FINAL REPORT

Workshop on Advanced Approaches to Quantify Denitrification

May 3-5, 2004
The Woods Hole Research Center
Woods Hole, Massachusetts, USA

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Workshop Objectives

During the last few decades, the introduction of reactive nitrogen (N) into the biosphere by food and energy production has exceeded rates of N fixation in native terrestrial ecosystems. Among the largest uncertainties about the human-dominated N cycle on all scales is the amount of reactive N that is converted back to N_2 during the last step of denitrification[1]. Without this knowledge, it is impossible to determine the rate of accumulation of reactive N in most environmental reservoirs, and thus impossible to assess its long-term consequences. Nitrogen interacts with carbon and other nutrient cycles and has numerous cascading effects on water and air quality and climate. An improved understanding of where, when, and how much reactive N is denitrified could contribute to finding solutions to the problems created by excessive reactive N in the environment.

Unfortunately, we have little knowledge about rates of denitrification across landscapes and waterscapes. We know the environmental conditions under which denitrification occurs, but reliable quantification of N_2 production in the field is still relatively rare. There are three primary reasons for lack of knowledge about N_2 production: (1) it is difficult to measure due to the high atmospheric background concentrations; (2) N_2 production rates are very heterogeneous in space and time; and (3) there is a lack of synergy between the scientific communities that determine N_2 production rates.

A three-day workshop was held on May 3-5, 2004, at The Woods Hole Research Center, Woods Hole, Massachusetts, USA, attended by about 50 participants from Africa, Asia, Europe, North America, South America, New Zealand, and Australia. Participants included experts on both aquatic and terrestrial ecosystems, with specialties ranging from molecular biology to ecosystem science, and from both junior (graduate students and Ph.D.'s in early stages of their research careers) and senior professional positions. The objectives of the workshop were:

- to evaluate the state of our knowledge of denitrification rates in a wide range of terrestrial and aquatic ecosystems;
- to compare methodologies that have been adopted by different scientific disciplines, with the expectation that collaborations across disciplines could yield methodological advances; and
- to chart out the current weaknesses and the actions needed to address those weaknesses for an improved global assessment of where, when, and how much reactive N is converted to N_2 in the biosphere.

This workshop was organized as an integral part of the International Nitrogen Initiative (INI), a joint project of SCOPE and IGBP. The overall goal of the INI is to optimize nitrogen's role in sustainable food and energy production, while minimizing negative effects on human health and the environment. Hence, this workshop is part of

[1] (Galloway et al. 2003. The nitrogen cascade. *BioScience* 53: 341-356)

an effort that has board implications for society by contributing knowledge to future management the global N cycle to meet objectives of both food security and environmental quality.

Workshop Design

To assist with selection of potential invitees, a matrix of ecosystems and methodological approaches was first developed (Table 1). An advisory committee of experts in the field, including Elizabeth Boyer, Mary Firestone, Anne Giblin, J. Wendell Gilliam, Peter Groffman, Luiz Martinelli, Lars Peter Nielsen, and Mary Scholes, assisted PIs Davidson and Seitzinger to fill this matrix with names of experts in those areas of research. The committee then helped the PIs selected about 50 potential invitees that included representatives of each cell in the matrix. Nearly all of those invited accepted their invitations. Only about 10% of the initial invitees declined and were replaced by others on the list.

Table 1. Matrix of ecosystem types and methodological approaches.

Ecosystem	Methodological Approach							
	C_2H_2 inhibi -tion	^{15}N tra- cer	^{15}N natural abundance	Direct N_2 production measure- ments	N_2/Ar ratios	Nutrient ratio stoichi- ometry	Ecosystem budget mass balance	Molecular approach- es (e.g., mRNA)
upland, native terrestrial								
upland agriculture								
wetlands, riparian buffers, flooded agriculture								
groundwater, lakes & reservoirs								
Rivers & estuaries								
Continental shelves & oceanic								

Two important considerations were superimposed over this matrix of ecosystem types and methodological approaches: (1) What are the appropriate scales over which N_2 production can and should be measured?; and (2) How can models be integrated with the measurements to improve mechanistic understanding of the processes and permit estimation of fluxes at larger spatial scales, longer temporal scales, and across a range of forecasted scenarios?

