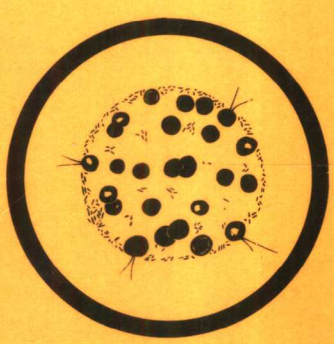
R. Campbell

# Microbial Ecology

Second edition



Basic Microbiology Volume 5

Blackwell Scientific Publications

BASIC MICROBIOLOGY

EDITOR: J. F. WILKINSON

VOLUME 5

# Microbial Ecology

R. CAMPBELL
BSc, MS, PhD
Department of Botany
University of Bristol

SECOND EDITION

BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD LONDON
EDINBURGH BOSTON MELBOURNE

Blackwell Scientific Publications
Editorial offices:
Osney Mead, Oxford, OX2 0EL
8 John Street, London, WC1N 2ES
9 Forrest Road, Edinburgh, EH1 2QH
52 Beacon Street, Boston
Massachusetts 02108, USA
99 Barry Street, Carlton
Victoria 3053, Australia



First published 1977
Second edition 1983

Set by Santype International Ltd
Salisbury

Printed and bound at
Alden Press Ltd, Oxford

DISTRIBUTORS

USA
Blackwell Mosby Book Distributors
11830 Westline Industrial Drive
St Louis, Missouri 63141
Canada
Blackwell Mosby Book Distributors
120 Melford Drive, Scarborough
Ontario, M1B 2X4
Australia
Blackwell Scientific Book Distributors
31 Advantage Road, Highett
Victoria 3190

British Library
Cataloguing in Publication Data

Campbell, R.
Microbial ecology.—2nd ed.—(Basic
microbiology; v.5)
1. Microbial ecology 2. Biotic
communities
I. Title II. Series
576'.15 QR100

ISBN 0-632-00988-8

# Microbial Ecology

# Preface to the second edition

This second edition has been enlarged to include some subjects, such as rumen microbiology, which were omitted in the first edition because of overlap with other books proposed for this series. There has also been a vast amount of material published on microbial ecology over the last few years and an attempt has been made to incorporate new approaches and ideas. I have tried to update and add material in proportion to the new information in the field. In some cases this has resulted in whole new sections (such as that on adhesion) whereas in others it has resulted in a better balance of material (algae and bacteria in lakes). I have again chosen to leave out detailed techniques for this information is readily available elsewhere and for most students the facts discovered are the most important part, provided that enough is known of the methodology to evaluate the reliability of the data.

Again I would like to acknowledge all those colleagues and authors whose ideas and interpretations I have used, without specific acknowledgement, in the text where references have been confined to general articles and reviews for further reading. In particular, I would like to thank Dr M.F. Madelin, A. Feest and T.M. Butt who made constructive comments on the manuscript, which was typed by Jean Hancock; Tim Colborn produced new figures and modified his previous ones and Sue Pettit gave great assistance in the library work involved.

# Preface to the first edition

This book is an attempt to describe the activities and the distribution of microorganisms on the basis of both the chemical transformations that they mediate and the environments in which they live. The physiological approach to microbial ecology has not been stressed because I think that there is a need to consider the subject in terms of communities of interacting organisms. This approach is hampered by the very uneven quality and quantity of information on the various groups of microorganisms and by the difference in emphasis made by the specialists who study each group. I have tried to give a balanced account which reflects the relative importance of algae, protozoa, bacteria and fungi in the different habitats.

I thank all my colleagues in the Departments of Botany and Bacteriology, Bristol University, who have endured my questions and requests for help. In particular, Professor D.C. Smith, Dr A. Beckett and S. Shales gave much advice during the preparation of the manuscript. I would also like to thank Tim Colborn who helped with many of the illustrations and Jean Hancock for all the typing. Finally, I would like to acknowledge all those whose papers and books have supplied ideas or information but who have not been specifically quoted in the text.

