Biogeosciences Discuss., 9, C1188–C1193, 2012
www.biogeosciences-discuss.net/9/C1188/2012/

Biogeosciences Discussions

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.

M. Dannenmann (Referee)

michael.dannenmann@kit.edu

Received and published: 14 May 2012

Felber et al. provide a study on denitrification from an agricultural site in Switzerland. Since denitrification is probably representing the most important and largest knowledge gap in N cycling, this topic is of high relevance and well in the scope of BG. The authors estimated denitrification by applying the acetylene inhibition technique in the presence of oxygen in their soil samples. In view of the systematic but irreproducible errors of this approach, I would usually tend to reject such a manuscript. However, large parts of the manuscript actually are dealing explicitely with the many and severe limitations of the method, providing a mostly accurate discussion of the issue. This is very useful,

since abundant studies have still been published in recent years simply applying this approach and providing denitrification estimates, which are likely severely but irreproducibly underestimated. Furthermore the authors conduct comparisons of N2O fluxes from acetylene incubations with field data of N2O fluxes (which is not valid as it was done, see below). Finally they come up with a lower bound estimate of denitrification and provide uncertainty estimates. In view of the discussion of both the denitrification measurement problem and the severe limitations of the acetylene inhibition technique, I consider this can be a valuable and publishable contribution, hopefully helping to better distribute the awareness on the limitations of acetylene inhibition approach in the scientific community and thus helping to avoid that in future acetylene studies are published simply providing denitrification rates as if they would be true. However, there are several issues which require attention and major revision. It must be even more clearly emphasized, that the present study cannot provide accurate denitrification rates. The authors are from my point of view still not critical enough in their discussion about the acetylene inhibition approach, but should even more point out the limitations rather than defend the acetylene method with the limitations of other, more modern methods. Limitations of other (actually more reliable methods) do not improve the accuracy of the acetylene method. I also felt very uncomfortable about the removal of "bad data", i. e. choosing only a subset of highest values out of the measurements. This very artificially and irreproducibly increased the denitrification estimates of the authors. It would be much more convincing to use all the data resulting in lower denitrification estimates and accept this as even more strongly underestimated data rather rather than conducting an artificial data tuning. Additionally, the comparison with field N2O flux measurements is not valid as it is done in the current approach, since field fluxes comprise both net N2O losses from nitrification and denitrification pathways. The latter discussion needs to be completely revised. Finally it has to be stated that the most useful comparison would have been a comparisons of acetylene results with a more modern method such as the 15N gas flux or the He incubation techniques rather than with these field chamber N2O measurements. And I felt that the references were not complete – I missed both some very recent studies as well as very old but nevertheless still very relevant studies. Given the authors can address these issues (and further issues named below under specific comments), I would welcome this manuscript to be published in Biogeosciences. Specific comments 2852 L3: Monitoring of N2 emissions at the field scale is not "impossible" but e. g. possible in agricultural systems when high amounts of 15N are added and gas samples for 15N analysis are taken by use of chambers. See e. g. Rolston et al. 1978, 1982. Abstract: I would name and discuss limitations of the acetylene technique here in the abstract, since I consider the (still not sufficiently) critical view of the method being the major strength of the manuscript. 2852 L25 There is widespread evidence that plants also use monomeric organic N forms (Näsholm et al. 2009, New Phytologist), but not only ammonium and nitrate, as the authors write here. 2853 L21. The authors write that "all known approaches suffer from a large degree of uncertainty". This may be partly misleading, since some non-acetylene approaches are providing indeed high precision in the measurements. Often, the problem rather is that N2 emissions are extremely variable at temporal and spatial scales, and that such measurements are often time-consuming allowing for minor temporal or spatial replication only. L23 Isotope-based approaches are already available to estimate total N2 losses and are in particular applicable for agricultural systems since several decades to measure fertilizer denitrification. 2854 L5: There are several more very well-constrained non-acetylene but isotope-based studies for agricultural soils, e. g. Rolston et al. 1978, Rolston et al. 1982 and Mosier et al. 1986. It would be important to cite these studies also (and discuss later), since they reveal different results as compared to the studies using the acetylene technique, namely higher higher N2:N2O ratios, indicating the limitations of the acetylene approach. A compilation of studies is provided by Schlesinger et al. 2009. L20: Incomplete inhibition of N2O reductase by acetylene has been recently demonstrated (Yu et al. 2010). Qin et al. 2012 observed incomplete acetylene inhibition in denitrification potential incubations even when 10 g of sieved soil was incubated. It appears questionable if this is only related to diffusion problems. L16ff: There are even more problems with acetylene than the ones the au-