During the first day of the workshop, we focussed on the columns of the matrix. The morning plenary session was devoted to summaries of the state of the art of methodologies:

Time	Speakers	Topic
8:30	Eric Davidson	Welcome and charge to the workshop
9:00	Peter Groffman & Lars Peter Nielsen	Acetylene inhibition and ^{15}N tracers for N_2 quantification
9:30	Klaus Butterbach-Bahl & Todd Kana	Direct N_2 quantification
10:30	Mark David & Anne Giblin	Ecosystem-scale measurements using nutrient stoichiometry, mass balance, and ^{15}N tracers
11:00	JK Bölhke & Mark Altabet	^{15}N natural abundance and environmental tracers
11:30	Mary Firestone & Mary Voytek	Molecular approaches

In the afternoon of the first day, Dr. Rachael Craig, National Science Foundation, presented information pertaining to NSF's programs on Research Coordination Networks (RCN) and Frontiers in Integrative Biology (FIBR). We asked workshop participants to be thinking about applications to these programs as they discussed the workshop topics.

We then broke into two discussion groups to addressed the methodological issues raised during the morning plenary session:

Group A: Technological advances for quantifying N_2 production

Group B: Application of methodologies to ecosystem studies

The first day ended with a poster session, in which participants were able to present their latest research results on denitrification.

During the second day, we focussed on the rows in the matrix (Table 1). The morning's plenary session was devoted to discussion group reports from the previous day and to presentations on integration of the state of knowledge about denitrification rates across landscapes and waterscapes:

8:30	Stevens/Perez	Group A discussion report
8:45	Melillo/Harrison	Group B discussion report
9:00	Richard Lowrance	Upland soils to small streams
9:30	Bruce Peterson	Small streams to estuaries
10:30	Sybil Seitzinger	Estuaries to open ocean
11:00	Elizabeth Boyer & Richard Alexander	Biogeochemical perspective on modeling denitrification
11:30	Wayne Skaggs	Agronomic perspective on modeling denitrification

The afternoon discussions of the second day focussed on what we know and do not know about denitrification across landscapes and waterscapes:

Group C: Integrating denitrification estimates from soils to streams
Group D: Integrating denitrification estimates from fresh to salt water

The third and final day of the workshop was devoted to reports of the previous day's discussion groups, discussion of the desirability of proposing a Research Coordination Network (RCN) on denitrification, and plans for writing and publishing synthesis papers in a special thematic section of a peer-reviewed journal.

Workshop Products

Peer-reviewed publications: The editor of *Ecological Applications* has invited us to coordinate a special feature section on the results of our workshop. Five synthesis papers with lead authors were identified at the workshop to be included in this invited feature section. Several plenary speakers and other workshop participants will be coauthors:

1. Introductory paper describing the need for advances in denitrification research and placing the topic in the broader perspective of human alteration of the N cycle. *Davidson et al.*
2. A systematic and comparative review of existing research approaches for quantifying denitrification in both terrestrial and aquatic ecosystems, and prospects for new methodological advances. *Groffman et al.*
3. New advances in molecular approaches to studying denitrification and how they might be integrated with quantitative measurements of rates. *Firestone and Voytek*
4. A comprehensive review and integration of estimates of denitrification rates across landscapes and waterscapes: where and how much N_2 is produced. *Seitzinger et al.*
5. Comparison of biogeochemical, hydrological, and agronomic approaches to modeling denitrification in the landscape and waterscape. *Alexander et al.*

These manuscripts will go through the normal peer review process and must meet the criteria of the journal for publication. In addition to these five synthesis papers, we have also opened a call for original research papers on denitrification topics by workshop participants. If accepted by the journal's peer review process, these papers will follow the invited feature section in the same issue of the journal.

Research Coordination Network (RCN) proposal: Workshop participants enthusiastically endorsed the need for an RCN devoted to denitrification. Because denitrification is studied by researchers from a wide array of disciplines, from molecular biology to ecosystem science, and from soil science to oceanography, many denitrification researchers do not routinely read the journals or attend the meetings where some of the important denitrification studies are presented. Not only does this lack of interdisciplinary exchange impede the spread of knowledge about denitrification, but it also acts as a barrier to applications of methodologies across disciplines. Hence, an RCN could play a critical role in the dissemination of information about methods and results for a broad audience of denitrification researchers. Future workshops could also be coordinated by RCN to follow up on the findings of this workshop and to focus on the specific questions raised during this workshop.

CoPIs Seitzinger and Davidson submitted a RCN proposal to the National Science Foundation on June 25, 2004. The proposed advisory committee for the RCN was drawn largely from the advisory committed established for this workshop. We proposed that a part-time post-doc located at Rutgers would coordinate the activities of the denitrification RCN and that the workshop web site (http://whrc.org/nitrogen/index.htm) be updated and modified to meet the RCN needs of a point of dissemination of recent results and methodological developments on denitrification.