# Contents

# 1 Introduction

Ecology is the study of the interrelations between organisms in the environments where they live. 'Natural' environments are usually considered, but also within the scope of ecology are the environments of cities and industrial complexes; indeed in most industrialized countries there are few areas left unaffected by man. Furthermore, man is often most interested in severely disturbed areas; he wants to know what happens to the organisms living in the soil when he practices intensive agriculture or how rivers cope with the effluents he pours into them. The recent interest in ecology has prompted research along two main lines, firstly to find out how the natural environments operate while there is yet time, and secondly to study the influence of man on these normal processes.

The ecology of microorganisms includes that of bacteria, fungi, protozoa and algae (see Further Reading 3, 9 and 10 for an outline of these groups), which range in size from less than a micrometre ($\mu$m) to at most a few tens of $\mu$m, and show a great diversity in their requirements for life and their tolerance of unfavourable conditions. They may be aerobic or anaerobic, heterotrophic or chemo- or photo-autotrophic. The basic principles and concepts of the ecology of microorganisms are similar to those already worked out for higher plants and animals, but microorganisms also present additional problems and difficulties. For example, they are too small to see with the naked eye; you cannot just go into a field and count the bacteria as you would go and survey the plants by identifying and measuring them. One of the major problems in microbial ecology is therefore methodology, and the resulting scarcity of reliable basic data on the distribution, numbers and productivity of microorganisms remains one of the restraints on the development of microbial ecology as a quantitative science. We will consider what methods are available later in this chapter.

The small size of microorganisms results in very intimate contact with their environment and they have a very high surface area to volume ratio; a large surface through which the environmental factors may act on the organism. Many of the heterotrophs also have extracellular enzymes which are not contained within the buffered and ionically controlled environment of the cell. Microorganisms are therefore particularly sensitive to changes in the levels of temperature, light, pH, organic and inorganic nutrients, carbon dioxide, oxygen, water, etc., and extreme levels may well have more effect on

microorganisms than they do on higher plants and animals which are in some ways more isolated from the environment.

It follows from the great diversity of morphology and chemical ability found in microorganisms that they can live, or at least survive, in a remarkable variety of habitats. They are present everywhere on earth where higher plants and animals exist and also in much less hospitable places such as the deep ocean sediments or the upper layers of the atmosphere. Some can survive the conditions in space and they have been transported to the moon by man and can survive there outside spacecraft for at least many months.

Microorganisms are generally so well dispersed that any that are capable of living and growing in a particular environment will most probably be there. Conversely, if a microorganism is not there then there is usually a good reason. It is therefore difficult to introduce a microorganism into an established environment where it does not at present exist. Any disturbance of the existing balance of biotic and abiotic factors may cause a temporary shift in the population but a new equilibrium will eventually be established. Communities, including microbial ones, are inherently stable but at the same time they are dynamic structures, and we will see examples of this apparent contradiction in later chapters.

## METHODS OF STUDY IN MICROBIAL ECOLOGY

It is not the object of this section to give detailed methods for these are readily available elsewhere (see Further Reading 1, 2, 4, 5, 7, 8, and 11), but rather to set out some general principles.

Microorganisms have to be observed with the microscope or made visible by culturing them into macroscopic colonies. Both these processes are time-consuming and the amount of material studied is usually very small in relation to the whole habitat, but the heterogeneity of microbial communities requires that to get representative data the sample should, in fact, be rather large. This is a basic conflict in the methodology of microbial ecology. In the past, microbial ecologists have often ignored the statistical approach, which is considered essential in normal ecology, but this is now changing and it is realized that a few hard-won figures, whose degree of precision can be estimated, are more valuable than large amounts of data which cannot be objectively compared or analysed. No matter which particular method is used, it is going to be a long and probably tedious job to get reliable data which express the relationships between different microorganisms or between them and their environment.

