

CONTENTS

<i>Contributors</i>	<i>xiii</i>
<i>Preface</i>	<i>xix</i>
<i>Volumes in Series</i>	<i>xxi</i>

Section I. Process Measurements in Natural Systems **1**

1. Strategies to Determine Diversity, Growth, and Activity of Ammonia-Oxidizing Archaea in Soil **3**

Graeme W. Nicol and James I. Prosser

1. Introduction	4
2. Common Methods	6
3. Community Composition and Diversity	15
4. Determining Growth and Abundance	21
5. Activity	23
6. Conclusions	29
References	31

2. Responses of Aerobic and Anaerobic Ammonia/Ammonium-Oxidizing Microorganisms to Anthropogenic Pollution in Coastal Marine Environments **35**

Huiluo Cao, Meng Li, Hongyue Dang, and Ji-Dong Gu

1. Introduction	36
2. Selection of Sampling Sites and Physicochemical Characterization	38
3. Molecular Ecological Characterization	39
4. Community Structure Analyses	44
5. Relationship Between the Community Structure and Physicochemical Parameters	50
6. Relationships Between the Community Change and the Environments	54
7. Conclusions	55
Acknowledgments	56
References	56

3. Molecular and Stable Isotope Methods to Detect and Measure Anaerobic Ammonium Oxidation (Anammox) in Aquatic Ecosystems	63
Bongkeun Song and Craig R. Tobias	
1. Introduction	64
2. Molecular Methods to Detect and Quantify Anammox Bacteria in Environmental Samples	66
3. Stable Isotope Methods to Measure Anammox Rates in Environmental Samples	77
Acknowledgments	84
References	84
4. Nitrogen Mineralization and Assimilation at Millimeter Scales	91
David D. Myrold, Jennifer Pett-Ridge, and Peter J. Bottomley	
1. Introduction	92
2. Microbial Habitats in Soil	92
3. Methodological Approaches	94
4. Conclusions	109
References	109
5. Measurement of Carbon Dioxide, Methane, Nitrous oxide, and Water Potential in Soil Ecosystems	115
Martin E. Brummell and Steven D. Siciliano	
1. Introduction	116
2. Soil Gas Probes	122
3. Flux Estimates to the Atmosphere Using Recirculating Chambers	125
4. Data Analysis	125
5. Soil Profile Analysis	127
6. FTIR Calibration	128
7. Correlations Between Profile Concentrations and Surface Flux in Field Measurements	129
8. Correlations Between FTIR and Traditional Water Activity Measures	132
9. Common Issues During FTIR Measurement	132
10. Conclusions	134
Acknowledgments	135
References	135

6. Source Determination of Nitrous Oxide Based on Nitrogen and Oxygen Isotope Tracing: Dealing with Oxygen Exchange	139
Dorien M. Kool, Jan Willem Van Groenigen, and Nicole Wrage	
1. Introduction	140
2. Experimental Approach	143
3. Data Evaluation for Nitrous Oxide Source Determination	145
4. Application of the ERR Principle in Nitrate Source Determination	150
5. Discussion, Applications, and Future Directions	152
References	156

Section II. Process Measurements in Biofilm Communities **161**

7. A Polyphasic Approach to Study Ecophysiology of Complex Multispecies Nitrifying Biofilms	163
Satoshi Okabe, Hisashi Satoh, and Tomonori Kindaichi	
1. Introduction	164
2. Microsensors	165
3. Microsensor Measurements	169
4. Estimation of Microbial Activities	169
5. Limitations of Microsensor Measurements	171
6. MAR-FISH	172
7. Methodology of MAR-FISH	172
8. Application of Microsensors and MAR-FISH to Nitrifying Biofilms	177
9. Ecophysiological Interaction Among Community Members	180
10. Conclusions	182
References	182
8. <i>In Situ</i> Techniques and Digital Image Analysis Methods for Quantifying Spatial Localization Patterns of Nitrifiers and Other Microorganisms in Biofilm and Flocs	185
Holger Daims and Michael Wagner	
1. Introduction	186
2. Theory of Spatial Arrangement Quantification by Digital Image Analysis	190
3. Protocols for the Spatial Arrangement Quantification of Nitrifiers	201
4. An Application Example: Spatial Analysis of Three Nitrifying Biofilm Populations	208
Acknowledgments	211
References	211

