ABSTRACT

Benthic nitrate reduction in estuaries: process rates and key functional genes for denitrification (DN), nitrate ammonification (DNRA) and anammox (AN).

David B. Nedwell
University of Essex

The rates of DN, DNRA and AN have been investigated at three sites along the nutrified Colne estuary, U.K. using ^{15}N isotope pairing techniques, together with seasonal changes. DNRA was favoured at low ambient nitrate concentrations, and denitrification at higher nitrate. AN accounted for at most 30% of N_2 formation in the upper estuary site, was occasionally detected in the middle estuary but not detected at the estuary mouth. Key functional genes (*nar, nap, nir, nrf*) for nitrate reduction, DN and DNRA were measured along the estuary and with depth in the sediment, and 16S rRNA for anammox bacteria. Similar work was carried out in three tropical estuaries in Thailand, Indonesia and Fiji, and the data from these tropical estuaries will be compared to those from the temperate Colne estuary.

Denitrification in Terrestrial Ecosystems

Peter M. Groffman
Cary Institute of Ecosystem Studies

Denitrification is a miserable process to study in terrestrial ecosystems. Methods for quantifying the process are lousy, variability in activity is absurdly high, and temporal and spatial scaling challenges are extreme. These difficulties are unfortunate, as the need for information on terrestrial denitrification is great. Global analysis suggest that the majority of denitrification,especially of anthropogenically produced nitrogen, occurs in the terrestrial environment (Seitzinger et al. 2006). At the regional scale, there is great interest in understanding and managing denitrification to reduce eutrophication in coastal waters (Van Breemen et al. 2002, Schaefer et al. 2009). And at the ecosystem/field scale, denitrification is a key controller of the nitrogen availability to plants and hydrologic exports to groundwater (Gentry et al. 2009).

Available methods for measuring terrestrial denitrification are problematic for a variety of reasons; they change substrate concentrations, disturb the physical setting of the process, lack sensitivity or are prohibitively costly in time and expense (Groffman et al. 2006). Most fundamentally, it is very difficult to quantify the dominant end-product (N_2) of denitrification given its high background concentration in the atmosphere. However, new techniques for direct measurement of N_2 flux are becoming more common (Swerts et al. 1995, Butterbach-Bahl et al. 2002), although these still rely on the use of extracted cores. ^{15}N-based *in situ* methods are suitable for sites with high soil nitrate concentrations, but more sensitive mass spectrometers will be needed for tracer-level studies in natural ecosystems (Stange et al. 2007).

Spatial and temporal variation in denitrification is high due to control of the process by multiple factors (oxygen, nitrate, carbon) that each vary in time and space (Parkin 1993). A particular challenge is that small areas (hotspots) and brief periods (hot moments) frequently account for a high percentage of N gas flux activity (Parkin 1987, McClain et al. 2003). These phenomena are challenging to account for in measurement, modeling and scaling efforts (Groffman et al. 2009).

However, all is not bleak. Recent advances in measurement methods (cited above), new conceptual approaches for addressing hotspot and hot moment dynamics (Groffman et al. 2009), and clever new remote sensing and geographic information system-based scaling methods (Ollinger and Smith 2005) suggest that we are poised to make great improvements in our understanding of terrestrial denitrification over the next 5 – 10 years. These improvements will increase our basic science understanding of a complex biogeochemical process and our ability to manage widespread nitrogen pollution problems.

Literature Cited

Butterbach-Bahl, K., G. Willibald, and H. Papen. 2002. Soil core method for direct simultaneous determination of N-2 and N_2O emissions from forest soils. Plant and Soil **240**:105-116.

Gentry, L. E., M. B. David, F. E. Below, T. V. Royer, and G. F. McIsaac. 2009. Nitrogen mass balance of a tile-drained agricultural watershed in east-entral Illinois. J Environ Qual **38**:1841-1847.

Groffman, P., K. Butterbach-Bahl, R. Fulweiler, A. Gold, J. Morse, E. Stander, C. Tague, C. Tonitto, and P. Vidon. 2009. Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry **92**:49-77.

Groffman, P. M., M. A. Altabet, J. K. Bohlke, K. Butterbach-Bahl, M. B. David, M. K. Firestone, A. E. Giblin, T. M. Kana, L. P. Nielsen, and M. A. Voytek. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications **16**:2091-2122.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, W. H. McDowell, and G. Pinay. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems **6**:301-312.

Ollinger, S. V. and M. L. Smith. 2005. Net primary production and canopy nitrogen in a temperate forest landscape: An analysis using imaging spectroscopy, modeling and field data. Ecosystems **8**:760-778.

