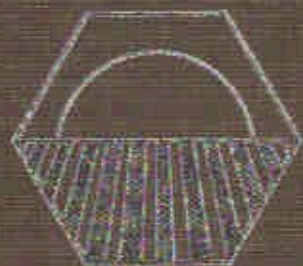


SSSA Book Series: 5



Methods of Soil Analysis
Part 2—Microbiological and Biochemical Properties

Methods of Soil Analysis

Part 2 Microbiological and Biochemical Properties

Editorial Committee: R. W. Weaver, chair
Scott Angle
Peter Bottomley
David Bezdicek
Scott Smith
Ali Tabatabai
Art Wollum

Managing Editor: S. H. Mickelson
Editor-in-Chief SSSA: J. M. Bigham

Number 5 in the Soil Science Society of America Book Series

Published by: Soil Science Society of America, Inc.

1994

CONTENTS

FOREWORD	xv
PREFACE	xvii
CONTRIBUTORS	xix
CONVERSION FACTORS FOR SI AND NON-SI UNITS	xxiii

Chapter 1 Soil Sampling for Microbiological Analysis **1**

A. G. WOLLUM, II

1-1 Principles	2
1-2 Methods	8
1-3 Sources of Error	12
1-4 Concluding Remarks	13
REFERENCES	13

Chapter 2 Statistical Treatment of Microbial Data **15**

TIMOTHY B. PARKIN AND JOSEPH A. ROBINSON

2-1 Characteristics of the Lognormal Distribution	16
2-2 Diagnosing Lognormality	17
2-3 Estimating Population Parameters from Sample Data	21
2-4 Selecting the Appropriate Location Parameter	25
2-5 Hypothesis Testing	28
2-6 Sample Number Requirements	31
2-7 Concluding Remarks	32
APPENDIX 1	34
APPENDIX 2	37
REFERENCES	38

Chapter 3 Soil Sterilization **41**

DUANE C. WOLF AND HORACE D. SKIPPPER

3-1 Principles	41
3-2 Moist Heat	42
3-3 Dry Heat	43
3-4 Gamma Irradiation	44
3-5 Microwave Irradiation	45
3-6 Gaseous Compounds	45
3-7 Nongaseous Compounds	47
3-8 Conclusions	49
REFERENCES	49

Chapter 4 Soil Water Potential **53**

K. J. McINNES, R. W. WEAVER, AND M. J. SAVAGE

4-1 Principles	54
4-2 Materials	55
4-3 Procedure	55
4-4 Comments	57
REFERENCES	57

Chapter 5 Most Probable Number Counts	59
PAUL L. WOOMER	
5-1 Principles	60
5-2 Methodology	65
5-3 Comments	77
ACKNOWLEDGMENT	78
REFERENCES	78
Chapter 6 Light Microscopic Methods for Studying Soil Microorganisms	81
PETER J. BOTTOMLEY	
6-1 Sampling of Soil for Microscopic Observation	81
6-2 Microscopic Enumeration of Total Bacteria in Soil	82
6-3 Determining the Proportion of Viable Soil Bacteria Using a Cell Elongation Assay	92
6-4 Determining the Proportion of Viable Soil Bacteria by Following the Reduction of Tetrazolium Dyes to Formazan	94
6-5 Microscopic Determination of the Mycelial Length of Soil Fungi	96
6-6 Determining the Proportion of Metabolically Active Fungal Mycelia by Following the Hydrolysis of Fluorescein Diacetate	98
6-7 Determining the Proportion of Metabolically Active Fungal Mycelia by Following the Reduction of Tetrazolium Dyes to Formazan	99
6-8 Determining the Weight of Soil Biomass from Microscopic Estimates of Biovolume	100
6-9 Microscopic Enumeration of Bacteria with Fluorescently Labeled Oligonucleotides Directed at Specific Regions of 16S Ribosomal RNA	101
ACKNOWLEDGMENT	104
REFERENCES	104
Chapter 7 Viruses	107
J. SCOTT ANGLE	
7-1 General Principles of Analysis	109
7-2 Phages	110
7-3 Enteric Viruses	114
REFERENCES	115
Chapter 8 Recovery and Enumeration of Viable Bacteria	119
DAVID A. ZUBERER	
8-1 Principles of Enumerating Soil Bacteria	120
8-2 Materials and Equipment	122
8-3 Collection and Preparation of Soil Samples	123
8-4 Release of Bacteria from Soils	124
8-5 Diluents Used in Recovery and Enumeration of Soil Bacteria	125
8-6 Preparation of Serial Dilutions	127
8-7 Plating Techniques	130
8-8 Media for Enumeration of Soil Bacteria	134
8-9 Analysis and Presentation of Plate Count Data	139
8-10 Conclusion	141
REFERENCES	142

