

PREFACE

Although not (yet) noticed by most politicians and environmental activists who focus on the carbon footprint, the global nitrogen cycle is the one most impacted by humankind. This has come about as the consequence of a major breakthrough in applied science, namely, the industrial production of ammonium-based fertilizer, synthetically “fixed nitrogen,” which has led to almost uncontrolled use worldwide to support the increasing demand for agricultural production. Despite being initiated by the great Sergey Winogradsky more than 100 years ago at the turn of the twentieth century—at the same time when Haber and Bosch invented the industrial synthesis of ammonia—research on the transformation of nitrogen compounds in an environmental context has been stagnant until about 10–15 years ago. In contrast, “reactive nitrogen species” have been a hot topic in biomedical research since quite some time, acknowledged by the Nobel Prize in Physiology and Medicine in 1998 for work on the role of nitric oxide as a signaling molecule in the cardiovascular system of animals. Many of the methods instrumental to the progress of this work were captured in *Methods in Enzymology* Volumes 436 and 437 (2008), entitled “Globins and Other Nitric Oxide-Reactive Proteins,” edited by Robert K. Poole, and Volumes 268 and 269 (1996), 301 (199), 359 (2002), 396 (2005), 440 (2008), and 441 (2008) entitled “Nitric Oxide, parts A to G,” edited by Enrique Cadenas and Lester Packer. The lack of progress in the environmental arena was mainly attributable to missing instrumentation, tools, and methods at the molecular level rather than underdeveloped theory. For instance, the recent discovery of anaerobic ammonia oxidation by bacteria (anammox Process: 1999; molecular verification: 2006) was predicted decades earlier by Engelbert Broda solely based on thermodynamics.

The idea for developing two volumes on “Research on Nitrification and Related Processes” came after a fabulous international meeting on the Nitrogen Cycle organized by the Agouron Institute in Scottsdale, Arizona, in October 2009 (<http://agi.org/pdf/nmtg-abstracts/NMTgAbstractsforWeb.pdf>), where I presented new insights into N-cycle evolution. The Agouron Nitrogen meeting succeeded another significant event in the N-cycle community: the first International Conference on Nitrification (ICoN1) organized by the Nitrification Network (<http://nitrificationnetwork.org/news%20and%20events.php#icon1>) under my stewardship in Louisville, Kentucky, in July 2009. ICoN1 was made possible by support from the U.S. National Science Foundation, the Gordon and Betty Moore

Foundation, and the University of Louisville. So, I was well informed about the existing wealth of new approaches and data, which led me to agree when John Abelson and Mel Simon generously invited me to edit a volume of *Methods in Enzymology* to cover research on reactive N in an environmental context.

Acceptance of the three-domain structure of the universal tree of life by the majority of biologists, pioneered by Carl Woese and George Fox, the brilliant concept of primer extension developed by Ray Wu (which is the basis of dideoxy-primer sequencing and PCR) and whole genome sequencing have paved the way for new hot areas in the (micro)biological sciences such as phylogenomics, molecular microbial ecology, and systems microbiology. Newly developed experimental procedures and improvements in instrumentation in context with theoretical work in the last decade have also finally changed our view on many aspects of microbial biogeochemical cycles, including the global nitrogen cycle. Many novel processes and the molecular inventory and organisms facilitating them have been discovered only within the last 5–10 years and this discovery continues today. These new findings suggest that assigning biogeochemical processes exclusively to specific cohorts of (micro)organisms (i.e., “Denitrifiers,” “Nitrifiers,” “Ammonifiers,” etc.) was ill-conceived. Rather, the modular mix and match of metabolic inventory cassettes in diverse genomic backgrounds over long evolutionary time periods ultimately led to an extant diversity of nitrogen cycle organisms, including those with significance to carbon and sulfur cycles. It is thus opportune for *Methods in Enzymology* to present, in two parts, a state-of-the-art update on the methods and protocols dealing with the detection, isolation, and characterization of macromolecules and their hosting organisms that facilitate nitrification and related processes in the nitrogen cycle as well as the challenges of doing so in very diverse environments: Ready-to-use methods for modelers, molecular ecologists, biogeochemists, ecophysiologicals, geneticists, environmental engineers, and biochemists are presented.

These two parts would not have been possible without the generous sharing and enthusiasm of so many of my colleagues and friends inside and outside the Nitrification Network. I thank them all, and also Delsy Retchagar and Sujatha Thirugnanasambandam (Elsevier, Chennai, India) and Zoe Kruze (Elsevier, Oxford, UK), for their help and advice in steering both parts to a successful and timely outcome. My particular gratitude and thanks go to my colleague and friend Lisa Yael Stein (University of Alberta-Edmonton, Canada), who has skillfully shared with me the task of coediting part B.

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