

Dec 14, 2018

Dear Dr. Myers,

We submit the manuscript herewith entitled “Measurement of the potential rates of dissimilatory nitrate reduction to ammonium (DNRA) based on  $^{14}\text{NH}_4^+ / ^{15}\text{NH}_4^+$  analysis by sequential conversion and detection with quadrupole GC/MS”.

- 1) All authors have read the submitted version of the manuscript and agree to submit the work to JoVE.
- 2) This work has not been published before and is not being considered for publication by another journal.
- 3) In this paper, we provide the detailed procedure for the measurement of potential DNRA rate and applied it to the salt marsh sediments. Briefly, the potential DNRA rate can be calculated from the  $^{15}\text{N}$ -labeled ammonium ( $^{15}\text{NH}_4^+$ ) accumulation rate in  $^{15}\text{N}$ -labeled nitrate ( $^{15}\text{NO}_3^-$ ) addition incubation. Determination of non-labeled and labeled ammonium concentrations is comprised of several sequential steps, including the collection and conversion of the ammonium. These steps are basically based on previous studies, we modified several procedures. Firstly, in the step of collecting  $\text{NH}_4^+$ , we shortened the incubation period and lowered temperature. Secondly, we made a few modifications to the method for conversion about preservation and preparation of the bacterial cells required in this procedure. These modifications allow to shorten the required time for a series of the experiments. In this method,  $\text{NH}_4^+$  is finally converted to  $\text{N}_2\text{O}$  and it enable to determine concentration and isotope ratio of  $\text{NH}_4^+$  by quadrupole GC/MS since  $\text{N}_2\text{O}$  has low atmospheric background. GC/MS is less expensive and easy to manage compared with IRMS, which has been typically used in  $^{15}\text{NH}_4^+$  studies. The representative result demonstrated the proposal procedures effective to determine the  $\text{NH}_4^+$  isotope ratio and concentration especially for samples with relatively high concentration of ammonium. Because of these, we believe that proposed method will gain broad attention from the scientific community and make a contribution to JoVE.

We appreciate if the manuscript could be reviewed and considered for publication.

Kind regards,

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