Geophysical Research Abstracts
Vol. 15, EGU2013-9950, 2013
EGU General Assembly 2013

Acetylene inhibition of N2O reduction in laboratory soil and groundwater denitrification assays: evaluation by 15N tracer and 15N site preference of N2O

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The measurement of denitrification in soils and aquifers is still challenging and often enough associated with considerable experimental effort and high costs. Against this background, the acetylene inhibition technique (AIT) applied in laboratory soil and groundwater denitrification assays is by far the most effective approach. However, this method has been largely criticized, as it is susceptible to underestimate denitrification rates and adds an additional carbon source to the substrates to be investigated.

Here we provide evidence that the AIT is not necessarily an inappropriate approach to measure denitrification, that its reliability depends on the drivers governing the process, and that the 15N site preference of N2O (SP) may serve as a tool to assess this reliability. Two laboratory batch experiments were conducted, where sandy aquifer material and a peat soil were incubated as slurries. We established (i) a standard anaerobic treatment by adding KNO_3 (10 mg N L-1), (ii) an oxygen treatment by adding KNO_3 and O_2 (5 mg L-1), and (iii) a glucose treatment by adding KNO_3 supplemented with glucose (200 mg C L-1). Both experiments were run under 10 % (v/v) acetylene atmosphere and as 15N tracer treatments using labeled $K15NO_3$ (60 atom % 15N).

In the case of the standard anaerobic treatments, we found a very good agreement of denitrification potential obtained by the AIT and 15N tracer methods. SP of N2O of the AIT samples from this treatment ranged between -4.8 and 2.6 ‰ which is indicative for N2O production during bacterial denitrification but not for N2O reduction to N2. In contrast, we observed substantial underestimation of denitrification by AIT for the glucose treatments compared to the 15N method, i.e. denitrification was underestimated by 36 % (sandy aquifer material) and 47 % (peat soil). SP of N2O of the AIT samples from this treatment ranged between 4.5 and 9.6 ‰ which suggests occurrence of bacterial N2O reduction. In the case of the oxygen treatments, we observed a very good agreement of denitrification potential obtained by the AIT and 15N tracer methods for the aquifer material, but a significant underestimation of 20 % in the AIT samples of the peat soil. The 15N site preference of N2O again mirrored this and ranged between -1.2 and -3.5 ‰ (aquifer material) and 5.5 and 11.0 (peat soil), respectively.

We conclude that the AIT can act as a reliable method in laboratory soil and groundwater bacterial denitrification assays, but our results suggest that this relies on substrate types and incubation conditions. Additional measurements of SP have potential to assess AIT efficacy and can help to reduce parallel time-consuming and expensive 15N tracer experiments.