SOIL FUNGI AND SOIL FERTILITY

S. D. GARRETT

Soil Fungi and Soil Fertility

S. D. GARRETT, Sc.D.

Fellow of Magdalene College, Cambridge, and University Reader in Mycology

PERGAMON PRESS
OXFORD · LONDON · PARIS · FRANKFURT
THE MACMILLAN COMPANY
NEW YORK

PERGAMON PRESS LTD.
Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London W.1

THE MACMILLAN COMPANY
60 Fifth Avenue, New York 11, New York

COLLIER-MACMILLAN (Canada) LTD.
132 Water Street South,
Galt, Ontario, Canada

GAUTHIER-VILLARS ED.
55 Quai des Grands-Augustins, Paris 6

PERGAMON PRESS G.m.b.H.
Kaiserstrasse 75, Frankfurt am Main

Library of Congress Card No. 63-12691

Set in 10 on 12 pt Baskerville and Printed in Great Britain by
THE BAY TREE PRESS, STEVENAGE, HERTS

PREFACE

THIS is a book about life in the soil. Firstly, it is an introduction to the science of soil microbiology through the study of soil fungi and their behaviour. Secondly, it is an introduction to the subject of mycology, because one cannot explain the behaviour of fungi in the soil without a preliminary account of fungi as living organisms. I have written this book primarily for university students taking courses in biology. Most students now come up to the university with much more knowledge of biology than was the case in my day, but there is still a minority to whom the subject is more or less a new one. In the writing of this book, therefore, I have endeavoured to assume only an essential minimum of previous biological knowledge.

The particular advantages of some knowledge of the behaviour of soil fungi as a guide to an understanding of the mechanisms of soil fertility will soon become apparent, I hope, to anyone reading this book. As universal agents of decay, from which new life continually arises and is nourished, the fungi make a unique contribution to the maintenance of soil fertility. Other soil micro-organisms share with the fungi in the biochemical promotion of these processes, but the contribution of soil fungi is made unique through the peculiarities of their physical organization. Some fungal species cause diseases of the root system in crop plants, and thus exercise an adverse effect on soil fertility so far as agriculture is concerned; about these also I have something to say. This book therefore introduces fungi as living organisms existing in the microbial community of the soil, rather than as cultures in the laboratory or as dead specimens in the herbarium. Throughout my account of these organisms, I have emphasized the parallels that exist between the behaviour of the community of plants and animals occupying the surface of the soil, and the behaviour of the microbial community living within the soil. Each of these communities is dependent upon the other, and so some knowledge of microbiology is essential for botanists and zoologists. In my attempt to convey this knowledge, I have endeavoured to give readers some idea of how mycologists and microbiologists go to work in choosing and pursuing research problems — down to factual details of the techniques employed. This is, I know, a somewhat unusual procedure in an introductory book, but to explain "how?" is often the best way of explaining "why?"; a mere cataloguing of results and conclusions at best leaves the reader with a feeling of unreality and, at its worst, is a rebuff to the inquiring mind.

It is my pleasant duty to acknowledge valued help in criticism from various colleagues. Firstly, to Dr. H. L. K. Whitehouse for reading Chapter IV, and for helpful discussions at various times on problems in fungal genetics. Secondly, to my two mycological colleagues, Dr. John Rishbeth and Dr. H. J. Hudson, who have rendered me an invaluable service by carefully reading the whole of the book in manuscript. I should also like to take this opportunity of acknowledging a debt to Dr. Rishbeth of much longer standing; his first-hand experience of root-infecting fungi has fortunately, though quite fortuitously, been complementary to my own, and has greatly enlarged my understanding of this group of organisms. In my own field of research, I certainly owe much more to Dr. Rishbeth than to anyone else. It is also a pleasure to acknowledge a still older debt to the present Head of the Cambridge Botany School, Professor Harry Godwin, whose lectures on plant ecology when I was a student first aroused my particular interest in this branch of botany, and later suggested an application to problems of microbial ecology in the soil. Lastly, I wish to thank my former research associates, some of whom are mentioned by name in these pages, for their efforts in our joint field of endeavour. Had it not been for them, I should have had a much less interesting story to tell about those

problems in soil microbiology with which I have been concerned.

I am particularly grateful to Mr. B. A. Golding for making three original drawings (Text-figs. 2, 3 and 6) and also for preparing the diagrams given in Text-figs. 1, 10 and 11. My thanks are further due to those authors and editors of journals who have allowed me to reproduce original illustrations; the source of these is acknowledged individually in the text.