Discussion Group Reports: Reports of the four workshop discussion groups follow.

Group A: Technological advances for quantifying N_2 production
Discussion leader: R. James Stevens
Rapporteur: Tibisay Perez

The discussion session of Group A addressed the following questions:

- What are the limitations of the current denitrification techniques?
- How could we improve and/or adapt existing techniques that have proven effective in one environment and at one scale for use in other environments and to address larger temporal and special scales?
- How can information about methodological development be shared more efficiently across disciplines and among both aquatic and terrestrial researchers? Would a Research Coordination network (RCN) work?

Five of the current methodologies to quantify emission estimates of N_2 derived from denitrification in different environments are: acetylene (C_2H_2) inhibition, ^{15}N labeling, ^{15}N natural abundance, N_2 direct determination and N_2 /Ar ratios. They have advantages, limitations and applications to different systems. However, little is known about their adaptations, if possible, to environments different from the ones for which they were originally developed. In this report we emphasize the concerns and new promising opportunities to overcome their limitations discussed by the researchers in Group A. Three other methods for measuring denitrification, namely, nutrient ratio stoichiometry, ecosystem mass balance, and molecular biology, were not discussed by the group.

Acetylene Inhibition: This technique was one of the first applied to determine denitrification rates, particularly in soil systems. Acetylene inhibits the N_2O reductase enzyme present in denitrifiers allowing the N_2O accumulate in the headspace above the soil core. The N_2O concentration difference between a control and C_2H_2-incubated soil allows the rate of N_2 production to be quantified. This technique has been widely applied in agroecosystems and works very well for soils with high nitrate content and where nitrification is not the sole source of nitrate. It has the advantage that is robust, simple and cheap. The problems with C_2H_2 inhibition are well recognized. The biggest disadvantages of adding C_2H_2 are: (1) It provides an additional source of carbon which can stimulate additional microbial activity and therefore overestimate denitrification rates; (2) It is affected by soil diffusivity which can cause uneven distribution of C_2H_2 and therefore non-uniform blocking of nitrification and nitrous oxide reductase throughout the soil; and (3) It blocks nitrification so for soils with low nitrate availability, denitrification could be underestimated. Another concern raised by the researchers is whether or not nitrous oxide reductase in fungi is blocked by C_2H_2.

^{15}N labeling : This technique is very robust and well developed for both aquatic and terrestrial ecosystems. The technique consists of adding nitrogen usually in the form of $^{15}NO_3^-$ to the study system and measuring the ^{15}N isotopic composition of the evolved N_2 by mass spectrometry. The N_2O and N_2 produced is also quantified by mass spectrometry and the measurement of the distribution of ^{15}N in the labeled N_2O and N_2 molecules used to quantify the amount of N_2O derived from nitrification and denitrification. Tracer ^{15}N studies have been undertaken at the watershed scale, using the analytical precision of natural abundance IRMS for improving detection limits.

The major limitations of this technique are nitrogen fertilization and non-uniform ^{15}N labeling, which could result in overestimation of the denitrification rates of the studied system. Creating a uniformly-labeled nitrate pool is less of an issue in aquatic studies than in soil studies. The nitrate concentration test that has been applied to sediment/water studies to check that the transport of ^{15}N-labeled nitrate was not limiting denitrification could also be applied to soils to check for uniform labeling of the nitrate pool. One suggested way of achieving uniform labeling in soil is to add ^{15}N-labeled N_2O. There are on-going analytical developments in mass spectrometric techniques to improve precision (e.g. Bergsma et al., 2001, *Env. Sci. Tech,* 35, 4307 4312).

^{15}N natural abundance method: The determination of denitrification by natural abundance is used mostly for ocean water profiles where the isotopic composition of N_2, N_2O, NO_3^- and NH_4^+ is determined at a particular water depth, and, by means of a mass balance, the formation of N_2 denitrification-derived is estimated. It has been used also for groundwater determination of denitrification by correlating the ^{15}N isotopic composition of NO_3^- with the residence time of the ground water mass. The greatest advantage is that is a non-invasive method and it can be combined with other techniques (such as isotope dating). The biggest limitations are: (1) it is very complex; (2) mixing problems could mislead interpretation of the isotopic results; and (3) there may be multiple sources of N that cannot be easily separated (i.e. anammox).

