# METHODS FOR STUDYING THE ECOLOGY OF SOIL MICRO-ORGANISMS

IBP HANDBOOK No. 19

# Methods for Studying the Ecology of Soil Micro-organisms

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# Foreword

The International Biological Programme is a world study of 'biological productivity and human adaptability', started in 1964 and lasting for a decade until 1974. Being a wide ranging programme it is divided into seven sections, of which the one concerned with terrestrial productivity (PT) has sponsored this present handbook.

For section PT there are already five other handbooks which provide guidance and advice on methods of research. They are on the primary production of forests (No. 2), on grasslands (No. 6), on the productivity of large herbivores (No. 7), on other terrestrial animals (No. 13), and on quantitative soil ecology (No. 18). This one on the ecology of soil micro-organisms is concerned with one of the most important branches of ecology, responsible for energy flow through the ecosystems of all biome or habitat types. It should be mentioned that two other IBP handbooks which have been sponsored by other sections also deal with the ecology of micro-organisms. One of these, from section PP, deals with root-nodule bacteria (No. 15), and the other which is still in the press is concerned with the bacteria of freshwater ecosystems.

The microbiology of natural ecosystems is a subject which is advancing rapidly, and is fraught with peculiar difficulties in its methodology. Even the problem of estimating biomass, which is relatively easy in most groups of organisms, is highly complex when it comes to bacteria or minute hyphae or fungi ranging through the soil. Thus the appearance of this book, which should help materially towards the comparability of results derived from different ecosystems in different parts of the world, is timely. It will be used not only during the remaining years of IBP, but doubtless for a considerable period thereafter. Indeed, as with all the other IBP handbooks, it is hoped that experience in its use will lead to the improvement of methods and so to a new, revised edition of the book before many years have passed.

Of the three authors, Professor D.Parkinson is Head of the Department of Biology and Professor of Microbiology in the University of Calgary, Alberta,

Canada. He is also the Theme Co-ordinator for decomposition processes in Section PT of the IBP, and is currently engaged in research on soil fungi as part of the large Canadian grassland IBP project at Matador, and also on soil micro-organisms and decomposition processes as part of the Canadian tundra IBP project, Devon Island. Dr T.R.G.Gray who was a student at Nottingham University is lecturer in Botany at the University of Liverpool (1960–1971) during which period he held a post-doctoral fellowship in soil science at the University of Minnesota for a year. He was editor (with Professor Parkinson) of the *Ecology of Soil Bacteria* (Liverpool University Press 1968) and author (with Dr Williams) of *Soil Micro-organisms* (Oliver & Boyd 1971). Dr S.T.Williams is lecturer in Botany at the University of Liverpool where he was formerly a student; he conducts research in soil microbiology, especially on actinomycetes. We are much indebted to these three biologists for a valued addition to the IBP handbook series.

August 1971 E.B.Worthington

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Of the many individuals who helped us, we would particularly like to thank Mrs Linda Whelan, who collected much of the information used in the book and helped to prepare the initial draft manuscript, and Mr John Bissett who made some constructive comments on chapter 3. Mrs V.Preuter was responsible for producing the typescript at all stages of its preparation and additional valuable help was received from Mrs W.Packer and Mrs. S.Collins.

Finally, we should like to thank all those microbiologists whose work we have quoted. Any errors in the description of their work are the responsibility of the authors of this manual.

# Contents

# 1
# Objectives

In *IBP News 9*, 1967, under outlines of the PT program, a restricted number of methods were recommended as applicable to soil microbiological studies in IBP PT projects. These techniques, considered by many to be minimal for IBP studies have been discussed in IBP Handbook 18. This present handbook attempts to survey methods which are in use in soil microbiological laboratories and therefore discusses a much wider range of techniques. However, it will be evident from comments in this handbook that soil microbiologists are not yet in a position to answer some of the important questions being asked by other members of IBP PT projects, e.g., questions regarding the rate of microbial cell production in soil and on the relative rates of metabolic activity of different components of the soil microflora in soil microhabitats. Thus, it is not possible to measure cell production without destroying the natural environment, and the metabolism of the various components of the soil biota (including roots) is not sufficiently different to allow distinction between the components, except in certain special cases (e.g., nitrogen fixing organisms). Moreover, there seems little immediate prospect of solving these problems. In view of this, workers may well ask whether effort should not be restricted to more generalized assessments of biological activity in relation to the decomposer cycle, e.g., measurement of rates of input and output of substrates and metabolites. Perhaps problems of the partition of energy between the soil components should be shelved, at least in the context of IBP, until more much-needed research has been carried out.