The most common methods count the numbers in some way, but this does not give information on whether the organisms were active or dormant and takes no account of size, so it is not possible to estimate the ecological significance of an organism from its numbers alone. The biomass in the environment tells more than the numbers but does not take account of the different metabolic rates in different organisms. Generally, the metabolic activity per unit mass decreases as the mass increases. It can be that small,

rapidly growing bacteria are equivalent in metabolic terms to very much larger but less active yeasts or protozoa. Thus, by the nature of what they measure, the different methods must give different estimates of the populations, though they should be correlated with each other.

There are three main ways of studying microbial populations in nature: by direct examination, by various cultural methods, and by trying to assess metabolic activity. Each of these will now be examined in turn.

## Direct examination

Direct examination involves the observation of the organisms, usually with the light microscope, and counting their numbers or measuring the length of filamentous organisms like fungi and some algae. The results (direct counts) can be expressed as numbers per unit area, volume or weight, and the size can be measured to give an estimate of biomass. Many different staining methods have been tried and dilution or concentration of samples is often necessary. There has recently been a great increase in the variety of techniques used, including phase, Nomarski interference and fluorescent microscopy. The latter may involve general staining of the organisms, e.g. with acridine orange, though many other fluorescent stains (fluorochromes) are available. The great advantage of fluorescence microscopy is that incident light is usually used (epifluorescence) and so microbes on opaque materials, such as soil, can be counted. Some of the fluorochromes are also specific for live versus dead organisms. The main problems with epifluorescence counting is general, non-specific background fluorescence which can make it difficult to see the organisms, and also the rapid fading of many of the colours so that the counting or photography has to be done quickly. The fluorescent stains may be made specific to a particular organism or strain by linking them to antibody produced by experimental animals injected with the required microorganisms. Apart from this last, rather specialized, method there is usually no way of identifying fungal or bacterial species during direct counting, though algae and protozoa are relatively easily identified. Electron microscopes, both transmission and scanning, have also been used for the direct examination of microorganisms (Further Reading 7).

The various direct examination methods have one further advantage over all cultural and metabolism measurements; they enable one to see where the microbes are in relation to soil crumbs, organic matter and even water films in some techniques (see Fig. 6.2). The precise position of a microorganism in relation to surfaces or to each other may be very important because of the variation in the microenvironment over very short distances (see Chapter 2).

## Cultural methods

There is a wide variety of methods involving the culturing of microorganisms. It may be necessary to dilute or concentrate the sample so that a reasonable number of organisms grow on each plate (dilution plate) or in each

tube. The basic assumption is that each propagule in the environment gives rise to one macroscopic colony which can be counted. The assumption is obviously open to criticism; the cells can stay in groups or microcolonies and the medium used will not be suitable for the growth of all microorganisms. Both these points give an underestimate, and frequently cultural counts are as little as 1 to 10% of direct counts in soil, and even less in water. This may be partly caused by counting dead as well as live cells in the direct method (Table 1.1). The other problem with culturing methods is that with fungi, and to some extent algae, the 'number' really has little meaning. There is no easy way of knowing whether the macroscopic fungal colony arose from a hypha or a spore, and if the former then how large the piece was (i.e. what its biomass was). Careful studies of the origin of fungal colonies have shown that most arise from spores which are dormant in the environment. Repeated counting by dilution plates, over a long period of time such as a year, of dormant microbial cells may give a reasonable estimate of the numbers and activity, for the spores have been produced by living mycelium and will over this timespan be a reflection of past, and an estimate of future, activity. The estimate is not good, however, for an instantaneous measure of activity. The problem of the sort of medium used and the environmental conditions provided, is a major one in all cultural studies. By definition obligate parasites will not grow on any media. Various incubation temperatures, both aerobic and anaerobic conditions, and different nutrient media have to be used in order to allow the growth of as many different organisms as possible. These problems of medium and the environmental conditions have been taken advantage of in various selective culture methods designed to isolate particular groups of organisms. Thus for algae there is usually no carbon source in the medium and the plates are incubated in the light; nitrogen-fixing organisms (Chapter 4) may be isolated on nitrogen-free medium where most heterotrophs will not grow. It is also possible to select for some organisms by

