

## Reply to anonymous referee no. 1

Dear Sir or Madam

Thank you for your careful reading and your supportive suggestions. We agree with most of them.

Indeed, the large amount of factor combinations was traded-off against a relatively short incubation duration which did not allow for the observation of the dynamics following the application of the biogas digestate (BD). But, as explained in our response to referee no. 2, our aim was not to study the dynamics, rather the effect of the different factors. We also argue in the other reply why we think the duration was appropriate to detect the peaks of the N<sub>2</sub>O and N<sub>2</sub> emissions. However, because you acknowledged our efforts to get such rare data, we assume that you agree with our study design (in opposite to the second referee).

Basically, we follow your suggestion to include the data of all days into the statistical analysis and to the figures of the emissions. We also included the DOC content after the incubation into the step wise regression selection. As a result, DOC replaced NO<sub>3</sub><sup>-</sup> content in the model for N<sub>2</sub>O and completed the models for N<sub>2</sub> and CO<sub>2</sub>.

However, the resulting models failed the Shapiro-Wilk test on normal distribution of the model residues. This might be a consequence of the increased number of data points which tend to reduce the reliability of tests on normal distribution in general. Hence, we decided to apply the skewness of the respective distributions as a measure, whereas a skewness of smaller than two is acceptable for normality assumptions (West et al., 1995) and all new models meet this assumption.

Thank you for your clue about the possibility of NO formation and loss, which we acknowledge now with the mechanism of NO<sub>2</sub>- accumulation in our discussion as you elucidated in lines 327 – 337.

In the following, we will reply to your specific comments. All corresponding changes are highlighted throughout the supplement pdf.

L. 1: Tune down the “simulated injection” story. I do not know how a band of injected slurry looks like in situ, but mixing it (evenly?) into dry (?) soil and packing it in a 200 cc cylinder does not seem to simulate conditions in a band of injected slurry at all. I get the point that different intensities of slurry (per cylinder) are a faint proxy for different row spacing in the field, if you look at it at the ha-scale. However, the study is conducted at the cm-scale and should be treated like this. Therefore, “simulated” should be removed from the title and the whole story should be tuned down throughout the text. It does not add much, anyway, because the study does not lend itself to making extrapolations to field-scale N losses. If you prefer to keep the stress on “simulated injection”, much more detail would be needed about how the slurry was mixed into the soil (soil moisture at mixing, bulk densities before and after mixing, etc.).

& L. 15. Insert “the latter” between application rate and proxy. Otherwise, the sentence does not make sense.

→ We agree that our set-up was inadequate to “simulate” injection and revised the story accordingly. Rather, we point now to the high nutrient concentration occurring in injection bands which we reproduced in our repacked

soil cores. To focus our manuscript more to “the cm-scale”, we changed the differentiation from the application rates per ha to mL per soil core (though, still referred to a hypothetical application of 160 kg BD-N ha<sup>-1</sup> in the methods section), but we discarded the spacing phrase: “Hence, we performed an incubation experiment with soil cores in a helium-oxygen atmosphere to examine the influence of soil substrate (loamy sand, clayey silt), water-filled pore space (WFPS; 35, 55, 75%) and application rate (0, 17.6 and 35.2 mL BD per soil core [250 cm<sup>3</sup>]) on the emissions of N<sub>2</sub>O, N<sub>2</sub> and CO<sub>2</sub> after the usage of high loads of BD” (now in lines 15 – 16). Throughout the MS, the terms “160 kg N ha<sup>-1</sup>” and “320 kg N ha<sup>-1</sup>” are substituted by “LOBD” (low BD concentration) and “HIBD” (high BD concentration), respectively.

L. 16. Remove “these”

→ removed (17)

L. 21. “Content in”

→ replaced (21)

L. 31: “emission and deposition ... are of environmental concern” & L. 32: rephrase: “e.g., through acidification and conversion to N<sub>2</sub>O”

→ phrase discarded to shorten the manuscript

L. 39: Slurry? Animal slurry?

→ phrase discarded

L. 40: mention NO here

→ done (40)

L. 41: how can an “overall effect ... [be] under debate”? What is under debate? The mechanism behind the effect, the “sign” of the effect, the effect strengths, or all together? Clarify.

→ phrase discarded

L. 44: Reduction of local O<sub>2</sub> availability. What are the processes/factors reducing O<sub>2</sub> availability? This paragraph seems to focus solely on denitrification. Mention also NH<sub>4</sub><sup>+</sup> and nitrification, which is a strong O<sub>2</sub> sink while being a potential source for N<sub>2</sub>O.

→ We rephrased the respective section: “On the one hand, high NH<sub>4</sub><sup>+</sup> concentrations in the injection band promote nitrification, which is a significantly O<sub>2</sub> consuming process releasing N<sub>2</sub>O (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<sub>2</sub> supply (Dell et al., 2011).” (44 – 47)

L. 46: skip “these”; in general restrict the use of “these”

→ OK

L. 55...and nitrification!

→ right, included (55)

L. 58 ff.: tune down the “row spacing”. Your study does not address row spacing. See comment above.

→ We discarded the row spacing story and mention it only in the methods section 2.2 to reason our approach.

L. 66: not the least

& L. 66. Methodological issues; be a bit more specific

→ phrase discarded

L. 69: generation = emission?

→ Yes, changed accordingly (64)

L. 70: CO<sub>2</sub> as an indicator for O<sub>2</sub> consumption. Not entirely free of problems. The slurry is full of bicarbonate, the soil is above pH 7. But better than nothing.

→ We added “[...] but with the restriction that inorganic sources could not be differentiated.” in lines 66.

