

Interactive  
Comment

## ***Interactive comment on* “The acetylene inhibition technique to determine total denitrification ( $N_2 + N_2O$ ) losses from soil samples: potentials and limitations” by R. Felber et al.**

**R. Well (Referee)**

reinhard.well@vti.bund.de

Received and published: 3 May 2012

### General comments

The paper addresses total denitrification fluxes from a grassland soil measured using the acetylene inhibition technique. A soil core laboratory incubation over 18 month is presented showing relatively low denitrification rates. The limitations of the method are discussed in detail and plausibility of data is evaluated by comparing  $N_2O$  fluxes of acetylated samples to N-balance losses of the same site and  $CO_2$  production to  $CO_2$  fluxes in the field. The authors claim to present minimum denitrification estimates for their site. As the authors explain measuring denitrification in the field is rather difficult

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



and until now not possible without substantial uncertainty. The bias originating from the acetylene ( $C_2H_2$ ) method is substantial. Therefore it is a difficult task for reviewers to decide whether results are suitable for publication. Although I see the method even more critical than the authors, it must be taken into account that there is a vast number of studies with the  $C_2H_2$  method that have been published without discussing the error potential of the method. In view of the authors claim to give a minimum estimate I find it appropriate to accept these data for publication even though they give little progress on the magnitude of denitrification in soils. My personal view is that the denitrification data presented do not lead to progress in understanding denitrification and quantifying the process in the studied soil due to the vast uncertainties. Nevertheless I consider the study as a valuable contribution to the discussion on the validity of the acetylene method. It is highly needed that  $C_2H_2$  studies are published which discuss the method critically, not only those which neglect the problems. There is a clear lack in studies comparing the  $C_2H_2$  method to more reliable techniques. Moreover, it is a fact there are numerous researchers (including many reviewers apparently) who use the method as if it would yield quantitative estimates of denitrification. Therefore this critical study is highly needed and should be published even though the extent of bias was not quantified.

But there the following points need to be addressed and make major review necessary.

$N_2O$  from nitrification in the field has not been taken into account. Therefore the comparison between  $N_2O$  fluxes in field chambers to  $N_2O$  fluxes of acetylated cores is not adequate. Hence, nothing can be said about the  $N_2O/N_2$  ratio of denitrification even in the days with measurable  $N_2O$  fluxes. But I suggest to constrain periods with probable dominance of denitrification (e.g. WFPS > 60-70%). In these periods the ratio of  $N_2O$  fluxes of acetylated and non-acetylated cores could be used as an estimate of the  $N_2O/(N_2+N_2O)$  ratio of denitrification. This can then be compared to literature data to illustrate the plausibility of results. Due to the underestimation of denitrification by the  $C_2H_2$  method, the  $N_2O/(N_2+N_2O)$  ratio should be overestimated. This had been

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

shown by an older review (Becker et al, 1990) revealing that C<sub>2</sub>H<sub>2</sub> studies yielded always high N<sub>2</sub>O/(N<sub>2</sub>+N<sub>2</sub>O) compared to other methods. Unfortunately, I am not aware of a more recent analysis of this type.

Specific comments and technical details

P2852 Title: this is misleading since the method is not evaluated, so potentials and limitations are not assessed but only discussed.

Line 2 The 15N<sub>2</sub> flux method has been used in the field (MOSIER, et al., 1986).

L 6 despite L26 N uptake is typically around  $\frac{1}{2}$  or 2/3 of fertilisation, so it is not a minor fraction of N addition

P2853 L3 delete “denitrified to” since N<sub>2</sub>O can also originate from other processes

L 15-23 Not all sources of bias are mentioned: Inhibition of N<sub>2</sub>O and NO<sub>3</sub> from nitrification Consumption of C<sub>2</sub>H<sub>2</sub> by heterotrophs Incomplete inhibition of N<sub>2</sub>O reduction in some soils

P 2854 L5-10 It would be better to refer to reviews here instead of randomly citing a handful of studies, eg. NIEDER et al. (1989) von RHEINBABEN (1990), Barton et al., 1999

P2856 L 1-4 details on the sampling device must be given. Please report on compaction (which is probable in the clayey soil at high moisture). Was an auger without liner used? I assume that this was the case because otherwise, the author’s aim to have short diffusion path would not be realised. If no liner was used, to which extent could soil cores be kept “intact” until transferal into the incubation chamber. I assume that cores easily broke at low soil moisture?

P2857 L 16 labour

L2858 L24-28 Why were the base runs not reported to estimate the N<sub>2</sub>/N<sub>2</sub>O ratio How about time courses of acetylated samples? This might reflect the reaching of complete

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



Interactive  
Comment

inhibition. It would be interesting to see if stable N<sub>2</sub>O fluxes after acetylation were reached earlier under drier conditions. It would be worth to report this, e.g by investigating scaled N<sub>2</sub>O fluxes (actual vs maximum N<sub>2</sub>O flux) over time after acetylation. If there was a relationship with moisture, it might be useful to show means of moisture classes here.