Chapter 9 Coliform Bacteria**145**

RONALD F. TURCO

9-1 Recovery and Enumeration of Fecal Coliforms from Soil	147
9-2 Detection and Enumeration of Total Coliforms	151
9-3 Rapid Test for Detection of <i>E. coli</i> in Soil	154
9-4 Direct Methods for Detection of <i>E. coli</i> in Soil	156
ACKNOWLEDGMENT	156
REFERENCES	157

Chapter 10 Autotrophic Nitrifying Bacteria**159**

EDWIN L. SCHMIDT AND L. W. BELSER

10-1 Enumeration by Most Probable Number	160
10-2 Diversity of Nitrifiers	163
10-3 Immunofluorescence Examination	165
10-4 Isolation of Nitrifiers	167
10-5 Maintenance of Pure Cultures	169
10-6 Nitrifying Activity in Soils	171
REFERENCES	176

Chapter 11 Free-living Dinitrogen-fixing Bacteria**179**

ROGER KNOWLES AND WILFREDO LASERNA BARRAQUIO

11-1 The Acetylene Reduction Assay	180
11-2 Methods for Dinitrogen Fixers in General	181
11-3 Methods for Azotobacteraceae	183
11-4 Method for Methanotrophs	186
11-5 Method for Hydrogen-Using Dinitrogen Fixers	188
11-6 Method for Cyanobacteria	189
11-7 Method for Photosynthetic Purple Nonsulfur Bacteria	191
11-8 Method for Clostridia	192
11-9 Method for Sulfate-Reducing Bacteria	194
REFERENCES	195

Chapter 12 Legume Nodule Symbionts**199**

R. W. WEAVER AND PETER H. GRAHAM

12-1 Nodule Collection and the Isolation of Symbionts	200
12-2 Cultivation of Nodule Symbionts	203
12-3 Maintenance of Cultures	205
12-4 Enumeration of Nodule Symbionts in Soil and Inoculants	206
12-5 Inoculants for Field Experimentation	210
12-6 Inoculation of Seed	213
12-7 Field Experimentation Involving Inoculation	215
12-8 Growth-Pouch Infection Assays	216
REFERENCES	218

Chapter 13 Anaerobic Bacteria and Processes	223
HEINRICH F. KASPAR AND JAMES M. TIEDJE	
13-1 Principles	224
13-2 Methods for Removal of Oxygen	226
13-3 Methods for Reduction of Media	229
13-4 Redox Indicators	231
13-5 Culture Methods	233
13-6 Enumeration Methods	235
13-7 Simple Method to Carry Out Anaerobic Incubations of Soil	240
ACKNOWLEDGEMENTS	241
REFERENCES	242
Chapter 14 Denitrifiers	245
JAMES M. TIEDJE	
14-1 Nitrate Reducing Processes	245
14-2 Key Physiological and Ecological Features of Respiratory Denitrifiers ...	250
14-3 Enumeration of Denitrifiers	251
14-4 Enumeration of Dissimilatory Nitrate to Ammonium Reducers	255
14-5 Denitrifier Enzyme Activity	256
14-6 Isolation of Denitrifiers	257
14-7 Confirmation of Respiratory Denitrification	259
14-8 Taxonomic Identification	265
ACKNOWLEDGMENT	265
REFERENCES	265
Chapter 15 Actinomycetes	269
E. M. H. WELLINGTON AND I. K. TOTH	
15-1 Enumeration, Enrichment, and Isolation	270
15-2 Isolation of Physiological Groups	279
15-3 Grouping and Identification of Actinomycetes	284
REFERENCES	287
Chapter 16 <i>Frankia</i> and the Actinorhizal Symbiosis	291
DAVID D. MYROLD	
16-1 Characteristics of <i>Frankia</i>	291
16-2 Isolation, Culturing, and Maintenance of <i>Frankia</i> Strains	294
16-3 Quantification and Differentiation of <i>Frankia</i> Strains	305
16-4 Characterization of <i>Frankia</i> in Symbiosis	309
16-5 Quantification in Soil	316
16-6 Conclusion	320
ACKNOWLEDGMENTS	320
REFERENCES	322
Chapter 17 Filamentous Fungi	329
DENNIS PARKINSON	
17-1 Qualitative Studies: Isolation Methods	330
17-2 Quantitative Methods	342
REFERENCES	347