L. 73: not entirely clear from the introduction what you base your hypothesis 3 on.

→ We added the following sentence into the introduction section: “In general, fine textured soils exhibit a lower gas diffusivity compared to coarse textured soils, which result regularly in higher denitrification activity in the former with higher N<sub>2</sub>O emission rates, but also a higher probability for the consecutive reduction to N<sub>2</sub> (Senbayram et al., 2014; Gu et al., 2013; Ball, 2013).” (56 – 59)

L. 76: treated with WFPS? Adjusted to WFPS

→ Changed accordingly (72)

L. 78: give volume of the cores

→ 250 cm<sup>3</sup> (75)

L. 82 to “gather” soil. Like in Hunter and Gatherers? Replace by “collect”

→ replaced (76 & 78)

L. 84: give some reasoning for why you dry the soils

→ “to facilitate adjustment of WFPS” (81)

L. 97: “with the respective quantity of water calculated based on”. Too many words!

→ We split the respective sentence into two: “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<sup>-1</sup> and reduced by the expected moisture input from subsequent addition of BD.” (94 – 96)

L. 99 ff.: see comment above. Does the mixing described here really simulate injection, the result of which I would figure as a band rather than a mixture?

→ No, you are right. As for your former comment, we adjusted the story towards the concentration of BD appearing in injection bands rather than simulating injection bands (section 2.2).

L. 109: wouldn't an apparatus like this typically involve the use of an additional empty reference chamber to infer N<sub>2</sub> leak rates, blank values, etc.? Please comment.

→ We used all six chambers because the tightness of one individual one kept empty does not allow concluding about the individual tightness of each of the other five chambers.

Generally, we calculated the fluxes from the concentration differences at the respective inlets and outlets. To reduce contamination with atmospheric air, the lids of every single chamber were purged permanently with helium. Additionally, we determined individual blank values every cycle before the measurements started by inserting aluminium blocks into the chambers. Since the obtained blank values were usually steady, we suggest that the chambers were tight. The blank values were subtracted from the values measured at the respective outlets (128 – 135).

L. 118: why “possibly”? N<sub>2</sub> measured by a TCD is by a factor 10<sup>3</sup> to 10<sup>4</sup> less precise than N<sub>2</sub>O by an ECD. That’s a fact.

→ We omitted “possibly” (115)

L. 118: So, what was the precision? Please give precision in ppm and in mg N<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>

→ We refer to Eickenscheidt et al. (2014) (115). See also our reply about precision/blank values above.

L. 128: Difference of gas concentrations. Between what? Inlet and outlet?

→ Yes, changed accordingly (127)

L. 130: How comes that the detection limit for N<sub>2</sub> in this study is one order of magnitude higher than the one given by Eickenscheidt et al. (2014)?

→ The “detection limits” given in the manuscript are actually derived from the blank values and, thus, do not represent the detection limit of the TCD. The actual detection limits are in accord with Eickenscheidt et al. (2014), see comment above on precision/blank values.

L. 130: Entirely unclear to me how you can check a detection limit daily in vessels filled with soil. Please explain.

→ Same misunderstanding about detection limit and blank values as above. The respective section was deleted.

L. 134: Any reasoning behind the use of 0.01 M KCl as an extractant? Wouldn’t it be more interesting to look at total exchangeable NH<sub>4</sub><sup>+</sup>?

→ No special reasoning since this was the standardised extraction method of the commissioned laboratory at Leibniz Centre for Agricultural Landscape Research e. V., annotated now. (139 – 140)

L. 143: Just out of curiosity: the “technical reasons” for omitting all these fluxes would be what? Influx of N<sub>2</sub> from the atmosphere?

→ No, the soil cores were not adjusted to the right WFPS and were, thus, not coherently useful.

L. 177: Replace “from” with “in”

→ phrase discarded

L. 185: Why not moving the CO<sub>2</sub> subchapter here?

→ OK (178 – 186)

L. 191: “. . .the other treatment means emitted between. . .”. Means cannot emit!

→ phrase discarded

L. 207 Fig. 4: Insert a line in figure 4, denoting the average detection limit for N<sub>2</sub>. No N<sub>2</sub> fluxes could be detected = N<sub>2</sub> fluxes were under the detection limit.

→ Inserted.

L. 210: Negligible amounts of N<sub>2</sub>. What is negligible? Replace by “small rates”. It is actually rates you are reporting.

→ Replaced (202)

L. 212: Awkward phrasing

→ Rephrased: “The clayey silt showed mean fluxes of up to 1.4 mg N<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> at 2 °C (all incubations with 75% WFPS) and up to 3.8 mg N<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> at 15 °C (75% WFPS with LOBD), but no fluxes in all BD treatments with 35% WFPS.” (203 – 205)

L. 214: Can a soil “present” emissions?

→ Rather not. Rephrased to: “emitted” (206).

L.254 ff.: Not so strange that temperature “shows . . . significant influences” if one warms a soil from 2 to 15°C. Did you ever consider to use the temperature difference to tentatively distinguish between chemical and biological processes? I would actually exclude temperature from the mixed model. It’s too obvious.

→ No, we did not consider to use the temperature for such a tentatively distinction, because it is out of the scope of our study. However, aren’t chemical processes also driven by temperature?

We tentatively set temperature as a random effect in the model for N<sub>2</sub>, which then had been eliminated during the step-wise regression section. But the resulting model had only a slightly improved AIC (decreased from 121 to 118). Thus, we decided to keep the temperature as a fixed effect, because its omitting resulted in virtually unchanged effects of the independent variables. However, we reduced the discussion about temperature in the revised manuscript.