Table 1.1. A comparison of the numbers of bacteria recorded by direct observations and by culturing on dilution plates.

| Source of sample | Ratio of direct counts to counts from dilution plates |
|---|---|
| Bacteria in soil and on roots (1): | |
| (a) On root surface, plant 17 weeks old | 2.6 |
| (b) Soil around roots, plant 17 weeks old | 3.1 |
| (c) Uncropped soil, 17 weeks after start of experiment | 9.2 |
| Bacteria on root surface, plant 12 weeks old (2) | 10.2 |
| Bacteria in marine and inshore waters (3): | |
| (a) Direct count on material trapped on a Millipore filter | 147.0 |
| (b) Direct count on a concentrated water sample | 2100.0 |

(1) Louw H.A. & Webley D.M. (1959) *Journal of Applied Bacteriology* **22**, 216–26.
(2) Rovira A.D., Newman E.I., Bowen H.J. & Campbell R. (1974) *Soil Biology and Biochemistry*, **6**, 211–16.
(3) Jannasch H.W. & Jones G.E. (1959) *Limnology and Oceanography* **4**, 128–39.

the use of antibiotics, by the aeration and temperature conditions employed or by pasteurizing the inoculum so that only spores survive. Having selected one or more media and incubation conditions, the sample may be spread on or mixed with the agar medium and the number of colonies counted after allowing time for them to grow; this is a dilution plate or spread plate. Alternatively, the sample may be diluted to such an extent that the chance of getting one organism in a small volume (e.g. 50 $\mu$l), is low: then, if several of these drops are placed individually on agar or in a tube or in dishes of medium the number of ones showing growth can be counted. From a knowledge of the dilution and the proportion of the drops which contained microorganisms, it is possible to calculate how many microbes there must have been in the original suspension. This 'most probable number' method, though less accurate than dilution plates, is especially useful with some selective media to isolate particular genera or physiological groups of organisms.

The great advantage of cultural methods is that they can give you an estimate of viable cells (viable under the conditions one provides) and they also provide a culture from which it may be possible to identify the fungi and bacteria. The attempted identification, especially of bacteria, may be very elaborate involving biochemical and morphological tests on hundreds or thousands of isolates and known reference cultures. This numerical taxonomic approach may often, however, not lead to identification of genera or species because of the lack of suitable identified reference strains from natural environments, and many isolates do not fall into any described taxon. A means of avoiding this problem, which has recently been used quite often, is to try to arrange the isolates into groups regardless of whether they have any names attached to them. This is known as principal component analysis or factor analysis (Further Reading 8) and again it depends upon large numbers of isolates each characterized by a considerable number of independent tests. Those isolates with many test responses in common, i.e. highly correlated test results, are set together in a correlation matrix, and from this new groupings of the isolates can be made. The groups do not necessarily bear direct relationships to the original test or to any particular taxa but they may be based on broad categories of tests such as nutritional characteristics (see p. 20 and Fig. 6.4).

Another way of looking at data from cultural studies is to look at microbial diversity. All sorts of diversity indices, often derived from higher plant and animal ecology, have been used; some of these are based on colony characters, simple cell morphology and staining; others are based on more complex, often biochemical, characterization of isolates. Such diversity indices give an idea of shifts or differences in community structure but are less rigorous than the numerical taxonomy or the factor analysis approaches.

All these cultural methods will allow counts to be made, but there are also methods which set out to give only a rough guide to numbers, or even just 'present' or 'absent'; these are the enrichment methods. They are used when the microorganisms under study are rare in the environment so that under any conditions that can be devised for plate counting they will be lost

amongst the many colonies of other organisms. The natural soil or water sample is enriched with a substrate that will favour the required organisms and when they have increased in numbers they can be isolated. For example, sulphur-oxidizing bacteria (Chapter 5) can be encouraged by the addition of sulphur and calcium phosphate to the soil, or sulphur reducers by incubating anaerobically in the presence of high levels of sulphate.

