## Contents

### 1 New Frontiers in Soil Microbiology:
**How To Link Structure and Function of Microbial Communities?**

*Blaž Stres, James M. Tiedje*

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

*Ingrid Kögel-Knabner*

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
## Contents

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  
  Lars R. Bakken, Åsa Frostegård  
  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  
  Paolo Nannipieri  
  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  
  Oladele A. Ogunseitan  
  5.1 Introduction .............................................................. 95  
  5.2 Rationale and Context of Soil Proteomics ..................... 96
### Contents

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

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
Christopher W. Schadt, Jizhong Zhou

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
Mike Manefield, Robert I. Griffiths, Mark J. Bailey, Andrew S. Whiteley

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
Martin Krsek, William H. Gaze, N.Z. Morris, Elizabeth M.H. Wellington

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
   Liliana Gianfreda, Pacifico Ruggiero

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

Kornelia Smalla, Holger Heuer

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

Jan Dirk van Elsas, Sarah Turner, Jack T. Trevors

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
Anne Mercier, Elisabeth Kay, Pascal Simonet

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
15.7 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
Jan Sørensen, Ole Nybroe

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
Mette Burmølle, Lars Hestbjerg Hansen, Søren J. Sørensen

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

- **Bioavailability**
  - Use of Biosensors To Measure Bioavailability of Metals in Soil
  - In Situ Versus Extract Measurements of Bioavailability

### 17.4 Bioavailability

- Use of Biosensors To Measure Bioavailability of Metals in Soil
- In Situ Versus Extract Measurements of Bioavailability

### 17.5 Detection of Microbial Interactions

- Production of Oxytetracycline
- Production of Communication Signals

### 17.6 Concluding Remarks

### References

---

### 18 Marker Genes As Tools To Study Deliberately Released Soil Bacteria

*Christoph C. Tebbe, Rona Miethling-Graff*

#### 18.1 Introduction: The Importance of Tagging Microbial Inoculants for Environmental Applications

#### 18.2 Genetic Tools for Tagging Inoculants

#### 18.3 Selective Markers

#### 18.4 Luminescence and Fluorescence Markers

#### 18.5 Objectives for Field Releases of Genetically Engineered Bacteria

#### 18.6 Field Release of *Sinorhizobium meliloti* L33 and L1 – A Case Study

#### 18.7 Evaluation of Strategies To Eliminate *S. meliloti* from Soil

#### 18.8 Conclusions: Biosafety and Usefulness of Small-Scale Field Release Studies with Marker Gene-Tagged Bacteria

### References

---

### Subject Index