9. Investigating <i>Nitrosomonas europaea</i> Stress Biomarkers in Batch, Continuous Culture, and Biofilm Reactors	217
Tyler S. Radniecki and Ellen G. Lauchnor	
1. Introduction	219
2. Identifying Stress Responses in Batch Bioreactors	222
3. Identifying Stress Responses in Continuous Growth Systems	233
4. Identifying Stress Responses in Biofilms	239
5. Conclusions	242
References	244
10. Nitrification of Raw or Used Water Using Expanded Bed Biofilm Reactor Technology	247
M. J. Dempsey	
1. Introduction	248
2. Design and Operation of EBBRs	252
3. Measurement of Nitrification Performance	259
4. Typical Bioreactor and Bioparticle Performance Data	262
5. Conclusions	265
References	266
11. Ammonia-Oxidizing Bacteria in Wastewater	269
Micol Bellucci and Thomas P. Curtis	
1. Introduction	270
2. Sampling	271
3. DNA Extraction	271
4. Quantitative PCR	272
5. Denaturing Gradient Gel Electrophoresis	273
6. Fluorescence <i>In Situ</i> Hybridization	275
7. Conclusion	283
References	284
Section III. Genomics, Metagenomics and Transcriptomics	287
12. Genomics for Key Players in the <i>N</i> Cycle: From Guinea Pigs to the Next Frontier	289
Patrick S. G. Chain, Gary Xie, Shawn R. Starkenburg, Matthew B. Scholz, Nicholas Beckloff, Chien-Chi Lo, Karen W. Davenport, Krista G. Reitenga, Hajnalka E. Daligault, J. Chris Detter, Tracey A. K. Freitas, Cheryl D. Gleasner, Lance D. Green, Cliff S. Han, Kim K. McMurry, Linda J. Meincke, Xiaohong Shen, and Ahmet Zeytin	

1. Introduction: The Genomic Guinea Pigs	290
2. Want a Genome? Library Preparation First!	294
3. Sequencing a Genome from Start to Finish	297
4. Après Sequencing	306
5. Outlook: The Next Frontier	312
Acknowledgments	313
References	313
13. Preparation of High-Molecular Weight DNA and Metagenomic Libraries from Soils and Hot Springs	319
Laila J. Reigstad, Rita Bartossek, and Christa Schleper	
1. Introduction	320
2. Protocols	324
3. Growing, Picking, Replicating, and Storage of the Fosmid Library	338
References	342
14. Characterizing Bacterial Gene Expression in Nitrogen Cycle Metabolism with RT-qPCR	345
James E. Graham, Nicholas B. Wantland, Mark Campbell, and Martin G. Klotz	
1. Why Study Bacterial Transformation of Reactive Nitrogen in the Environment?	346
2. Nucleic Acids as Markers of N-Metabolic Activity	347
3. Gene Expression in Bacteria That Facilitate Reactive Nitrogen Transformations	356
4. Future Directions of the Approach	364
Acknowledgments	365
References	366
15. The Utility of Functional Gene Arrays for Assessing Community Composition, Relative Abundance, and Distribution of Ammonia-Oxidizing Bacteria and Archaea	373
B. B. Ward and N. J. Bouskill	
1. Introduction	374
2. DNA Microarrays: Introduction to Microarrays	375
3. Probe Selection	375
4. Target Preparation	379
5. Array Printing, Hybridization, and Scanning	380
6. Factors That Influence Hybridization Results	381
7. Array Applications	384

8. Possibilities and Limitations	389
9. Detailed Protocol for Functional Gene Microarrays Using Oligonucleotide Probes	390
References	394

Section IV. Biochemistry and Proteomics **397**

16. Structure and Function of Formate-Dependent Cytochrome c Nitrite Reductase, NrfA **399**

Oliver Einsle

1. Introduction	400
2. Isolation of NrfA	401
3. Crystallization of NrfA and the NrfHA Complex	402
4. Structure Solution by Multiple-Wavelength Anomalous Dispersion	404
5. Structure of NrfA	405
6. Heme Group Arrangement	408
7. Active Site Architecture	410
8. Substrate Complexes, Activity Assays, and Reactivity	411
9. Reaction Mechanism	413
10. Electron Transfer Systems	415
Acknowledgments	418
References	418

17. Detection and Characterization of a Multicopper Oxidase from *Nitrosomonas europaea* **423**

Thomas J. Lawton and Amy C. Rosenzweig

1. Introduction	423
2. Identification, Detection, and Isolation	426
3. Characterization and Crystallization	430
Acknowledgments	431
References	431

18. Assessing Variability in Gel-Based Proteomic Analysis of *Nitrosomonas europaea* **435**

Emily O. Burton and William J. Hickey

1. Introduction	436
2. Materials and Methods	440
3. Results and Discussion	445
Acknowledgments	460
References	460

19. Nitrogen Metabolism and Kinetics of Ammonia-Oxidizing Archaea	465
Willm Martens-Habbena and David A. Stahl	
1. Introduction	466
2. Strain Cultivation and Analytical Methods	468
3. Microrespirometry Setup	470
4. Stoichiometry and Kinetics of Ammonia Oxidation of <i>N. maritimus</i> and AOB	474
5. Variability of Kinetic Constants in AOB and AOA	480
6. Summary and Conclusions	482
Acknowledgments	483
References	483
<i>Author Index</i>	489
<i>Subject Index</i>	515