Chapter 18 Vesicular-Arbuscular Mycorrhizal Fungi	351
DAVID M. SYLVIA	
18-1 Quantification of Vesicular-Arbuscular Mycorrhizal Propagules in Soil ..	352
18-2 Quantification of Vesicular-Arbuscular Mycorrhizal Colonization in Roots	353
18-3 Quantification of Vesicular-Arbuscular Mycorrhizal External Hyphae	357
18-4 Recovery of Vesicular-Arbuscular Mycorrhizal Fungal Spores	360
18-5 Identification of Vesicular-Arbuscular Mycorrhizal Fungi	360
18-6 Assessment of Growth Response and Selection of Effective Isolates	361
18-7 Production and Use of Vesicular-Arbuscular Mycorrhizal Inocula	366
18-8 Monoxenic Cultures for Basic Research	372
REFERENCES	372
Chapter 19 Isolation of Microorganisms Producing Antibiotics	379
JEFFRY J. FUHRMANN	
19-1 General Principles	380
19-2 Microbiological Media	382
19-3 Preparation of Inocula	384
19-4 Dual Culture Detection Methods	385
19-5 Culture Filtrate Methods	389
19-6 Screening Methods	393
19-7 Methods for Selected Classes of Compounds	396
19-8 Concluding Comments	402
REFERENCES	403
Chapter 20 Microbiological Procedures for Biodegradation Research	407
DENNIS D. FOCHT	
20-1 The Enrichment Culture	409
20-2 Isolation of Pure Cultures	412
20-3 Maintenance of Cultures	413
20-4 Growth in Liquid Cultures	415
20-5 Preparation of Washed Cell Suspensions	418
20-6 Preparation and Use of Cell-Free Extracts	419
20-7 Oxygen Consumption	421
20-8 Chloride Determination	423
20-9 Conclusion	424
REFERENCES	424
Chapter 21 Algae and Cyanobacteria	427
F. BLAINE METTING, JR.	
21-1 Identification of Soil Algae and Cyanobacteria	428
21-2 Direct Methods for Enumeration	432
21-3 Indirect Methods for Enumeration	440
21-4 Methods for Isolation and Purification of Microalgal Cultures	444
21-5 Methods for Growth and Storage of Microalgal Cultures	447
21-6 Methods for Estimating Photosynthesis	453
21-7 Methods for Measuring Cyanobacterial Dinitrogen Fixation	454
21-8 Methods for Studying Endosymbiotic Cyanobacteria in Cycad Roots	454
ACKNOWLEDGMENT	455
REFERENCES	456

Chapter 22 Nematodes	459
	RUSSELL E. INGHAM
22-1 Nematode Sampling	461
22-2 Extraction of Nematodes from Soil	469
22-3 Extraction of Nematodes from Plant Material	477
22-4 Microscopic Observation and Identification of Nematodes	479
22-5 Nematode Identification	482
REFERENCES	487
Chapter 23 Protozoa	491
	ELAINE R. INGHAM
23-1 First Consideratons	491
23-2 Protozoan Ecology	494
23-3 Methods of Enumeration	498
23-4 Identification	509
23-5 Summary	511
ACKNOWLEDGMENT	511
REFERENCES	512
Chapter 24 Arthropods	517
	ANDREW R. MOLDENKE
24-1 Principles	518
24-2 Methods	518
24-3 Processing the Extracted Biota Sample	528
24-4 Biota Identification	531
24-5 Preservation and Archiving	532
24-6 Archiving	533
24-7 Rearing	535
24-8 Statistical Methods to Analyze Diversity	538
REFERENCES	539
Chapter 25 Carbon Utilization and Fatty Acid Profiles for Characterization of Bacteria	543
	A. C. KENNEDY
25-1 Characterization of Bacteria	544
25-2 Carbon Source Utilization	544
25-3 Fatty Acid Analysis	551
REFERENCES	553
Chapter 26 Multilocus Enzyme Electrophoresis Methods for the Analysis of Bacterial Population Genetic Structure	557
	B. D. EARDLY
26-1 Principles	559
26-2 Methods	562
REFERENCES	572

