

THE ENIGMA OF PROGRESS IN DENITRIFICATION RESEARCH

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Abstract. Humans have dramatically increased the amount of reactive nitrogen (primarily ammonium, nitrogen oxides, and organically bound N) circulating in the biosphere and atmosphere, creating a wide array of desirable products (e.g., food production) and undesirable consequences (e.g., eutrophication of aquatic ecosystems and air pollution). Only when this reactive N is converted back to the chemically unreactive dinitrogen (N₂) form, do these cascading effects of elevated reactive N cease to be of concern. Among the quantitatively most important processes for converting reactive N to N₂ gas is the biological process of classical denitrification, in which oxides of nitrogen are used as terminal electron acceptors in anaerobic respiration. This Invited Feature on denitrification includes a series of papers that integrate our current state of knowledge across terrestrial, freshwater, and marine systems on denitrification rates, controlling factors, and methodologies for measuring and modeling denitrification. In this paper, we present an overview of the role of denitrification within the broader N cycle, the environmental and health concerns that have resulted from human alteration of the N cycle, and a brief historical perspective on why denitrification has been so difficult to study.

Despite over a century of research on denitrification and numerous recent technological advances, we still lack a comprehensive, quantitative understanding of denitrification rates and controlling factors across ecosystems. Inherent problems of measuring spatially and temporally heterogeneous N₂ production under an N₂-rich atmosphere account for much of this slow progress, but lack of interdisciplinary communication of research results and methodological developments has also impeded denitrification research. An integrated multidisciplinary approach to denitrification research, from upland terrestrial ecosystems, to small streams, river systems, estuaries, and continental shelf ecosystems, and to the open ocean, may yield new insights into denitrification across landscapes and waterscapes.

Key words: anammox; denitrifying bacteria; dinitrogen; eutrophication; nitrate; nitrogen cycle; nitrogen oxides.

DENITRIFICATION: A KEY PROCESS OF THE NITROGEN CYCLE

The triple bond between two N atoms renders the dinitrogen molecule (N₂), which comprises about 80% of the earth's atmosphere, extremely stable and unreactive. Only with large inputs of energy (e.g., biological nitrogen fixation, lightning, the Haber process of commercial fertilizer production, compression within the internal combustion engine) can the N₂ molecule be converted to N forms that are reactive in the atmosphere and the biosphere. Eventually, reactive forms of N are converted back into N₂, mostly via the nitrate reduction process of denitrification and the recently recognized anaerobic ammonium oxidation anammox process in

marine sediments and during some types of sewage treatment (Arrigo 2005).

This Invited Feature on denitrification presents a series of papers covering our current state of knowledge on denitrification rates, factors that control these rates, and methods for measuring and modeling denitrification in both aquatic and terrestrial ecosystems. The objective of this introductory paper is to place the process of denitrification into the broader context of N cycling research and societal concerns regarding human alteration of the N cycle.

A basic understanding of denitrification

Denitrifying bacteria were first isolated in 1886 (Payne 1981), and denitrification has been studied intensively by microbiologists, ecologists, oceanographers, agronomists, and engineers during the last half century. Classical denitrification is the microbial production of nitric oxide (NO), nitrous oxide (N₂O), and N₂ from nitrate and nitrite and is a facultative anaerobic process.

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In a seminal work on denitrification, Nömmik (1956) used mass spectrometry to quantify NO, N₂O, and N₂ emissions from soils in laboratory incubations receiving K¹⁵NO₃. He systematically varied one variable while holding others constant, including temperature, water content, oxygen, aggregate size, pH, organic C, nitrite, nitrate, and iron. This research in the middle of the 20th century has formed the basis of our current understanding of the roles of electron donors (organic C) and electron acceptors (oxygen and nitrogen oxides) as regulators of denitrification rates and relative proportions of gaseous end products.

We know that denitrification occurs where carbon and nitrate are available as substrates and where oxygen is scarce or absent, and that it occurs in almost all terrestrial, freshwater, coastal, and some oceanic ecosystems, as well as human-engineered systems. Some of the environmental conditions under which high rates of denitrification have been observed include partially or fully saturated soils and aquatic sediments and low oxygen waters. However, we lack reliable quantitative estimates of denitrification rates for many systems and knowledge of how these rates vary temporally and spatially across landscapes and waterscapes. Despite a broad understanding of the multiple environmental factors that control rates of denitrification, we have limited ability to integrate this knowledge to construct and validate robust and predictive numerical models of denitrification (Boyer et al. 2006, Seitzinger et al. 2006).