N_2 direct measurement method: The determination of the N_2 produced via denitrification can be achieved by direct determination if all the atmospheric-derived N_2 is removed from soils. To achieve such a goal it is necessary to use gas-tight chambers that allow the N_2 to be flushed out with helium and then proceed with incubation of the soil to measure the N_2 evolved. A recent development allows the determination of N_2 directly via gas chromatography using a Helium Ionization Detector The highest advantage of this technique is that allow the determination of very low denitrification rates (1 kg ha^{-1} yr^{-1}). The major limitations are the difficulty associated with the generation of a leak proof chamber and also the method is not easily scaled up due to the fact that it needs to be done on soil cores and not in the field.

N_2/Ar method: This method consists on the determination of the N_2/Ar ratio via Membrane Introduction Mass Spectrometry (MIMS). The method consists of a capillary membrane contacting the liquid or sediment (in aqueous phase). The diffused gases in the membrane are transferred to a high vacuum system where a quadrupole mass spectrometer measures the relative abundance of nitrogen and argon. This technique has been widely used in sediments and aquatic systems and it has the advantage that can integrate over large spatial and temporal scales. However, little is know about its potential for use in other system such as soils. The entire group agreed that this technique is under-exploited and that efforts must be directed towards its application to other systems. The following questions need to be answered: Can it be applied to soils by developing novel inlet systems for MIMS? Does it the sensitivity necessary to be used via micromet techniques to measure N_2 fluxes directly at the field scale? John Freney measured N_2/Ar ratios 15 years ago in a soil profile and could detect denitrification. Why was this approach not pursued?

Future directions for methodological developments would be facilitated by the following:

- Define the precision needed for detecting N_2/Ar method from soils by MIMS.
- Evaluate applicability of N_2/Ar method for terrestrial ecosystem and its potential for use in combination with ^{15}N labeling methods.
- A Research Coordination Network would be used for assembling a mini-workshop to conduct a feasibility analysis N_2/Ar measurements by MIMS in soils. The RCN could also help coordinate methods inter-comparisons.

Group B: Application of methodologies to ecosystem studies
Discussion leader: Jerry Melillo
Rapporteur: John Harrison

In our discussion we addressed the following questions:

- Is the time ripe for assembling a research group that addresses denitrification from several disciplines and methodologies, as in a Frontiers in Integrative Biological Research (FIBR)-like project? If so, what might that project look like?
- How can we apply existing tools and combinations of tools in a more integrated way across landscapes and waterscapes?
- What sets of measurements (for denitrification and other parameters) complement each other best and in which environments?

- How do we get at controlling factors and move toward an integrated measurement/modeling approach from microbasins to large river basins, to global assessments?

Discussion group B came to an early consensus that there were several important and unresolved research issues related to ecosystem-scale denitrification that could be addressed through a FIBR-like project. There was a general consensus that there have not been whole-system studies with denitrification measurements using multiple techniques at the same site and time. It was generally agreed that much could be gained by such a study. Participants also agreed that there have not been any studies measuring denitrification in a whole system moving downstream from terrestrial to aquatic environments. Participants raised the issue that such studies are essential if we are to address critical questions such as: What are regional and global rates of denitrification? Where is denitrification occurring? How do humans affect rates of denitrification? And how might we optimize N, water, and soil management to maximize agricultural productivity and minimize environmental impacts?

To begin answering these questions and to lay the groundwork for future studies our group proposed a "flagship" study of denitrification. This study would encompass an entire catchment, thereby greatly exceeding the scale of other previous studies and allowing for an integrated understanding of the relative importance of different systems with respect to denitrification. The study would include multiple measurements of denitrification using different techniques at the same sites and times in order to allow for method inter-comparison and development. Discussion participants suggested that it would be productive to focus our flagship study on a system where denitrification rates were likely to be high and hydrology relatively simple (e.g. an irrigated agricultural system with high N-inputs or a constructed wetland) in order to facilitate denitrification measurements and N-budgeting efforts.

It was generally agreed that there is currently no single technique for quantitative measurement of denitrification that works at all sites or at all times. Discussion participants also thought that the considerable uncertainty associated with the use of any single technique was substantial. For these reasons, participants recommended that multiple measures of denitrification be employed simultaneously. In our flagship study, we discussed applying various techniques, including: whole system scale ^{15}N addition, N_2:Ar measurements, ^{15}N natural abundance, mass balance N-budgeting, and acetylene (C_2H_2) inhibition. Participants thought that whole-system ^{15}N addition, while promising, faced challenges such as rapid disappearance of applied ^{15}N and limitations associated with discrete sampling techniques. There was substantial interest in the application of the N_2:Ar MIMS method at the ecosystem scale. However, significant engineering and methodological challenges must be overcome before the N_2:Ar method can be applied to measure denitrification effectively in soils at all, let alone at the ecosystem scale.

