

Contents

1 New Frontiers in Soil Microbiology:	
How To Link Structure and Function of Microbial Communities?	1
<i>Blaž Stres, James M. Tiedje</i>	
1.1 Introduction:	
A Framework for the Structure–Function Grand Challenge...	1
1.2 Microbial Community Structure:	
One-Half of the Structure–Function Paradigm	3
1.3 The Other One-Half:	
Functional Traits of Microbial Communities	6
1.4 Newer Approaches for Linking Function	
with Phylogeny and Structure	9
1.5 Future Challenges	12
References	17
2 Chemical Structure of Organic N and Organic P in Soil	23
<i>Ingrid Kögel-Knabner</i>	
2.1 Introduction	23
2.2 Biological Forms of Organic N and P That Enter Soils	24
2.2.1 Proteins and Polypeptides	25
2.2.2 Amino Sugar Polymers	27
2.2.3 DNA and RNA	28
2.2.4 Minor N-Containing Molecules	29
2.2.5 Teichoic Acids.....	29
2.2.6 Inositol Phosphates	31
2.2.7 Phospholipids.....	31
2.3 Techniques To Analyse Soil Organic Nitrogen	32
2.3.1 Hydrolysis.....	33
2.3.2 Analytical Pyrolysis and Thermochemolysis	34
2.3.3 Solid-State ^{15}N NMR Spectroscopy.....	35
2.3.4 X-ray Absorption Near-Edge	
Structure Spectroscopy	36
2.4 Forms of Organic N in Soil Organic Matter	37

2.5	Techniques To Analyse Organic P in Soils	39
2.5.1	Sequential Extraction and Separation.....	40
2.5.2	^{31}P NMR Spectroscopy	40
2.6	Forms of Organic P in Soils	42
2.7	Summary.....	43
	References	43
3	Nucleic Acid Extraction from Soil	49
	<i>Lars R. Bakken, Åsa Frostegård</i>	
3.1	Introduction	49
3.2	Lysis and Extraction	51
3.2.1	Cell Rupture Depends on Cell Type and Growth.....	51
3.2.2	Bead Beating, Efficiency and Bias	52
3.2.3	Grinding	56
3.2.4	Freeze/Thaw	57
3.2.5	Enzymatic Lysis	57
3.2.6	Chemical Agents	59
3.2.7	Extraction for Metagenome Libraries	59
3.3	Purification.....	60
3.4	RNA Extraction	62
3.5	Cell Extraction	63
3.5.1	Dispersion	64
3.5.2	Separation.....	65
	References	67
4	Role of Stabilised Enzymes in Microbial Ecology and Enzyme Extraction from Soil with Potential Applications in Soil Proteomics	75
	<i>Paolo Nannipieri</i>	
4.1	Introduction	75
4.2	Evidence for the Presence of Stabilised Enzymes in Soil.....	77
4.3	Extraction of Enzymes from Soil.....	79
4.4	The Role of Stabilised Enzymes in Soil Microbial Ecology	82
4.5	Proteomics	83
4.6	Soil Proteomics	85
4.7	Conclusions	89
	References	90
5	Soil Proteomics: Extraction and Analysis of Proteins from Soils	95
	<i>Oladele A. Ogunseitan</i>	
5.1	Introduction	95
5.2	Rationale and Context of Soil Proteomics	96