Why we need to understand denitrification better

A comprehensive, quantitative understanding of denitrification rates and controlling factors across ecosystems is important for many reasons. Nitrogen plays a critical role as a limiting nutrient in many ecosystems on earth, including many forests, wetlands, some lakes, many estuaries, continental shelves, and oceanic ecosystems (Vitousek and Howarth 1991, National Research Council 2000, Rabalais 2002). Over geological time scales, the balance between nitrogen fixation and denitrification is thought to have affected global biogeochemical cycles of carbon (Falkowski 1997). More recently, human activities have increased the conversion of N₂ to reactive forms of N, and, as a consequence, N is accumulating in the environment locally, regionally, and globally.

Introduction of reactive N into the biosphere by humans now exceeds the rate of biological nitrogen fixation in native terrestrial ecosystems (Galloway et al. 2004). This increased reactive N is due primarily to N fertilizer production and fossil fuel combustion used to support the food and energy demands of a rapidly expanding human population. Increased inputs of NO and other nitrogen oxides to the atmosphere can increase tropospheric ozone formation, reduce atmospheric visibility, and increase acid deposition. Increased N deposition can acidify soils, streams, and lakes and can alter forest productivity (Matson et al. 2002),

although the effects of these N inputs on the carbon cycle in forests remain controversial (Houghton et al. 1998, Nadelhoffer et al. 1999). Increased inputs of N to aquatic ecosystems from atmospheric deposition, sewage, and agricultural runoff can cause eutrophication and a range of associated effects, including damage to fisheries in coastal ecosystems (Rabalais 2002). The formation of N₂O during nitrification and denitrification in all systems results in tropospheric warming and stratospheric ozone depletion (Prather et al. 2001). While human health certainly benefits from the salutary effects of food production made possible by N fertilization, it is also negatively affected by several diseases that have been linked to air- and water-borne N (Townsend et al. 2003). These undesirable “cascading effects” (Galloway et al. 2003) of reactive N moving through aquatic and terrestrial ecosystems and the atmosphere do not stop until the reactive N is eventually converted back to N₂, primarily through the processes of denitrification and anammox.

A great deal of attention has been directed recently to the intermediate gaseous products of denitrification—NO and N₂O (Bouwman et al. 1995, Hall et al. 1996, Seitzinger and Kroeze 1998, Davidson et al. 2000, Davidson and Mosier 2004)—because of their importance in processes of ozone production and consumption and radiative forcing (Prather et al. 2001). Interest in these pressing environmental concerns may partly account for less attention being directed to the final N₂ product of denitrification in terrestrial ecosystems, where data on emissions of NO and N₂O are far more abundant than are data on N₂ emissions. However, the last step of denitrification (the microbial production of N₂) is also critically important, because it is a permanent sink for reactive N in the environment. In contrast to terrestrial ecosystems, concern over eutrophication and drinking water quality has motivated denitrification research in aquatic ecosystems to address denitrification from a broader perspective than trace gas emissions (Seitzinger et al. 1984, Brettar and Rheinheimer 1992, McMahon et al. 1999, Mulholland et al. 2004, Savage et al. 2004, Fear et al. 2005).

The amount of reactive N that is converted back to N₂ during the last step of classical denitrification and in the anammox reaction is by far the largest uncertainty of the N cycle on all scales (Galloway et al. 2004). Despite the large number of denitrification studies (7236 publications since 1975 listed for denitrification in Environmental Sciences and Pollution Management Index), there are still only a few locations with measurements adequate to quantify denitrification rates and how they vary at a range of spatial and temporal scales. Narrowing this uncertainty is critical, for without this knowledge, it is impossible to determine the rate of accumulation of reactive N in most environmental reservoirs or to assess the long-term consequences of the continued introduction of newly fixed N into the environment. In addition to improved technologies in

agriculture, industry, and transportation to minimize unnecessary introduction of reactive N into the environment, 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.

An example of using knowledge about denitrification to help avoid excess reactive N reaching unintended targets comes from the fields of agronomy and stream and wetland ecology. For obvious reasons, agronomists and farmers do not want denitrification in the rooting zone to reduce availability of N to crops. Unfortunately, it is difficult, and almost impossible, to obtain near 100% efficiency of crop use of fertilizer (Cassman et al. 2002), and so a substantial fraction of applied N often leaves the crop rooting zone as nitrate, dissolved organic N, or as trace gas emissions. Once nitrate leaches from the rooting zone, however, denitrification can be encouraged by management practices to reduce excess N where it is unwanted (Seitzinger et al. 2006). For example, riparian buffer strips and/or wetlands have been proposed as management options to decrease N inputs to rivers from agricultural runoff and consequently downstream coastal ecosystems (Gilliam 1994, Gold et al. 2001). Additional examples include denitrification as an important component of many coastal ecosystem models used to develop watershed nutrient reduction plans (Cercio 2000). Denitrification is also used in advanced wastewater treatment facilities to decrease N release to the environment.