L. 256. Q10 is just a metrics for temperature response. It is not reserved to biological processes.

→ We now omit Q10 and rephrased to: “increased metabolic activity” (234)

L. 257: Temperature is not a proxy. It’s a driver!



→ Changed accordingly (235)

L. 258: CO<sub>2</sub> is not a product of O<sub>2</sub>!

→ rephrased to: “resulting from respiration of O<sub>2</sub>” (236)

L. 267: “many N<sub>2</sub>-producing processes”. How many are there?

→ Not so many. Actually it’s denitrification as coupled nitrification-den., nitrifier den., co-den. and chemo-den. (Butterbach-Bahl et al., 2013). We rephrased: “N<sub>2</sub>O is the direct precursor of N<sub>2</sub> in denitrification” (245).

L. 274: 35% WFPS should be more than enough for substrate diffusion in the soil. This is not a good argument for explaining low fluxes at this soil moisture.

→ We omitted this argument and refer to the presumably well aeration in these treatments (253).

L. 278: such moderate amounts of N?

→ We rephrased to “commonly applied amounts of BD-N (i.e., 160 kg N ha<sup>-1</sup>)” (255 – 356).

L. 279: I see your point, but also you mixed the BD into the soil, didn’t you’?

→ Yes, we mixed it into the soil, but we aim at the substrate concentration (in injection slits) in contrast to a mixing in the soil surface (see reply to your comment on line 1) (256 – 257).

L. 283: highly amended

→ revised (261)

L. 284. Do not use “where” as a relative pronoun, unless it deals with a place

→ OK, rephrased as a separate sentence “Accordingly, Anthonisen et al. (1976) found an [...]” (262).

L. 286: “relatively high concentrations of NH<sub>3</sub>”. Use the NH<sub>4</sub> – NH<sub>3</sub> partitioning coefficients given by Venterea et al. (2015) together with your slurry pH and calculate the apparent NH<sub>3</sub> concentration.

→ We calculated the apparent NH<sub>3</sub> concentration according to Emerson et al. (1975) with 15 °C under the assumption that all extractable soil NH<sub>4</sub> is in solution, because we do not know the sorption capacity of our soils and, further, Equation (3) in Venterea et al. (2015) resulted in unrealistic high numbers. The corresponding section reads now: “The application rate in the treatments with HIBD amounted to approximately 500 mg NH<sub>4</sub><sup>+</sup>-N (kg soil)<sup>-1</sup> (Fig. 3) which correspond to 25.8 mg NH<sub>3</sub>-N (kg soil)<sup>-1</sup> at 15 °C if we use the pH of the BD and assume that all extractable NH<sub>4</sub><sup>+</sup>-N was in solution (Emerson et al., 1975).” (264 – 265)

L. 287: An argument? Or rather the reason?

→ Rather the reason (266)

L. 290: this is a good argument (clay fixation), which you probably would not observe to the same extent if the slurry was placed as a band in the soil

→ We discussed this issue in lines 271 – 272: “However, since we mixed the BD with the soil, we would expect a lower NH<sub>3</sub> fixation in tubular injection slits in situ, resulting in probably lower N<sub>2</sub>O and N<sub>2</sub> fluxes from clayey soils.”

L. 294: If you really think that nitrifiers reduce more NO<sub>2</sub>- than denitrifiers, then please cite the original literature, and not some review

→ This was a misunderstanding. We aimed at the preferred reduction of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  compared to  $\text{N}_2\text{O}$  during denitrification. In general,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  are preferably reduced compared to  $\text{N}_2\text{O}$  during denitrification sequence since the energy yield of each reduction step decreases from  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$  (Koike and Hattori, 1975). Additionally, the reaction rate of reduction is higher for  $\text{NO}_3^-$  and  $\text{NO}_2^-$  than for  $\text{N}_2\text{O}$ , which results in an accumulation of  $\text{N}_2\text{O}$ , if  $\text{NO}_3^-$  concentration is not limited (Betlach and Tiedje, 1981). However, the discarded the discussion on this issue.

L. 299: Consider also AOB - NOB decoupling due to  $\text{NH}_4$  inhibition of NOB

→ We added a phrase about this important hint: “Actually, high  $\text{NH}_4^+$  loads in conjunction with an increased pH favour  $\text{NO}_2^-$  accumulation, because  $\text{NO}_2^-$  oxidising bacteria are less resilient against high concentrations of  $\text{NH}_3$  than  $\text{NH}_3$  oxidising bacteria (Anthonisen et al., 1976). This  $\text{NO}_2^-$  should have protonated then partly to the toxic and unstable  $\text{HNO}_2$ , which drives biological and chemical production of  $\text{NO}$  and  $\text{N}_2\text{O}$  for detoxification (Venterea et al., 2015).” (273 – 276)

L. 302: it certainly was

→ reformulated accordingly: “we suggest a dominant role of nitrifier denitrification” (277).

L. 304: one verb too many

→ phrase discarded

L. 302: How about writing: “Different effects of soil diffusivity on  $\text{N}_2\text{O}$  and  $\text{N}_2$  fluxes”

→ Good idea, thanks!

L. 314: why do we need saturated conductivity here? You mixed the slurry into the soil. There shouldn't be any gravitational flow

→ phrase discarded

L. 315. Let\*s\*

→ changed accordingly (293)

L. 316. “inferior diffusion characteristics”, rephrase

→ rephrased to “relatively poor diffusion characteristics” (294)

L. 327: “reasonable establishment”, rephrase

→ rephrased to: “sufficient establishment” (306)

L. 328: ensured a moderate diffusional constraint!

→ rephrased accordingly (307)

L. 337: if you really think that NO<sub>3</sub><sup>-</sup> availability affected the N<sub>2</sub>/(N<sub>2</sub>+N<sub>2</sub>) ratio, cite the original literature.

→ We refer now to Senbayram et al. (2012) (316).

L. 346: “reasonable stocks of NO<sub>3</sub><sup>-</sup>“?

→ “sufficient stocks” (321)

L. 349: proposed

→ changed (325)

L. 351 ff. nice discussion about the role of C, but why did you not try to correlate total N gas flux with CO<sub>2</sub> or DOC?