CONTENTS

CHAPTER ONE

INTRODUCTION

Mycologists do not expect other people to know the meaning of this word; they are accustomed to being met with a blank look if they venture to give their profession or recreation, as the case may be, its proper name. An explanation that mycology is the science of fungi does not always prove much more enlightening. Yet even the least observant amongst us must be familiar with certain manifestations of these common organisms, though education as well as observation are required to connect the blue and green moulds on oranges, lemons and cheese, the white or grey furry outgrowths on bread, jam or meat left too long in a damp larder, the mushrooms in the fields and the toadstools in the woods. All these consist of the physical bodies of various kinds, or species, of fungi, and all the commoner kinds have been minutely observed, described and finally named by mycologists. The fungi constitute a natural group of organisms that is of great scientific and aesthetic interest to students of nature; in a more practical way, they affect all of us, as the living agents concerned in the causation of such various processes as the fermentation of wines and beers, the ripening of cheeses, the production of antibiotics like penicillin and of other industrial products such as citric acid and vitamins, the decay of timber, the spoilage of leather and textiles, the production of many kinds of disease in cultivated plants and the maintenance of soil fertility through the decomposition of dead plant remains in the soil. Although other kinds of organisms may be associated with them, it is the fungi that play the pioneering and often the principal part in the processes listed above, as well as in some others.

This book will be concerned with the part played by soil fungi in the building up and maintenance of soil fertility. By soil fertility is meant the productivity of a soil in terms of actual or potential yields of crops; although a scientific term, it is an anthropocentric one, inasmuch as it describes any particular soil in terms of its practical value to man. Soil consists of a complex aggregation of mineral particles of various kinds and sizes together with organic matter in different stages of decay; it holds air and water like a sponge, though this simile is an over-simplification of the complex air- and water-holding properties of soil, which have been intensively studied. Because soil contains organic matter, derived from the remains of dead plants and animals, it supports a rich and varied *microflora* of decay-producing micro-organisms, and an equally varied *microfauna* of microscopic animals. An admirably clear and comprehensive introduction to soil science has been provided by G. V. Jacks (1954) in his book *Soil*.

Although we shall be chiefly concerned here with the particular role of soil fungi in the maintenance of soil fertility, much of what will be said about the soil fungi can be applied to soil micro-organisms in general. The fungi, in fact, will be used to exemplify the nature of the various microbiological processes occurring in soil. These processes affect soil fertility in various ways; beneficially, through decomposition of plant and animal remains so that nutrients in a form suitable for absorption by the roots of green plants are made available for use all over again; adversely, inasmuch as fungi are the most important cause of diseases of the root system in crop plants. For such studies, the fungi have one outstanding advantage over the other two main groups in the soil microflora (bacteria and actinomycetes); because of their physical peculiarities, different species of fungi can be identified with comparative speed and virtual certainty.

The various microbiological processes that contribute, positively or negatively, to soil fertility are carried out by mixed communities of micro-organisms living side by side in the soil. Some of these processes, such as the decomposition of cellulose (a major structural component of plant tissues) or the conversion of one form into another of the essential nutrient, nitrogen, can be carried out by a single species of micro-organism. Most of these organisms can be isolated from the soil as pure cultures, and grown on suitable nutrient media in the laboratory. From experiments with such pure cultures, a great deal has been learnt about the precise biochemical mechanisms of these transformations, and, incidentally, about biochemical mechanisms in general; many biochemical processes are common to micro-organisms, higher* plants and higher* animals. For such studies with pure cultures, which are now the particular province of the microbiological biochemist, bacteria have so far been used more than have fungi.

Such laboratory studies of single organisms in pure culture are an obvious and essential pre-requisite for understanding their behaviour in the complex microbial community of the soil. But although this is an essential first step to understanding, it is a first step only; micro-organisms may behave in one way in pure culture, and in another way in a mixed community, in which factors of association and competition come into play. That branch of biology that is concerned with the study of mixed communities of organisms, in relation to the *habitat* in which they live, is called *ecology*. It follows from what I have said above, therefore, that this book will be an account of the *microbial ecology of the soil*, using fungi as illustrative examples. There is only one science of ecology, although it is divided up into the various branches of microbial, plant, animal and even human ecology, in order of increasing complexity of the organisms being studied. All these organisms, from the lowliest to the highest, are living together in a state of nature, and so the separation of the various branches of ecology one from another is no more than an essential but regrettable administrative convenience for study and for the organization of knowledge. Underlying the great

* This term "higher" is used in such a context, throughout this book, in its usual evolutionary sense.

diversity of organisms, and a corresponding and quite essential diversity in the methods whereby they are studied, certain broad and general unifying principles of ecology as a whole can be discerned. Unfortunately, the minds of scientists are much preoccupied with the consideration of ways and means, as an essential prelude to discovery; for this reason, different kinds of ecologists do not find communication with one another much easier than it is between different kinds of scientists in general. Wide differences in approaches to a problem and in techniques can constitute a barrier at least as formidable as a foreign language.