In addition to denitrification measurements, it was generally agreed that ancillary measurements would be required, both to aid in the understanding of controls on denitrification and to place denitrification in context with other biogeochemical transformations and cycles. Discussion participants suggested including (at minimum) the following ancillary measurements in the flagship study: other N cycle processes including assimilatory NO_3^- reduction, nitrification, N-uptake by plants and microbes, and mineralization, hydrological parameters, carbon cycle processes, and microbial community composition (though in a very targeted manner). Recent advances in wireless data transmission should be applied so that numerous sensors of key environmental parameters are networked and logged at time intervals that are meaningful to variation in denitrification and its controlling factors. One participant suggested that the group measure as many potentially important environmental variables as possible, effectively over-determining the system to avoid missing an important controlling variable. Other members thought it would be worthwhile to spend sampling energy on potentially integrative parameters (e.g. cellular enzymes).

It was generally agreed that though the flagship study would likely facilitate a quantum leap forward in the scientific community's understanding of denitrification across the landscape, further studies would certainly be necessary if understanding of denitrification were to be generalized to the regional or global level. Potential follow-up studies suggested by participants included comparisons of denitrification rates and controls in a range of environments, including rural-urban, temperate-tropical, and pristine-anthropogenically influenced comparisons. Discussion participants also thought that modeling should constitute a critical component of any potential follow-up studies.

Group C: Integrating denitrification estimates from soils to streams
Discussion leaders: Wendell Gilliam and Louis Shipper
Rapporteur: Madura Kulkarni

The discussion group was posed a number of questions by workshop organizers. After some preliminary discussion this preliminary list was reduced to two key questions that were addressed by the group:

- What do we know about denitrification rates at landscape scales, and conversely what are our areas of weakness?
- What study is needed to address our information needs.

What do we know, what do we not know?

The general consensus was that the international community has some useful information on landscape rates of denitrification for most environments. However, it was clear that information across different ecosystems is not balanced and there are serious questions about the validity of some of the techniques used to determine denitrification rates. Ecosystems/regions that were poorly represented included: tropical forests, tile drained areas, arid environments, groundwater and possibly irrigated agriculture. It is likely that this is not an exhaustive list and many others regions/environments would also be underrepresented.

How well do we need to know denitrification rates?

Under some situations it may be sufficient to know denitrification rates +/- 10 kg N ha^{-1} y^{-1}, perhaps for rates above 20 kg N ha^{-1} y^{-1}. In other cases, however, it would be necessary to be able to determine whether denitrification rates were either 5 or 10 kg N ha^{-1} y^{-1}. Determining total maximum daily loads is an example of when such resolution would be desired. If daily loads of nitrogen to a forest through atmospheric deposition were in question, a difference of 5 kg N ha^{-1} y^{-1} could be very important and of significant economic/environmental cost. Further, where such areas of low denitrification rates are extensive, even slight differences between estimates of small rates of denitrification could quickly multiply and prove to be important at regional/continental/global scales. The consensus was that currently, in terrestrial ecosystems, this level of precision is beyond current methods being applied, although some methods, e.g., N_2/Ar ratios and direct N_2 fluxes, are promising. These methods need further testing and comparison to other techniques (see below).

Although it was thought that we generally had information on denitrification rates in different terrestrial environments, it was pointed out that many of these measurements were made using either the acetylene block technique or mass balance approaches. In some cases these methods are used to evaluate the effectiveness of one another, which may lead to confirmation of our current prejudices. However, both of these methods have important constraints and limitations. Generally, it is considered that the acetylene blockage approach (most widely used to report denitrification rates in terrestrial systems) underestimates denitrification rates and is particularly questionable in systems with low rates of denitrification.

In the plenary presentation by Richard Lowrance, he reviewed about 1500 total citations on terrestrial denitrification in Agricola, of which less than 120 studies reported annual rates, and most of these used acetylene inhibition. He pointed out that we need more estimates using other techniques to corroborate the acetylene inhibition estimates. The existing data demonstrate clear control of denitrification by soil texture, landscape position, available C, and N enrichment. It is clear that other methods besides acetylene inhibition need to be tested to determine denitrification rates in systems where nitrate concentrations were low. At this point, discussion focussed on the type of study that might best improve our information on denitrification in terrestrial systems.

What study is needed to determine information needs?