→ Honestly, we do not know ourselves why we have not correlated them. But we catch it up and found a very good correlation for the clayey silt ( $R^2 = 0.93$ ,  $p = 0.001$ ), when the treatments with 35 % WFPS (which showed virtually no N emissions) were omitted. We included this finding into the respective section: “Accordingly, there is also a good correlation between cumulated CO<sub>2</sub> and N<sub>2</sub>O + N<sub>2</sub> fluxes for the same period from the clayey silt ( $R^2 = 0.93$ ,  $p = 0.001$ ), when the treatments with 35 % WFPS (which showed virtually no N emissions) are omitted (Fig. 7). However, there was no such a correlation for the loamy sand. This confirms the interactive effect of diffusivity (induced by both the soils and WFPS) and C availability on the emissions of N<sub>2</sub>O and N<sub>2</sub>.” (336 – 341)

L. 352. There is only one denitrification process that depends on the availability of organic carbon, namely denitrification.

→ That’s right, changed accordingly (334).

L. 368: “a tendency of fostered N<sub>2</sub>O reduction”; rephrase

→ phrase discarded

L. 370: here Q<sub>10</sub> pops up again. To what end? If you want to use Q<sub>10</sub> values, then try to calculate them from your emission rates at 2 and 15°C.

→ We omitted the reference to Q<sub>10</sub> (cf. L. 256)

L. 380: claggy? Do you mean cloggy?

→ Yes, cloggy, but section discarded

L. 388: larger loss and higher ratios

→ Changed accordingly (357)

L. 392: Appropriate denitrifying community? Denitrifiers are ubiquitous and comprise some 5 to 20% of any soil microbial community. Any reason why there should be no appropriate denitrifying community?

→ To be honest, no. So we omit this phrase now.

## References

- Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S.: Nitrous oxide emissions from soils: how well do we understand the processes and their controls?, *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 368, doi:10.1098/rstb.2013.0122, 2013.
- 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.
- Senbayram, M., Chen, R., Budai, A., Bakken, L., and Dittert, K.: N<sub>2</sub>O emission and the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) 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.
- 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 N<sub>2</sub>O production, *Scientific Reports*, 5, 12153 EP -, 2015.
- 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, Thousand Oaks, 56–75, 1995.

## Reply to anonymous referee no. 2

Dear Sir or Madam

We acknowledge your reasonable doubts regarding our manuscript and accept that there are, from an *in situ* point of view, methodically conditioned pitfalls, which prevent conclusions about the dynamics of short-term emissions of N<sub>2</sub>O, N<sub>2</sub> and CO<sub>2</sub> after soil amendments with ‘injected’ biogas digestate (BD). However, in the following, we would like to respond to your major points of criticism and argue why our approach should be accepted as an opportunity to get insights into relevant human induced biogeochemical processes, which are very difficult to examine with any other approach.

Indeed, the ambition of our study was not to draw general conclusions about the dynamics over the whole period of increased emissions after the amendment. Rather, we aimed to detect the importance of different factors (soil texture, water-filled pore space and high nutrient concentrations resulting from spacing between injection slits) on the short-term emissions potentials, especially of N<sub>2</sub>, after amendments with BD, which has never been done before.

The determination of N<sub>2</sub> emissions from soils is a delicate matter since the atmosphere consists to a large part of N<sub>2</sub>; hence, it is difficult to detect concentration changes in amounts relevant for soil processes. Generally, there still exist only two appropriate methods to tackle this challenge: <sup>15</sup>N labelling and the use of an N<sub>2</sub> free artificial headspace (Lewicka-Szczebak et al., 2014). Up to now, both methods, used separately or in combination, are only applicable for laboratory incubations. Recent efforts to determine N<sub>2</sub> fluxes on field sites confirmed the need to continue the development of an *in situ* method (Lewicka-Szczebak et al., 2017). Since BD is a rather heterogeneous substrate, <sup>15</sup>N labelling of ammonium would end up in a relative uneven distribution of the latter. Nevertheless, to establish a device for the helium oxygen method is also a protracted issue due to the efforts needed to achieve complete tightness against contamination from air born N<sub>2</sub>. In other words, we were able to perform an incubation to determine N<sub>2</sub> fluxes from soils and such data have a high value. Moreover, such measurements on BD are generally rare and we are the first to provide data on N<sub>2</sub> emissions from such high concentrations as they appear after injective application. Further, by applying the N<sub>2</sub>/(N<sub>2</sub> + N<sub>2</sub>O) ratio to field measurements, an estimation of N<sub>2</sub> emissions *in situ* might be possible.

Generally, the initial phase, i.e., the first week after fertilizer application, is crucial for N<sub>2</sub>O emissions (Dobbie et al., 1999; Kaiser and Heinemeyer, 1996) and most probably also for N<sub>2</sub> because the same processes are involved. Regarding BD, first N<sub>2</sub>O peaks were observed within the first and third day in incubation experiments, which indicate a rather immediate reaction also for N<sub>2</sub> at least *in vitro* (Köster et al., 2011; Köster et al., 2015;

Senbayram et al., 2009). Therefore, we deduce our consecutive two days of measurements as appropriate. Nevertheless, these former studies recorded a second plateau of N<sub>2</sub>O emission consistently after around two weeks, which would imply an incubation duration of at least three weeks. Transferred to our incubation, this would mean a duration of at least 27 weeks, which involve a huge logistical effort for systems maintaining a nearly N<sub>2</sub> free headspace. However, these authors operated at a higher WFPS than we in our study. At a lower 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. On the bottom line, the efforts for a study of the dynamics of N<sub>2</sub> emissions harmonised along the lines of your criticism were out of our scope and are probably more appropriate for projects lasting for years that focus this single issue. However, we observed ourselves only a few days of increased N<sub>2</sub>O emissions in field trials under optimal conditions (Hagemann et al., 2016). Hence, we are convinced that the duration of measurements we have chosen are appropriate to get a first feeling for the impact of the different conditions of soil texture, water-filled pore space and applied BD concentration.