IMPEDIMENTS TO A BETTER UNDERSTANDING OF DENITRIFICATION

Three major impediments to a comprehensive understanding of denitrification are (1) the large background N₂ concentrations in air and water, making it difficult to analytically detect small increases in N₂ concentrations derived from denitrification; (2) the large spatial and temporal heterogeneity of N₂ production in most environments; and (3) a lack of communication among the scientific disciplines that are interested in N₂ production rates, which has impeded technology transfer, innovation, and sharing of knowledge of the denitrification process.

While the first two impediments are the nature of the denitrification process that we must learn to cope with, the third is one we can do something about. Addressing analytical and sampling difficulties could be facilitated by more interdisciplinary collaboration. However, because denitrification is studied by researchers from such a wide array of disciplines, from molecular biology to ecosystem science, and from soil science to oceanography, many denitrification researchers do not routinely attend the same scientific conferences and do not routinely read journals where denitrification studies outside their discipline are published. This lack of interdisciplinary exchange impedes the spread of knowledge about denitrification and also acts as a barrier to

applications of, and advances in, methodologies across disciplines.

A number of advances in direct quantification of denitrification have been made, but there has sometimes been a 10–15 year lag in the transfer of new technologies, particularly between terrestrial and aquatic scientists (Groffman et al. 2006). For example, N₂ production rates measured directly by gas chromatography have been made in aquatic sediments for over 20 years (Seitzinger et al. 1980, Devol 1991), but this approach has only recently begun to be used in soils (Butterbach-Bahl et al. 2002, Cardenas et al. 2003). Membrane inlet mass spectrometry (MIMS) for quantifying N₂ production based on changes in N₂:Ar ratios was also developed over 10 years ago (Kana et al. 1994) and has been used to measure denitrification in sediments from a range of aquatic ecosystems and anoxic waters (Kana et al. 1998, An et al. 2001, Laursen and Seitzinger 2001, Harrison 2003), including integrated measurements over spatial scales of kilometers in streams (Laursen and Seitzinger 2002, McCutchan et al. 2003). It has not yet been applied to terrestrial soils, although there may be considerable potential, provided that some technological challenges can be overcome. An advance in measuring N₂ fluxes from terrestrial ecosystems would be particularly significant, given that denitrification in terrestrial soils is estimated to be the largest global sink for natural and anthropogenic land-based N sources (Galloway et al. 2004, Seitzinger et al. 2006) and that N budgets for terrestrial ecosystem studies are seldom balanced.

In addition to recent advances in methods to quantify denitrification, a number of other pathways of N₂ production have been identified, including anammox, aerobic denitrification, and N₂ production coupled to manganese, iron, and sulfur cycling (Luther et al. 1997, Thamdrup and Dalsgaard 2002, Zehr and Ward 2002, Arrigo 2005). The applicability of existing methods to quantify these pathways is not fully known. Considerable advances in molecular methods to identify the different species of bacteria capable of N₂ production have also been made (Groffman et al. 2006), and there is a need to integrate these with N₂ flux measurements in ecosystems. Large scale, integrative direct measures of denitrification and anammox are also needed for continental shelves, given that they are the single largest N sink in marine systems and that they remove substantial amounts of anthropogenic land-based N as well (Seitzinger et al. 2006).

Quantitative, processed-based knowledge of the relationships between rates of denitrification and controlling factors across the range of terrestrial and aquatic ecosystems is critical to understanding “hot spots” and “hot times” of N removal beyond local study sites (Groffman and Crawford 2003, McClain et al. 2003, Boyer et al. 2006, Seitzinger et al. 2006). Developing these relationships is challenging not only because of the difficulties of quantifying denitrification, but also

because of the temporal and spatial variability in controlling factors and hence denitrification rates. Denitrification measurements have often been made at scales of centimeters or less, often in the laboratory, removed from in situ conditions. Hence, many studies of denitrification are not suitable for developing models to scale measurements up to ecosystem levels. For example, a survey of published denitrification measurements in rivers identified only a few studies (<10) in which measurements of other processes were sufficient to build even a regression model of denitrification appropriate for application at the whole river network scale (Seitzinger et al. 2002). On the other hand, terrestrial ecosystem modelers have developed mechanistic representation of controlling factors of denitrification that have been used to scale-up denitrification estimates (Del Grosso et al. 2001, Li 2000, Potter et al. 1996), but lack of data on N_2 production and emission has impeded validation of the total denitrification estimates derived from these models. This difficulty in scaling up laboratory and field studies combined with the scarcity of field data on N_2 production have led to our current poor quantitative understanding of in situ rates of denitrification and controlling factors at ecosystem scales.