Cultural methods are widely used in microbial ecology, particularly for bacteria, and they are best for comparative studies where it is the relative number of similar species that are important rather than a complete enumeration of the population.

### Metabolic activity measurement

The third group of methods attempts to assay the metabolic activity of microorganisms in the environment. If the size of the population is known then the turnover rate or generation time (the time between successive generations) may be estimated by measuring the rate at which tritiated thymidine is incorporated into the DNA of the population. Similarly, the rate of consumption of various substrates may be measured by using radioactive tracers, but the amount of the compound under study and the route by which it is broken down in the environment must be known. Attempts have been made to assay the activity of particular common enzymes; the assumption is that all the actively metabolizing cells possess them and use them to an equal degree. Dehydrogenase has been widely used but there is some doubt as to whether it is closely correlated with microbial activity. In soil, urease is usually highly correlated with microbial biomass. The results are confused by the complexity of natural systems with many possible substrates and other conflicting biochemical reactions taking place at the same time. An enzyme assay that works well in laboratory studies usually shows a much more variable response with normal populations in less well-defined natural habitats. Furthermore, many enzymes are adsorbed to particulate matter and may not reflect the microbial populations present.

The amount of ATP (adenosine triphosphate) in the environment has also been used to assess microbial activity; it is assumed that ATP occurs only in living cells and that ATP content of organisms is approximately the same or is known for the particular ones under consideration. This is by no means true and, furthermore, the ATP content of particular organisms can vary with, for example, the phosphorus level in the environment. High levels of organic matter can interfere with the ATP assay. Various studies have shown, however, that ATP levels are quite well correlated with, though not necessarily easily converted to, biomass of microorganisms in some environments. The level of ATP is difficult to measure under natural conditions but in the laboratory it is a very sensitive assay (about $10^{-5}$ $\mu$g ATP $l^{-1}$ can be detected).

The biomass and activity of algae may be measured by estimating the

amount of chlorophyll and other pigments and this is usually done spectrophotometrically after extraction with organic solvents.

The most widely used method of measuring activity has been to estimate the total respiration, and photosynthesis for algae, as changes in oxygen or carbon dioxide levels. This has met with some success though there are problems such as the occurrence of anaerobic microhabitats, or the use by some microorganisms of compounds other than oxygen, e.g. nitrate, as electron acceptors. Careful studies have shown that a major problem for metabolic measures is disturbance when the material is brought into the laboratory for assays to be done. For example, just the rearrangement of the soil during collection can increase the respiration rate as new substrates are brought into contact with the enzymes and the aeration conditions are changed. There are, however, some methods of measuring the respiration rates of relatively undisturbed soil cores in the laboratory or the field.

The soil respiration rate can also be used in an indirect way to measure soil biomass. All organisms are first killed by fumigation, usually with chloroform, and the soil is then reinoculated with a small amount of 'live' soil. There is a great increase in the soil respiration, when compared with a non-fumigated control, as the dead organisms are decomposed and this increase is related to the biomass of the original organisms. The relationship is not, however, a simple one, for it is necessary to estimate how much of the dead biomass is decomposed over the time, usually one or two weeks, that the