Parkin, T. B. 1987. Soil microsites as a source of denitrification variability. Soil Science Society of America Journal **51**:1194-1199.

Parkin, T. B. 1993. Spatial variability of microbial processes in soil - a review. Journal of Environmental Quality **22**:409-417.

Schaefer, S., J. Hollibaugh, and M. Alber. 2009. Watershed nitrogen input and riverine export on the west coast of the US. Biogeochemistry **93**:219-233.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications **16**:2064-2090.

Stange, C. F., O. Spott, B. Apelt, and R. W. B. Russow. 2007. Automated and rapid online determination of N-15 abundance and concentration of ammonium, nitrite, or nitrate in aqueous samples by the SPINMAS technique. Isotopes in Environmental and Health Studies **43**:227-236.

Swerts, M., G. Uytterhoeven, R. Merckx, and K. Vlassak. 1995. Semicontinuous measurement of soil atmosphere gases with gas-flow soil core method. Soil Science Society of America Journal **59**:1336-1342.

Van Breemen, N., E. W. Boyer, C. L. Goodale, N. A. Jaworski, K. Paustian, S. P. Seitzinger, K. Lajtha, B. Mayer, D. Van Dam, R. W. Howarth, K. J. Nadelhoffer, M. Eve, and G. Billen. 2002. Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry **57**:267-293.

NITROGEN. Agouron meeting, Scottsdale, AZ, October 14-19, 2009

Denitrifying eukaryotes

Lars Peter Nielsen. Department of Microbiology, Institute of Biological Sciences, Aarhus University, Ny Munkegade. Building 1540, DK-8000 Aarhus C, Denmark. E-mail: biolpn@biology.au.dk

Species of foraminifera inhabiting anoxic marine sediment have been found to accumulate nitrate and perform complete denitrification to dinitrogen (1). Out of 58 probed species 38 were found to accumulate nitrate in a recent survey of marine habitats spanning from arctic to tropical climates and from river mouths and estuaries to open sea and ocean oxygen minimum zones (2). Nitrate storaging species were widespread among the different taxonomic groups of foraminifera (Figure 1) and all investigated species of Gromia, a Rhizarian taxon outside of the foraminifera, contained nitrate as well.

Microscoping investigation and molecular probing of *Globobulimina turgid (formerly named G. pseudospinescens)* excluded that bacterial symbionts could be the drivers of denitrification (1), and the genetics, biochemistry, and cellular locations of foraminiferal denitrification have not been resolved yet. The fungi *Fusarium oxysporum*, however, has been shown to perform partial denitrification to nitrous oxide, and a gene has been isolated that is homologous to the bacterial nirK gene responsible for encoding copper-containing nitrite reductase (NirK) (3). This observation and the diversity of denitrifying eukaryotes showing up now lend significant support to the intriguing idea, that the ancestral protomitochondrion of all eukaryotes was a denitrifying bacterium (3). Lateral transfer of the entire denitrification gene assembly at an early stage of eukaryote evolution or repeatedly later could be another option, and a more general screening for denitrification genes and reminiscences of them in eukarya might be required to clear out the origin and evolution of the denitrification trait.

The production of dinitrogen by foraminifera in marine sediment has been estimated from denitrification rate measurements on individuals incubated in the laboratory and field data of cell abundance of denitrifying foraminifera (2,4). The combined results from several locations suggest that foraminifera might compare with prokaryotes in removing combined nitrogen in marine sediments. Further evaluation of prokaryotic versus eukaryotic denitrification probably requires measures in situ. Present methods of measuring benthic denitrification vary greatly in their ability to include or exclude foraminiferal denitrification, and combinations of methods may therefore be valuable (2,4).

Contrary to known denitrifying prokaryotes, foraminifera are not dependent on continuous, diffusive supply of nitrate from the oxic to the anoxic zone. The foraminifera can take up nitrate in the oxic zone when available and bring it with them into the anoxic zone in quantities that can sustain denitrification for weeks (1). No similar storage and transport mechanism is known for oxygen, which therefore may not be that superior as electron acceptor as assumed in models of benthic carbon mineralization. Modeling of benthic denitrification is further complicated by the occurrence of prokaryotes reducing nitrate to ammonium rather than dinitrogen.

The challenge is evident in sediments underlying oxygen minimum zones, where nitrate is the major terminal electron acceptor and large sulfur bacteria reducing it to ammonium compete directly with foraminifera (Figure 2) (4). The two groups of nitrate reducers seem to have a poorly predictable complementary distribution, both on local spatial scales and on inter-annual temporal scales, possibly related to El Niño events.