This portion of the session built on a discussion group from the previous day and attempted to determine the desired characteristics of a study needed to answer more specific questions. The discussion group identified a number of desirable characteristics for the study site:

- Approximately 1 km^2 in size for intensive comparison of methods nested within a larger catchment for latter potential expansion of the study. The catchment should include a range of land types and be connected via a stream network to an estuary to the open ocean to allow integration of a range of studies to determine catchment scale denitrification rates

- The initial site should be a "hotspot" of denitrification (likely high inputs of nitrogen and potentially irrigated). A major question is whether the newer methods are sufficiently sensitive to accurately detect denitrification and a 'hotspot' would give a greater chance of initial success.
- The site should have already been studied or still part of an existing study where a number of important characteristics are well described including: hydrology, nitrogen mass balance or previous assessment of denitrification using existing methods. Understanding the hydrology of the system will be key to understanding N losses, and so previous work on hydrologic characterization of a study site would be very desirable.
- While only one or two such integrated studies might be initially established, the design should allow for transfer to other regions to cover a range of denitrification rates within different geomorphological and hydrological settings. Different soil types/textures and land-uses should also be eventually covered. Common protocols should be developed so that information transfer is possible between sites.
- A range of ancillary measurements need to be made, including: hydrology, labile carbon cycling and other components of nitrogen cycling.
- It is expected that the data generated will be used to test/improve models of denitrification. Similarly, models will identify gaps in knowledge and identify further data requirements. A range of models may be used to address different questions.

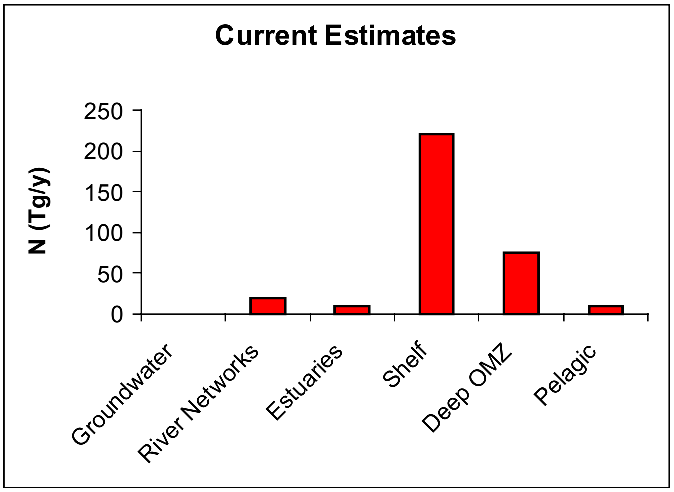
Group D: Integrating denitrification estimates from fresh to salt water
Discussion leader: Anne Giblin
Rapporteur: Kevin Kroeger

The group reviewed the importance of understanding denitrification rates in freshwater and in marine waters and pointed out that the importance varies substantially with location. For groundwater, the loss of N is important for human health and for understanding and preventing eutrophication. In estuarine waters denitrification is of interest for the role that nitrogen plays in controlling primary production and eutrophication. In the continental shelf and in ocean oxygen minimum zones (OMZ), an understanding of denitrification is needed to understand global N and C cycles and for looking at climate feedbacks. Of primary interest is to understand the controls on denitrification and what are the affects of perturbations – both past and future changes. We need to know the controls on not only the loads but on the responses of the systems to those changes in loads, positive and negative feedbacks, and nonlinearities

The group addressed two major questions:

- What is the current state of knowledge about the amount of denitrification taking place as water moves from fresh to salt water; and
- How good are our estimates and what are the major holes in our understanding?

As a starting point we reviewed the estimates presented by Sybil Seitzinger in the plenary session for denitrification rates in rivers, estuaries, shelf sediments and in oxygen minimum zones.



There are considerable uncertainties and common problems with trying to estimate denitrification at regional or global scales based on existing data:

1) The estimates are made on very few sites. For rivers and estuaries, the great majority of the measurements we are aware of are concentrated in US and Europe. Most are in the temperate zone, fewer in subtropics, and tropics very poorly represented. Currently, 40% of world's fertilizer is being used in S. Asia and in China, and use in these regions is projected to increase significantly. We need more measurements in these areas to know if the models which are being developed are applicable to these other areas.

2) In many cases the methods do not distinguish between direct and coupled denitrification. The controls on these two pathways are very different. Our ability to make "predictive models" may be limited by not having this information.

3) Many budgets have not adequately addressed N_2 fixation, and in some cases even our direct measurements look at the balance between fixation and denitrification. For true system level understanding, and for better model development, we need to separate them.