Therefore, this handbook does not aim to provide a complete survey of methods in soil microbiology or production ecology but concentrates on methods which microbial ecologists have found useful. Complete standardization of methodology in studies of soil microbial ecology is not desirable and methods must be chosen and modified which attempt to provide data relevant to the soils and organisms being studied. It is hoped that this handbook will aid in the preliminary selection of suitable methods for ecological investigations, particularly in institutions where soil microbiological studies are not

currently in progress. Workers in such institutions may have limited access to current literature and background information and so considerable detail is provided for many of the methods. In describing them, attempts have been made to provide a detailed description of the more important methods plus references to relevant literature, to outline clearly the purposes (general and specific) of each method, to provide a clear statement (wherever possible) on the uses and limitations of the methods and to discuss how the techniques may be tested.

Methods concerned specifically with root-nodule bacteria have been omitted since these are described comprehensively in IBP Handbook 15 (Vincent, 1970). Similarly, techniques concerned with the estimation of nitrogen fixation by micro-organisms have not been described here since they are being dealt with by workers in the Production Processes Section of IBP.

# 2

# Habitat Description

## 2.1 Selection of site

The planning of a project and its sampling program must be preceded by a proper survey of the site under study, and the collection of data on the environment and its changes. Some of the considerations necessary in the selection of forest sites are given in IBP Handbook No. 2 (Newbould, 1967).

## 2.2 Selection of sampling areas

In considering a range of terrestrial ecosystems (forest, grassland, desert, tundra) the following generalizations may be useful. Within the selected study area the need for three basic site units must frequently be recognized:

1. An undisturbed zone: the initial phases of environmental research programs are frequently exploratory, the unrestricted activities of researchers could result in the destruction of the environment under study.
2. A zone, adequate in area for multi-disciplinary research where sampling procedures are restricted to those of a non-destructive nature.
3. An extensive zone in which sampling techniques are not restricted.

Suitable entry-exit routes to the plots must be established to avoid indiscriminate movement of workers over the study area.

Following the mapping and delineation of study plots all determinations (physical, chemical and biological) should be made on a proper statistical basis.

## 2.3 Selection of environmental factors to be measured

In all studies in soil microbial ecology, investigations must normally be made on the soil(s) under study and on the environmental factors to which the microbe(s) are subjected. The amount of such data will vary according to the

nature and scope of the microbiological project and the number of workers (and their analytical expertise) available to assist in its compilation.

It is impossible to give a standard list of habitat data to be collected but it must be emphasized that sufficient data should be provided to allow other workers to recognize the major features of the environment under investigation. The uncoordinated compilation of habitat data which has no relevance to the aims and applicability of the project merely represents a profligate use of available laboratory facilities.

In planning this work the following scheme may be a useful starting point for the selection of the relevant factors to be studied.

### 2.3.1 Soil factors

a. *topography* } these studies should give data on the degree of
b. *soil profile description* } gross variation in the soil under study.
c. *chemical analysis*: the degree of detail will depend on the requirements of the project; minimally it would probably involve assessment of total organic matter content.
d. *particle size analysis*: studies varying from simple mechanical analysis to the characterization of clay minerals and mineral components.
e. *pH*: the gross methods usually applied in these measurements give little or no data on the pH state at the microhabitat level.
f. *moisture status*: the determinations may vary from moisture content determinations (on an oven dry weight basis) on freshly taken soil samples to determinations of soil moisture characteristics (pF curves).
g. *temperature, $CO_2$ and $O_2$ status*: such measurements are frequently made on a continuous basis (using automated apparatus) over the whole experimental period.

### 2.3.2. Biotic factors

a. *Above ground*
   (i) Description of the pattern of the higher plant community (communities).
   (ii) Dominant and associated green plant species: determined at each of the seasons.
   In the course of (i) and (ii) the peculiarities in the vegetation and its distribution should be noted.
   (iii) Vegetational history (if possible): previous cultivation, burning or successional stages.

