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## Measurement of undisturbed di-nitrogen emissions from aquatic ecosystems

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Increased production of reactive nitrogen (Nr) from atmospheric di-nitrogen (N2) during the last century has greatly contributed to increased food production1-4. However, enriching the biosphere with Nr through N fertilizer production, combustion, and biological N2 fixation has also caused a series of negative effects on global ecosystems 5,6, especially aquatic ecosystems7. The main pathway converting Nr back into the atmospheric N2 pool is the last step of the denitrification process, i.e. the reduction of nitrous oxide (N2O) into N2 by microorganisms7,8. Despite several attempts9,10, there is not yet an accurate, fast and direct method for measuring undisturbed N2 fluxes from denitrification in aquatic sediments at the field scale11-14. Such a method is essential to study the feedback of aquatic ecosystems to Nr inputs1,2,7.

Here we show that the measurement of both N2O emission and its isotope signature can be used to infer the undisturbed N2 fluxes from aquatic ecosystems. The microbial reduction of N2O increases the natural abundance of 15N-N2O relative to 14N-N2O ( $\delta$ 15N-N2O). We observed linear relationships between  $\delta$ 15N-N2O and the logarithmic transformed N2O/(N2+N2O) emission ratios. Through independent measurements, we verified that the undisturbed N2 flux from aquatic ecosystems can be inferred from measurements of N2O emissions and the  $\delta$ 15N-N2O signature. Our method allows the determination of field-scale N2 fluxes from undisturbed aquatic ecosystems, and thereby allows model predictions of denitrification rates to be tested. The undisturbed N2 fluxes observed are almost one order of magnitude higher than those estimated by the traditional method, where perturbation of the system occurs, indicating that the ability of aquatic ecosystems to remove Nr may have been severely underestimated.