There are also some specific problems with some of these estimates:

River Basin Estimates:

1) The current estimate of 20Tg by Seitzinger is based upon simple assumptions of losses. The actual measurements are few and span 4 orders of magnitude. At some point the estimate is constrained by what goes on land and what we measure in terms of river export to ocean but this assumes steady state. There could be large storages which may be temporary. Wajih Naqvi estimated that N loss or storage could be as high as 5 Tg for Southern Asia alone.

2) The river basin estimates ignore or "black box" groundwater. There is direct groundwater discharge to the ocean and there can be significant N storage in groundwater. How much is stored and how much is being processed? For groundwater the issue of availability of electron donors is very important. This may be influenced by flow path, interaction with riparian zones, aquifer characteristics (soil type).

3) Most temperate freshwaters are P limited. What is the role of P in controlling N losses within river networks? Will our finding from the temperate zone be relevant in the tropics?

4) What is the role of dams, reservoir, freshwater wetlands, and how may this change over time?

5) There is some data suggesting that streams draining agricultural lands have a lower % loss than predicted by models. First order loss terms may not work in highly loaded systems, and models may need more mechanisms, such as a Michaelis-Menten kinetics or other formulation.

6) Are annual time scale adequate or do we need shorter time scales?

7) There has been a large loss of stream gauging stations. In the US, USGS has lost or cut back measurements by 30%. Similar losses have been seen worldwide. This may compromise our ability in the future to make robust predictions.

8) More partnerships between academics and federal agencies needed for research of this scale.

Estuaries

1) There was agreement that the data constrains the estuarine number to >5 Tg/y, but it has to be less than 60 Tg, which is the global river N export. Although some systems may import oceanic N, this is not considered to alter the estimated range.

2) Current models of estuarine denitrification rely on simple relationships between residence time and loading. These log/log relationships are not adequate for understanding how systems respond to change; we need a more mechanistic understanding.

3) Differences between measurements in different systems suggest that benthic microalgae and organic matter inputs from land may play an important role in setting estuarine denitrification rates.

4) A number of participants thought we would make progress by developing a better estuarine classification scheme which also includes aspects of the geomorphology and hydrology beyond Tr, that are based upon controlling processes.

5) What is the role of saline wetlands? Some studies have suggested they are large sinks and have denitrification rates but they also have high rates of N_2 fixation. Do they function as sinks, transformers, or sources?

Continental Shelf Sediments

1) The current estimate of 225 Tg is not at all constrained. It is based on only about 12 sets of measurements worldwide, and biased to upwelling areas. Other lines of evidence are suggesting that the estimate is of the right order of magnitude, but the range is very large 100-400 Tg (Devol-Codispoti). This is the largest global number and it is important to improve our understanding of the magnitude and controls on it to understand shelf productivity and the role of the oceans in global N and C cycling.

2) Exciting new processes which produce N_2 may be important (such as anammox) but this will require us to rethink our understanding of controls on the system.

3) The group discussed whether or not there were other ways to set constraints with isotopes (perhaps NH_4), or by looking for a N_2/Ar anomaly.

Oxygen Minimum Zones

1) The current estimate of 75 Tg is constrained by the measured nitrate anomaly and oceanography. This may be our best number but the estimate assumes a standard denitrification mechanism. This number could go up as high as 150 Tg with anammox.

2) The largest unknowns are on the controls of the zones themselves. How are the zones formed, and what are the feedbacks which control zone size and stability? We have some general principles but an incomplete understanding.

3) The paleo-record shows that some of the OMZ are very sensitive to climate change but again, the way in which they will respond in the future is not well understood. These sites are a large global N sink, again we need to know how they control global N and C over large time scales.

Slope and Pelagic Sediments

1) There have been very few studies in pelagic sediments but denitrification probably cannot be too much larger than 10 Tg. Some studies suggest no NO_3 depletion in porewater, suggesting no denitrification, while others see small depletions suggesting low rates. The pelagic site is probably too small to be concerned with in the context of global N cycling.

Holes in the data sets:

1) We have no estimates of denitrification in slope sediments.

2) Denitrification losses in waste treatment plants (WTP), were not included in this estimate. Worldwide, most WTP are not tertiary plants designed for denitrification. However, it was suggested that the possible limits of the current and future contribution of these plants be examined.

How to make progress:

1) We have to model mechanistically– measurements can only squeeze the error bars so small. We need a more mechanistic understanding of the processes, and we need to see if under represented systems follow previous trends.

