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N_2O and N_2 losses from simulated injection of biogas digestate depend mainly on soil texture, moisture and temperature

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Abstract. Biogas digestate (BD) is increasingly used as organic fertiliser, but has a high potential for NH_3 losses. Its proposed injection into soils as a counter-measure has been suggested to promote the generation of N_2O, leading to a potential trade-off. Furthermore, the effect on N_2 losses after injection of BD into soil has not yet been evaluated. We performed a simulated BD injection experiment in a helium-oxygen atmosphere to examine the influence of soil substrate (loamy sand, clayey silt), water-filled pore space (WFPS; 35, 55, 75%), temperature (2°C, 15°C) and application rate (0, 160, 320 kg N ha^{-1}) as a proxy for row spacing of injection on the emissions of N_2O, N_2, and CO_2. To determine the potential capacity for these gaseous losses, we incubated under anaerobic conditions by purging with helium for the last 24 h of incubation. N_2O and N_2 emissions as well as the $N_2/(N_2O+N_2)$ ratio depended on soil type and increased with WFPS and temperature, indicating a crucial role of soil gas diffusivity for the formation of these gases in agricultural soils. However, the emissions did not increase with the application rate of BD, i.e. a broader spacing of injection slits, probably due to an inhibitory effect of the high NH_4^+ content of BD. Our results suggest that the risk of N_2O and N_2 losses even after injection of relatively large amounts of BD seems to be small for dry to wet sandy soils and acceptable when regarding simultaneously reduced NH_3 emissions for dry silty soils.

1 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (Myhre et al., 2013), with agriculture being the largest single source of anthropogenic N_2O emissions, contributing about 4.1 Tg N_2O-N yr^{-1} or 66% of total gross anthropogenic emissions mainly as a result of mineral nitrogen (N) fertiliser and manure application (Davidson and Kanter, 2014). The generation of nitrogen gas (N_2) is of agronomic interest in terms of nutrient management, since these gaseous losses may imply a significant loss of N from the soil/plant system (Friedl et al., 2016; Cameron et al., 2013). However, from an environmental stance, N_2 is innocuous and, thus, the preferred type of gaseous N-loss from soil (Davidson et al., 2015). Further, emission and deposition of ammonia (NH_3) is of environmental concern, e.g., through acidification and conversion to N_2O (Ferm, 1998; Mosier et al., 1998). In general, the improvement of N use efficiency and thus the decrease of N losses in crop production are paramount in the presence of challenges like food security, environmental degradation and climate change (Zhang et al., 2015).

In Germany, the increased demand for renewable energy sources like methane from biogas plants entails an expanded amount of digestion residues (biogas digestate, BD) used as organic amendment in agriculture (Möller and Müller, 2012). Digestion in biogas reactors increases pH and the proportion of ammonium (NH_4^+) and narrows the C to N ratio due to the depletion of labile C fractions of the feedstock (Möller and Müller, 2012). Compared to undigested amendments like slurry, these altered chemical properties may promote biochemical reactions in the soil that are responsible for the formation of gaseous N species like N_2O, N_2 and especially NH_3 (Nkoa, 2013). In general, the overall effect of BD on gaseous N losses from soil is still under debate (Möller, 2015).

Significant losses of N as NH_3 can 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 due to a reduction of local O_2 availability (Wulf et al., 2002; Velthof and Mosquera, 2011; Webb et al., 2010) and the occurrence of high nutrient concentrations in the injection band (Dell et al., 2011). Hence, these conditions during the initial phase after injection of BD foster microsites favourable for microbial denitrification, which may promote also the formation of N_2 due to anaerobic conditions (Köster et al., 2015).

There is a wealth of biotic and abiotic processes in soils that produce N_2O and N_2, most of which are enhanced by anaerobic or at least hypo-aerobic conditions (Butterbach-Bahl et al., 2013). Also the amounts and the relative share of N_2 and N_2O in the overall gaseous N emissions depend – among other factors like the favoured reduction of NO_3^- rather than N_2O as alternative electron acceptor – on the degree of O_2 restriction (Firestone and Davidson, 1989). Soil physical and biotic factors (i.e. diffusion and consumption of O_2) as well as their interactions control the aerobic status of a soil. Diffusion of O_2 depends on the porosity of the soil substrate in conjunction with water-filled pore space (WFPS), while O_2 is consumed by heterotrophic respiration which depends on mineral N content, carbon (C) availability as well as on temperature (Ball, 2013; Uchida et al., 2008; Maag and Vinther, 1999). Simultaneously, the supply of substrates for microorganisms is determined by liquid diffusion rates in soil water and, thus, by WFPS (Blagodatsky and Smith, 2012; Maag and Vinther, 1999). However, nutrient concentrations and WFPS should theoretically be controlled especially by the row spacing between the injection bands, since for a given amount of BD per area, a wider spacing requires a higher concentration of BD application in the band and *vice versa* with consequences for microbial activity and O_2 availability within the band. There are studies that examined the effect of manure injection depth on N_2O emissions (Webb et al., 2010), but we are not aware of studies on the effect of row spacing in general as well as application rate as a proxy in particular.

Hence, there is a general lack of knowledge about effects of manure injection on gaseous N-losses and especially about the effects of BD and row spacing and how they interact with O_2 limiting factors like soil texture and WFPS, as well as temperature and heterotrophic respiration. These knowledge gaps are caused not least by methodological issues with the determination of N_2 fluxes. Thus, we applied the helium-oxygen (He-O_2) incubation technique

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(Butterbach-Bahl et al., 2002) in a laboratory experiment to evaluate the effect of above suggested factors on the generation of N_2O and N_2 after simulated injection of BD into 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 hypothesised that (1) N_2O and N_2 emissions will increase with WFPS and temperature, (2) these gaseous N losses will also be affected by BD application rate, i.e. injection row-spacing, and (3) the fine textured clayey silt will induce higher gaseous N losses than the coarse loamy sand.

2 Material and Methods

2.1 Selected soils, sampling of soil cores and biogas digestate

Two soils were selected and both were treated with three levels of WFPS and three quantities of BD (Table 1), resulting in 18 factor combinations with three repetitions each. Temperature was increased from 2 °C during the first two days to 15 °C for the last three days of the incubation. Intact soil cores (diameter 0.072 m, height 0.061 m) were taken with sample rings in the range from 0–0.10 m depth from two sites with different textures, i.e. sandy loam and clayey silt. The sandy loam samples were gathered from a stagnic luvisol (IUSS Working Group WRB, 2006) located in Gülzow (North-East 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 gathered 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.

Both sites have been cultivated with similar crop rotations used as feedstock for biogas production and have been amended with biogas digestate for the past nine 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 sudangrass (*Sorghum* × *drummondii*) instead of sorghum.