5.3	Methodology for Soil Proteomics	99
5.3.1	Extraction Methods	99
5.3.2	Analytical Methods	101
5.4	Case Studies and Emerging Issues in Soil Proteomics	104
5.4.1	Glomalin.....	105
5.4.2	Soil Proteins as Metal Biosensors	108
5.4.3	Prospects for Proteomic Analysis of Soil Microbial Communities.....	110
	References	112
6	The Various Sources and the Fate of Nucleic Acids in Soil	117
	<i>Wilfried Wackernagel</i>	
6.1	Introduction	117
6.2	Release of DNA from Organisms	118
6.2.1	Bacteria	118
6.2.2	Plants	119
6.2.3	Other Organisms.....	121
6.3	Presence of DNA in Soil	122
6.4	Distribution of Extracellular DNA in Soil	124
6.4.1	The Binding of DNA to Soils	124
6.4.2	The Distribution of DNA to Liquid and Solid Soil Phases	125
6.5	Persistence of DNA in Soil.....	126
6.5.1	Protection of Mineral-Associated DNA against DNases	126
6.5.2	Degradation Kinetics of Introduced DNA in Soil	128
6.5.3	Methods to Assay the Persistence of Functional DNA in Soils	129
6.5.4	Persistence and Spread of Plant DNA in Agricultural Field Plots.....	130
6.5.5	Long-Term Field Persistence of Plant DNA in Cellular Material or as Free DNA.....	131
6.6	The Extracellular Gene Pool Hypothesis.....	132
6.7	Conclusions	133
	References	134
7	Stabilization of Extracellular DNA and Proteins	
	by Transient Binding to Various Soil Components	141
	<i>Kaare M. Nielsen, Luca Calamai, Giacomo Pietramellara</i>	
7.1	Introduction	141
7.2	DNA Interactions with Purified Soil Components	143
7.2.1	DNA Interactions with Sand	143
7.2.2	DNA Interactions with Clay Minerals	143

7.2.3	Natural Transformation of Bacteria with DNA Adsorbed or Bound to Clays.....	145
7.2.4	DNA Interactions with Humic Substances.....	146
7.2.5	Natural Transformation in the Presence of Humic Substances.....	146
7.3	Protein Interactions with Purified Soil Components.....	147
7.3.1	Protein Interactions with Clay Minerals	147
7.3.2	Protein Interactions with Humic Substances	148
7.4	Interactions of DNA, Combined with Other Cellular Substances, with Pure Soil Components	148
7.4.1	DNA-Protein Interactions	149
7.4.2	Adsorption of DNA-Protein Complexes on Different Soil Components	149
7.5	DNA Interactions with Natural Soils	150
7.6	Protein Interactions with Natural Soils.....	151
7.7	Concluding Remarks	152
	References	153
8	Assessing Bacterial and Fungal Community Structure in Soil Using Ribosomal RNA and Other Structural Gene Markers	159
	<i>George A. Kowalchuk, Barbara Drigo, Etienne Yergeau, Johannes A. van Veen</i>	
8.1	Introduction	159
8.2	The General Choices in Molecular Analysis of Soil-Borne Microbial Communities.....	160
8.2.1	Community Structure Versus Diversity	161
8.2.2	The Benefits and Limitations of PCR-Based Approaches	163
8.2.3	DNA Versus RNA Targets.....	164
8.2.4	Cloning Inventories Versus Community Profiling Methods.....	165
8.3	General Approaches for Microbial Community Description ..	167
8.3.1	The rRNA Approach	167
8.3.2	Other General Markers for In Situ Determinations of Microbial Community Structure	170
8.4	Group-Specific Microbial Community Analyses	172
8.4.1	rRNA Approaches Focused on Specific Phylogenetic Groups	173
8.4.2	Specific Approaches Based on Specialised Functional Genes	177
8.5	Concluding Remarks	179
	References	180

9 Advances in Microarray-Based Technologies for Soil Microbial Community Analyses	189
<i>Christopher W. Schadt, Jizhong Zhou</i>	
9.1 Introduction	189
9.2 Types of Environmental Microarrays	190
9.3 Important Issues in Microarray Analysis	190
9.3.1 Specificity	190
9.3.2 Sensitivity	192
9.3.3 Quantitation	193
9.4 Applications of Different Formats of Microarrays	194
9.4.1 Phylogenetic Oligonucleotide Arrays (POAs)	194
9.4.2 Functional Gene Arrays (FGAs)	196
9.4.3 Community Genome Arrays (CGAs)	197
9.5 Conclusions and Future Perspectives	200
References	201
 10 Stable Isotope Probing:	
A Critique of Its Role in Linking Phylogeny and Function	205
<i>Mike Manefield, Robert I. Griffiths, Mark J. Bailey, Andrew S. Whiteley</i>	
10.1 Introduction	205
10.2 Polar Lipid Derived Fatty Acid Based Stable Isotope Probing (PLFA-SIP)	207
10.3 DNA- and RNA-Based Stable Isotope Probing (NA-SIP)	208
10.4 Alternative Stable Isotope Based Approaches	211
10.5 Radioactive Isotope Based Approaches	212
10.6 Notes on Isotopic Enrichments	213
10.7 Conclusions	213
References	214
 11 Gene Detection, Expression and Related Enzyme Activity in Soil	217
<i>Martin Krsek, William H. Gaze, N.Z. Morris, Elizabeth M.H. Wellington</i>	
11.1 Introduction	217
11.2 Molecular Detection of Functional Genes in Soil	220
11.2.1 Introduction	220
11.2.2 Antibiotic Biosynthesis Genes	222
11.2.3 Detection of Antibiotic and Heavy Metal Resistance Genes	224
11.2.4 Nutrient Cycle Genes; the Nitrogen Cycle	227
11.2.5 Biodegradation of Soil Polymers and C1 Compounds	228
11.2.6 Bioremediation Activity	230