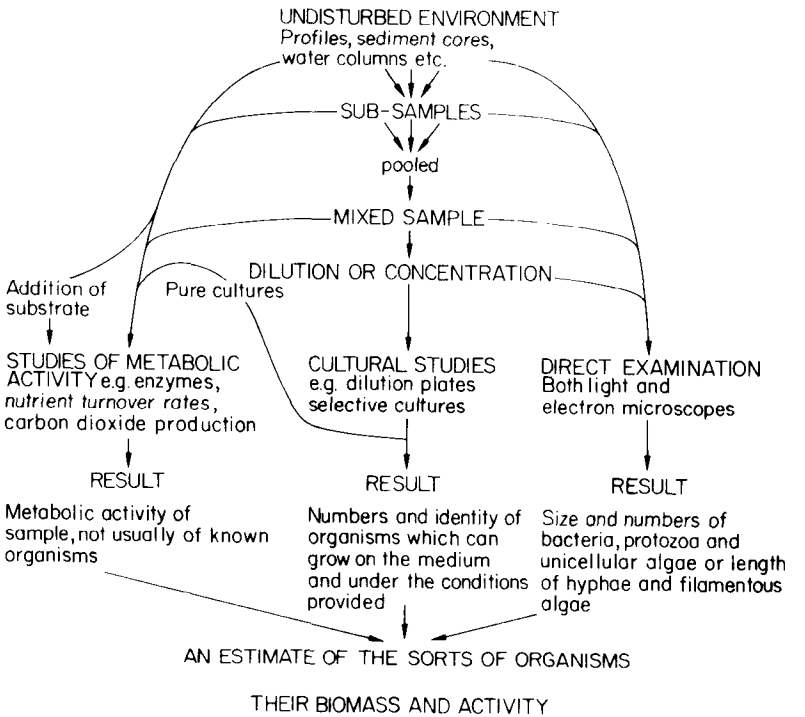



**Fig. 1.1** Summary of the possible methods of studying the ecology of microorganisms.

respiration is measured. Cell wall polymers of fungi and the exoskeletons of arthropods may take many months to be used up. The amount decomposed in two weeks is generally taken to be 40 or 50% of the original soil biomass.

All these methods of assaying metabolic activity usually yield no information on the species present or their distribution in the environment.

This brief review of the general methods (Fig. 1.1) may sound rather depressing. Nevertheless it is possible to get useful information by combining several methods, provided that their limitations are realized. Careful consideration of the precise questions to be answered about a particular environment, and the constraints which the environment imposes, can usually lead to the selection of acceptable methods. Many valid comparisons can be made even if absolute values cannot always be put on microbial populations. If microbial ecologists had waited for the perfect technique nothing would have been learnt; the incentive to produce better methods is the need to measure particular activities and to solve problems.

## MODELS IN MICROBIAL ECOLOGY

A rather different approach to the study of microbial ecology has been to set up artificial, usually simplified, environments in the laboratory. For example, the conversion of ammonium to nitrate (Chapter 4) is an important process involving several groups of microorganisms and it has been studied in soil and water which have been sterilized and then inoculated with known populations of microorganisms. The rate of the reaction under different conditions can be studied by sampling the system and doing chemical analyses. Small parts of the environment may be taken into the laboratory and studied as 'microcosms'. These may be aquaria or flasks of soil and leaf litter or many other things. This makes life more convenient than actually having to venture out into the natural world and it allows neater sampling programmes and statistical treatment. Comparisons with the real environment are always necessary and may show good agreement, as in many terrestrial litter decomposition studies. Fresh waters and a marine situation are, however, very difficult to mimic, largely because of inescapable effects of the scale of the environment studied.

Similarly, by the use of chemostats, it is possible to study the way in which defined mixtures of microbes interact with each other within the controlled environment (Further Reading 11) or to determine the growth rates, nutrient requirements and optimum conditions for particular organisms. Chemostats also allow the enrichment and isolation of organisms, or mixtures of organisms, after prolonged periods at limiting nutrients: such conditions cannot be produced in enriched batch cultures. However, chemostats are rapidly stirred, usually fairly homogeneous liquid cultures; whether data obtained under such conditions are relevant to heterogeneous environments with particulate material and a complex, changing microbial population is open to considerable doubt. Perhaps we are learning a lot about the microbial ecology of chemostats and not much about the ecology of microorganisms in

their normal environment. This is a general criticism of laboratory study and great care must be taken in transferring data from controlled laboratory to unknown field conditions. Generation times of microorganisms are often much longer in natural environments; laboratory studies give times of minutes or a few hours for bacteria (30 minutes for *Escherichia coli,* two hours for *Leucothrix mucor*) but they may be many hours or days in the natural environment (12 hours for *E. coli,* 11 hours for *L. mucor*; see also p. 83). Furthermore, because of the diversity of microhabitats, there can be much more variation in generation times within a natural community than in the more uniform conditions of culture.