The biogas digestate 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% 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 with the respective quantity of water calculated based on the bulk density and an assumed particle density of 2.65 g cm^{-1}, reduced by the expected moisture input from subsequent addition of BD. Since injection bands *in situ* have a thickness comparable to the sample rings we used, the soil cores were mixed with the biogas digestate and finally repacked to simulate the concentration of BD in soil after injection. The amounts of added BD were calculated with an assumed injection of 160 kg N ha^{-1} into soil with row spaces of 0.15 and 0.30 m, which are common ranges used by injection machinery. The application rate per band of 160 kg N ha^{-1} at 0.30 m spacing would correspond to the concentration of BD resulting from the injection of 320 kg N ha^{-1} at 0.15 m spacing. Thus, for convenience, we will denote the different levels of BD by amount based on a row spacing of 0.15 m. 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.

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 (Scholefield et al., 1997; Butterbach-Bahl et al., 2002). 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 moderately (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 headspaces for 72 h to remove residues of N_2 from soil cores. After this pre-incubation, during the following two days, the headspace concentration of N_2O and CO_2 was measured once daily in the morning. To compensate for a possibly lower precision of the detector for N_2 in relation to the detector for N_2O and CO_2, 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 two days. Finally, the He/O_2 gas mixture was substituted by pure He and, following 24 h of acclimatisation, gas measurements were carried out once again (Figure 1) to determine the current potential for N_2O and N_2 generation in a completely anaerobic soil matrix. The settings of the chromatographs for gas analyses are described in Eickenscheidt et al. (2014). Gas fluxes were calculated according to Eq. (1):

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

where f is the flux (N_2 and CO_2: mg m^{-2} h^{-1}, N_2O: µg m^{-2} h^{-1}), M the molar mass in g mol^{-1} (N_2: 28, CO_2: 44, N_2O: 44), p the air pressure (Pa), v the air flow (L h^{-1}), R the gas constant (8.31 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).

The lowest detectable fluxes were checked every day for each vessel and were on average 0.427 ($1\sigma = 0.271$) mg N_2-N m^{-2} h^{-1}, 3.6 (3.1) µg N_2O-N m^{-2} h^{-1} and 0.918 (0.693) mg CO_2-C m^{-2} h^{-1}. Estimated fluxes from the soil cores smaller than the respective detection limits 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 (soil to extract ratio 1:4) and analysed for NH_4^+ and nitrate (NO_3^-) by spectrophotometry according to DIN ISO 14256 with a continuous flow analyser 'CFA-SAN', Skalar Analytical B.V., the Netherlands and for DOC by combustion according to DIN ISO 10694 with an analyser 'RC 612', Leco Instruments GmbH, Germany.

2.5 Statistical analysis

All statistical analyses were done using R version 3.2.3 (R Core Team, 2015) with the data of the consecutive measuring days two and four (2 and 15 °C, respectively, under He-O_2 atmosphere) to allow for establishment of a new flux equilibrium on day three after temperature change and, thus, minimising the effect of time. Data from the vessels with the factor combination of 35% WFPS and 160 kg N ha^{-1} with clayey silt had to be omitted due to technical reasons during sample preparation. For the final period of pure He headspace, some gas concentration data are missing due to logistical reasons. For the loamy sand this affects all WFPS levels with 160 kg N ha^{-1} (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 package 'lmerTest' (Kuznetsova et al., 2015) 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^- 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^+ and DOC were omitted since they showed high autocorrelation with the amount of BD applied. The individual soil core was set as random effect with regard to lack of independence of consecutive measurements. The model responses for N_2, N_2O and CO_2 where log transformed (ln[value + 1]) since these 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 'An Information Criterion'). Shapiro-Wilk normality test ($\alpha = 0.05$) was applied to check residuals for normal distribution. Three outliers, identified by 'Scale-Location' plots, were omitted in each model for N_2 as well as for N_2O and five outliers were discarded in the model for $N_2/(N_2+N_2O)$ product ratio to obtain normality. For mixed effect models, the *p*-values of the summaries were calculated with *t*-test based on Satterthwaite's approximations.

Cumulated gas fluxes were estimated with a bootstrap method using function 'auc.mc' of R package 'flux' version 0.3-0 (Jurasinski et al., 2012) for the R statistical software version 3.2.3 (R Core Team, 2015). In short, the fluxes for the period of aerobic headspace were cumulated in 100 iterations, while for each run 2 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 kg soil approximated for the sandy loam 247.0 mg (160 kg N ha^{-1}) and 494.0 mg (320 kg N ha^{-1}), and for the clayey silt 266.0 mg (160 kg N ha^{-1}) and 532.0 mg (320 kg N ha^{-1}). The NO_3^- content of BD was negligible. After incubation, the recovered NH_4^+-N contents increased with the level of amendment with BD in both soils and were not affected by WFPS, with the exception of treatments of clayey silt with 35% WFPS (Fig. 2). In the loamy sand, the mean amounts of NH_4^+-N per kg soil ranged from 8.5 to 10.0 mg (no amendment), from 170.4 to 185.6 mg (160 kg N ha^{-1}) and from 273.7 to 314.0 mg (320 kg N ha^{-1}). In the clayey silt, NH_4^+-N contents per kg soil reached only 1.8 to 8.8 mg (no amendment), 89.7 to 98.9 mg (160 kg N ha^{-1}) and 146.8 to 194.0 mg (320 kg N ha^{-1}) and, thus, roughly half the amounts of the clayey silt. However, in contrast to the loamy sand, the clayey silt showed also substantial NO_3^- contents between 25.7 (35% WFPS without amendment) and 49.8 mg NO_3^--N (kg soil)$^{-1}$ (55% WFPS with 160 kg N ha^{-1}). Negligible amounts of NO_3^-

were detected in the loamy sand after incubation: except for a mean value of 2.4 mg NO_3^--N (kg soil)$^{-1}$ from the unamended treatment with 75% WFPS, the values of all other treatments ranged between 0.2 and 0.5 mg. The amounts of recovered DOC increased with the application rate of BD, but with different magnitudes for both soils. While mean values from 38.6 (55 % WFPS without amendment) to 500.1 mg DOC per kg soil (75 % WFPS, 320 kg N ha^{-1}) were determined for the loamy sand after incubation, lower mean values from 18.9 (55 % WFPS without amendment) to 358.1 mg (35 % WFPS, 320 kg N ha^{-1}) were found in the clayey silt, where the respective second highest values were considerably lower for both soils (loamy sand: 362.2 mg for 75 % WFPS with 160 kg N ha^{-1}, clayey silt: 105.9 mg for 75 %WFPS with 320 kg N ha^{-1}) (Table 4).