P2862 L10-14 report methodical details on d<sub>15</sub>N and d<sub>18</sub>O, report sample size and sampling procedure I assume the d<sub>18</sub>O is vs air-O<sub>2</sub>? Please add this information Small changes over time may be due to sampling if samples were extracted without pressure equilibration in the chambers If there were no pressure changes due to sampling then the results could also reflect diffusive exchange of soil gas and chamber gas. This should be discussed.

P2863 L 13 no NO<sub>3</sub> data are reported. It is thus not clear how NO<sub>3</sub> can be used to explain anything in this case.

L14-16 comparison with chambers is not possible because chamber N<sub>2</sub>O (as well as N<sub>2</sub>O of non-actylated cores) can contain N<sub>2</sub>O from nitrification. You might check the data set for periods where N<sub>2</sub>O from nitrification can be assumed to be negligible (e.g WFPS > 60-70%, see Davidson & Oremland (1993)

P2865 L 1-7 How about isotopes of flux events, why are they not shown? See comment above on possible sampling artefacts and diffusive exchange. It is also not clear to me what these results imply with respect to the data set. Is it to illustrate the existence of N<sub>2</sub>O reduction? But most N<sub>2</sub>O reduction can be assumed to occur to N<sub>2</sub>O produced before it is emitted and not with N<sub>2</sub>O diffusing into the soil. The coincidence of no flux and change in isotopes does not prove that there were N<sub>2</sub> fluxes. This section must be deleted or a more profound discussion on methods, the aims and the various possible interpretations is needed. Consider sampling artefacts: for N<sub>2</sub>O analysis typically at least 100mL air is needed. Collecting 8 times 100 ml in a closed chamber without venting causes large convective N<sub>2</sub>O fluxes from the soil. Even with venting, the sampling

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

inevitably causes small pressure changes in the chamber which causes some convection. The changes in  $\delta^{15}\text{N}$  are really small, only  $<1$  per mil. This is small compared to potential differences between  $\delta^{15}\text{N}$  of air  $\text{N}_2\text{O}$  and of soil-derived  $\text{N}_2\text{O}$  of up to approx. 50 per mil. Why should  $\delta^{15}\text{N}$  decrease with time while  $\delta^{18}\text{O}$  increases? Air- $\text{N}_2\text{O}$  is typically 44 per mil vs SMOW. Soil emitted  $\text{N}_2\text{O}$  is typically below 30 per mil if there is not strong  $\text{N}_2\text{O}$  reduction like in very wet soils. So  $\delta^{18}\text{O}$  should also decrease in this study. This discrepancy must be addressed. It is not clear why uncertainties of enrichment factors are mentioned since the isotopic fingerprint of soil-derived  $\text{N}_2\text{O}$  can not be estimated from the data. Overall, this part must be deleted or a far more detailed discussion is needed.

L 19-24 It is not possible to say anything about the effect of elevated  $\text{O}_2$  due to aeration of the cores. Denitrification is far more sensitive to changes in pore space  $\text{O}_2$  than respiration. Denitrification occurs in anaerobic microsites which can have a small fraction of the total soil volume. Hence, aeration may have completely inhibited denitrification with only small changes in respiration. This must be discussed.

Please add a section discussing the consequences of blocking  $\text{N}_2\text{O}$  from nitrification. Did you check time courses of  $\text{N}_2\text{O}$  to track when full inhibition of  $\text{N}_2\text{O}$  reductase might have been reached? It would be helpful to analyse the data-set in this respect and check whether increasing soil moisture lead to slower establishment of steady state fluxes (see above).

P2868 Reliability: -Please distinguish between uncertainty and systematic errors -The sad reality is that there are no comprehensive studies evaluating the  $\text{C}_2\text{H}_2$  method at field-like conditions and comparison between methods were often not done under comparable conditions. For example some of the comparisons between  $^{15}\text{N}$  and  $\text{C}_2\text{H}_2$  studies compared acetylated fluxes with emitted  $^{15}\text{N}$ -tracer-derived  $\text{N}_2+\text{N}_2\text{O}$ , neglecting the emission of  $\text{N}_2+\text{N}_2\text{O}$  from soil-borne non-labeled  $\text{NO}_3$  in the  $^{15}\text{N}$  tracer studies. Russow's results and Bollman et al are contradictive since Bollman et al measured lowering of  $\text{N}_2\text{O}$  fluxes in presence of  $\text{C}_2\text{H}_2$ . Note that Murray & Knowles is a slurry study