(iv) Animal effects: e.g., grazing.
(v) Input of organic matter: i.e., tree litter fall and production of ground vegetation, animal parts and faeces.

b. *Below ground*
(i) Root distribution in soil profile.
(ii) Movement of soil animals.
(iii) Input of organic matter: root production, soil animals (faeces, bodies, etc.).

### 2.3.3 Meteorological factors

a. *Solar radiation*: essential for studies on energy flow and productivity.
b. *Rainfall*
c. *Temperature*

It may be desirable to consider meteorological factors at both macro- and micro-levels.

No reference has been made to the procedural details for obtaining habitat data. Reference to standard works will provide the required data, e.g., Black *et al.* (1965); Metson (1956); Piper (1944); Newbould (1967).

# 3
# Sampling

## 3.1 Collection of samples from the field

Samples may be collected from the field for one of two main reasons. They may be required only as a source of micro-organisms, e.g. for isolation of antibiotic producers, or they may be needed for studies of the natural state of the soil.

In the former case, very few sampling precautions are necessary for in most cases it will not matter whether the sample has been disturbed or altered so as to cause changes in the microflora, providing that these changes have not led to wholesale elimination of all or part of the microflora. Even soil returned to the laboratories, sieved and air dried, and stored for some months, may be useful in such studies. The method described by Clark (1965) is suitable for this type of study. Here a thoroughly mixed gross sample is taken and a large amount, e.g. 2 lb, is placed in a polyethylene bag or a sealed, waxed cardboard container. This may be taken to the laboratory, only taking care not to expose it to undue heating or drying. The sample may be used at once, or stored at 4°C for up to two weeks. Prior to use, the sample may be passed through a 10-mesh sieve to homogenize the material.

However, if conclusions concerning the native environment are to be drawn from the results, it is usually necessary to try to preserve the sample in such a way that it is in the same physical, chemical and biological state when it is used as it was when it was taken from the soil. Complete stability of the sample is not likely to be achieved but one should be aware of the major changes which can occur, and it is clear that the method outlined above will not be suitable.

The most obvious change that can take place is a change in temperature. Air temperature and laboratory air temperature are frequently very different from soil temperature. Dark soils exposed to the sun may be considerably hotter than the atmosphere while frozen soils will be colder than ordinary laboratory temperatures. Two approaches have been made to this particular

problem. Some workers prefer to refrigerate their samples, arguing that multiplication of cells will be slowed down. While this is undoubtedly true there have been very few studies on the effect of low temperatures on the death rate of soil micro-organisms and so we cannot be sure that the microflora remains stable. Other workers attempt to keep their samples at the temperature at which they were sampled, which is most easily achieved by keeping them in some sort of thermos flask. This is probably most satisfactory unless some other factor, which was limiting activity, has become non-limiting in the sample, e.g. aeration, in which case substantial and unnatural growth might result.

Amongst the other factors which are most likely to change in the sample are aeration and water content, with the most marked changes occurring near the surface of the sample. For this reason, it is often advisable to take a large soil block back to the laboratory and sample it again immediately prior to use. Even large blocks may change, however, especially if one is dealing with waterlogged soils for the excess water may easily drain away into the bottom of the soil container. In this case it may be best to take relatively small samples and keep them in plastic bags. The nature of the bags may be changed to suit the sample. If it is aerobic, then polyethylene (Stotzky *et al.*, 1962) should be used, which allows for passage of air but not water vapour. If the samples are anaerobic, impervious plastics or glass will be suitable.

Biological changes could also result from the contamination of specimens. Contamination may be from the air, from the sampling tool or soil container, or from soil one wishes to exclude from the sample. Contamination from the air is likely to be of little importance since its microbial content is very much smaller than soil. Its effect may also be countered by sub-sampling a large soil block in the laboratory, using strict aseptic technique. Contamination from sampling devices or soil might be more serious, but once again can be countered by sub-sampling if it is felt that gross contamination has occurred. In some cases, such contamination might be difficult to detect, i.e., the accidental mixing of soil on an exposed profile by careless digging, so care must be taken even before the removal of the sample.

The importance of all the changes dealt with so far may be aggravated or minimized by the duration of the storage period. It is likely that the average generation time of members of the soil microflora is long, except in soils with freshly added, readily available nutrients, e.g., rhizosphere soils and some leaf litters, so that even if a change takes place, it will be small if the sample is used soon after sampling. No accurate information on the permissible