.6 a
4	He–O_2	15	75	High BD	0 ± 0 b	3.3 ± 0.8 a
5	He	15	35	No BD	3.3 ± 0.4 d	0 ± 0 c
5	He	15	35	Low BD	NA	NA
5	He	15	35	High BD	22.9 ± 5.7 b	1.8 ± 0.1 c
5	He	15	55	No BD	6 ± 2.2 cd	1.8 ± 0.2
5	He	15	55	Low BD	NA	9.5 ± 2.7 a
5	He	15	55	High BD	35.1 ± 8.6 a	5.1 ± 1.8 bc
5	He	15	75	No BD	9.2 ± 0.4 c	1.9 ± 0.1 c
5	He	15	75	Low BD	NA	4.8 ± 1.6 bc
5	He	15	75	High BD	26.8 ± 1.1 b	6.7 ± 0.8 b

The Supplement related to this article is available online at https://doi.org/10.5194/soil-3-161-2017-supplement.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. We thank the editor Karsten Kalbitz and the three anonymous referees for their careful reading, critical comments and valuable suggestions. We are very grateful to Heinrich Graf von Bassewitz and Matthias Haß from Gut Dalwitz for their straightforward support with substrate from their anaerobic digester. We thank Madlen Pohl from the ZALF, Institute for Landscape Biogeochemistry, Müncheberg, Germany, most sincerely for managing the laboratory analyses of the soil samples. The joint research project underlying this report was funded by the German Federal Ministry of Food and Agriculture under the funding identifier 22007910.

Edited by: Karsten Kalbitz
Reviewed by: three anonymous referees

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