Our method is up to date for the investigation of soil related N<sub>2</sub> emissions and has been applied successfully by several working groups, e.g., Köster et al. (2011) and Wang et al. (2011). For this method it is mandatory to keep the vessels at low temperature at the beginning: since we wanted to record the *de novo* production of N<sub>2</sub>O and N<sub>2</sub>, these two days without measurements are needed to rid the soil of this gases by diffusion into the virtually nitrogen free headspace. We specified the methods accordingly: “[...] to remove residues of N<sub>2</sub> from soil cores by diffusion, including a restricted N<sub>2</sub> production by decreased microbial activity” (lines 112 – 113). Thus, such a low temperature at the beginning is also obligate to reach a static state with no restricted N gas production. As a result, we had to perform a shift in temperature anyway and we just extended this period of low temperature for an additional check for emissions activity during these conditions, which also gave us the opportunity to have a comparison of the effects these two different temperature regimes. Wang et al. (2011) and Wang et al. (2013), respectively, also presented a similar course of temperature shift. However, the temperature dependence of microbial processes like denitrification is well known (Phillips et al., 2015) and should no longer be emphasised in the revised manuscript.

Finally, we chose to switch to the headspace from an oxic state to anaerobicity to determine the current potential for N<sub>2</sub>O and N<sub>2</sub> generation in a completely anaerobic soil matrix, which is important to get a clue about actual potential for these gaseous N losses after BD application. We included the latter to the manuscript: “[...] to determine the generation of N<sub>2</sub>O and N<sub>2</sub> in a completely anaerobic soil matrix. The latter step is important to get a clue about the actual potential for these gaseous N losses after highly concentrated BD application” (lines 120 – 121). Further, the microbes in soil associated with the production of N<sub>2</sub>O and N<sub>2</sub> are able to react fast to changing environmental conditions by utilising existing enzymes within minutes or by *de novo* synthesis within 4 – 8 hours (Rudaz et al., 1991). Wang et al. (2011, 2013) showed in similar studies to ours that the emission of N<sub>2</sub> and N<sub>2</sub>O peaked within less than 24 hours after switching their headspace from oxic to anaerobic conditions, which emphasise our study design as appropriate. Thus, the immediate and strong increase of N<sub>2</sub> emissions in the



sandy soil and the obvious changes in the silty soil on the last day of our study represent very likely the emission potential.

However, your concerns about the duration of our study might be a critical issue; but again, our aim was not to study the dynamics after BD application. Rather it was to estimate the effect of the differing conditions on gaseous  $N_2$  losses. Moreover, the fluxes from the anaerobic headspace indicate that we captured the actual potentials. On the one hand, we observed no changes of  $N_2O$  in the clayey silt, which suggest no further increase would have awaited if we had extended the incubation period. The increased  $N_2$  emissions showed ‘only’ the potential, which would have aroused if the soil cores had been complete completely anaerobic. The latter has, however, no implications for mineral soils since such conditions are unlikely to occur ‘in reality’. On the other hand, the extremely increased  $N_2$  emissions from the loamy sand verify that this soil permitted abundantly oxygen diffusion, which let us assume a prevention of a possible emission increase in the former oxic headspace. Hence, we are convinced, again, that the duration of our measurements were appropriate to distinguish the effect of the examined conditions. Nevertheless, we will discuss the latter assumptions with respect to the incubation duration in the final section “5 Relevance and implications”

Further, we did not reproduce injection of BD per se, but rather the conditions, which would establish in soils after this application method with regard to the high nutrient concentrations: “repacked to reach nutrient concentrations comparable to that in injection bands” (114). We also emphasised this statement in the abstract, now: “[...] the effect of high nutrient concentrations on  $N_2$  losses as they may appear after injection of BD [...]” (13 – 14), “Hence, we performed an incubation experiment with soil cores in a helium-oxygen atmosphere to examine the influence of soil substrate (loamy sand, clayey silt), water-filled pore space (WFPS; 35, 55, 75%) and application rate (0, 17.6 and 35.2 mL BD per soil core [250 cm<sup>3</sup>]) on the emissions of  $N_2O$ ,  $N_2$  and  $CO_2$  after the application of high loads of BD” (14 – 17) and, finally, “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 as appearing after injection [...]” (22 – 24). Nevertheless, a reproduction of injection as done by Markfoged et al. (2011) is quiet impossible for our method due to the huge airtight apparatus which would be needed. This would be, again, rather an issue for a long-term study. However, to treat the present study like an immediate incorporation of a slurry band as recommended, would assume indeed unrealistic high application rates. Nevertheless, we practiced injection of BD also in a field study (Fiedler and Jurasinski, 2015) and did not observe “very heterogeneous distribution of BD” as you stated in your review (which is another issue than the heterogeneity within BD). Additionally, we varied these nutrient concentrations with respect to a differencing spacing between injections rows, which result in a doubling of the application rate with a doubling of the row spacing to get the same applied amount per area, e.g. 160 kg N ha<sup>-1</sup>. Thus, yes, we had mixed twice as much for the 320 kg N ha<sup>-1</sup> treatment. However, we renamed the treatments (low BD and high BD, respectively) and gave the actual BD application per soil core (see above).