3.2 N_2O fluxes

The mean N_2O fluxes from the loamy sand at 2 °C under the He-O_2-atmosphere were virtually zero and, thus, negligible (Fig. 3, Day 2 in Table A1). This was similar at 15°C with the exception of 35% WFPS without digestate (0.1 mg N_2O-N m^{-2} h^{-1}, Fig. 3, Day 4 in Table A1). The clayey silt showed much larger fluxes than the loamy sand: even at 2 °C, up to 1.5 mg N_2O-N m^{-2} h^{-1} were detected (55% WFPS with 160 kg N ha^{-1}). After shifting the temperature to 15 °C, the same factor combination had a mean flux of 6.2 mg N_2O-N m^{-2} h^{-1} and the other treatment means emitted between 1.0 and 3.0 mg N_2O-N m^{-2} h^{-1} with the exception of incubations with 35% WFPS, where fluxes were smaller. The sand showed weak N_2O emissions, independent of temperature and WFPS as well as the amount of BD application. In contrast, the emissions of the clayey silt increased with temperature and were highest with intermediate WFPS and amount of BD, i.e. 55% and 160 kg N ha^{-1}, respectively. Surprisingly, at 15 °C, increasing the amount of BD up to 320 kg N ha^{-1} did not increase the observed N_2O efflux; rather it decreased the efflux significantly ($p < 0.05$, Tuckey's HSD) at 55% and also, but not significantly, at 75% WFPS (Fig. 3, Table A1). However, this effect was not noticed at 35% WFPS due to generally low emissions at this moisture level. According to the linear mixed model for N_2O fluxes in aerobic conditions, WFPS, amount of digestate, temperature, NO_3^- 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 and 320 kg N ha^{-1} (11.7 mg N_2O-N m^{-2} h^{-1}). The same soil showed a tendency of decreasing N_2O fluxes with increasing WFPS. Fluxes were largest with an amendment of 160 kg N ha^{-1}. 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 A1). So, the anaerobic headspace induced a change only in the loamy sand by increasing emissions.

3.3 N_2 fluxes

From the loamy sand, no N_2 fluxes were detected at 2 °C under He-O_2-atmosphere (Fig. 4, Day 2 in Table A2). Under the same conditions, the clayey silt showed mean fluxes from zero (all incubations with 35% WFPS) to 1.4 mg N_2 m^{-2} h^{-1} (all incubations with 75% WFPS). After increasing the temperature to 15 °C, again, the sandy loam released mostly negligible amounts of N_2, except for 0.5 mg m^{-2} h^{-1} with 55% WFPS and 320 kg N ha^{-1} (Fig. 4, Day 4 in Table A2). In contrast, up to 3.8 mg N_2 m^{-2} h^{-1} (75% WFPS with 160 kg N ha^{-1}) were detected in the clayey silt, where, however, also no fluxes were detected in all incubations with 35% WFPS. Put simply, temperature had a small effect on N_2 emissions from the sandy loam, but WFPS and amount of BD showed no consistent influence. In contrast, the clayey silt presented clearly increasing emissions with increasing temperature, WFPS and also with the application of BD, where a raise from 160 up to 320 kg N ha^{-1} at 15 °C, however, resulted in slightly, but not significantly ($p > 0.05$, Tuckey's HSD), decreased fluxes (Fig. 4, Table A2). The summary of the linear mixed model for N_2 fluxes under aerobic conditions revealed that soil type, WFPS and N_2O flux had significant ($p < 0.001$) effects on N_2 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 from 3.3 (35% WFPS without N) to 35.1 mg N_2 m^{-2} h^{-1} (55% with 320 kg N ha^{-1}), where the fluxes from amended treatments were always higher than fluxes from the unamended ones (Fig. 2, Day 5 in Table A2). For the clayey silt, compared with aerobic atmosphere, mean fluxes increased slightly to 1.9 mg N_2 m^{-2} h^{-1} in unamended treatments and more remarkably to 9.3 mg N_2 m^{-2} h^{-1} in amended ones, still not reaching the amounts observed for the sandy loam. This implies that the N_2 emissions were increased from both soils under anaerobic headspace conditions, but the loamy sand exhibited a much more intense reaction.

3.4 $N_2/(N_2 + N_2O\text{-}N)$ product ratio

No clear trend of the product ratio of $N_2/(N_2 + N_2O\text{-}N)$ was found for incubations of the loamy sand. However, there was a clear distinction of the ratios for this soil under aerobic 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, where the highest ratios (up to 0.8) were found in treatments without digestate and at least 55% WFPS. The digestate-amended treatments showed mostly ratios around or above 0.5, with exception of the 35%

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WFPS treatments, which had ratios close to zero. According to the linear mixed model, the product ratio under aerobic conditions was affected significantly ($p < 0.001$) by soil type, amount of digestate and temperature (Table 5).

3.5 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 the loamy sand compared with the clayey silt (Table A3). In general, the mean fluxes from the loamy sand increased with the amount of digestate from 8.3 to 57.6 (aerobic atmosphere at 2°C), from 34.0 to 168.7 (aerobic at 15 °C) and from 11.2 to 87.9 mg CO_2-C m^{-2} h^{-1} (anaerobic at 15°C), but showed no obvious pattern with WFPS. Although the mean fluxes from the clayey silt were also always smallest in the unamended treatments, there was no clear trend of fluxes with the amount of amendment. There was a slight trend of decreasing fluxes with increasing WFPS 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 and temperature had significant ($p < 0.001$) effects (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

The overall N_2O fluxes corresponded well with those from other studies with similar incubation conditions and application rates of BD in terms of N ha^{-1} (Severin et al., 2015; Senbayram et al., 2012; Köster et al., 2015). However, the latter studies assumed a distribution of BD into soil by a cultivator, which implies a smaller concentration of BD compared to its occurrence in injection slits. 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 controllers of N_2O generation (Maag and Vinther, 1999), showed significant influences. Both are physical (by gas diffusion) and biological (by Q_{10} and consequently O_2 consumption by respiration) proxies for O_2 availability, respectively (Maag and Vinther, 1999; Ball, 2013). Accordingly, the CO_2 flux (as respiration product of O_2) generally increased with temperature and was also identified as significant by regression selection.

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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 A1) 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 five days of their incubation. Although the amount of BD in terms of applied N (250 kg ha^{-1}) was comparable, Köster 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), most likely due to the direct influence of soil structure on diffusivity and, thus, the supply with O_2 (Balaine et al. 2016; Butterbach-Bahl et al. 2013). N_2O flux showed also a significant effect during regression selection for N_2. N_2O is the direct precursor of N_2 in many N_2-producing processes and, hence, the flux of the latter depends on the availability of the former. However, temperature showed no significant effect.

