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Application note 015

Determining Sources and Cycling of Nitrate in an Agricultural Catchment Using $\delta^{15}N$ and $\delta^{18}O$ in Nitrate

Understanding the nitrogen cycle is increasingly important. Humans have more than doubled the annual global production of reactive nitrogen, negatively impacting water quality, and terrestrial and coastal ecosystems. Agricultural activity is a major source of anthropogenic reactive nitrogen. It accounts for 75% of anthropogenic fixed nitrogen and comes primarily from the production of nitrogen fertilisers.

However, a poor understanding of the nitrogen cycle in agricultural environments results in the loss of nearly three quarters of agricultural nitrogen to water and the atmosphere rather than contributing to food production. Nitrification and denitrification play key roles in the loss of agricultural nitrogen. Nitrification transforms ammonium to nitrate. Nitrate is the form of fixed nitrogen which is most readily lost, via denitrification in gaseous forms, or as dissolved nitrate exported in streams.

KEYWORDS: nitrogen and oxygen isotopes in nitrate, hydrology, agriculture, denitrification.

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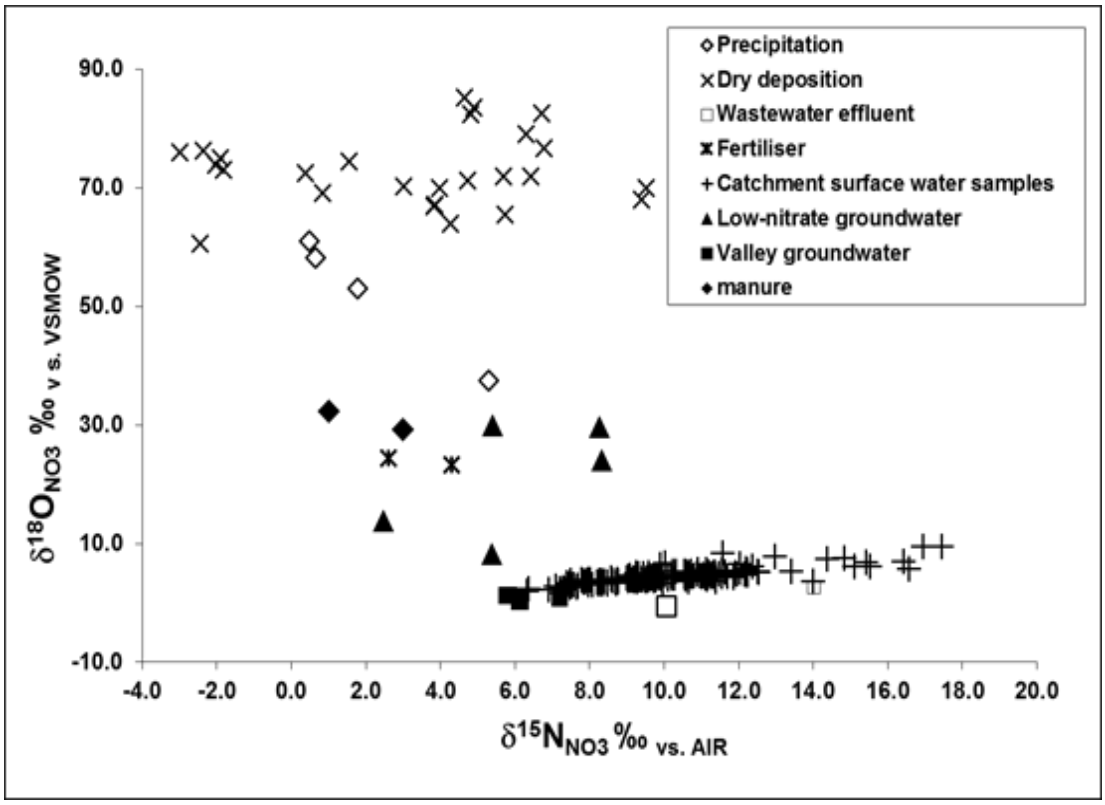
The Wensum catchment in East Anglia, eastern UK, is a 570 km^2 lowland agricultural watershed. Over 90% of the land in the catchment is classified as especially vulnerable to nitrate contamination. Diffuse pollution from nitrogen fertilisers and manure in addition to sewage effluent discharge results in a high level of export of dissolved nitrate from the catchment via the River Wensum, a 75 km Chalk limestone stream.

Dual isotope analysis was used to determine the isotopic composition of sources of nitrate and the effects of partial denitrification of nitrate in the hyporheic zone of the river Wensum seen in mass dependent isotopic fractionation. Denitrification accounts for the loss of up to 30% nitrate nitrogen from the river by the catchment outlet, via transfer to the atmosphere (Wexler et al. 2011; 2012).



Method

Samples are filtered using 0.22 micron cellulose acetate filter units, and frozen at -15 °C prior to analysis. Nitrate in samples is converted to nitrous oxide using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002). The denitrifier method uses the biochemical activity of a naturally occurring single strain bacterial denitrifier, Pseudomonas chlororaphis subsp. aureofaciens (ATCC #13985); a facultative anaerobe which, under low oxygen conditions, uses nitrate and nitrite as electron acceptors during anaerobic respiration. The strain lacks nitrous oxide reductase activity, so the denitrification pathway is truncated at the nitrous oxide step, meaning that nitrate and nitrite are quantitatively converted to nitrous oxide. Nitrous oxide isotopic composition is measured against a laboratory cylinder reference gas on a Sercon GEO 20:20 continuous flow gas chromatograph isotope ratio mass spectrometer, with a TG II prep system, a customised Gilson autosampler, and an open split interface. Nitrous oxide is extracted and purified from sample vials before isotopic analysis. Sample vials contain media, bacterial cells, and river water, with trace gases including nitrous oxide.

Vials are purged with helium using a double needle, and the helium + vial gas mixture is cleaned by a reverse flow Nafion drier and a magnesium perchlorate trap to remove water, a Carbosorb trap to remove carbon dioxide and a Supelco F trap to remove volatile organic compounds. A stationary loop immersed in a dry-ice/ethanol trap removes residual water. The nitrous oxide is trapped and focussed in a cryogenic trap consisting of two steel loops immersed in liquid nitrogen. After cryofocussing the nitrous oxide is passed through a Varian Poraplot/Q pre-column to separate any further interfering compounds. Finally the nitrous oxide passes through a HP-PLOT/Q GC column kept at 30 °C in a GC oven to separate of carbon dioxide, before passing the carbon dioxide and nitrous oxide peaks to the mass spectrometer. The GEO 20:20 mass spectrometer measures masses 44, 45, and 46. The 45/44 and 46/44 ratios are calculated by the Sercon Callisto operational software. The international reference materials IAEA-NO_3, USGS 34 and USGS 35 are prepared alongside samples and used to calibrate results, with a long term precision of 0.5 ‰ for both isotopes.

References

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GEO 20-20 IRMS, at the Science Analytical Facilities, University of East Anglia

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