“Oh no” was the immediate response from the audience to this presentation on denitrification by eukaryotes , reflecting a frustration in facing yet another overlooked complexity in the marine nitrogen cycle. The understanding of eukaryotic life in anoxic environments, however, is probably being clarified and simplified by this discovery. Eventually the same may happen to our understanding of nitrogen cycling, in particular if the special mechanisms of eukaryotic nitrate reduction provide more consistences in rate measurements, mass balances, and isotope data.

References:

1. Risgaard-Petersen, N., Langezaal, A. M., Ingvardsen, S., Schmid, M. C., Jetten, M. S. M., Op den Camp, H. J. M., Derksen, J. W. M., Pina-Ochoa, E., Eriksson, S. P., Nielsen, L. P., et al. (2006) Evidence for complete denitrification in a benthic foraminifer. Nature 443, 93-96.
2. Ochoa, E.P., Høgslund, S., Geslin, E., Cedhagen, T., Revsbech, N.P., Nielsen, L.P., Magali Schweizer, M., Jorissen, F., Rysgaard, S., Risgaard-Petersen, N. (2010) Widespread occurrence of nitrate storage and denitrification among Foraminifera and Gromiida. Proceedings of the National Academy of Science of the United States of America, 107, 1148-1153.
3. Kim, S.W., Fushinobu, S.,Zhou, S.M., Wakagi, T., Shoun, H.(2009) Eukaryotic nirKgenes encoding copper-containing nitrite reductase: Originating from the Protomitochondrion? Appl. Environ. Microbiol. 75, 2652–2658.
4. Høgslund, S., Revsbech, N. P., Cedhagen, T., Nielsen, L. P., & Gallardo, V. A. (2008) Denitrification, nitrate turnover, and aerobic respiration by benthic foraminiferans in the oxygen minimum zone off Chile. J. Exp. Mar. Biol. Ecol. 359, 85-91.

Figures:

Figure 1. Shell collection of denitrifying foraminifera. The scaling is variable. (SEM photos: C. Fontanier, SIAM, University of Angers, France; T.Cedhagen, Aarhus University, Denmark. Artwork: Nils Risgaard-Petersen, Aarhus University.)

Figure 2. View of intact sea floor collected in the OMZ off Chile and maintained in laboratory flume with anoxic water. Denitrifying foraminifera are seen as conical bodies at elevated sites where they collect nitrate from the overlying water in competition with large filamentous sulfur bacteria, *Thioploca* sp. The dominant foraminifera at the surface are *Nonionella* cf. *stella*. being about 0.2 mm in length. Function of the numerous, smaller filamentous bacteria is yet to be resolved (Photo: Lars Peter Nielsen).

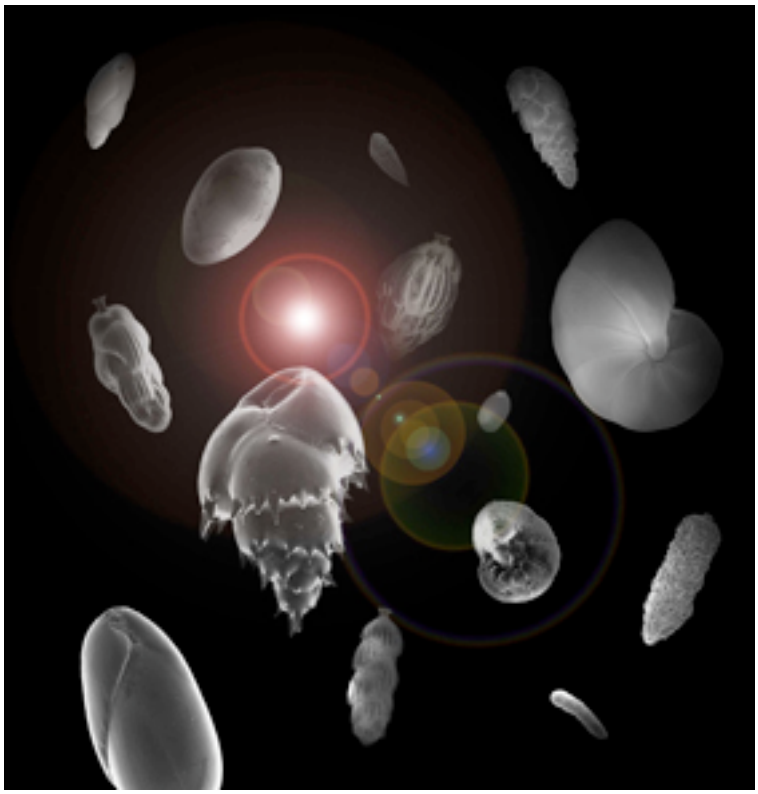

Figure 1

Figure 2