The $N_2/(N_2+N_2O)$ 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 the loamy sand to generally adverse conditions for the formation of N_2O and N_2. Contrary, the influence of WFPS apparently mirrored favourable conditions in the clayey silt (Table 5). Simultaneously, with increasing WFPS, the reduction of N_2O accelerates as an alternative electron acceptor under reduced O_2 supply (Benckiser et al., 2015). Nevertheless, a sufficient soil moisture is required to supply NH_4^+, NO_3^- and DOC for microbial activity (Blagodatsky and Smith, 2012) and may be the reason why no or rather small fluxes of the investigated gaseous N species were generally found in our treatments with 35% WFPS.

In our study, one treatment (clayey silt, 55% WFPS, 160 kg N ha^{-1}) showed exceptionally large mean N_2O fluxes of up to 7.1 mg N m^{-2} h^{-1} (Fig. 3, Table A1). This could be evidence that injection of such moderate amounts may favour much larger losses of N_2O compared to an even distribution of BD in soils due to larger substrate concentration in injection slits. However, with higher amendments (i.e. 320 kg N ha^{-1}), we observed surprisingly partially significant ($p < 0.05$, Tuckey's HSD) reductions of N_2O and a decreasing tendency of N_2 emissions (Table A1, Table A2). 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 reason for the rather smaller emissions of high amended (320 kg N ha^{-1}) treatments might be the inhibitory effect of NH_3 on nitrification, where Anthonisen et al. (1976) found an inhibition by concentrations from 0.1 to 150 mg NH_3 L^{-1}. Since the application rate in the treatments with 320 kg N ha^{-1} amounted to approximately 500 mg NH_4^+ (kg soil)$^{-1}$ (Fig. 3) and, thus, relative high concentrations of NH_3 in the rather alkaline milieu of BD (Möller and Müller, 2012), we consider this inhibitory effect as an argument for the missing increase of N_2O and N_2. Additionally, the amount of NH_4^+ fixed as NH_3 by soil organic matter increases with pH and, moreover, this fixed NH_3 is not readily extractable by the KCl method we have applied

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(Kissel et al., 2008). This is consistent with the observation of generally higher N_2O and N_2 fluxes from the clayey silt since clay increases the sorption capacity of soils for NH_4^+ and may, thus, reduce the inhibitory effect on nitrification (Kissel et al., 2008).

An increasing application of BD tended also to decrease the $N_2/(N_2+N_2O)$ ratio, but this effect was also not significant ($p > 0.05$, Tuckey's HSD). In general, NO_2^- and NO_3^- from oxidation of applied NH_4^+ are preferably reduced compared to N_2O during nitrifier-denitrification and denitrification, respectively (Benckiser et al., 2015). Consequently, the share of N_2O builds up with the supply of BD.

The inhibitory effect is in line with the strong influence of NO_3^- content of the soils after incubation (Table 5), suggesting a dominant role of denitrifying processes in the generation of N_2O (Butterbach-Bahl et al., 2013). Indeed, 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). Notably, in contrast to the clayey silt, no or negligible concentrations of NO_3^- were found in all treatments with loamy sand. Although we have not determined NO_2^-, we speculate that it was a substantial source for reduction by nitrifier denitrification, especially during the anaerobic headspace conditions at the end of the incubation. Actually, high NH_4^+ loads in conjunction with alkaline conditions are typical for BD (Möller and Müller, 2012) favour NO_2^- accumulation and may be the reason for the relatively small NO_3^- recovery in both soils (van Cleemput and Samater, 1995).

4.2 Differentiated effects on N_2O and N_2 fluxes controlled by diffusivity

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 denitrifying processes 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; Letey et al., 1980; Ball, 2013). Soils with a coarser texture like the loamy sand have a higher proportion of macro-pores and thus a higher saturated conductivity and gas diffusion compared with fine textured soils like the clayey silt we used (Groffman and Tiedje, 1991). This let us expect conditions that are more favourable for N_2O and N_2 generation in the latter due to inferior 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.

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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 electron acceptor (Parkin and Tiedje, 1984). We acknowledge e.g. DNRA and anammox as possible additional sources of N_2O and N_2, respectively, under these conditions, but we were not able to quantify their contribution. The anaerobic headspace induced a considerable increase of N_2O fluxes in the loamy sand, but not in the clayey silt. Concurrently, the N_2 fluxes increased in both soils, but pronounced, 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 reasonable establishment of anaerobic microsites, while the clayey silt ensured at least a moderate gas diffusion to maintain hypo-aerobic conditions. However, the large production rates indicate that also the loamy sand harboured the necessary microbial community able to generate N_2 as soon as the atmospheric conditions become favourable. In general, only N_2O fluxes from treatments with negligible fluxes during the previous aerobic period increased under anaerobic conditions. This included all treatments with loamy sand and the highly amended clayey silt with 35% WFPS (Fig. 3, Table A1). At the same time, there was a reduction of N_2O fluxes in all other clayey silt treatments. However, when we take a closer look at the simultaneous changes of N_2 fluxes after atmosphere change, virtually all of these treatments showed increased rates. Hence, there was an enhanced reduction of N_2O to N_2, which is reflected in the increased $N_2/(N_2 + N_2O)$ 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 additionally by small NO_3^- availability (Fig. 2) and a high availability of C (Table 4), which promoted the reduction of N_2O to N_2 (Benckiser et al., 2015). Alternatively, the much smaller increase of N_2 fluxes from the clayey silt could have resulted from depleted mineral N stocks (NO_3^- and NH_4^+) due to the previous gaseous N losses during the course of incubation. 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 160 kg N ha^{-1} and 55% WFPS, which was roughly 3.5% of the calculated NH_4^+-N applied with BD (Fig. 2). Thus, we found no evidence for any shortage of substrate in the clayey silt during the subsequent anaerobic headspace conditions. On the other hand, the $N_2/(N_2+N_2O)$ 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), which point to still reasonable stocks of NO_3^- in the latter (Benckiser et al., 2015). 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 supposed as the main reason for the differing N_2O and N_2 fluxes.