Approaches to modeling denitrification vary widely, including using mass balance, stoichiometry, regression analysis, and process-based algorithms (Middleburg et al. 1996, Potter et al. 1996, Seitzinger and Giblin 1996, Alexander et al. 2000, Li 2000, An and Joye 2001, Del Grosso et al. 2001, Deutsch et al. 2001, Seitzinger et al. 2002; see review by Boyer et al. 2006). However, comparison of model formulations beyond the original ecosystem type (e.g., terrestrial soils vs. aquatic sediments; oceanic oxygen minimum zones vs. anoxic waters in lakes and rivers; freshwater vs. saltwater wetlands, and so on) have not generally been conducted. Much could be learned from comparisons among model formulations and from interactions among the community of scientists that are measuring and modeling denitrification from different scientific disciplines.

OVERCOMING THE IMPEDIMENTS

An interdisciplinary workshop

This Invited Feature in *Ecological Applications* is the product of an effort to begin bridging the disciplinary gaps in research on denitrification. The review papers herein emanate from the combined efforts of participants in a recent workshop on "Advanced Approaches to Measuring Denitrification: Integrating Landscapes to Waterscapes" held on 2–5 May 2004 at the Woods Hole Research Center, Woods Hole, Massachusetts, USA (see *Acknowledgments* for a list of supporting agencies). About 50 participants from six continents attended the workshop, which was organized as an integral part of the International Nitrogen Initiative (INI), a joint project of the Scientific Committee on Problems of the

Environment (SCOPE) and the International Geosphere–Biosphere Program (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 was part of an effort that has broad implications for society by contributing knowledge to future management of the global N cycle to meet objectives of both food security and environmental quality.

The major objectives of the workshop were (1) to evaluate the state of knowledge regarding denitrification rates in a wide range of terrestrial and aquatic ecosystems; (2) to compare methodologies that have been adopted by different scientific disciplines, with the expectation that collaborations across disciplines could yield methodological advances; and (3) 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.

Review papers on denitrification across landscapes and waterscapes

The paper by Seitzinger et al. (2006) takes on the formidable task of addressing the first workshop objective: developing a synthesis of denitrification from upland terrestrial ecosystems, to small streams, river systems, estuaries and continental shelf ecosystems, and to the open ocean. More specifically, that paper focuses on commonalities across ecosystems in temporal and spatial linkages of denitrification with nitrate sources, and ecosystem scale controlling factors. They also developed the first spatially explicit global view of denitrification rates across the full array of ecosystem types using GIS-based modeling approaches.

The paper by Groffman et al. (2006) addresses the second workshop objective, providing a comprehensive overview of the methodologies used to study denitrification by scientists from a broad range of disciplines. While both of these papers address the strengths and weaknesses mentioned in the third workshop objective, a third paper by Boyer et al. (2006) provides a modeling perspective on how our understanding of denitrification derived from field and laboratory studies are integrated into conceptual and numerical modeling frameworks. Both successes and limitations of such modeling efforts are explored. These review papers, and a companion set of original research papers, demonstrate the considerable progress that has been made in denitrification research during the last few decades.

Looking back and looking ahead for progress

Despite the impressive recent advances, it is particularly humbling to reflect on a description of the status of denitrification research 50 years ago. In a review paper entitled "The Enigma of Soil Nitrogen Balance

Sheets,” F. E. Allison (1955) made the following observations:

“... regardless of years of research, an accurate soil nitrogen balance sheet for a field soil can seldom be drawn up.”

“... we usually lack quantitative data ... because of the experimental difficulties encountered in obtaining them.”

“In order to obtain accurate values for some sources of gains and losses it is necessary to make the experimental conditions artificial. The experimenter then wonders how closely the data obtained apply under field conditions.”

“Although the main mechanisms of loss are probably known, quantitative data relating to each type of loss are certainly inadequate.”

Unfortunately, those observations are as apt today as they were in 1955. To be sure, advances have been made in the intervening five decades on understanding the factors that control classical biological denitrification, although the basic understanding of electron donors and acceptors provided by Nömmik (1956) still stands. While new technological advances hold promise that the next few decades will yield more rapid progress in the study of denitrification, technology alone is unlikely to solve the problem. Denitrification is a process that inherently requires integration across disciplines and scales. Our multidisciplinary workshop reached the following conclusions:

1) No single methodological approach—or “silver bullet”—will solve the enigma of balancing nitrogen budgets for ecosystems. Rather, novel combinations of several promising methodological techniques should be applied to field studies in an integrated manner.

2) Because hydrologic flows of water through soils, groundwater, sediments, and streams are key to understanding denitrification within watersheds, integrated studies 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.

3) 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 should be made within the context of other biological, chemical, and physical processes within the ecosystem.

Denitrification remains under-sampled relative to its spatial and temporal heterogeneity in the environment and its ecological and societal importance. 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. Such efforts are urgently needed to improve understanding and quantification of denitrification, to promote our ability to manage reactive nitrogen in the biosphere for the positive benefits of food and energy production, and to avoid harmful effects of excess reactive nitrogen on water quality, air quality, and human health.

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