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

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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 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 cm³]) on the emissions 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 $N_2/(N_2O+N_2)$ ratio depended on soil type and increased with WFPS indicating a crucial role of soil gas diffusivity for the formation of nitrogenous gases in agricultural soils. However, the emissions did not increase with the application rate of BD probably due to an inhibitory effect of the high NH_4^+ content in BD. Our results suggest a larger potential for N_2O formation in the fine-textured clayey silt compared to the coarse loamy sand after applying high concentrations of BD like after injection. However, the loamy sand showed basically a large potential for N_2 formation under anaerobic headspace conditions. Nevertheless, our results show the need for further investigations on the dynamics and the duration of the observed effects and their significance for field conditions.

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 such gaseous losses may imply a significant loss of N from the soil/plant system (Friedl et al., 2016, 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). 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).

Digestion residues (biogas digestate, BD) from biogas plants are used as organic amendment in agriculture. But, compared to undigested amendments, digestion results in an increased pH, a higher proportion of ammonium

(NH_4^+) and a narrowed C to N of the BD (Möller and Müller, 2012). This 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 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 (Wulf et al., 2002; Velthof and Mosquera, 2011). On the one hand, high NH_4^+ concentrations in the injection band promote nitrification, which is a significantly O_2 consuming process releasing N_2O (Christensen and Rowe, 1984). On the other hand, increased amounts of C in the injection band also promote respiration and, thus, additionally deplete the 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 may promote also 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, carbon (C) availability as well as on temperature, most of which are enhanced by anaerobic 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; Uchida et al., 2008; Maag and Vinther, 1999). In general, fine textured soils with higher clay contents exhibit a lower gas diffusivity compared to coarse textured soils, which result regularly in higher denitrification activity in the former with higher N_2O emission rates, but also a higher probability for the consecutive reduction to N_2 (Senbayram et al., 2014; Gu et al., 2013; Ball, 2013).

There is a general lack of knowledge about the effects of high BD concentrations 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 ($He\text{-}O_2$) incubation technique (Butterbach-Bahl et al., 2002) in a laboratory experiment to evaluate the effect of above suggested factors 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), but with the restriction that inorganic sources could not be differentiated. We hypothesised that (1) N_2O and N_2 emissions will increase with WFPS, (2) this gaseous N losses will also be

affected by BD application rate, i.e. the hypothetical concentration resulting from injection, 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 adjusted to 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 7.2 cm, height 6.1 cm, volume 250 cm^3) 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 collected 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 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 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. The respective quantities of water were calculated based on the bulk density, an assumed particle density of 2.65 g cm^{-1} and 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 that in injection bands. 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 m (narrow injection bands with low BD concentration, LOBD) and 0.30 m (wide injection bands with high BD concentration, HIBD), which are common ranges used by injection machinery and which correspond to 17.6 and 25.3 mL BD, respectively, 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.

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 by diffusion, including a restricted N_2 production by decreased microbial activity. 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 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 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 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):

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

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) between inlet and outlet of a vessel.

To enhance the tightness against atmospheric N_2 contamination, the lids of the incubation vessels were purged permanently with helium. We obtained blank values by inserting aluminium blocks into the vessels before each measurement cycle. Since this blank values were usually steady with means of 1.9 (1σ = 0.9) ppm N_2, 349.6 (1σ = 11.4) ppb N_2O and 353.9 (1σ = 13.5) ppm CO_2, we suggest that the vessels were tight. Derived from the blank values, lowest detectable fluxes were on average 0.427 (1σ = 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}. 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 (soil to extract ratio 1:4, standardised extraction method of the commissioned laboratory at Leibniz Centre for Agricultural Landscape Research e. V.) 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 measuring days under 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

148 headspace, some gas concentration data are missing due to logistical reasons. For the loamy sand, this affects all
149 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
150 clayey silt the treatment 35% WFPS without amendment (N_2O and CO_2).
151 To account for repeated measurement of vessels, linear mixed effect models were applied with package
152 'lmerTest' version 2.0-33 (Kuznetsova et al., 2016) for fluxes of each gas type. The three pseudo-replicated
153 fluxes from the N_2 measurements of each vessel were averaged for each day to obtain the same number of
154 observations as for N_2O and CO_2 fluxes. The fixed structure of models included soil type, WFPS, amount of
155 digestate, temperature, NO_3^- and DOC contents after incubation as well as the fluxes of N_2O (in the model for
156 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
157 high autocorrelation with the amount of BD applied. The individual soil cores in the vessels were set as random
158 effect (nested within the week of incubation and with allowance for a variable slope of the effect each day) with
159 regard to lack of independence of consecutive measurements. The model responses for N_2, N_2O and CO_2 where
160 log transformed (ln[value + 1]) since gas fluxes from soils usually show lognormal distributions (Kaiser et al.,
161 1998). The function 'step' was used for automatic backward selection of models based on AIC (Akaike's 'An
162 Information Criterion'). The skewness (γ) was calculated with R package 'moments' version 0.14 (Komsta and
163 Novomestky, 2015) to check residuals for normal distribution and $|\gamma| \leq 2$ was assumed as appropriate (West et
164 al., 1995). For mixed effects models, *p*-values of the ANOVA (type 2) were calculated based on Satterthwaite's
165 approximation)
166 Cumulated gas fluxes were estimated with a bootstrap method using function 'auc.mc' of R package 'flux'
167 version 0.3-0 (Jurasinski et al., 2014) for the R statistical software version 3.2.3 (R Core Team, 2015). In short,
168 the fluxes for the period of aerobic headspace were cumulated in 100 iterations, while for each run 2 fluxes were
169 omitted randomly. Then, the resulting data were used to calculate means and standard deviations.

170 **3 Results**

171 **3.1 Soil NH_4^+, NO_3^- and DOC contents**

172 The calculated application of NH_4^+-N from BD per kg soil approximated for the sandy loam 247.0 mg (LOBD)
173 and 494.0 mg (HIBD), and for the clayey silt 266.0 mg (LOBD) and 532.0 mg (HIBD). The NO_3^- content of BD
174 was negligible. In general, the NH_4^+ content of the soils after incubation increased with digestate application
175 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 the loamy sand compared with the clayey silt (Table A1). In general, the mean fluxes from the 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 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 as well as 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 ($p < 0.05$, Tuckey'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 induced a change only 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 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 clearly increasing fluxes with increasing temperature and WFPS. However, the application raise from LOBD up to HIBD at 15 °C, resulted in slightly, but not significantly ($p > 0.05$, Tuckey'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 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). The mean fluxes from the 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 $N_2/(N_2 + N_2O\text{-}N)$ 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% 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).