Chapter 27 Spontaneous and Intrinsic Antibiotic Resistance Markers	575
CHARLES HAGEDORN	
27-1 Selection of Spontaneous Antibiotic Resistant Strains	577
27-2 Selection of Intrinsic Antibiotic Resistant Strains	581
27-3 Evaluation of SAR and IAR Strains	585
REFERENCES	589
Chapter 28 Serology and Conjugation of Antibodies	593
S. F. WRIGHT	
28-1 Antibodies	594
28-2 Adsorption of Cross-Reactive Antibodies from Polyclonal Antiserum	601
28-3 Purification of Antibodies from Antiserum	602
28-4 Conjugation of Antibodies with Fluorescein Isothiocyanate	604
28-5 Storage of Antibodies and Antibody Conjugates	606
28-6 Immunoassays	606
REFERENCES	616
Chapter 29 Whole-Cell Protein Profiles of Soil Bacteria by Gel Electrophoresis	619
DIPANKAR SEN	
29-1 Principles	620
29-2 Methods	621
29-3 General Comments	630
REFERENCES	633
Chapter 30 Plasmid Profiles	635
IAN L. PEPPER	
30-1 Plasmid Chromosome Relationships	635
30-2 Plasmid Profile Analysis	638
30-3 Applications of Plasmid Profile Analyses for Soil Bacteria	644
REFERENCES	644
Chapter 31 DNA Fingerprinting and Restriction Fragment Length Polymorphism Analysis	647
M. J. SADOWSKY	
31-1 DNA Fingerprinting	648
31-2 RFLP Analyses	656
REFERENCES	662
Chapter 32 Nucleic Acid Probes	665
A. V. OGRAM AND D. F. BEZDICEK	
32-1 Probe Selection	667
32-2 Isolation and Purification of Fragments to be Used as Probes	669
32-3 Labeling of Probes	671

32-4 Hybridization	679
32-5 Detection Systems	684
REFERENCES	686
Chapter 33 Marking Soil Bacteria with <i>lacZY</i>	689
T. E. STALEY AND D. J. DRAHOS	
33-1 Principles	691
33-2 Materials	693
33-3 Procedure	696
33-4 Exconjugant-Type Isolate Vigor	700
33-5 Marker Stability	701
33-6 Recovery from Nonsterile Soil	702
33-7 Attributes and Deficiencies	703
33-8 Comments	704
REFERENCES	705
Chapter 34 Detection of Specific DNA Sequences in Environmental Samples via Polymerase Chain Reaction	707
IAN L. PEPPER AND SURESH D. PILLAI	
34-1 Theory	709
34-2 Primer Design and Amplification Protocol	710
34-3 Optimization of Amplification	714
34-4 Identification of Amplified Products	717
34-5 Quality Control	717
34-6 Specificity of Amplification	719
34-7 Sensitivity of Amplification	720
34-8 Applications in Environmental Microbiology	721
REFERENCES	725
Chapter 35 Isolation and Purification of Bacterial DNA from Soil	727
WILLIAM E. HOLBEN	
35-1 General Considerations	729
35-2 Bacterial Fractionation Approach for Recovery of Bacterial Community DNA	731
35-3 Direct Lysis Approach for the Recovery of Total Bacterial Community DNA	733
35-4 Bacterial Fractionation Protocol	736
35-5 Direct Lysis Protocol	741
35-6 Fractionation of DNA Gradients, Final Purification and Quantitation of Bacterial Community DNA	743
ACKNOWLEDGMENTS	750
REFERENCES	750
Chapter 36 Microbial Biomass	753
W. R. HORWATH AND E. A. PAUL	
36-1 Soil Sampling, Preparation, and Storage	754
36-2 Physiological Methods	754