Likewise we tuned our conclusions down to the immediate observed effects and omit now the speculations about the overall emissions in field situations and recommend further investigations on this issue: “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.” (25 – 26)

Regarding the emission units, we would prefer to keep the reference to area. First, we would lose some comparability with other studies (e.g., Eickenscheidt et al., 2014) and any, though rough, estimation of area-related N<sub>2</sub> emissions would become impossible. Second, if we were going to use a reference to mass, then a reference to dry matter of BD or the amount of applied N might be more useful, because the amount of soil does not change within each of both soil treatments. However, as our results show already now, there is no effect of the amount of BD on the emission rates. A new calculation may result in decreased rates per unit BD, but this finding could be discussed as is. Third, every approach that relies on a hypothetical mass of soil (or on area), which would be affected directly by BD application, has to be kept speculative as long as there is no comparable research on the effect of different injection techniques *in situ*.

Overall, we acknowledge some methodical pitfall we need to discuss, but we do not think that they justify such a rigorous rejection of our manuscript. First, the duration of our study was appropriate to catch the first emission peak: we did not examine the dynamics (which could last weeks), rather than the effect of different environmental conditions on the actual potential of N<sub>2</sub> and N<sub>2</sub>O emissions. Second, we simulated the BD concentration in soil after injection rather than the injection band per se, which would not be realisable for an available Helium-Oxygen incubation system. However, we can comprehend your doubts about the limited relationships to real conditions in the field and would weaken our conclusions we draw, but it is difficult to perform such studies *in situ*, because current methods does not permit a direct measurement of N<sub>2</sub> fluxes hereunder. Therefore, the use of an artificial atmosphere is prerequisite to reproduce basic numbers, e.g., for an extrapolation to field by N<sub>2</sub>O/(N<sub>2</sub> + N<sub>2</sub>O) product ratios.

Yours faithfully

Sebastian Fiedler and co-authors

## References

Dobbie, K. E., McTaggart, I. P., and Smith, K. A.: Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors, *J. Geophys. Res.*, 104, 26891–26899, doi:10.1029/1999JD900378, 1999.

- Eickenscheidt, T., Heinichen, J., Augustin, J., Freibauer, A., and Drösler, M.: Nitrogen mineralization and gaseous nitrogen losses from waterlogged and drained organic soils in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest, *Biogeosciences*, 11, 2961–2976, doi:10.5194/bg-11-2961-2014, 2014.
- Fiedler, S. R. and Jurasinski, G.: Schlussbericht zum Verbundvorhaben: Potenziale zur Minderung der Freisetzung von klimarelevanten Spurengasen beim Anbau von Energiepflanzen zur Gewinnung von Biogas: Teilvorhaben: Messung des Gasaustauschs und Ermittlung der Klimawirkung sowie C-Bilanzen auf dem Standorten Gülzow (Mecklenburg-Vorpommern), University of Rostock, Rostock, 2015.
- Hagemann, U., Augustin, J., Prescher, A.-K., Kage, H., Glatzel, S., Jurasinski, G., Mühling, K. H., Stichnothe, H., Drösler, M., Bethwell, C., Knieß, A., and Neukam, D.: Schlussbericht zum Verbundvorhaben: Potenziale zur Minderung der Freisetzung von klimarelevanten Spurengasen beim Anbau von Energiepflanzen zur Gewinnung von Biogas, Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF) e.V., Mückeberg, 2016.
- Kaiser, E. A. and Heinemeyer, O.: Temporal changes in N<sub>2</sub>O-losses from two arable soils, *Plant and Soil*, 181, 57–63, doi:10.1007/BF00011292, 1996.
- 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 N<sub>2</sub>O emissions compared to cattle slurry by affecting rate and product stoichiometry of denitrification – An N<sub>2</sub>O isotopomer case study, *Soil Biology and Biochemistry*, 84, 65–74, doi:10.1016/j.soilbio.2015.01.021, 2015.
- Lewicka-Szczebak, D., Augustin, J., Giesemann, A., and Well, R.: Quantifying N<sub>2</sub>O reduction to N<sub>2</sub> based on N<sub>2</sub>O isotopocules – validation with independent methods (helium incubation and <sup>15</sup>N gas flux method), *Biogeosciences*, 14, 711–732, doi:10.5194/bg-14-711-2017, 2017.
- Lewicka-Szczebak, D., Well, R., Köster, J. R., Fuß, R., Senbayram, M., Dittert, K., and Flessa, H.: Experimental determinations of isotopic fractionation factors associated with N<sub>2</sub>O production and reduction during denitrification in soils, *Geochimica et Cosmochimica Acta*, 134, 55–73, doi:10.1016/j.gca.2014.03.010, 2014.
- Markföged, R., Nielsen, L. P., Nyord, T., Ottosen, L. D. M., and Revsbech, N. P.: Transient N<sub>2</sub>O accumulation and emission caused by O<sub>2</sub> depletion in soil after liquid manure injection, *Eur J Soil Sci*, 62, 541–550, doi:10.1111/j.1365-2389.2010.01345.x, 2011.
- Phillips, R. L., McMillan, A. M., Palmada, T., Dando, J., and Giltrap, D.: Temperature effects on N<sub>2</sub>O and N<sub>2</sub> denitrification end-products for a New Zealand pasture soil: *New Zealand Journal of Agricultural Research*, *New Zealand Journal of Agricultural Research*, 58, 89–95, doi:10.1080/00288233.2014.969380, 2015.
- Rudaz, A. O., Davidson, E. A., and Firestone, M. K.: Sources of nitrous oxide production following wetting of dry soil, *FEMS Microbiology Letters*, 85, 117–124, doi:10.1111/j.1574-6968.1991.tb04703.x-i1, 1991.
- 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.
- Wang, R., Willibald, G., Feng, Q., Zheng, X., Liao, T., Brüggemann, N., and Butterbach-Bahl, K.: Measurement of N<sub>2</sub>, N<sub>2</sub>O, NO, and CO<sub>2</sub> Emissions from Soil with the Gas-Flow-Soil-Core Technique, *Environ. Sci. Technol.*, 45, 6066–6072, doi:10.1021/es1036578, 2011.