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

under anaerobic condition and that Bollman state their reported effect is only effective in presence of O<sub>2</sub>! So anaerobic slurries are probably not affected by this artefact. Overall, the reliability of the C<sub>2</sub>H<sub>2</sub> method must be discussed more open he. From my view the authors argue a bit too much in favour which is not supported by the proven error potential of this method. Moreover aeration effect of the small core incubation must be discussed here: if C<sub>2</sub>H<sub>2</sub> reaches denitrifying microsites, the same can apply for O<sub>2</sub>. The authors did not attempt to conduct an independent check for the incubations using incubations under He/O<sub>2</sub> or with the <sup>15</sup>N<sub>2</sub> method. Because this was not done, little can be said about reliability of the results. Groffman's statement (2006) on the "reasonably robust" nature of the C<sub>2</sub>H<sub>2</sub> method in NO<sub>3</sub>-rich soils is hardly proven, the NO decomposition problem reported by Bollman & Conrad, 19997 (although mentioned in that paper) is neglected in his statement.

P2869 L8 It is not clear what uncertainty is addressed here. But please distinguish between uncertainty and systematic underestimation (which applies for the acetylene method). It is not suitable to compare the uncertainty range of observed denitrification estimates (which specifies only the uncertainty arising from different ways to calculate fluxes as far as I understand) to the range of other studies which are not subject to systematic underestimation.

L 10-22 It is not clear to me why differences in results are addressed here. Clearly, the true denitrification rates of different soils have large ranges, but this is not the point here. So these paragraphs should be deleted.

L24f, N<sub>2</sub>O/N<sub>2</sub> ratio It is not adequate to compare core fluxes to chamber fluxes (see also previous comments on N<sub>2</sub>O from nitrification and on core aeration and disturbance). Instead, the ratios should be derived from core data only. Only if cores without C<sub>2</sub>H<sub>2</sub> did not emit N<sub>2</sub>O, these samples preclude calculation of the N<sub>2</sub>O/N<sub>2</sub> ratio. In Table 1 core fluxes without C<sub>2</sub>H<sub>2</sub> should be shown in addition to chamber data.

P 2870 L 7-14 This is a highly relevant comparison. I agree that in principle this might

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

give insight in the reliability of the C<sub>2</sub>H<sub>2</sub> data. But of course accurate N budgets are very difficult to obtain. I have not read the paper so I can't comment on this in detail. But from my view this comparison is only useful here if all N-fluxes had been measured. As far as I can see this was not the case for symbiotic or non-symbiotic N<sub>2</sub>-fixation and NH<sub>3</sub>-uptake by plants. Together these inputs can easily amount to 50 -100 kg N/ha/a or even more. So this budget approach seems to include higher uncertainty than shown here. A more detailed discussion of the budget approach is thus needed.

I suggest to compare the denitrification data also to expected rates for this soil and land-use type. For example, the authors might refer to an empirical classification scheme for denitrification in agricultural soils in Germany (Gäth et al., 1997) where the study would fall in the class of medium denitrification rates (30 to 50 kg N/ha/a) for soils with loamy to clayey texture and with stagnic properties.

References Barton et al., 1999 Aust J Soil Res 37, 1073f Becker, K. W., H. Hoper, et al. (1990). "Rates of denitrification under field conditions as indicated by the acetylene inhibition technique - a critical review." Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 60(25-30; Proceedings): International Workshop on Denitrification. Soil, Rhizosphere and Aquifer, Giessen, FRG, March 17-19 1989.) Davidson, E. A. and R. S. Oremland (1993). "Soil water content and the ratio of nitrous oxide to nitric oxide emitted from soil." Biogeochemistry of global change: radiatively active trace gases GÄTH, S., ANTONY, F., BECKER, K.-W., GERIES, H., HÖPER, H., KERSEBAUM, C. & NIEDER, R. (1997): Bewertung der standörtlichen Denitrifikationsleistung und des Mineralisations-/Immobilisationspotenzials von Böden und Bodennutzungssystemen. – Mitt. Dt. Bodenkdl. Ges. 85: 1373–1376. MOSIER, A.R., W.D. GUENZI & E.E. SCHWEIZER (1986): Soil losses of dinitrogen and nitrous oxide from irrigated crops. Soil Sci Soc. Am. J. 50: 344 - 348. NIEDER, R., G. SCHOLLMAYER & J. RICHTER (1989): Denitrification in the rooting zone of cropped soils with regard to methodology and climate: a review. Biol. Fert. Soils 8: 219 - 226, RHEINBABEN, W.v. (1990): Nitrogen loss from agricultural soils through denitrification - a critical evaluation. Zeitschr.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

Pflanzenern. Bodenkd. 153, 157 – 166

---

Interactive comment on Biogeosciences Discuss., 9, 2851, 2012.

**BGD**

9, C953–C960, 2012

---

Interactive  
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

C960