225 # 4 Discussion

226 ## 4.1 Increased BD application rate did not increase N_2O and N_2 losses probably due to inhibitory effect
227 of high NH_4^+ concentrations

228 The overall N_2O fluxes corresponded well with those from other studies with similar incubation conditions and
229 application rates of BD in terms of N ha^{-1} (Severin et al., 2015; Senbayram et al., 2012; Köster et al., 2015).
230 However, the latter studies assumed a distribution of BD into soil by a cultivator, which implies a smaller
231 concentration of BD compared to its occurrence in injection slits. Although we observed differences in N_2O
232 emissions between soils, soil type was not confirmed as a significant effect. Nevertheless, WFPS and
233 temperature, which are well known controllers of N_2O generation (Maag and Vinther, 1999), showed significant
234 influences. Both are physical (by gas diffusion) and biological (by increased metabolic activity and consequently
235 increased O_2 consumption by respiration) drivers for O_2 availability, respectively (Maag and Vinther, 1999; Ball,
236 2013). Accordingly, the CO_2 flux (resulting from respiration of O_2) generally increased with temperature and
237 was also identified as significant by regression selection.
238 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
239 considerably smaller than the mean fluxes of up to 13.0 mg m^{-2} h^{-1} observed by Köster et al. (2015) during the
240 first five days of their incubation. Although the amount of BD in terms of applied N (250 kg ha^{-1}) was
241 comparable, Köster et al. (2015) used a higher WFPS of 90%, which may have increased the generation of N_2. In
242 contrast to N_2O emission rates, the observed N_2 fluxes depended not only on WFPS, but also on soil type (Table
243 5), most likely due to the direct influence of soil structure on diffusivity and, thus, the supply with O_2 (Balaine et
244 al. 2016; Butterbach-Bahl et al. 2013). N_2O flux showed also a significant effect during regression selection for
245 N_2. N_2O is the direct precursor of N_2 in denitrification and, hence, the flux of the latter depends on the
246 availability of the former. However, temperature showed no significant effect.
247 The $N_2/(N_2+N_2O)$ ratios were significantly determined only by soil type and WFPS: while no clear trend was
248 observable for the loamy sand, there was a pronounced effect in the clayey silt (Fig 4). We attribute the lack of a
249 trend in the loamy sand to generally adverse conditions for the formation of N_2O and N_2. Contrary, the influence
250 of WFPS apparently mirrored favourable conditions in the clayey silt (Table 5). Simultaneously, with increasing
251 WFPS, the reduction of N_2O accelerates as an alternative electron acceptor under reduced O_2 supply (Tiedje,
252 1988). Accordingly, no or rather small fluxes of the investigated gaseous N species were generally found in our
253 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 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 surprisingly partially significant ($p < 0.05$, Tuckey's HSD) reductions of N_2O and a decreasing tendency of N_2 emissions (Table A2, Table 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 reason for the rather smaller emissions of highly amended HIBD treatments might be the inhibitory effect of NH_3 on nitrification. Accordingly, Anthonisen et al. (1976) found an inhibition by concentrations from 0.1 to 150 mg NH_3 L^{-1}. The application rate in the treatments with HIBD amounted to approximately 500 mg NH_4^+-N (kg soil)$^{-1}$ (Fig. 3) which correspond to 25.8 mg NH_3-N (kg soil)$^{-1}$ at 15 °C if we use the pH of the BD and assume that all extractable NH_4^+-N was in solution (Emerson et al., 1975). Hence, we consider this inhibitory effect as the reason for the missing increase of N_2O and N_2. Additionally, due to the increased pH of BD (Möller and Müller, 2012), the amount of NH_4^+ fixed as NH_3 by soil organic matter increases and, moreover, this fixed NH_3 is not readily extractable by the KCl method we have applied (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). However, because we mixed the BD with the soil, we would expect a lower NH_3 fixation in tubular injection slits *in situ*, resulting in probably lower N_2O and N_2 fluxes from clayey soils.

Actually, high NH_4^+ loads in conjunction with an increased pH favour NO_2^- accumulation, because NO_2^- oxidising bacteria are less resilient against high concentrations of NH_3 than NH_3 oxidising bacteria (Anthonisen et al., 1976). This NO_2^- should have protonated then partly to the toxic and unstable HNO_2, which drives biological and chemical production of NO and N_2O for detoxification (Venterea et al., 2015). Although we have not determined 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, indicating 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), calling for taking account of NO measurements in future studies.

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, 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 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 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 such 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 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, when we take a closer look at the simultaneous changes of N_2 fluxes after atmosphere change, virtually all of the respective treatments showed increased rates. Hence,

312 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)
313 and points to intensified reduction of N_2O due to the lack of oxygen (Parkin and Tiedje, 1984). The much larger
314 N_2 fluxes from the loamy sand compared to the clayey silt might have been caused additionally by small NO_3^-
315 availability (Fig. 2) and a high availability of C (Table 4), which promoted the reduction of N_2O to N_2
316 (Senbayram et al., 2012). Further, we found no evidence for any shortage of substrate in the clayey silt during
317 the subsequent anaerobic headspace conditions. However, the cumulated fluxes of both N_2 and N_2O amounted to
318 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
319 was roughly 3.5% of the calculated NH_4^+-N applied with BD (Fig. 2). On the other hand, the $N_2/(N_2+N_2O)$ ratios
320 increased only slightly (Fig. 5) and, in contrast to the loamy sand, there were still significant N_2O fluxes in the
321 clayey silt (Fig. 3), which point to still sufficient stocks of NO_3^- in the latter (Senbayram et al., 2012). In fact, the
322 NO_3^- stock was greater in the clayey silt than in loamy sand after incubation (Fig. 2). Thus, we suggest that the
323 gas fluxes were unaffected by the change to anaerobic headspace in the clayey silt due to already low O_2
324 concentrations as a result of poor diffusivity. In conclusion, distinct gas diffusivities of both soils can be
325 proposed as the main reason for the differing N_2O and N_2 fluxes.
326 In interaction with soil diffusivity, also respiration affects the aerobicity of a soil matrix by concurrent
327 consumption and formation of O_2 and CO_2, respectively. Depending on microbial availability of carbon,
328 respiration could be indicated approximately by DOC, though not all DOC might be readily degradable (Cook
329 and Allan, 1992). Generally, the DOC contents after our incubation increased with application rate of BD (Table
330 4), but the DOC contents were always smaller in the clayey silt. This might reflect a stronger sorption of C and
331 thus a lower availability for respiration in the clayey silt compared to the loamy sand (Kaiser and Guggenberger,
332 2000). If we compare the DOC values with the cumulated flux rates of CO_2 over the period of aerobic
333 headspace, we find a good regression fit ($R^2 = 0.91$, $p < 0.001$) for both soils (Fig. 6) indicating a sufficient
334 availability of C from BD for respiration and, thus, implicitly also for denitrification (Reddy et al., 1982).
335 Moreover, as increased DOC enhanced respiration (Table A1), it consequently affected O_2 consumption and,
336 thus, also the emergence of anaerobic microsites (Azam et al., 2002). Accordingly, there is also a good
337 correlation between cumulated CO_2 and $N_2O + N_2$ fluxes for the same period from the clayey silt ($R^2 = 0.93$, $p =$
338 0.001), when the treatments with 35 % WFPS (which showed virtually no N emissions) are omitted (Fig. 7).
339 However, there was no such a correlation for the loamy sand. This confirms the interactive effect of diffusivity
340 (induced by both the soils and WFPS) and C availability on the emissions of N_2O and N_2, which, nevertheless,
341 interacted with the inhibitory effect of high NH_4^+ loads on nitrification (see chapter 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 two days without a repeated increase later, regardless the amount of applied BD. Thus, we assume a single peak shortly after application holds also true for our incubation as well. We assume also the measurements after only 24 hours of anaerobicity in the headspace as representative for the emission potential since Wang et al. (2011; 2013) showed in similar studies to ours that the emission of N_2 and N_2O peaked within less than 24 hours after switching their headspace from aerobic to anaerobic conditions.