In interaction with soil diffusivity, also respiration affects the aerobicity of a soil matrix by concurrent consumption and formation of O_2 and CO_2, respectively. Similarly to N_2O and N_2 generation by denitrifying processes, respiration depends on the microbial availability of carbon as well. Although anaerobic digestion reduces readily degradable organic matter in BD, a 'labile' fraction usually remains, but the biodegradability of the respective residual organic carbon is variable, depending on the origin of BD (Askri et al., 2015). However, DOC could be used as an approximate indicator for microbial availability of carbon, though not all DOC might be readily degradable (Cook and Allan, 1992). Generally, the DOC contents after our incubation increased with application rate of BD (Table 4), but the DOC contents were always smaller in the clayey silt both in the not amended and especially in the amended treatments. This might reflect a stronger sorption of C and thus a lower availability for respiration in the clayey silt compared to the loamy sand (Kaiser and Guggenberger, 2000). If we compare the DOC recoveries with the cumulated flux rates of CO_2 over the incubation cycle, 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 processes (Reddy et al., 1982). Moreover, as increased DOC enhanced respiration (Table A3), it consequently affected O_2 consumption and, thus, also the emergence of anaerobic microsites (Azam et al., 2002), preferably in the highly amended treatments. Although CO_2 fluxes were mostly higher in the treatments with 320 kg compared to 160 kg N ha^{-1}, this behaviour was not generally reflected in the emissions of N_2O and N_2 which might be a result of the inhibitory effect of high NH_4^+ loads on nitrification (see chapter 4.1). However, the $N_2/(N_2O+N_2)$ ratios implied a tendency of fostered N_2O reduction due to a shortage of alternative electron acceptors like O_2 in the highly amended treatments. Additionally, temperature influenced indirectly the aerobic status of the soils due to increased microbial activity (Q_{10}) and, thus, respiration (Maag and Vinther, 1999).

4.3 No indications for BD induced short-term priming effect

We further checked for a short-term priming effect after amendment with BD as suggested recently by Coban et al. (2015). After balancing cumulated net CO_2-C-fluxes (difference between amended and unamended treatments) against the calculated DOC-C application with BD for the period of aerobic headspace, we found no evidence for a short-term priming effect. In the loamy sand with 160 kg N ha^{-1}, between 76% (35% WFPS) and 103% (75% WFPS) of the DOC-C had been respired (data not shown). In the respective treatments with 320 kg N ha^{-1}, the CO_2-C losses ranged from 47% (35% WFPS) to 76% (75% WFPS). By contrast, only between 11% (320 kg N ha^{-}

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^{1}) and 42% (160 kg N ha^{-1}) has been respired in the clayey silt (both at 55% WFPS). However, if one would consider the period after BD application for a longer time than we, the light loamy sand may be vulnerable for C losses after BD application than the claggy clayey silt.

5 Conclusions

As hypothesised, N_2O and N_2 emissions as well as the $N_2/(N_2O+N_2)$ ratio increased with WFPS and temperature, most probably due to restricted supply and enhanced consumption, respectively, 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, respectively, on nitrification, which is found typically in biogas digestates. However, the $N_2/(N_2O+N_2)$ ratio tended to decrease with application rate as supposed, probably due to a copious supply with NO_2^- and NO_3^- from oxidised BD-NH_4^+. Confirming our third hypothesis, the fine textured clayey silt induced higher 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 injection of BD. However, the loamy sand showed a large potential for N_2 formation under anaerobic headspace conditions, indicating the occurrence of an appropriate denitrifying community. In summary, the risk of N_2O and N_2 losses even after injection of relatively large amounts of BD seems to be small for dry to wet sandy soils and acceptable when regarding simultaneously reduced NH_3 emissions for dry silty soils. However, further investigations are needed in regard to study different types of soil and BD, the duration of the observed effects and their reliability for field conditions.

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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

Table 2: Characteristics of both soils. Texture and mean values with standard deviations (in brackets) 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 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 TitraMaster85, 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 analyser “CFA-SAN”, Skalar Analytical B.V., the Netherlands, performed according to ISO 14256

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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 (cf. Fig. 1). The factors were combined by (1) soil (loamy sand: LS, clayey silt: CS), (2) amount (kg) of applied N from digestate per ha and (3) WFPS (%).

Week	Factor combination 1	Factor combination 2
1	LS - 0 N - 35%	LS - 0 N - 55%
2	LS - 0 N - 75%	LS - 160 N - 35%
3	LS - 160 N - 55%	LS - 160 N - 75%
4	LS - 320 N - 35%	LS - 320 N - 55%
5	LS - 320 N - 75%	CS - 0 N - 35%
6	CS - 0 N - 55%	CS - 0 N - 75%
7	CS - 160 N - 35%	CS - 160 N - 55%
8	CS - 160 N - 75%	CS - 320 N - 35%
9	CS - 320 N - 55%	CS - 320 N - 75%

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Table 4: Mean recovered DOC values from soils after incubation with standard deviations in brackets 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
0	35	41.4 (2.7)	18.9 (1.1)
	55	38.6 (3.1)	19.8 (1.4)
	75	43.7 (1.4)	19.0 (1.8)
160	35	197.4 (20.7)	n.a.
	55	190.5 (19.3)	68.3 (12.7)
	75	362.2 (40.0)	63.2 (9.6)
320	35	316.8 (25.3)	358.1 (26.3)
	55	312.5 (14.3)	94.8 (13.6)
	75	500.1 (33.4)	105.9 (14.8)

n.a.: data not available

Table 5: Significant fixed effects with *P*-values (*t*-test) of the linear mixed models for estimated fluxes of N_2, N_2O, $N_2/(N_2+N_2O)$ product ratio and CO_2 in aerobic He-O_2 atmosphere with soil type, water-filled pore space (WFPS), amount of digestate, temperature, NO_3^- content of soil after incubation as well as fluxes of N_2O and CO_2 as possible independent variables. The respective vessels in the incubation system were set as random effect, which was always significant.

Response	Fixed effects						
	Soil type	WFPS	Digestate amount	Temperature	NO_3^- soil post	N_2O flux	CO_2 flux
N_2	< 0.001	< 0.001	†	†	†	< 0.001	†
N_2O	†	< 0.001	< 0.001	< 0.001	< 0.001	*	< 0.001
$N_2/(N_2+N_2O)$	< 0.001	< 0.001	†	†	†	*	†
CO_2	<0.001	†	<0.001	<0.001	†	†	*