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 N<sub>2</sub>, N<sub>2</sub>O and NO, *Plant and Soil*, 363, 175–189, doi:10.1007/s11104-012-1264-x, 2013.

### Reply to anonymous referee no. 3

Dear Sir or Madam

Thank you for your thoughts and recommendations on our manuscript. We follow your doubt that the chosen laboratory design does not allow for a sound evaluation of the risk of injection of BD on field scale N<sub>2</sub>O and N<sub>2</sub> losses. In agreement to the other two referees, we have tuned our conclusions to the immediate effects of the investigated factors (soil, amount of BD and WFPS) on gas emissions at the laboratory scale. We omit now the speculations about the overall emissions in field situations and recommend further investigations on this issue.

We suggest our chosen levels of WFPS as rather appropriate since we do not expect a saturation of 100 % WFPS even for the soil surface after precipitation at least for coarse sandy soils as the one we used. However, even if we suggest a wet soil which could reach saturated conditions after additional rain following fertilisation as you prospected, we argue that it is not recommended to use machinery on such wet soils at all. Moreover, the BD is rather not placed into the first few millimetres directly under the surface by injection (we still refer to injection since this technique let us still expect such high concentrations of BD as we applied).

To come to an accommodation regarding to your suggestion on a short communication, we discarded the following phrases, sentences and passages to shorten the manuscript. Line numbers refer to the revised manuscript uploaded on July 4th 2017.

L 2 – 3: „depend mainly on soil texture and moisture”

L 34 – 35: “Further, emission of ammonia (NH<sub>3</sub>) is of environmental concern, e.g., due to acid deposition or conversion to N<sub>2</sub>O (Ferm, 1998; Mosier et al., 1998).

L 38 – 39: “In Germany, the increased demand for renewable energy sources like methane entails an expanded amount of”

L 42: like animal slurry

L 44 – 45: “In general, the effect strengths of BD on gaseous N losses from soil is still under debate (Möller, 2015).

L 57 – 58: “like the favoured reduction of  $\text{NO}_3^-$  rather than  $\text{N}_2\text{O}$  as alternative electron acceptor”

L 60 – 63: “Diffusion of  $\text{O}_2$  depends on the porosity of the soil substrate in conjunction with water-filled pore space (WFPS), while  $\text{O}_2$  is consumed by heterotrophic respiration which depends on mineral N content, carbon (C) availability as well as on temperature.”

L 66 – 71: “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, though high within injection bands, nutrient concentrations and WFPS should theoretically increase further with the row spacing between the injection bands, if a given amount of BD per area is assumed. We are not aware of studies addressing the effect of such high BD concentrations.”

L 74 – 78: “The indicated knowledge gaps are caused not the least by methodological constraints with the direct determination of  $\text{N}_2$  fluxes due to the high background level of  $\text{N}_2$  in the atmosphere, while indirect applications like acetylene-based methods and  $^{15}\text{N}$  tracers are unfavourable since the former implicates serious underestimations and the latter has rather high detection limits (Groffman et al., 2006).”

L 114 – 117: “The mixing was done for methodical reasons since the available space in the incubation vessels was limited and, hence, ‘real’ injection not feasible. However, injection bands have actually a thickness comparable to the sample rings we used.”

L 193 – 202: “After incubation, the recovered  $\text{NH}_4^+\text{-N}$  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  $\text{NH}_4^+\text{-N}$  per kg soil ranged from 8.5 to 10.0 mg (no amendment), from 170.4 to 185.6 mg (LOBD) and from 273.7 to 314.0 mg (HIBD). In the clayey silt,  $\text{NH}_4^+\text{-N}$  contents per kg soil reached only 1.8 to 8.8 mg (no amendment), 89.7 to 98.9 mg (LOBD) and 146.8 to 194.0 mg (HIBD) and, thus, roughly half the amounts of the clayey silt. However, in contrast to the loamy sand, the clayey silt showed also substantial  $\text{NO}_3^-$  contents between 25.7 (35% WFPS without amendment) and 49.8 mg  $\text{NO}_3^-\text{-N}$  (kg soil) $^{-1}$  (55% WFPS with LOBD). Negligible amounts of  $\text{NO}_3^-$  were detected in the loamy sand after incubation: except for a mean value of 2.4 mg  $\text{NO}_3^-\text{-N}$  (kg soil) $^{-1}$  in the unamended treatment with 75% WFPS, the values of all other treatments ranged between 0.2 and 0.5 mg.”

L 204 – 209: “While mean values from 38.6 (55 % WFPS without amendment) to 500.1 mg DOC per kg soil (75 % WFPS, HIBD) were determined for the loamy sand after incubation, lower mean values from 18.9 (55 % WFPS without amendment) to 358.1 mg (35 % WFPS, HIBD) 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 208 LOBD, clayey silt: 105.9 mg for 75 %WFPS with HIBD).”

L 212 – 212: “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<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> 213 (anaerobic at 15°C)”

L 214 – 215: “Although the mean fluxes from the clayey silt were also always smallest in the unamended treatments,

L 222 – 229: “This was similar at 15°C with the exception of 35% WFPS without digestate (0.1 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> , Fig. 3, Day 4 in Table A2). The clayey silt showed much larger fluxes than the loamy sand: even at 2 °C, up to 1.5 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> were detected (55% WFPS with LOBD). After shifting the temperature to 15 °C, the same factor combination had a mean flux of 6.2 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> and the other treatments emitted in mean between 1.0 and 3.0 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> with the exception of incubations with 35% WFPS, where fluxes were smaller. The sand showed weak N<sub>2</sub>O 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 LOBD, respectively”

L 232 – 233: “However, this effect was not noticed at 35% WFPS due to generally low emissions at this moisture level.”