However, as hypothesised, N_2O and N_2 emissions as well as the $N_2/(N_2O+N_2)$ 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, respectively, on nitrification, which are found typically in biogas digestates (BD). Nevertheless, 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 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. Nevertheless, further investigations are needed in regarding the dynamics and the duration of the observed effects and their reliability for field conditions.

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371 **References**

372 Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., and Srinath, E. G.: Inhibition of Nitrification by Ammonia
373 and Nitrous Acid, Journal (Water Pollution Control Federation), 48, 835–852, 1976.
374 Azam, F., Müller, C., Weiske, A., Benckiser, G., and Ottow, J.: Nitrification and denitrification as sources of
375 atmospheric nitrous oxide – role of oxidizable carbon and applied nitrogen, Biology and Fertility of Soils,
376 35, 54–61, doi:10.1007/s00374-001-0441-5, 2002.
377 Balaine, N., Clough, T. J., Beare, M. H., Thomas, S. M., and Meenken, E. D.: Soil Gas Diffusivity Controls N2O
378 and N2 Emissions and their Ratio, Soil Science Society of America Journal, 80, 529–540,
379 doi:10.2136/sssaj2015.09.0350, 2016.
380 Ball, B. C.: Soil structure and greenhouse gas emissions: a synthesis of 20 years of experimentation, Eur J Soil
381 Sci, 64, 357–373, doi:10.1111/ejss.12013, 2013.
382 Blagodatsky, S. and Smith, P.: Soil physics meets soil biology: Towards better mechanistic prediction of
383 greenhouse gas emissions from soil, Soil Biology and Biochemistry, 47, 78–92,
384 doi:10.1016/j.soilbio.2011.12.015, 2012.
385 Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S.: Nitrous oxide
386 emissions from soils: how well do we understand the processes and their controls?, Philosophical
387 Transactions of the Royal Society of London B: Biological Sciences, 368, doi:10.1098/rstb.2013.0122,
388 2013.
389 Butterbach-Bahl, K., Willibald, G., and Papen, H.: Soil core method for direct simultaneous determination of N2
390 and N2O emissions from forest soils, Plant and Soil, 240, 105–116, doi:10.1023/A:1015870518723, 2002.
391 Cameron, K. C., Di, H. J., and Moir, J. L.: Nitrogen losses from the soil/plant system: a review, Ann Appl Biol,
392 162, 145–173, doi:10.1111/aab.12014, 2013.
393 Christensen, J. P. and Rowe, G. T.: Nitrification and oxygen consumption in northwest Atlantic deep-sea
394 sediments, Journal of Marine Research, 42, 1099–1116, doi:10.1357/002224084788520828, 1984.
395 Cook, B. D. and Allan, D. L.: Dissolved organic carbon in old field soils: Total amounts as a measure of
396 available resources for soil mineralization, Soil Biology and Biochemistry, 24, 585–594, doi:10.1016/0038-
397 0717(92)90084-B, 1992.
398 Davidson, E. A. and Kanter, D.: Inventories and scenarios of nitrous oxide emissions, Environmental Research
399 Letters, 9, 105012, 2014.
400 Davidson, E. A., Suddick, E. C., Rice, C. W., and Prokopy, L. S.: More Food, Low Pollution (Mo Fo Lo Po): A
401 Grand Challenge for the 21st Century, Journal of Environmental Quality, 44, doi:10.2134/jeq2015.02.0078,
402 2015.
403 Dell, C. J., Meisinger, J. J., and Beegle, D. B.: Subsurface Application of Manures Slurries for Conservation
404 Tillage and Pasture Soils and Their Impact on the Nitrogen Balance, Journal of Environmental Quality, 40,
405 doi:10.2134/jeq2010.0069, 2011.
406 Eickenscheidt, T., Heinichen, J., Augustin, J., Freibauer, A., and Drösler, M.: Nitrogen mineralization and
407 gaseous nitrogen losses from waterlogged and drained organic soils in a black alder (Alnus glutinosa (L.)
408 Gaertn.) forest, Biogeosciences, 11, 2961–2976, doi:10.5194/bg-11-2961-2014, 2014.

Emerson, K., Russo, R. C., Lund, R. E., and Thurston, R. V.: Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature, Journal of the Fisheries Research Board of Canada, 32, 2379–2383, doi:10.1139/f75-274, 1975.

Firestone, M. K. and Davidson, E. A.: Microbiological basis of NO and N2O production and consumption in soil, in: Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere, Andreae, M. a. S. D. (Ed.), Wiley, Chichester, 7–21, 1989.

Friedl, J., Scheer, C., Rowlings, D. W., McIntosh, H. V., Strazzabosco, A., Warner, D. I., and Grace, P. R.: Denitrification losses from an intensively managed sub-tropical pasture – Impact of soil moisture on the partitioning of N2 and N2O emissions, Soil Biology and Biochemistry, 92, 58–66, doi:10.1016/j.soilbio.2015.09.016, 2016.

Groffman, P. M. and Tiedje, J. M.: Relationships between denitrification, CO2 production and air-filled porosity in soils of different texture and drainage, Soil Biology and Biochemistry, 23, 299–302, doi:10.1016/0038-0717(91)90067-T, 1991.

Gu, J., Nicoullaud, B., Rochette, P., Grossel, A., Hénault, C., Cellier, P., and Richard, G.: A regional experiment suggests that soil texture is a major control of N2O emissions from tile-drained winter wheat fields during the fertilization period, Soil Biology and Biochemistry, 60, 134–141, doi:10.1016/j.soilbio.2013.01.029, 2013.

Jurasinski, G., Koebsch, F., and Hagemann, U.: flux: Flux rate calculation from dynamic closed chamber measurements, 2014.

Kaiser, E. A., Kohrs, K., Kucke, M., Schnug, E., Heinemeyer, O., and Munch, J. C.: Nitrous oxide release from arable soil: Importance of N-fertilization, crops and temporal variation, Soil Biology and Biochemistry, 30, 1553–1563, doi:10.1016/S0038-0717(98)00036-4, 1998.

Kaiser, K. and Guggenberger, G.: The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils, Organic Geochemistry, 31, 711–725, doi:10.1016/S0146-6380(00)00046-2, 2000.

Kissel, D. E., Cabrera, M. L., and Paramasivam, S.: Ammonium, Ammonia, and Urea Reactions in Soils, in: Nitrogen in Agricultural Systems, Agronomy Monographs, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, 101–155, 2008.

Komsta, L. and Novomestky, F.: moments: Moments, cumulants, skewness, kurtosis and related tests, 2015.