† Variable eliminated during stepwise regression selection

* Variable was not included into original regression

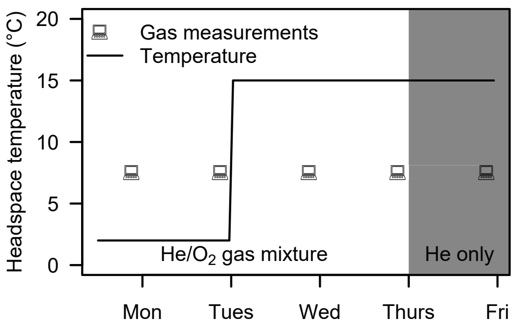
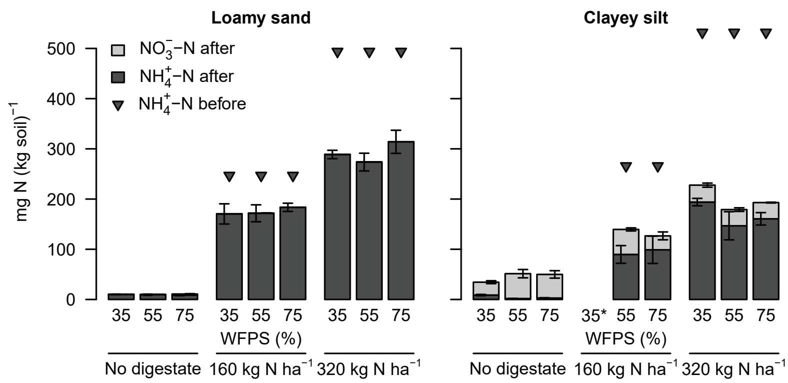


Figure 1: Course of incubation and gas measurements with respect to atmosphere and temperature of the headspace after two days of pre-incubation at 2 °C in He/O_2 gas mixture. Gas concentrations of the headspace were determined on five consecutive days, i.e. Monday to Friday in the morning. After the first two 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.



563 **Fig. 2: Ammonium and nitrate contents from loamy sand and clayey silt after incubation with different water-filled pore spaces (WFPS, %) and amounts of digestate (kg N ha^{-1}). 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, calculated amounts of ammonium applied with biogas digestate are shown by triangles. One treatment (*) was omitted from all analyses due to technical reasons.**

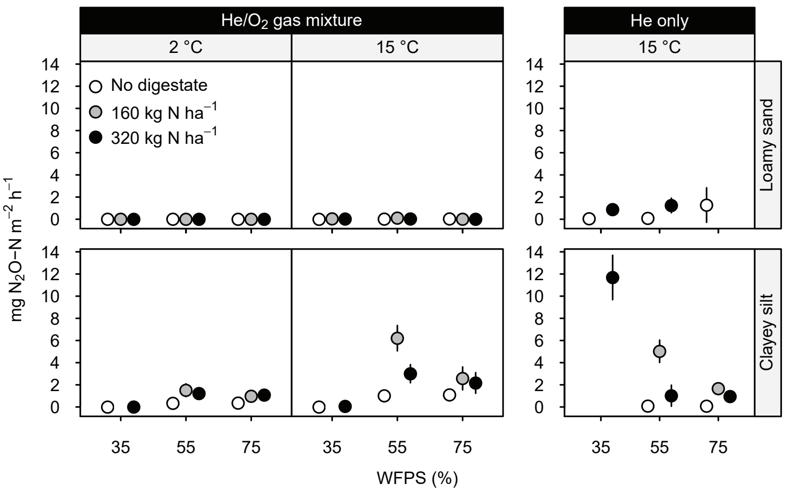


Fig. 3: Mean N_2O fluxes (mg N m^{-2} h^{-1}) from a loamy sand and a clayey silt incubated under different water-filled pore spaces (WFPS, %) with different amounts of digestate (kg N ha^{-1}). Shown are values measured on the second and fourth day of incubation (i.e. after two days with 2 and 15 °C, respectively) under aerobic He-O_2 atmosphere as well as day five (15 °C) under anaerobic He atmosphere. Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. Under 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 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, where mean flux increased strongly in the treatment with 320 kg digestate-N ha^{-1}.

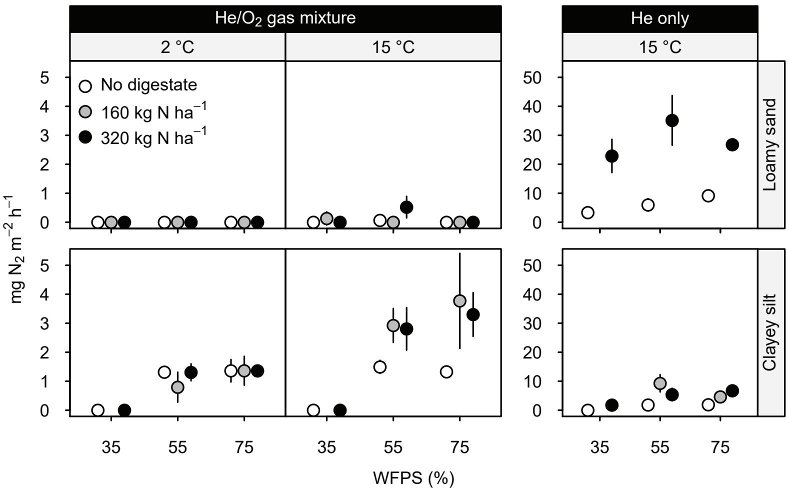


Fig. 4: Mean N_2 fluxes (mg m^{-2} h^{-1}) from a loamy sand and a clayey silt incubated under different water-filled pore spaces (WFPS, %) with different amounts of digestate (kg N ha^{-1}). Shown are values measured on the second and fourth day of incubation (i.e. after two days with 2 and 15 °C, respectively) under aerobic He-O_2 atmosphere as well as day five (15 °C) under anaerobic He atmosphere. Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. Under 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 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.

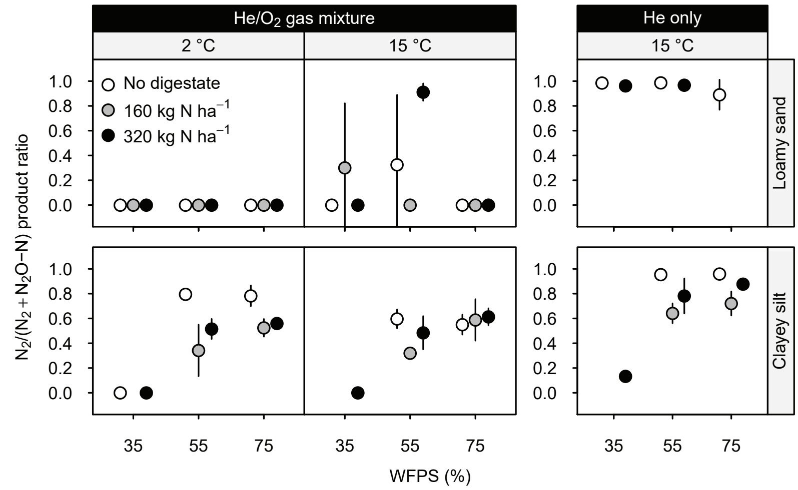
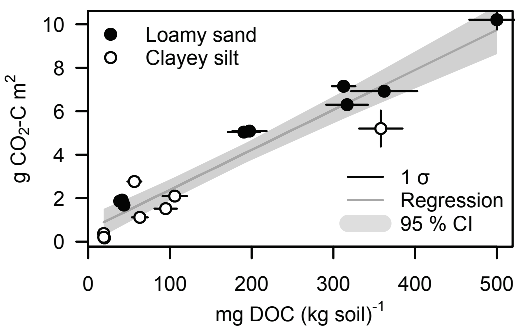


Fig. 5: Mean $N_2/(N_2 + N_2O\text{-}N)$ product ratio from a loamy sand and a clayey silt incubated under different water-filled pore spaces (WFPS, %) with different amounts of digestate (kg N ha^{-1}). Shown are values measured on the second and fourth day of incubation (i.e. after two days with 2 and 15 °C, respectively) under aerobic He-O_2 atmosphere as well as day five (15 °C) under anaerobic He atmosphere. 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, irrespectively 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.