11.2.7 Molecular Detection of Functional Gene Signatures for Detecting Pathogens in Soil.....	232
11.3 Expression of Functional Genes in Soil.....	235
11.3.1 Introduction – Methods for the Detection of mRNA in Soil	235
11.4 Linking Enzyme Activity to Gene Expression	244
11.4.1 Introduction.....	244
11.4.2 Decomposer Activity and Bioremediation.....	244
11.5 Conclusions	246
References	246
12 Enzyme Activities in Soil	257
<i>Liliana Gianfreda, Pacifico Ruggiero</i>	
12.1 Introduction	257
12.2 Type, Distribution, Location and Properties	257
12.3 Factors Affecting Soil Enzyme Activities	266
12.4 Measurement of Soil Enzyme Activities.....	267
12.5 Soil Functioning as Determined by Enzyme Activity.....	276
12.5.1 Degradation of Litter and Enzyme Activities in Forest Soil	276
12.5.2 Effect of pH	278
12.5.3 Effect of Stresses	278
12.6 Effects of Land Management Practices on Soil Enzyme Activities.....	282
12.6.1 Nitrogen Fertilisation	282
12.6.2 Organic Amendments	284
12.6.3 Tillage, Cropping and Other Managements	286
12.7 Relationship Between Enzyme Activities and Soil Physical Properties and Soil Depth	288
12.7.1 Distribution of Enzyme Activities with Soil Particles.....	288
12.8 Effects of Transgenic Plants and Recombinant Microorganisms on Soil Enzyme Activities.	
The Potential Role of Rhizosphere Enzyme Activities	290
12.9 Relationship Between Enzyme Activities and Their Substrates or Products in Soil.....	292
12.10 Enzymes as Decontaminating Agents.....	293
12.11 Enzyme Activities as Indicators of the Functional Status of the Soil Community.....	294
12.12 Future Challenges	297
References	297

13 How to Assess the Abundance and Diversity of Mobile Genetic Elements (MGE) in Soil Bacterial Communities?	313
<i>Kornelia Smalla, Holger Heuer</i>	
13.1 Introduction	313
13.2 Cultivation-Dependent Techniques: MGE in Bacterial Isolates from Soil	316
13.2.1 Plasmids Detected in Soil Bacteria by Plasmid Isolation	316
13.2.2 MGE Detected in Soil Bacteria by Probing or PCR.....	317
13.2.3 Sequencing of MGE in Bacterial Isolates Allows Insight into MGE-Encoded Traits and Their Evolution.....	318
13.3 Genome Sequencing of Soil Bacterial Isolates.....	319
13.4 Cultivation-Independent Methods.....	320
13.4.1 Microscopic Detection of Phages	320
13.4.2 Capturing of MGE by Exogenous Isolation in Biparental and Triparental Matings	321
13.4.3 PCR-Based Detection of MGE	325
13.5 Conclusions	326
References	326
14 Bacterial Conjugation in Soil	331
<i>Jan Dirk van Elsas, Sarah Turner, Jack T. Trevors</i>	
14.1 Introduction	331
14.2 Experimental Approaches to Studying HGT via Conjugation.	336
14.2.1 Conjugation Systems	336
14.2.2 Soil Microcosm Versus Field Studies	337
14.2.3 What Did We Learn from Donor-to-Recipient Gene Transfer Studies?	338
14.2.4 What Did We Learn from Donor-to-Indigenous-Bacteria Transfer Studies?.....	340
14.3 Conjugative Transfer to Total (Potentially Non-culturable) Bacteria.....	342
14.3.1 What Did We Learn from Studies on In Situ Conjugative Gene Transfer?	342
14.3.2 What Did We Learn from Sequence Analyses of Soil/Phytosphere Bacteria?	343
14.4 Conclusions	347
References	349