Köster, J. R., Cárdenas, L., Senbayram, M., Bol, R., Well, R., Butler, M., Mühling, K. H., and Dittert, K.: Rapid shift from denitrification to nitrification in soil after biogas residue application as indicated by nitrous oxide isotopomers, Soil Biology and Biochemistry, 43, 1671–1677, doi:10.1016/j.soilbio.2011.04.004, 2011.

Köster, J. R., Cárdenas, L. M., Bol, R., Lewicka-Szczebak, D., Senbayram, M., Well, R., Giesemann, A., and Dittert, K.: Anaerobic digestates lower N2O emissions compared to cattle slurry by affecting rate and product stoichiometry of denitrification – An N2O isotopomer case study, Soil Biology and Biochemistry, 84, 65–74, doi:10.1016/j.soilbio.2015.01.021, 2015.

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B.: lmerTest: Tests in Linear Mixed Effects Models, 2016.

Letey, J., Jury, W. A., Hadas, A., and Valoras, N.: Gas Diffusion as a Factor in Laboratory Incubation Studies on Denitrification1, Journal of Environmental Quality, 9, doi:10.2134/jeq1980.00472425000900020012x, 1980.

Maag, M. and Vinther, F. P.: Effect of temperature and water on gaseous emissions from soils treated with animal slurry, Soil Science Society of America Journal, 63, 858–865, 1999.

Möller, K. and Müller, T.: Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review, Eng. Life Sci., 12, 242–257, doi:10.1002/elsc.201100085, 2012.

Myhre, G., Shindell, D., Bréon, F. M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J. F., Lee, D., and Mendoza, B.: Anthropogenic and natural radiative forcing, in: Climate Change 2013: The Physical Science Basis.: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M. (Eds.), Cambridge University Press, Cambridge, United Kingdom, New York, NY, USA, 659–740, 2013.

Nkoa, R.: Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review, Agron. Sustain. Dev., 1–20, doi:10.1007/s13593-013-0196-z, 2013.

Parkin, T. B. and Tiedje, J. M.: Application of a soil core method to investigate the effect of oxygen concentration on denitrification, Soil Biology and Biochemistry, 16, 331–334, doi:10.1016/0038-0717(84)90027-0, 1984.

Quakernack, R., Pacholski, A., Techow, A., Herrmann, A., Taube, F., and Kage, H.: Ammonia volatilization and yield response of energy crops after fertilization with biogas residues in a coastal marsh of Northern Germany, Agriculture, Ecosystems & Environment, 160, 66–74, doi:10.1016/j.agee.2011.05.030, 2012.

R Core Team: R: A Language and Environment for Statistical Computing, Vienna, Austria: https://www.R-project.org/, 2015.

Reddy, K. R., Rao, P. S. C., and Jessup, R. E.: The Effect of Carbon Mineralization on Denitrification Kinetics in Mineral and Organic Soils1, Soil Science Society of America Journal, 46, 62–68, doi:10.2136/sssaj1982.03615995004600010011x, 1982.

Scholefield, D., Hawkins, J. M. B., and Jackson, S. M.: Development of a helium atmosphere soil incubation technique for direct measurement of nitrous oxide and dinitrogen fluxes during denitrification, Soil Biology and Biochemistry, 29, 1345–1352, doi:10.1016/S0038-0717(97)00021-7, 1997.

Senbayram, M., Chen, R., Budai, A., Bakken, L., and Dittert, K.: N2O emission and the N2O/(N2O + N-2) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations, Agriculture, Ecosystems & Environment, 147, 4–12, doi:10.1016/j.agee.2011.06.022, 2012.

Senbayram, M., Chen, R., Mühling, K. H., and Dittert, K.: Contribution of nitrification and denitrification to nitrous oxide emissions from soils after application of biogas waste and other fertilizers, Rapid Commun. Mass Spectrom., 23, 2489–2498, doi:10.1002/rcm.4067, 2009.

Senbayram, M., Chen, R., Wienforth, B., Herrmann, A., Kage, H., Mühling, K. H., and Dittert, K.: Emission of N2O from Biogas Crop Production Systems in Northern Germany, BioEnergy Research, 1–14, doi:10.1007/s12155-014-9456-2, 2014.

Severin, M., Fuss, R., Well, R., Garlipp, F., and van den Weghe, H.: Soil, slurry and application effects on greenhouse gas emissions, Plant, Soil and Environment, 61, 344–351, 2015.

Tiedje, J. M.: Ecology of denitrification and dissimilatory nitrate reduction to ammonium, in: Biology of anaerobic microorganisms, Zehnder, A. J. B. (Ed.), John Wiley and Sons Inc, New York, 179–244, 1988.

Uchida, Y., Clough, T. J., Kelliher, F. M., and Sherlock, R. R.: Effects of aggregate size, soil compaction, and bovine urine on N2O emissions from a pasture soil, Soil Biology and Biochemistry, 40, 924–931, doi:10.1016/j.soilbio.2007.11.007, 2008.

Velthof, G. L. and Mosquera, J.: The impact of slurry application technique on nitrous oxide emission from agricultural soils, Agriculture, Ecosystems & Environment, 140, 298–308, doi:10.1016/j.agee.2010.12.017, 2011.

Venterea, R. T., Clough, T. J., Coulter, J. A., Breuillin-Sessoms, F., Wang, P., and Sadowsky, M. J.: Ammonium sorption and ammonia inhibition of nitrite-oxidizing bacteria explain contrasting soil N2O production, Scientific Reports, 5, 12153 EP -, 2015.

Wang, R., Feng, Q., Liao, T., Zheng, X., Butterbach-Bahl, K., Zhang, W., and Jin, C.: Effects of nitrate concentration on the denitrification potential of a calcic cambisol and its fractions of N2, N2O and NO, Plant and Soil, 363, 175–189, doi:10.1007/s11104-012-1264-x, 2013.

Wang, R., Willibald, G., Feng, Q., Zheng, X., Liao, T., Brüggemann, N., and Butterbach-Bahl, K.: Measurement of N2, N2O, NO, and CO2 Emissions from Soil with the Gas-Flow-Soil-Core Technique, Environ. Sci. Technol., 45, 6066–6072, doi:10.1021/es1036578, 2011.

Webb, J., Pain, B., Bittman, S., and Morgan, J.: The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—A review, Special section Harvested perennial grasslands: Ecological models for farming's perennial future, 137, 39–46, doi:10.1016/j.agee.2010.01.001, 2010.

West, S. G., Finch, J. F., and Curran, P. J.: Structural equation models with nonnormal variables: Problems and remedies, in: Structural equation modeling: Concepts, issues, and applications, Hoyle, R. H. (Ed.), Sage, Thousend Oaks, 56–75, 1995.

Wulf, S., Maeting, M., and Clemens, J.: Application technique and slurry co-fermentation effects on ammonia, nitrous oxide, and methane emissions after spreading: II. Greenhouse gas emissions, Journal of Environmental Quality, 31, 1795–1801, 2002.

Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., and Shen, Y.: Managing nitrogen for sustainable development, NATURE, 528, 51–59, 2015.

515 **Table 1: The examined factors soil texture, water-filled pore space (WFPS), and amount (i.e., concentration) of**
516 **nitrogen (N) applied with biogas digestate (BD) with their respective levels applied in the present study, resulting in**
517 **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

518

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

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%

Table 4: Mean DOC values from soils, measured 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: ANOVA table (type 2, *p*-values calculated based on Satterthwaite's approximation) of the linear mixed effects 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. Soil type, water-filled pore space (WFPS), amount of digestate, temperature, NO_3^- and DOC content of soil after incubation as well as fluxes of N_2O and CO_2 were set as possible independent variables. The individual soil rings were set as 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.

	Fixed effects							
Response	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	†	0.001	< 0.001	†
N_2O	†	< 0.001	< 0.001	< 0.001	†	< 0.001	*	< 0.001
$N_2/(N_2+N_2O)$	0.005	0.004	†	†	†	†	*	†
CO_2	<0.001	†	<0.001	<0.001	†	0.007	†	*

† 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.

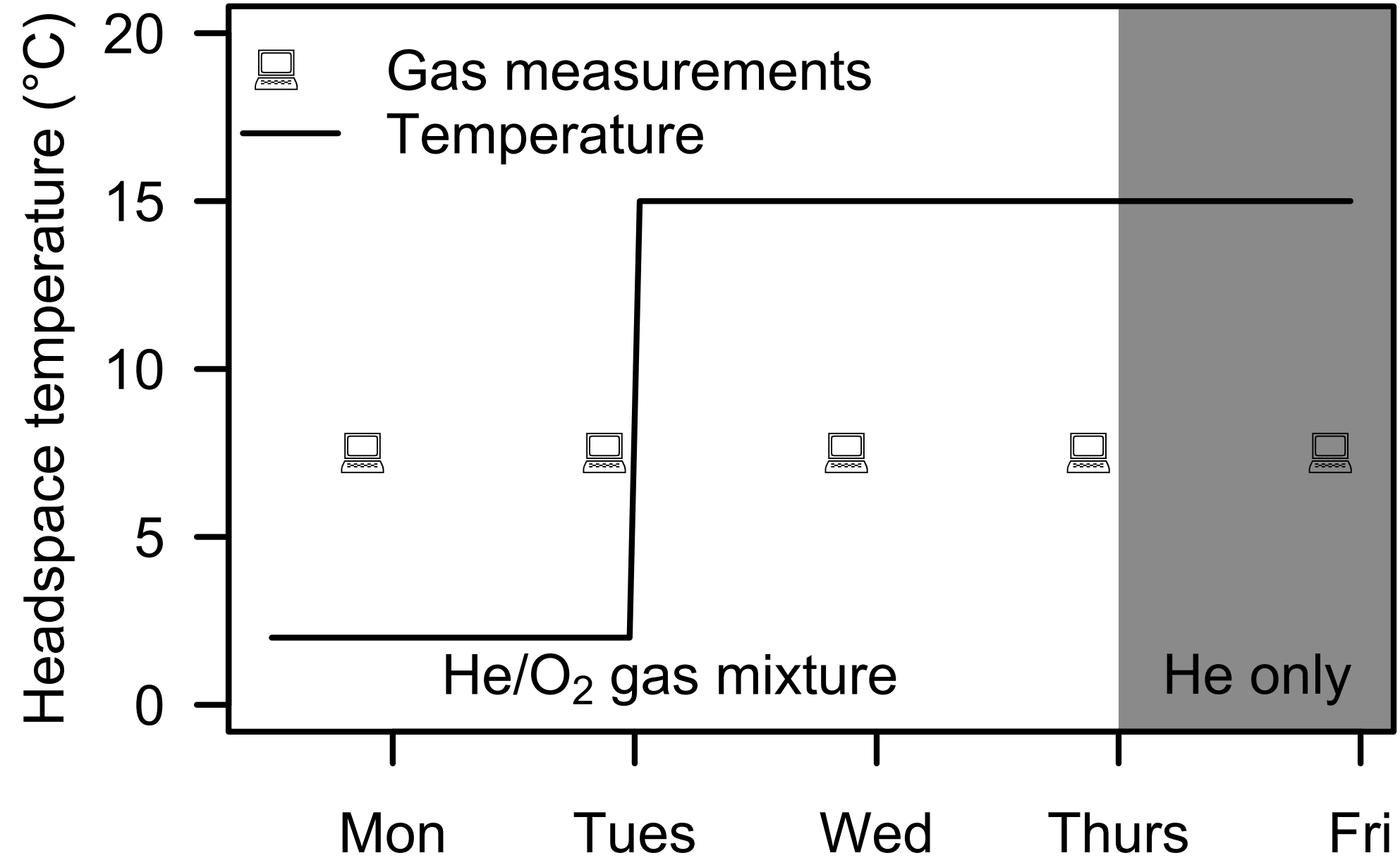

Headspace temperature (°C)
20
15
10
5
0
Gas measurements
Temperature
He/O2 gas mixture
He only
Mon
Tues
Wed
Thurs
Fri

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 (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, calculated amounts of ammonium applied with biogas digestate are shown by triangles. One treatment (*) was omitted from all analyses due to technical reasons.