595 **Fig. 6: Regression between DOC recoveries (mg per 100 g soil) after the incubation and the respective cumulated CO_2**
596 **emissions (g C m^{-2}) during the period of aerobic headspace with their standard deviations and confidence interval**
597 **(95%). If error bars are not visible, they are smaller than the symbols of the means. Both soils showed increasing**
598 **emissions with increasing soil DOC contents as well a good regression fit (R^2 = 0.91, p < 0.001).**

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Table A1: 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 (WFPS, %), amounts of digestate (kg N ha^{-1}) as well as different temperature regimes (°C) under aerobic (He-O_2) and anaerobic (He) atmosphere. 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 (%)	kg N ha^{-1}	mg N_2O-N m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He-O_2	2	35	0	0 ± 0	0 ± 0 c
1	He-O_2	2	35	160	0 ± 0	NA
1	He-O_2	2	35	320	0 ± 0	0 ± 0 c
1	He-O_2	2	55	0	0 ± 0	0.3 ± 0.1 c
1	He-O_2	2	55	160	0 ± 0	1.7 ± 0.4 a
1	He-O_2	2	55	320	0 ± 0	1.1 ± 0.1 b
1	He-O_2	2	75	0	0 ± 0	0.4 ± 0.1 c
1	He-O_2	2	75	160	0 ± 0	1 ± 0.1 b
1	He-O_2	2	75	320	0 ± 0	1 ± 0.2 b
2	He-O_2	2	35	0	0 ± 0	0 ± 0 d
2	He-O_2	2	35	160	0 ± 0	NA
2	He-O_2	2	35	320	0 ± 0	0 ± 0 cd
2	He-O_2	2	55	0	0 ± 0	0.3 ± 0.1 bc
2	He-O_2	2	55	160	0 ± 0	1.5 ± 0.6 a
2	He-O_2	2	55	320	0 ± 0	1.2 ± 0.2 a
2	He-O_2	2	75	0	0 ± 0	0.4 ± 0.1 bc
2	He-O_2	2	75	160	0 ± 0	1 ± 0.1 ab
2	He-O_2	2	75	320	0 ± 0	1.1 ± 0.2 a
3	He-O_2	15	35	0	0 ± 0 cd	0 ± 0 c
3	He-O_2	15	35	160	0 ± 0 abc	NA
3	He-O_2	15	35	320	0 ± 0 ab	0 ± 0 c
3	He-O_2	15	55	0	0 ± 0 bcd	0.8 ± 0.2 c
3	He-O_2	15	55	160	0 ± 0 bcd	7.1 ± 0.9 a
3	He-O_2	15	55	320	0 ± 0 a	3.5 ± 0.7 b
3	He-O_2	15	75	0	0 ± 0 ab	0.8 ± 0.2 c
3	He-O_2	15	75	160	0 ± 0 d	3.2 ± 0.7 b
3	He-O_2	15	75	320	0 ± 0 cd	3 ± 0.9 b
4	He-O_2	15	35	0	0 ± 0 b	0 ± 0 c
4	He-O_2	15	35	160	0 ± 0 ab	NA
4	He-O_2	15	35	320	0 ± 0 ab	0.1 ± 0.1 c
4	He-O_2	15	55	0	0 ± 0 b	1 ± 0.2 bc
4	He-O_2	15	55	160	0.1 ± 0.1 a	6.2 ± 1.1 a
4	He-O_2	15	55	320	0 ± 0 ab	3 ± 0.8 b

4	He-O_2	15	75	0	0 ± 0 ab	1.1 ± 0.3 bc
4	He-O_2	15	75	160	0 ± 0 b	2.6 ± 1 b
4	He-O_2	15	75	320	0 ± 0 b	2.2 ± 0.9 b
5	He	15	35	0	0.1 ± 0	NA
5	He	15	35	160	NA	NA
5	He	15	35	320	0.9 ± 0.1	11.7 ± 2 a
5	He	15	55	0	0.1 ± 0	0.1 ± 0 c
5	He	15	55	160	NA	5 ± 1 b
5	He	15	55	320	1.2 ± 0.7	1.4 ± 0.8 c
5	He	15	75	0	1.3 ± 1.6	0.1 ± 0 c
5	He	15	75	160	NA	1.7 ± 0.3 c
5	He	15	75	320	NA	1 ± 0.3 c

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Table A2: Mean N_2 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 (WFPS, %), amounts of digestate (kg N ha^{-1}) as well as different temperature regimes (°C) under aerobic (He-O_2) and anaerobic (He) atmosphere. 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 (%)	kg N ha^{-1}	mg N_2 m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He-O_2	2	35	0	0 ± 0	0 ± 0 bc
1	He-O_2	2	35	160	0 ± 0	NA
1	He-O_2	2	35	320	0 ± 0	0.1 ± 0.1 bc
1	He-O_2	2	55	0	0 ± 0	1.5 ± 0.3 a
1	He-O_2	2	55	160	0 ± 0	1.5 ± 0.3 a
1	He-O_2	2	55	320	0 ± 0	1.5 ± 0 a
1	He-O_2	2	75	0	0 ± 0	1.2 ± 1.1 a
1	He-O_2	2	75	160	0 ± 0	1.5 ± 0.2 a
1	He-O_2	2	75	320	0 ± 0	1.7 ± 0.4 a
2	He-O_2	2	35	0	0 ± 0	0 ± 0 c
2	He-O_2	2	35	160	0 ± 0	NA
2	He-O_2	2	35	320	0 ± 0	0 ± 0 c
2	He-O_2	2	55	0	0 ± 0	1.3 ± 0.1 a
2	He-O_2	2	55	160	0 ± 0	0.8 ± 0.5 b
2	He-O_2	2	55	320	0 ± 0	1.3 ± 0.3 a
2	He-O_2	2	75	0	0 ± 0	1.4 ± 0.4 a
2	He-O_2	2	75	160	0 ± 0	1.4 ± 0.5 a
2	He-O_2	2	75	320	0 ± 0	1.4 ± 0.1 a
3	He-O_2	15	35	0	0 ± 0 b	0 ± 0 e
3	He-O_2	15	35	160	0 ± 0 b	NA
3	He-O_2	15	35	320	0.1 ± 0.1 ab	0 ± 0 e
3	He-O_2	15	55	0	0 ± 0 b	1.8 ± 0.3 cd
3	He-O_2	15	55	160	0 ± 0 b	2.3 ± 0.4 bc
3	He-O_2	15	55	320	0 ± 0 b	2.5 ± 0.2 ab
3	He-O_2	15	75	0	0.2 ± 0.3 a	1.5 ± 0.2 d
3	He-O_2	15	75	160	0 ± 0 b	3 ± 0.9 a
3	He-O_2	15	75	320	0 ± 0 b	2.6 ± 0.8 ab
4	He-O_2	15	35	0	0 ± 0 b	0 ± 0 c
4	He-O_2	15	35	160	0.1 ± 0.2 b	NA
4	He-O_2	15	35	320	0 ± 0 b	0 ± 0 c
4	He-O_2	15	55	0	0.1 ± 0.1 b	1.5 ± 0.2 b
4	He-O_2	15	55	160	0 ± 0 b	2.9 ± 0.6 a
4	He-O_2	15	55	320	0.5 ± 0.4 a	2.8 ± 0.7 a