CONTENTS	xiii
36-3 Chemical Methods	763
36-4 Comparison of Methods	770
REFERENCES	771
Chapter 37 Soil Enzymes	775
M. A. TABATABAI	
37-1 Principles	778
37-2 Factors Affecting Rates of Enzyme Reaction	778
37-3 Assay of Enzymes in Soil	790
REFERENCES	826
Chapter 38 Carbon Mineralization	835
L. M. ZIBILSKE	
38-1 General Principles	836
38-2 Experimental Principles	836
38-3 Field Methods	841
38-4 Laboratory Methods	850
REFERENCES	859
Chapter 39 Isotopic Methods for the Study of Soil Organic Matter Dynamics	865
DUANE C. WOLF, J. O. LEGG, AND THOMAS W. BOUTTON	
39-1 Decomposition of ¹⁴ C-Labeled Organic Matter in Soils	866
39-2 ¹³ C Natural Abundance Technique: Background and Principles	875
39-3 Decomposition of ¹⁵ N-Labeled Organic Matter in Soils	887
39-4 Extraction of Labeled Organic Fractions in Studies of Soil Organic Matter Dynamics	893
39-5 Conclusions	900
REFERENCES	901
Chapter 40 Practical Considerations in the Use of Nitrogen Tracers in Agricultural and Environmental Research	907
R. D. HAUCK, J. J. MEISINGER, AND R. L. MULVANEY	
40-1 Preparing ¹⁵ N-Labeled Materials	909
40-2 Field Study Techniques	919
40-3 Preparing for and Measuring Nitrogen-Isotope Ratio	935
40-4 Sources of Nitrogen-15 Supply and Analytical Service	942
REFERENCES	943
Chapter 41 Nitrogen Availability Indices	951
L. G. BUNDY AND J. J. MEISINGER	
41-1 Current Status of Nitrogen Availability Indices	951
41-2 Methods	955
REFERENCES	979

Chapter 42 Nitrogen Mineralization, Immobilization, and Nitrification	985
STEPHEN C. HART, JOHN M. STARK, ERIC A. DAVIDSON, AND MARY K. FIRESTONE	
42-1 Measurement of Gross Nitrogen-Transformation Rates	987
42-2 Field Methods for Estimating Net Rates of Nitrogen Transformations ...	999
42-3 Laboratory Methods for Estimating Net Nitrogen Transformation Rates	1006
42-4 Laboratory Methods for Assessing Nitrification	1009
42-5 Coda	1015
REFERENCES	1016
Chapter 43 Dinitrogen Fixation	1019
R. W. WEAVER AND SETH K. A. DANSO	
43-1 Acetylene Reduction	1019
43-2 Nitrogen Difference	1025
43-3 Nitrogen-15 Isotope Techniques	1030
43-4 Use of Dinitrogen-15 Gas	1038
REFERENCES	1043
Chapter 44 Measuring Denitrification in the Field	1047
A. R. MOSIER AND LEIF KLEMEDTSSON	
44-1 Methods	1048
44-2 Experimental Protocols	1049
44-3 Problems with Gas Sampling and Storage Containers	1061
44-4 Gas Diffusion Problems	1062
REFERENCES	1062
Chapter 45 Sulfur Oxidation and Reduction in Soils	1067
M. A. TABATABAI	
45-1 Sulfur Oxidation	1068
45-2 Sulfate Reduction	1071
REFERENCES	1076
Chapter 46 Iron and Manganese Oxidation and Reduction	1079
WILLIAM C. GHIORSE	
46-1 Iron-Depositing and Manganese-Oxidizing Heterotrophs	1081
46-2 Iron-Oxidizing Autotrophs	1086
46-3 Iron- and Manganese-Reducing Heterotrophs	1090
ACKNOWLEDGMENT	1094
REFERENCES	1094
Subject Index	1097