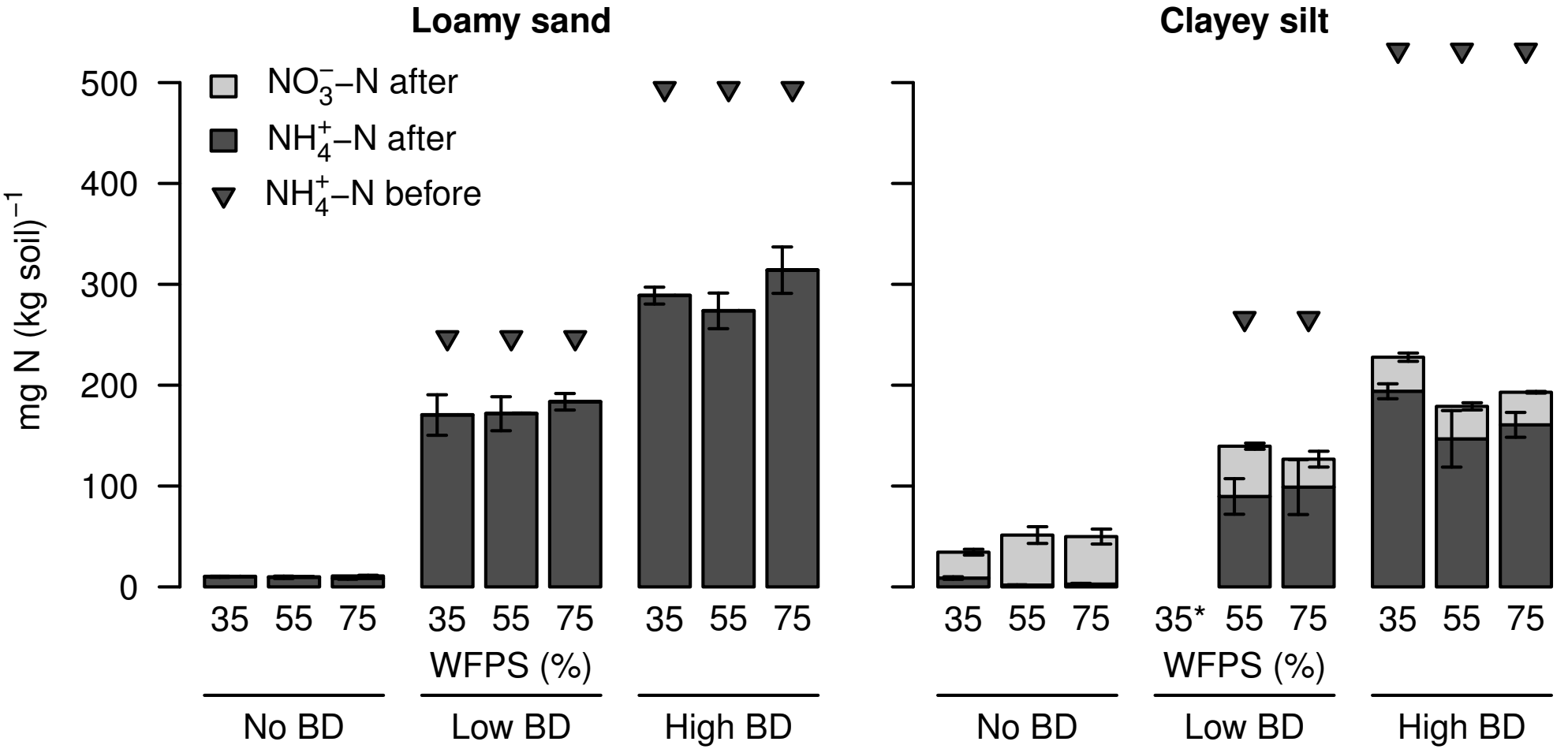

Loamy sand
Clayey silt
NO_3^-–N after
NH_4^+–N after
NH_4^+–N before
mg N (kg soil)$^{-1}$
500
400
300
200
100
0
35 55 75 35 55 75 35 55 75
WFPS (%)
No BD
Low BD
High BD
35 55 75 35* 55 75 35 55 75
WFPS (%)
No BD
Low BD
High BD

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 (0 mL per sample ring: 'No BD', 17.6 mL: 'Low BD' and 35.2 mL: 'High BD'). The first till the fourth day of the incubation were measured in an aerobic He-O_2 headspace (with two days at 2 °C followed by another two days at 15 °C) while on the fifth day measurements where 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 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}.

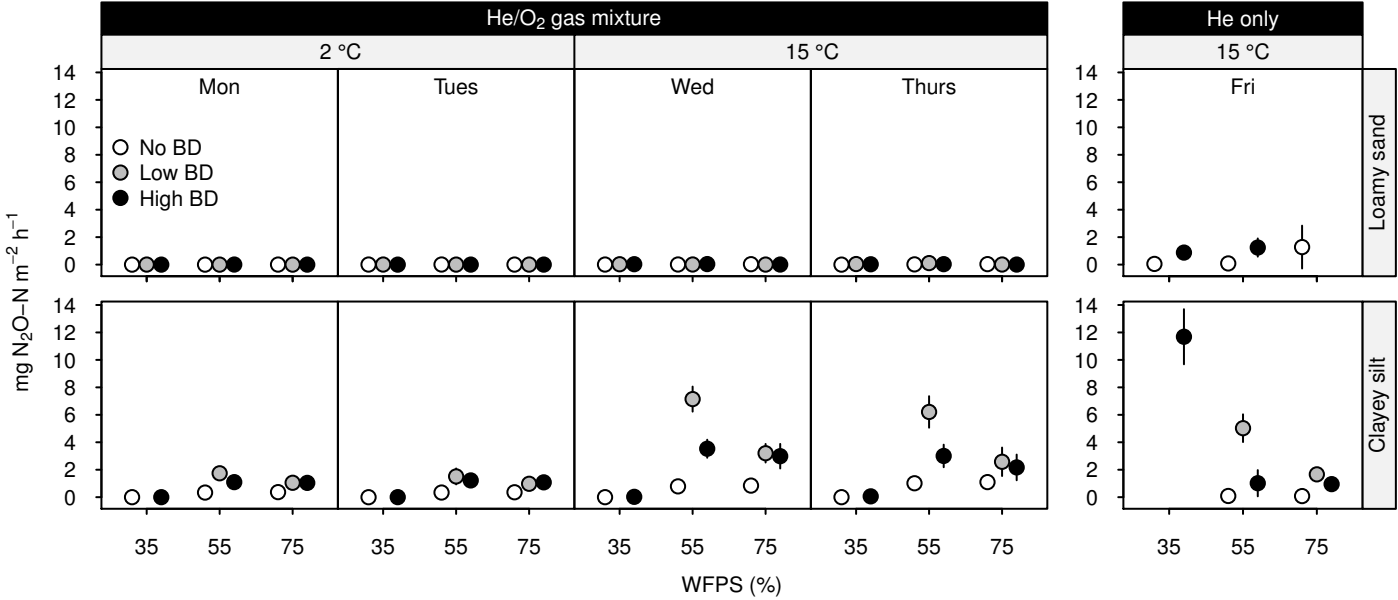

He/O2 gas mixture
He only
2 °C
15 °C
15 °C
Mon
Tues
Wed
Thurs
Fri
No BD
Low BD
High BD
Loamy sand
Clayey silt
mg N2O–N m−2 h−1
0
2
4
6
8
10
12
14
35
55
75
WFPS (%)

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 (0 mL per sample ring: 'No BD', 17.6 mL: 'Low BD' and 35.2 mL: 'High BD'). The first till the fourth day of the incubation were measured in an aerobic He-O_2 headspace (with two days at 2 °C followed by another two days at 15 °C) while on the fifth day measurements where 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 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.