CHAPTER TWO

MICROBIAL INHABITANTS OF THE SOIL

MICROBIAL cells, most of which can be distinguished only under the microscope, are measured in units of microns (μ); a micron is one-thousandth of a millimetre. This is probably the most general statement that can be made about micro-organisms. If all their characteristics, i.e., form, structure, behaviour and mode of existence, are taken into account, then micro-organisms can claim to show as wide a diversity as "macro-organisms". Individual micro-organisms may consist of single microscopic cells, which can be either static or motile (i.e., self-propelled). Or the individual organism can consist of a number of cells, often very large, organized into filaments or into a tissue. In the higher fungi, a fruiting body is built up of a number of kinds of tissue into an organ with a high degree of efficiency in the function that it performs. Such organs, popularly known as mushrooms and toadstools, are remarkably efficient machines for the daily production and dispersal of many millions of microscopic spores, whereby the fungus is carried by the wind in the same way as are the seeds of many species of flowering plant. Leaving aside the question of efficiency in function, many species of toadstool greatly exceed in size many species of flowering plant.

The non-biologist may well ask, therefore, why the toadstool-producing fungi are classified as micro-organisms, seeing that flowering plants, too, are constructed of microscopic units or cells — even though their cells are considerably larger than those of micro-organisms. It thus becomes necessary to explain that, in classifying either the Fungi or any other group of organisms, the most reliable criteria are afforded by those characteristics that comparative study has shown to be the most fundamental in the evolutionary sense of this word. The toadstool-producing fungi are in their form and structure, their behaviour and mode of existence, most certainly fungi; the great majority of the known species of fungi can truly be described as "micro-organisms", even though in the aggregate many of them can be seen as "moulds" by the naked eye; therefore the toadstool-producers must be called "micro-organisms" too. As will be described later, that part of a toadstool fungus that lives and feeds within the soil is similar in general appearance and behaviour to the underground parts of other fungi. Nevertheless, mycologists do recognize a degree of distinction by referring to the toadstool-producing fungi as "macrofungi" and to the others as "microfungi" or "moulds".

Micro-organisms, like macro-organisms, can be classified into plants and animals. Amongst microbiologists, the game of "Animal, vegetable or mineral?" has given rise to some considerable controversies as to whether certain groups of micro-organisms properly belong to the botanist or to the zoologist, and there may be no final answer to this question. The large group of pathogenic agents which cause infectious disease of various kinds in both plants and animals, and are known as "viruses", can no longer be considered as autonomous micro-organisms. Accounts of them are still included in text-books of microbiology, because microbiologists still continue to act as nursemaids even though the baby, like that in *Alice in Wonderland*, no longer seems to be what it was once thought to be.

There is, indeed, no single criterion that can be used to separate all known species of plant from all known species of animal. Green plants derive their energy from sunlight, which through the agency of the chlorophyll pigments in their leaves is used for the photosynthesis of carbohydrate from carbon dioxide and water. Animals, on the other hand, derive both their energy and their body-building materials from the intake and digestion of organic substances contained in the bodies of plants and of other animals. Some plants, however, are partial parasites like mistletoe (*Viscum* spp.) and obtain part of their food material from a host plant. Others, like the climbing leafless dodders (*Cuscuta* spp.), completely lack chlorophyll and are wholly parasitic on other plants. Other non-chlorophyllous flowering plants, including a number of orchids, were at one time thought to live directly on organic material in the soil, but are now known to be parasitic on various species of soil fungi. Amongst micro-organisms classified in the plant kingdom, the fungi live like animals on pre-formed organic material, either as saprophytes on dead material or as parasites on living plants or animals. Most bacteria are similarly either saprophytic or parasitic; some species, however, possess chlorophyll and live by photosynthesis; others obtain the necessary energy for their life-processes by the promotion of energy-yielding (exothermic) oxidations of inorganic substances, such as iron and sulphur compounds. This wide variety of nutrition thus affords no general criterion that can be used to separate plants from animals.

Animals, in distinction to plants, are usually thought of as motile organisms, moving freely about in search of food or in escape of enemies or unfavourable conditions of the environment; some animals (e.g., sea-anemones, sponges and corals), however, are as static as plants for the major part of their life-cycles. On the other hand, many microscopic aquatic or marine plants are motile either for part or for the whole of their active lives, so that the criterion of motility does not constitute a valid distinction between the plant and animal kingdoms. The most useful general distinction that can be made between plants and animals is that (with few exceptions) the protoplasm of plant cells is bounded by a rigid cell wall, which is permeable only to substances in aqueous solution. Microscopic unicellular animals like the amoeba have no such cell wall, and their protoplasm can therefore enfold and digest solid food particles in a way that plant cells cannot do. In more highly organized animals, the ingestion of solid food has been facilitated through the evolution of a mouth leading into an alimentary tract, in which digestion is carried out. This process of internal digestion in animals is more efficient than the process of external digestion in such saprophytic plants as fungi and bacteria, which excrete digestive ferments or *enzymes*, whereby solid food is rendered soluble and can then pass through the cell wall. By this process of external digestion, the organism is enabled to absorb part, and perhaps the major part, of the soluble products through its cell wall, but it inevitably loses part of the yield to other micro-organisms growing in the immediate vicinity of its operations.