15 Horizontal Gene Transfer by Natural Transformation in Soil Environment	355
<i>Anne Mercier, Elisabeth Kay, Pascal Simonet</i>	
15.1 Introduction	355
15.2 Mechanisms of Horizontal Gene Transfer	356
15.3 In Situ Regulation of Natural Transformation in Bacteria.....	357
15.4 Natural Transformation: An Unexpected Widespread Gene-Transfer Mechanism in Bacteria?	358
15.5 Bacterial Competence Development in Soil.....	360
15.6 Gene Transfer in the Environment by Alternate Genetic Transformation-Related Mechanisms?	362
15.7 Persistence of Extracellular DNA in Soil	362
15.8 Development of Methods To Investigate Gene Transfer.....	363
15.9 Gene Transfer by Natural Transformation from Transgenic Plants to Bacteria – A Possible Event?	365
15.10 Concluding Remarks	366
References	366
 16 Reporter Genes in Bacterial Inoculants Can Monitor Life Conditions and Functions in Soil	375
<i>Jan Sørensen, Ole Nybroe</i>	
16.1 Introduction to Reporter Bacteria	375
16.2 Applications of Reporter Bacteria in Soil	379
16.2.1 Non-specific Reporters of Metabolic Activity.....	379
16.2.2 Semi-specific Reporters of Stress	380
16.2.3 Reporters of Bacterial Growth (Ribosome Synthesis). .	381
16.2.4 Reporters of Nutrient Limitation	382
16.2.5 Reporters for Specific Carbon and Nitrogen Sources..	383
16.2.6 Reporters for Oxygen Limitation (Anaerobiosis)	384
16.2.7 Reporters of Aromatics and Their Degradation.....	384
16.3 Current and Future Trends	388
References	391
 17 Reporter Gene Technology in Soil Ecology; Detection of Bioavailability and Microbial Interactions	397
<i>Mette Burmølle, Lars Hestbjerg Hansen, Søren J. Sørensen</i>	
17.1 Introduction	397
17.2 Reporter Genes.....	398
17.2.1 Reporter Genes Encoding Luciferases.....	398
17.2.2 Reporter Genes Based on Chromogenic Detection	400
17.2.3 Reporter Genes Encoding Fluorescence	401
17.2.4 Reporter Genes Encoding Ice-Nucleation Activity	404

17.3	Whole-Cell Biosensors.....	404
17.4	Bioavailability	409
17.4.1	Use of Biosensors To Measure Bioavailability of Metals in Soil	410
17.4.2	In Situ Versus Extract Measurements of Bioavailability	411
17.5	Detection of Microbial Interactions	412
17.5.1	Production of Oxytetracycline.....	412
17.5.2	Production of Communication Signals	413
17.6	Concluding Remarks	414
	References	415
18	Marker Genes As Tools To Study Deliberately Released Soil Bacteria <i>Christoph C. Tebbe, Rona Miethling-Graff</i>	421
18.1	Introduction: The Importance of Tagging Microbial Inoculants for Environmental Applications	421
18.2	Genetic Tools for Tagging Inoculants	422
18.3	Selective Markers.....	425
18.4	Luminescence and Fluorescence Markers	426
18.5	Objectives for Field Releases of Genetically Engineered Bacteria	429
18.6	Field Release of <i>Sinorhizobium meliloti</i> L33 and L1 – A Case Study	431
18.7	Evaluation of Strategies To Eliminate <i>S. meliloti</i> from Soil	437
18.8	Conclusions: Biosafety and Usefulness of Small-Scale Field Release Studies with Marker Gene-Tagged Bacteria	438
	References	439
	Subject Index	449