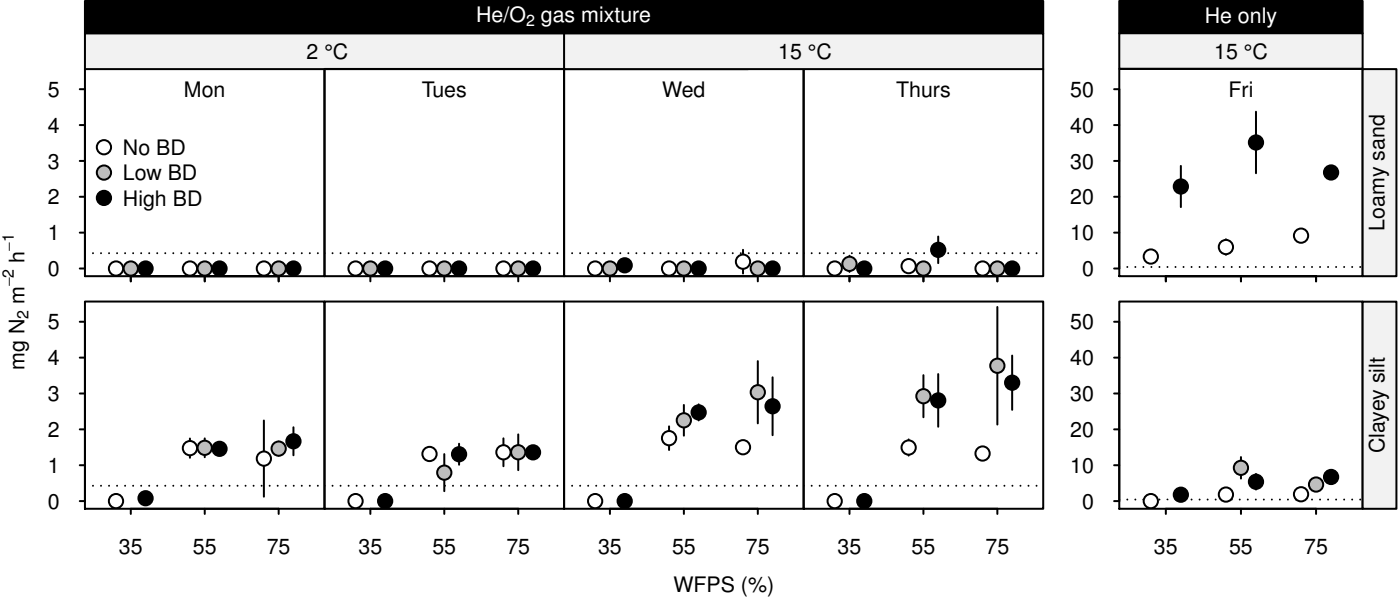
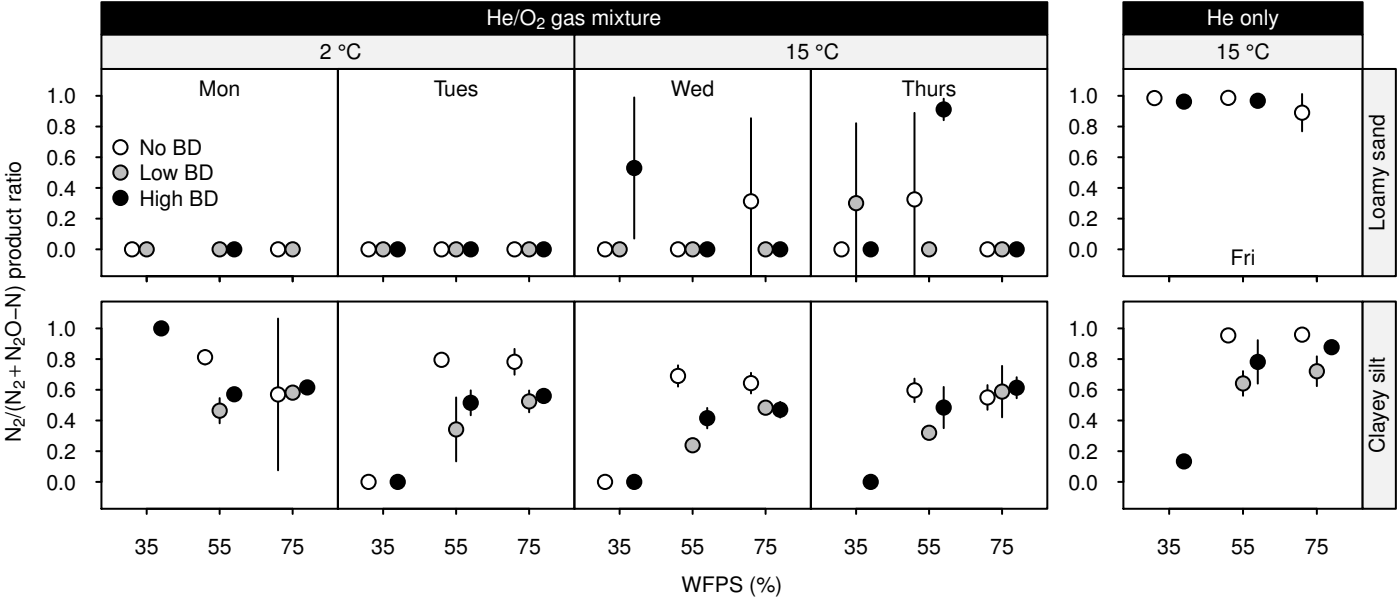

He/O2 gas mixture
He only
2 °C
15 °C
15 °C
Mon
Tues
Wed
Thurs
Fri
No BD
Low BD
High BD
Loamy sand
Clayey silt
mg N2 m−2 h−1
WFPS (%)
35
55
75
0
1
2
3
4
5
10
20
30
40
50

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 (0 mL per sample ring: 'No BD', 17.6 mL: 'Low BD' and 35.2 mL: 'High BD'). The first till the fourth day of the incubation were measured in an aerobic He-O_2 headspace (with two days at 2 °C followed by another two days at 15 °C) while on the fifth day measurements where 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, 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.


He/O2 gas mixture
He only
2 °C
15 °C
15 °C
Mon
Tues
Wed
Thurs
Fri
No BD
Low BD
High BD
Loamy sand
Clayey silt
N2/(N2+N2O–N) product ratio
0.0
0.2
0.4
0.6
0.8
1.0
35
55
75
WFPS (%)

584 **Fig. 6: Regression between DOC (mg per 100 g soil) measured after the incubation and the respective cumulated CO_2**
585 **emissions (g C m^{-2}) during the period of aerobic headspace with their standard deviations and confidence interval**
586 **(95%). If error bars are not visible, they are smaller than the symbols of the means. Both soils showed increasing**
587 **emissions with increasing soil DOC contents as well a good regression fit ($R^2 = 0.91$, $p < 0.001$).**