2) Whole system ^{15}N additions provide a mechanistic understanding of the entire N cycle, not just a loss rate.

3) The next step is to incorporate a multi-element approach: P may prove to be very important, especially in the river network; the role of C, O in most aquatic systems also needs to be known.

4) The group questioned whether we have a fully constrained budget for C, N, P and O (S) in any system? Are we now at the point where it is possible in aquatic systems using new tools such as acoustic doppler profiling current meters and continuous measurements of nutrients and oxygen to make accurate budgets?

5) Can we also exploit long term moorings to get better budgets? Do we need new or better sensors, or can we simply use automated sampling and standard chemistry? A better classification system (typology) could be useful in choosing sites for complementary studies for both the river network and the estuarine studies. Measurements need to be made in a way that will inform mechanistic models of linked elements.

6) Future studies need to measure and distinguish between all N_2 producing processes (coupled nitrification/denitrification, direct denitrification, anammox).

Next Steps:

The group thought the time was right for a large scale project on "How element interactions affect DNF across different systems". The goal would be to make measurements at multiple sites organized around a typological classification. This will be difficult to accomplish but one model would be to have multiple funding sources, organized under a RCN to coordinate data and standardize protocols. This would require an international effort to be assured all key ecosystem types were covered.

Summary of Major Findings of the Workshop

In a review paper entitled "The Enigma of Soil Nitrogen Balance Sheets" F.E. Allison reported the state of knowledge on denitrification in 1955 (*Advances in Agronomy* 7:213-251):

"… regardless of years of research, an accurate soil nitrogen balance sheet for a field soil can seldom be drawn up……Although the main mechanisms of loss are probably known, quantitative data relating to each type of loss are certainly inadequate."

Unfortunately, those words are as apt in 2004 as they were in 1955. To be sure, advances have been made in the intervening five decades on understanding the factors that control denitrification. New technological advances also hold promise that the next few decades will yield more rapid progress in the study of denitrification. For example, recent developments in molecular approaches hold promise for improved understanding of variation in the populations of organisms that are the agents of denitrification. Recent advances in gas chromatography and mass spectroscopy also hold promise for improved sensitivity of direct N_2 quantitation and isotopic measurements. Nevertheless, denitrification remains under-sampled relative to its spatial and temporal heterogeneity in the environment. Although technological advances will undoubtedly improve measurements of denitrification, technology alone is unlikely to solve the problem. Denitrification is a process that inherently requires integration across disciplines and scales. Therefore, in addition to simply calling for methodological improvements, and more studies in more places, the workshop participants highlighted the following major points of consensus:

- Measurements of N_2/Ar ratios in soils, perhaps now possible using Membrane Introduction Mass Spectrometry (MIMS), has been underutilized in terrestrial ecosystems. Modifications are needed so that the MIMS technology that was developed for aquatic ecosystems can be applied to soils and subsoils. This effort may be especially fruitful in the near term.
- Although MIMS is one example of excellent potential for a new advancement in studying terrestrial denitrification, there is no single methodological approach -- or "silver bullet" -- that will solve the enigma of balancing nitrogen budgets for ecosystems. Rather, a novel combination of several promising methodological techniques reviewed during this workshop should be applied in an integrated manner in one or more "flagship" studies. Once this novel integrated approach is applied successfully in a few flagship studies of terrestrial watersheds and saltwater ecosystems, the most successful components of those studies should be applied elsewhere.
- Hydrologic flows of water through soils, groundwater, sediments, and streams are key to understanding denitrification. Integrated studies that include several methodological approaches and that measure denitrification at numerous points along the continuum from upland soils to rivers and estuaries should be applied first to

study areas where the hydrology is already well characterized and/or is relatively simple.

- Development of quantitative, processed-based knowledge of the relationships between rates of denitrification and controlling factors at scales relevant to ecosystems require that denitrification measurements be made within the context of other biological, chemical and physical processes within the ecosystem.
- Because each methodological approach has its strength at particular spatial and temporal scales, new advancements in quantification of denitrification will require new integrative efforts across disciplines to simultaneously combine multiple approaches across landscapes and waterscapes. For Allison's characterization of denitrification research in 1955 not to be equally apt in 2055, interdisciplinary coordination among denitrification researchers and integrated ecosystem-scale studies will be necessary in the coming years to foster new advances in the understanding and quantification of denitrification.
- Improved understanding and quantification of denitrification will promote our ability to manage reactive nitrogen in the biosphere and to avoid harmful effects of excess reactive nitrogen on water quality, air quality, and human health.

Appendix: List of Workshop Participants

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