4	He-O_2	15	75	0	0 ± 0 b	1.3 ± 0.2 bc
4	He-O_2	15	75	160	0 ± 0 b	3.8 ± 1.6 a
4	He-O_2	15	75	320	0 ± 0 b	3.3 ± 0.8 a
5	He	15	35	0	3.3 ± 0.4 d	0 ± 0 c
5	He	15	35	160	NA	NA
5	He	15	35	320	22.9 ± 5.7 b	1.8 ± 0.1 c
5	He	15	55	0	6 ± 2.2 cd	1.8 ± 0.2
5	He	15	55	160	NA	9.5 ± 2.7 a
5	He	15	55	320	35.1 ± 8.6 a	5.1 ± 1.8 bc
5	He	15	75	0	9.2 ± 0.4 c	1.9 ± 0.1 c
5	He	15	75	160	NA	4.8 ± 1.6 bc
5	He	15	75	320	26.8 ± 1.1 b	6.7 ± 0.8 b

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Table A3: 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 (WFPS, %), amounts of digestate (kg N ha^{-1}) as well as different temperature regimes (°C) under aerobic (He-O_2) and anaerobic (He) atmosphere. 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 (%)	kg N ha^{-1}	mg CO_2-C m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He-O_2	2	35	0	6.8 ± 2.4 cd	0 ± 0 c
1	He-O_2	2	35	160	22 ± 3.5 bcd	NA
1	He-O_2	2	35	320	23.3 ± 9.3 bc	22.8 ± 2.8 ab
1	He-O_2	2	55	0	6 ± 0.7 d	4.6 ± 7.9 bc
1	He-O_2	2	55	160	34.4 ± 3.1 b	34.5 ± 11.6 a
1	He-O_2	2	55	320	28 ± 3.2 b	15.9 ± 3.4 abc
1	He-O_2	2	75	0	9.4 ± 1.4 cd	0 ± 0 c
1	He-O_2	2	75	160	37.5 ± 6 b	15.5 ± 12.1 abc
1	He-O_2	2	75	320	68.3 ± 12.1 a	24.5 ± 2.7 a
2	He-O_2	2	35	0	9.8 ± 3.5 c	1.3 ± 1.4 b
2	He-O_2	2	35	160	23 ± 3.9 bc	NA
2	He-O_2	2	35	320	30.9 ± 2.2 b	22.2 ± 2.4 a
2	He-O_2	2	55	0	8.7 ± 1.5 c	0.6 ± 1 b
2	He-O_2	2	55	160	33.4 ± 0.9 b	27.6 ± 12.3 a
2	He-O_2	2	55	320	35.9 ± 2.7 b	14.4 ± 1.9 ab
2	He-O_2	2	75	0	8.3 ± 1.5 c	0 ± 0 b
2	He-O_2	2	75	160	31.9 ± 3 b	13 ± 9.3 ab
2	He-O_2	2	75	320	57.6 ± 14.8 a	18.3 ± 4 a
3	He-O_2	15	35	0	42.5 ± 4.5 c	6.7 ± 0.7 b
3	He-O_2	15	35	160	114.3 ± 12.2 b	NA
3	He-O_2	15	35	320	149.5 ± 9.4 b	130.9 ± 105 a
3	He-O_2	15	55	0	41.3 ± 3.5 c	3.2 ± 0.4 b
3	He-O_2	15	55	160	108.7 ± 10.1 b	57.8 ± 12.2 bc
3	He-O_2	15	55	320	162.1 ± 9.6 b	26.8 ± 0.7 bc
3	He-O_2	15	75	0	44.1 ± 9.8 c	3.2 ± 0.7 b
3	He-O_2	15	75	160	150.4 ± 19 b	26.4 ± 11.8 bc
3	He-O_2	15	75	320	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	160	114.3 ± 6.4 b	NA
4	He-O_2	15	35	320	156.9 ± 15.4 a	65.7 ± 2.2 a
4	He-O_2	15	55	0	48 ± 3.4 c	4.2 ± 0.2 d
4	He-O_2	15	55	160	109 ± 14.4 b	51.2 ± 15.1 ab
4	He-O_2	15	55	320	177.7 ± 7.5 a	26.6 ± 2.3 cd

4	He-O_2	15	75	0	34 ± 7.8 c	6.7 ± 4 d
4	He-O_2	15	75	160	168.7 ± 0.4 a	22.1 ± 14.8 cd
4	He-O_2	15	75	320	166.3 ± 23.1 a	34.1 ± 5.7 bc
5	He	15	35	0	11.2 ± 0.6 d	NA
5	He	15	35	160	54.8 ± 9.3 c	NA
5	He	15	35	320	149.3 ± 3.9 a	45.8 ± 2.1 a
5	He	15	55	0	13.6 ± 1.9 d	3.4 ± 0.6 c
5	He	15	55	160	55.2 ± 4.4 bc	32 ± 11.4 ab
5	He	15	55	320	164.5 ± 3.5 a	15.2 ± 10.7 bc
5	He	15	75	0	20.9 ± 2.3 d	3.6 ± 0.1 c
5	He	15	75	160	75 ± 7.3 b	20.6 ± 8.5 bc
5	He	15	75	320	NA	26.1 ± 2.6 ab