The final 'simplification' is the study of microbial ecology by constructing mathematical models of environments, usually using a computer (Further Reading 6). The complex natural systems are broken down into many small, more simple, subsystems and the interactions between and within these are defined in terms of mathematical equations. Usually the models are dynamic so that changes can be studied in relation to time as the various controlling parameters are altered. Basic data are required on the levels of each factor incorporated and its rate of change under the conditions to be considered. These models do several things for the microbial ecologist: firstly, and perhaps most important, they force a consideration in detailed, mathematical terms, of just what factors might be important in controlling populations and how these might be linked. Secondly, models demand basic data and they usually show the considerable deficiencies which exist in the subject under study. The useful output from models never improves upon the quality of data in the input. Thirdly, they generate hypotheses or predictions which can then be tested by experiment: they can therefore point the way to useful areas of study. There are, however, very few models of microbial populations and communities in natural environments (see p. 98). There are many examples where decomposition by microbes features in some large-scale ecological balance sheet, but usually these say nothing about the microorganisms themselves. Alternatively, there are very detailed mathematical treatments of growth of this or that microbe under a variety of conditions in a chemostat. Those models of the microbial population dynamics in natural habitats are rare and severely limited by the available data.

Having discussed the methods that are available, and their reliability, the next chapter is concerned with how the results that they yield fit in with the general concepts of ecology that have been constructed, mostly with higher plants and animals in mind.

## FURTHER READING


1 AARONSON S. (1970) *Experimental Microbial Ecology*. Academic Press, New York.
A recipe book.

2 ATLAS R.M. & BARTHA R. (1981) *Microbial Ecology: Fundamentals and Applications*. Addison-Wesley, Massachusetts, California & London.
An excellent general text, also good for methods.

3 BROCK T.D. (1979) *Biology of Micro-organisms*. 3rd edn. Prentice-Hall, New Jersey.
Good general text, with a lot of ecology.

4 BURNS R.G. & SLATER J.H. (1982) *Experimental Microbial Ecology*. Blackwell Scientific Publications, Oxford.
Comprehensive reviews.
5 JONES J.G. (1979) *A Guide to Methods for Estimating Microbial Numbers and Biomass in Fresh Water*. Freshwater Biological Association, Ambleside, England.
Detailed methodology.
6 MAY R.M. (ed.) (1976) *Theoretical Ecology : Principles and Application*. Blackwell Scientific Publications, Oxford.
A good treatment of computer modelling, though not much microbiology.
7 ROSSWALL T. (ed.) (1973) *Modern Methods in the Study of Microbial Ecology*. Bulletin 17, Ecological Research Committee, Stockholm. Swedish NFR.
Reviews and research papers on methods.
8 ROSSWALL T. & KVILLNER E. (1979) Principal components and factor analysis for the description of microbial populations. *Advances in Microbial Ecology* **2**, 1–48.
The basis of the method and its application.
9 STANIER R.Y., ADELBERG E.A. & INGRAHAM J.L. (1976) *General Microbiology*. 4th edn. MacMillan, London.
An excellent text, especially for description of non-medical bacteria.
10 WILKINSON J.F. (1975) *Introduction to Microbiology*. 2nd edn. Basic Microbiology Vol. 1. Blackwell Scientific Publications, Oxford.
Introductory text.
11 VELDKAMP H.E. (1977) Ecological studies with the chemostat. *Advances in Microbial Ecology* **1**, 59–94.
General review of the method.