The classification of micro-organisms into the plant and animal kingdoms, like the finer sub-divisions of classification within each kingdom, thus has to be based upon a number of criteria considered together. The distinction between micro-organisms as either plants or animals is important, not only because it is fundamental but also because it has affected the way in which microbiology has developed. In the past, microbiologists have usually been trained for research *either* as botanists *or* as zoologists. Few have been trained to the requisite level in both disciplines, and fewer still have been able to do effective work in both fields of research concurrently. Nowadays, more and more microbiologists are entering the subject by way of biochemistry and/or genetics, because micro-organisms have many advantages as subjects for research in these sciences.

Because I myself was originally trained as a botanist, I am not competent to discuss (except in very general terms) those groups of soil micro-organisms classed in the animal kingdom. For our present purposes, however, a brief mention of the animal groups will suffice, just so as to keep in mind that no one group of soil micro-organisms can be considered in isolation. The activities of one group are continuously affected by those of other groups, and also by the activities of higher plants and animals living and moving within and upon the soil.

PROTOZOA AND OTHER MEMBERS OF THE SOIL MICROFAUNA

The phylum Protozoa is estimated to contain some 30,000 species. All species are unicellular (some protozoologists prefer to regard them as acellular), and the more highly evolved species have attained the highest degree of unicellular organization known to exist. Although soluble food materials can be absorbed if available in the environment, members of the Protozoa typically feed in the manner of animals, by the ingestion of solid food particles. The bulk of this food material consists of the living cells of bacteria, microscopic algae and other micro-organisms, including smaller protozoa. The precise method of feeding depends upon the cellular organization of the particular genus, and will be described below. The widespread occurrence of protozoa in the soil must affect population numbers of other soil micro-organisms, and particularly those of bacteria. Realization of this fact was the basis of the hypothesis proposed by Russell and Hutchinson (1909) some 50 years ago at the Rothamsted Experimental Station, to explain the phenomenal increase in bacterial numbers that they found to follow "partial sterilization" of the soil by treatment with steam or toxic chemicals, for eradication of insect pests and root disease fungi. Russell and Hutchinson suggested that the rapid rise in bacterial numbers was due to removal of a natural check on their increase, through killing of all or most of the predatory protozoa by the partial sterilization treatment. Further experiments later demonstrated that any effect of partial sterilization upon numbers of protozoa did not provide the whole or even the major part of the explanation for the observed rise in bacterial numbers; this is now thought to be due to a variety of causes, and particularly to liberation of nutrients from large numbers of microbial cells killed by the treatment. Nevertheless, the hypothesis of Russell and Hutchinson performed a useful service by drawing general attention to the predator/prey relationship between populations of protozoa and bacteria in the soil.

The free-living (i.e. non-parasitic) members of the Protozoa are classified into three groups: Sarcodina (creeping forms), Mastigophora (flagellates) and Ciliophora (ciliates). *Amoeba proteus* is a simple amoeboid protozoan in the group Sarcodina; it consists of a mass of cytoplasm, more or less rounded when at rest, with a large central nucleus and a conspicuous contractile vacuole, thought to be osmoregulatory in function, because at intervals it moves to the surface of the amoeba and discharges its contents. The amoeba moves along over a surface, changing its shape as it does so and extending finger-like projections known as pseudopodia. The pseudopodia engulf bacteria and other microbial cells, which are digested in a "food vacuole" within the cytoplasm; indigestible residues are eventually expelled.

The most specialized and highly organized species of protozoa are to be found in the Ciliophora, in which is included the well-known genus *Paramoecium*. The outside of the cell is covered with short, hair-like extensions known as cilia. The organism is propelled through soil moisture films in soil-inhabiting species by the coordinated, rhythmical beating of the cilia, in much the same way as a boat is propelled by banks of oars. Another series of cilia lines a groove along one side of the cell leading to a mouth opening, into which the cilia sweep small microbial cells and other food particles. Such particles are enclosed within a food vacuole, which follows a regular track around the cell, finishing up at the anal pore, whence undigested material is discharged. Osmoregulation is effected by means of two contractile vacuoles, one at either end of the elongated cell and each at the centre of a system of radial canals, which drain the surrounding cytoplasm into the contractile vacuole. The two contractile vacuoles discharge their contents through the cell membrane alternately.