L 245 – 247: “After increasing the temperature to 15 °C, again, the sandy loam released mostly negligible rates of N<sub>2</sub>, except for 0.5 mg m<sup>-2</sup> h<sup>-1</sup> with 55% WFPS and 320 kg N ha<sup>-1</sup> 246 (Fig. 4, Day 4 in Table A3).”

L 256 – 257: “from 3.3 (35% WFPS without N)”

L 258: “were always higher than fluxes from the unamended ones”

L 260 – 261: “not reaching the amounts observed for the sandy loam. This implies that the N<sub>2</sub> emissions were increased from both soils under anaerobic headspace conditions”

L 320 – 328: “An increasing application of BD tended also to decrease the N<sub>2</sub>/(N<sub>2</sub>+N<sub>2</sub>O) ratio, but this effect was also not significant ( $p > 0.05$ , Tuckey’s HSD). In general, nitrite (NO<sub>2</sub><sup>-</sup>) and NO<sub>3</sub><sup>-</sup> are preferably reduced compared to N<sub>2</sub>O during denitrification sequence since the energy yield of each reduction step decreases from NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>O and the reaction rate of reduction is higher for NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> than for N<sub>2</sub>O (Betlach and Tiedje, 1981; Koike and Hattori, 1975). Hence, increasing application rates of BD increase the availability NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> from NH<sub>4</sub><sup>+</sup> oxidation which, consequently, decreases N<sub>2</sub>O reduction. However, in field situations, sooner or later an important fraction of this NH<sub>4</sub><sup>+</sup> will be nitrified and can lead to further N<sub>2</sub>O and N<sub>2</sub> emissions if the WFPS is at sufficient levels. The inhibitory effect is in line with the strong influence of NO<sub>3</sub><sup>-</sup> content of the soils after incubation (Table 5).”

L 339 – 340: “Notably, in contrast to the clayey silt, no or negligible concentrations of NO<sub>3</sub><sup>-</sup> were found in all treatments with loamy sand.”

L 342 – 344: “Actually, high NH<sub>4</sub><sup>+</sup> loads in conjunction with alkaline conditions are typical for BD (Möller and Müller, 2012), which favour NO<sub>2</sub><sup>-</sup> accumulation and may be the reason for the relatively small NO<sub>3</sub><sup>-</sup> recovery in both soils (van Cleemput and Samater, 1995).”

L 366 – 368: “However, the large production rates indicate that also the loamy sand harboured the necessary microbial community able to generate N<sub>2</sub> as soon as the atmospheric conditions become favourable.”

L 377 – 379: “Alternatively, the much smaller increase of N<sub>2</sub> fluxes from the clayey silt could have resulted from depleted mineral N stocks (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) due to the previous gaseous N losses during the course of incubation”

L 390 – 393: “Similarly to N<sub>2</sub>O and N<sub>2</sub> generation by denitrification, 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).”

L 396 – 397: “both in the not amended and especially in the amended treatments.”



L 407 – 409: “Although CO<sub>2</sub> fluxes were mostly higher in the treatments with 320 kg compared to LOBD, this behaviour was not generally reflected in the separate emissions of N<sub>2</sub>O and N<sub>2</sub> which might be a result of the”

L 410 – 413: “However, the N<sub>2</sub>/(N<sub>2</sub>O+N<sub>2</sub>) ratios implied a tendency of N<sub>2</sub>O reduction due to a shortage of alternative electron acceptors like O<sub>2</sub> in the highly amended treatments. Additionally, increasing temperature also influenced indirectly the aerobic status of the soils due to increased microbial activity and, hence, respiration.”

L 414 – 423: “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<sub>2</sub>-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 LOBD, 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<sup>-1</sup>, the CO<sub>2</sub>-C losses ranged from 47% (35% WFPS) to 76% (75% WFPS). By contrast, only between 11% (320 kg N ha<sup>-1</sup>) and 42% (LOBD) 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 would, the light loamy sand may be vulnerable for C losses after BD application than the cloggy clayey silt.”

L 426 – 427: “since we have data for at most two days of static conditions in terms of temperature and headspace aerobicity.”

L 428 – 433: “Generally, the initial phase, i.e., the first week after fertilizer application, is crucial for N<sub>2</sub>O emissions (Dobbie et al., 1999) and most probably also for N<sub>2</sub> because the same processes are involved. Köster et al. (2011; 2015) and Senbayram et al. (2009) observed in incubation experiments N<sub>2</sub>O peaks within the first and third day, which indicate a rather immediate reaction also for N<sub>2</sub> at least in vitro. Nevertheless, the former studies recorded a second plateau of N<sub>2</sub>O emission consistently after around two weeks, though, at very high WFPS.”

L 436 – 456: “Moreover, on the one hand, we observed no changes of N<sub>2</sub>O in the clayey silt under anaerobic headspace, which suggest no further increase would have awaited if we had extended the incubation period with aerobic headspace. The increased N<sub>2</sub> emissions on the last day showed the potential, which would have arisen if the soil cores had been completely anaerobic. The latter has, however, no implications for mineral soils since

such conditions are unlikely to occur in situ. On the other hand, the extremely increased N<sub>2</sub> emissions from the loamy sand on the last day verify that this soil permitted abundantly oxygen diffusion, which let us assume no appearances of possible second emission increases in the former aerobic headspace. [...] microbes associated with the production of N<sub>2</sub>O and N<sub>2</sub> in soils are able to react fast to changing environmental conditions by utilising existing enzymes within minutes or by de novo synthesis within 4 – 8 hours (Rudaz et al., 1991).”

Yours faithfully

Sebastian Fiedler and co-authors