588

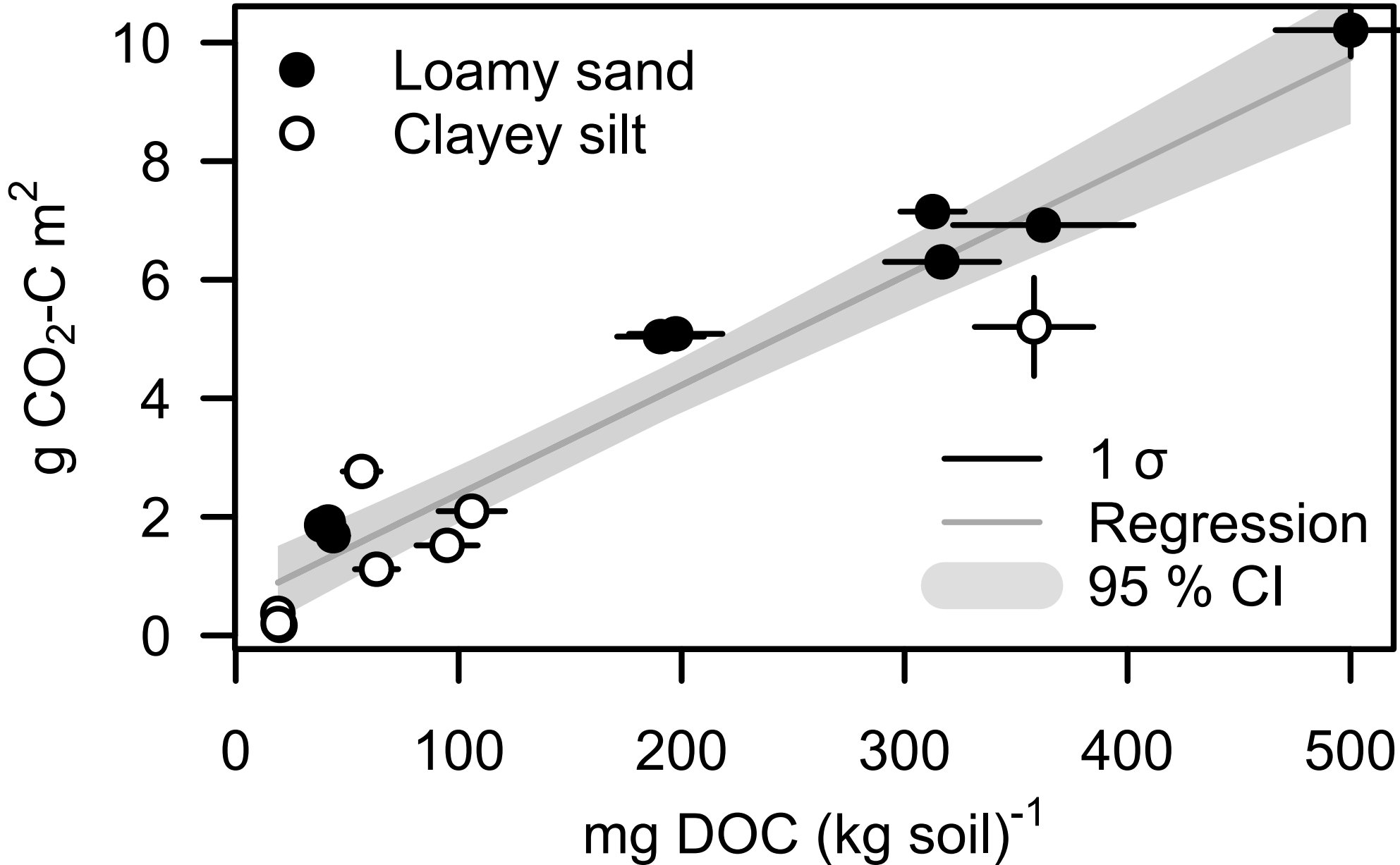

Loamy sand
Clayey silt
1 σ
Regression
95 % CI
g CO2-C m2
mg DOC (kg soil)-1
0
2
4
6
8
10
0
100
200
300
400
500

Fig. 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 of CO_2 and the N gas species shows a good regression fit of $R^2 = 0.93$, ($p = 0.001$).

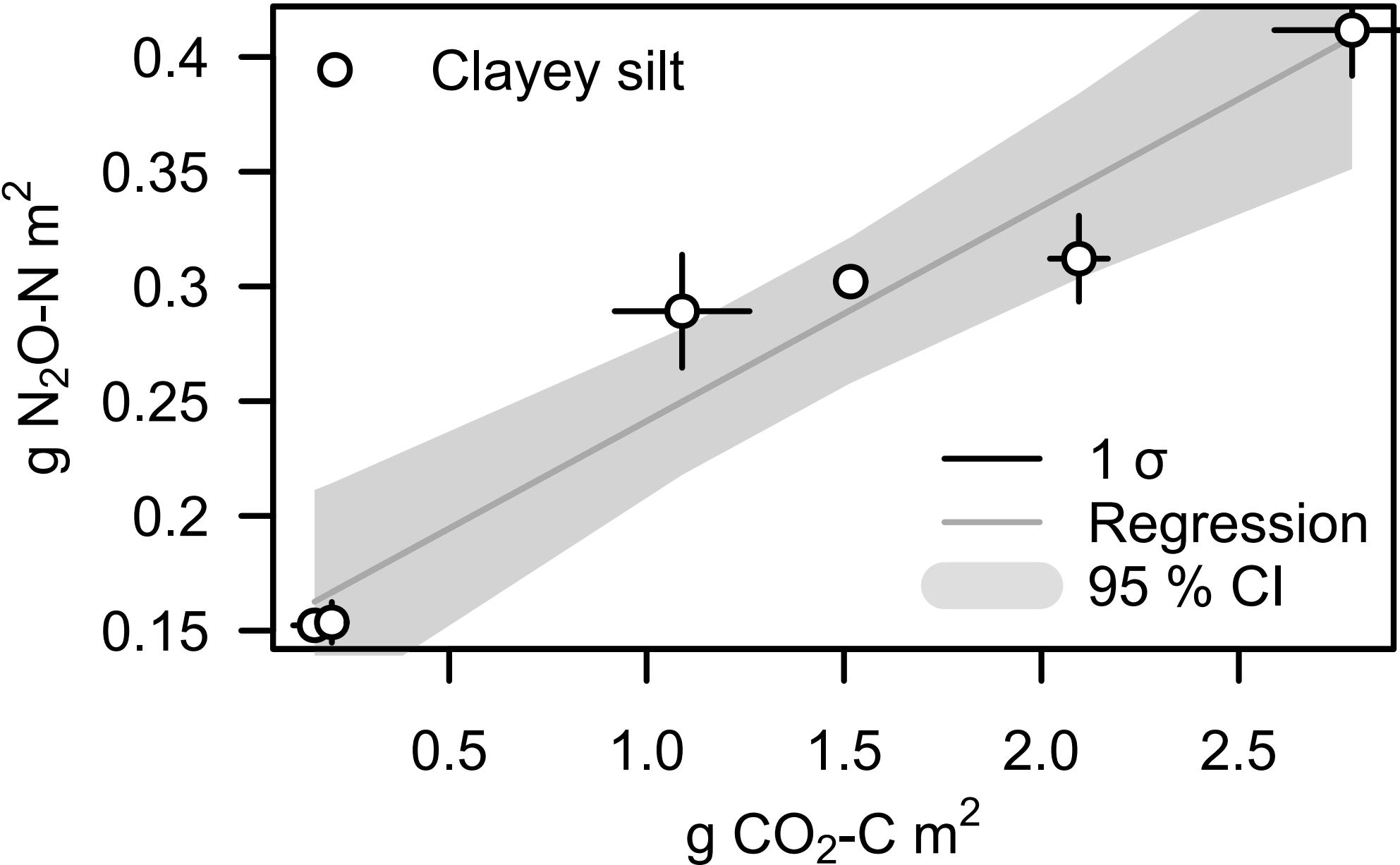

Clayey silt
1 σ
Regression
95 % CI
0.4
0.35
0.3
0.25
0.2
0.15
g N2O-N m2
0.5
1.0
1.5
2.0
2.5
g CO2-C m2

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 (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

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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 (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 A3: 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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