

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.

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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

C953

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

C954

shown by an older review (Becker et al, 1990) revealing that C₂H₂ studies yielded always high N₂O/(N₂+N₂O) 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 15N₂ 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 $\frac{2}{3}$ of fertilisation, so it is not a minor fraction of N addition

P2853 L3 delete "denitrified to" since N₂O can also originate from other processes

L 15-23 Not all sources of bias are mentioned: Inhibition of N₂O and NO₃ from nitrification Consumption of C₂H₂ by heterotrophs Incomplete inhibition of N₂O 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 N₂/N₂O ratio How about time courses of acetylated samples? This might reflect the reaching of complete

C955

inhibition. It would be interesting to see if stable N₂O fluxes after acetylation were reached earlier under drier conditions. It would be worth to report this, e.g by investigating scaled N₂O fluxes (actual vs maximum N₂O 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 d¹⁵N and d¹⁸O, report sample size and sampling procedure I assume the d¹⁸O is vs air-O₂? 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 NO₃ data are reported. It is thus not clear how NO₃ can be used to explain anything in this case.

L14-16 comparison with chambers is not possible because chamber N₂O (as well as N₂O of non-actylated cores) can contain N₂O from nitrification. You might check the data set for periods where N₂O 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 N₂O reduction? But most N₂O reduction can be assumed to occur to N₂O produced before it is emitted and not with N₂O diffusing into the soil. The coincidence of no flux and change in isotopes does not prove that there were N₂ 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 N₂O analysis typically at least 100mL air is needed. Collecting 8 times 100 ml in a closed chamber without venting causes large convective N₂O fluxes from the soil. Even with venting, the sampling

C956

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 N_2O and of soil-derived N_2O of up to approx. 50 per mil. Why should $\delta^{15}\text{N}$ decrease with time while $\delta^{18}\text{O}$ increases? Air- N_2O is typically 44 per mil vs SMOW. Soil emitted N_2O is typically below 30 per mil if there is not strong N_2O 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 N_2O 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 O_2 due to aeration of the cores. Denitrification is far more sensitive to changes in pore space 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 N_2O from nitrification. Did you check time courses of N_2O to track when full inhibition of N_2O 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 C_2H_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}N and C_2H_2 studies compared acetylated fluxes with emitted ^{15}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 NO_3 in the ^{15}N tracer studies. Russow's results and Bollman et al are contradictive since Bollman et al measured lowering of N_2O fluxes in presence of C_2H_2 . Note that Murray & Knowles is a slurry study

C957

under anaerobic condition and that Bollman state their reported effect is only effective in presence of O_2 ! So anaerobic slurries are probably not affected by this artefact. Overall, the reliability of the C_2H_2 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_2H_2 reaches denitrifying microsites, the same can apply for O_2 . The authors did not attempt to conduct an independent check for the incubations using incubations under He/O_2 or with the $^{15}\text{N}_2$ 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_2H_2 method in NO_3 -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, $\text{N}_2\text{O}/\text{N}_2$ ratio It is not adequate to compare core fluxes to chamber fluxes (see also previous comments on N_2O from nitrification and on core aeration and disturbance). Instead, the ratios should be derived from core data only. Only if cores without C_2H_2 did not emit N_2O , these samples preclude calculation of the $\text{N}_2\text{O}/\text{N}_2$ ratio. In Table 1 core fluxes without C_2H_2 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

C958

give insight in the reliability of the C₂H₂ 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 N₂-fixation and NH₃-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.

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C959

Pflanzenern. Bodenkdl. 153, 157 – 166

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

C960