thors name, i. e. utilization of C2H2 as a substrate for denitrification if C is limiting, and inhibition of nitrate ammonification, the extra pair of electrons that would have been used to reduce N2O to N2 can increase reduction of NO3. Furthermore, it should be clarified here that the major problem of NO scavenging is only occurring under presence of oxygen. Thus, this problem is – to our current state of knowledge - not affecting the determination of potential denitrification. Finally, acetylene may inhibit gross nitrification, thus affecting denitrification rates when nitrate is limiting. These issues are partly discussed later, but may be also mentioned here. L24 The authors write that "clearly, there is at present no scientific consensus as to the reliability and adequacy of the C2H2 inhibition technique." Despite of the large amount of acetylene studies on denitrification still published in the last decade, I would rather say that there is clearly sufficient published knowledge that the acetylene method used for denitrification measurements at least in the presence of oxygen is severely and irreproducibly biased and therefore not reliable. Clearly this is ignored by many studies even without discussing the limitations (the present study does a much better job) of the acetylene method, but ignoring the published knowledge has from my point of view nothing to do with scientific consensus on the method. In the following sentence, the authors write that the study investigates the plausibility of total N losses from a grass land obtained by the acetylene inhibition method by comparing to field N2O measurements. However, this comparison is biased by the neglection of N2O production via nitrification pathways. In order to test the plausibility of the acetylene method, it would be much more straightforward to conduct a comparison with modern methods such as the Helium soil core flushing technique or with isotope-based methods (15N2 and 15N2O measurements). 2861 L1ff The authors "filtered" their dataset, removing data showing smaller N2O emissions under presence of acetylene than without acetylene. However, as the authors correctly write, it could have happened that results with other samples showing the expected higher N2O production in acetylene treated than in control samples could be even be more biased. Therefore this seems to be a rather random, hardly reproducible tuning of the data. 2862 L 10 how was delta18O and -15N measured? L25ff

Here, the authors compare field N2O emissions (coming from nitrification and denitrification pathways) and acetylene laboratory incubations under presence of oxygen, providing more or less underestimated sums of N2+N2O emissions from denitrification. Please clarify this for the reader – and – what can you derive from this comparison? Fig. 3c needs a y axis break. 2864 L20ff Field fluxes can also be higher due to N2O emerging from nitrification pathways. Thus, fertilizer addition may also have stimulated N2O emission from nitrification, and N2O fluxes are not only "controlled by available N and accessible energy . . . for the denitrifying microbial communities." But also by substrate for nitrifiers. It would have supported conclusions about substrate and N2O production when there had been measurements on soil mineral N and extractable C. P 2866 L20 I have concerns about the selection of the three highest N2+N2O fluxes only. By the neglection of low measurements represents a quite artificial, unreproducible manipulation of the dataset. This cannot really be justified by the assumption that C2H2 diffusion may have been best in these cores. This is rather a selection of a subset of the measurements of a strongly biased method which fits best in the expectations and thus is not really appropriate. Here, the authors selected 3 out of 7 measurements – to further increase the denitrification rates one could measure 20 samples and take the largest 3 rates? There is an unknown interference of varying method-inherent underestimation by NO scavenging, nitrification inhibition etc. as well as spatial and temporal variability of fluxes. I do not think that this problem can be addressed by neglecting low fluxes. It would be more straightforward to take the acetylene method values as they are and compare them to more modern methods for denitrification measurements such as isotope-based methods or the He incubation method and then think about underestimation correction factors – these could then be compared with other studies. L25ff: The comparison between in situ chamber measurements of N2O and C2H2 lab incubations is not only an issue of spatial variability across sampling/chamber spots and N2O consumption in the soil profile. The chamber measurements include both N2O emission from nitrification and denitrification pathways, while nitrification was inhibited by the acetylene addition in the lab. Figure 3c) inadequate y axis scaling makes it im-

possible to follow N2O dynamics at the lower flux rates, which are dominating almost always. Add an axis break. Literature cited Yu, K., Seo, D.C. DeLaune, R.D. 2010 Incomplete Acetylene Inhibition of Nitrous Oxide Reduction in Potential Denitrification Assay as Revealed by using 15N-Nitrate Tracer. Comm. Soil Sci. Plant Anal. 41, 2201-2210. Näsholm et al. 2009, Uptake of organic nitrogen by plant. New Phytologist 182, 31-48. Rolston, D.E., Hoffman, D.L., Toy, D.W. 1978 Field measurements of denitrification: I. Flux of N2 and N2O. Soil Sci. Soc. Am. J. 42, 863-869. Rolston, D.E., Sharpley, A.N., Toy, D.W. Broadment, F.E. 1982 Field measurements of denitrification. III. Rates during irrigation cycles. Soil Sci. Soc. Am. J.. 46, 289-296. Mosier, A.R., Guenzi, W.D. Schweizer, E.E. 1986 Soil losses of dinitrogen and nitrous oxide from irrigated crops in Northeastern Colorado. Soil Sci. Soc. Am. J. 50, 344-348. Qin S, Hu C, Oenema O, 2012, Quantifying the underestimation of soil denitrification potential as determined by the acetylene inhibition method. Soil Biology and Biochemistry 47, 14-17. Schlesinger et al 2009 On the fate of anthropogenic nitrogen. PNAS, Supplementary material, 10.1073/pnas.0810193105PNAS Supporting Information

---

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