Environmental Microbiology

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Preface

The nature and scope of the text

The environment in which we live has to a large extent been determined by the activities of innumerable organisms interacting with each other and with their immediate surroundings. From the point of view of the microbiologist, it is obvious that microbial activity has a great part to play in the continuing maintenance of conditions suitable for other forms of life on this planet. There has therefore always been an awareness of the need for a good understanding of how microorganisms react in the environment, and this has been heightened from time to time as detrimental microbial activities become evident under certain conditions. The need for a good understanding has recently assumed a new importance as the era of microbial manipulation dawns—microbiology has always been a practical discipline, and the possibilities of beneficial modification on a global scale may be within our grasp.

The growing interest in environmental microbiology can be gauged from the increase in relevant undergraduate teaching. However, one of the most serious problems confronting the student is the dearth of appropriate texts. In part this is a reflection of the plethora of potential subject matter. The study of the relationships of microorganisms with each other and with their environments—"microbial ecology"—constitutes a subject area which is far from precisely circumscribed, and each researcher or teacher has his own personal notion of which topics are appropriate. It follows that the scope of any book purporting to fall within this general area will be the subject of dispute. In the end, what is included is the subjective choice of the author.

Texts do, however, often evolve from teaching experience with particular groups of students. In our opinion, "microbial ecology" is best taught at senior undergraduate level following a basic training in microbiology, and we have assumed that the readers of this book will have some knowledge of the major microbial groups and will be familiar with the special features of prokaryote structure and function. However, we believe that the text is

intelligible to those biologists with only a rudimentary knowledge of microbiology.

What kinds of environment are appropriate for consideration in a text of this kind? It is possible to argue indefinitely about what constitutes a "natural habitat"; for example, animals represent important and significant microbial habitats, and the relationship between man and microbial populations is important enough to be considered as a separate and distinct topic. However, the microbial population inhabiting animals may matter less in the long term maintenance of the biosphere than do the activities of certain groups of microorganisms such as sulphur oxidizers in soils and waters (7.1). We therefore consider the animal to be outside the scope of this book, except for a discussion of certain interrelationships in the rumen which have parallels elsewhere (4.8, 9.4).

It is clear that soil and water represent by far the most significant microbial habitats, in terms both of microbial biomass and microbial activity. It is beyond dispute that these habitats constitute "natural environments", and that microbial activity within these habitats is essential to the maintenance of the environment. A substantial section of the text is therefore concerned with the nature of microbial activity within soil and water. Further sections deal with the effects of microbial activity within these main habitats in both the short term and the long term, and how microorganisms in these habitats react under certain exceptional circumstances imposed by the "pollutant" activities of man.

Although existing texts cover some aspects of these topics, no single text at present covers all the aspects in detail. We have here attempted to fill the gap. Another important function of a book directed at senior undergraduates is to point the reader towards relevant literature. The breadth of the subject matter covered in this text is such that certain topics are inevitably not considered in great detail, but the reader should be able to find appropriate references leading to the detailed information in the literature. Particular care has been taken in the selection of recent key articles and books, and in compiling reference lists which are both concise and comprehensive.

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W.D.G./P.E.L.

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INTRODUCTION

THE SIGNIFICANCE OF MICROORGANISMS IN THE ENVIRONMENT

The role of microorganisms in providing inorganic compounds at a valence state appropriate for assimilation into higher plants is well understood, and the largely microbially-mediated nitrogen and sulphur cycles (6.1, 7.1) are often quoted and discussed. Less attention is given to the contribution of microorganisms to the continuing decomposition and mineralization of carbon compounds, other than a cursory acknowledgement of their contribution to decay.

In fact, in most ecosystems, the largest part of the organic production is not consumed and utilized by herbivores or carnivores, but becomes part of a pool of dead organic material (detritus). For the continued balance of the biosphere it is necessary that such a pool should remain in a steady state. Herbivore consumption decreases in importance with increasing size of the primary producers (see table), partly because higher organisms do not decompose a number of higher-plant polymers (5.4, 5.5), but partly also because macrophytes have an inappropriate carbon : nitrogen ratio. Animals at all trophic levels (with the exception of ruminants) require a diet with a C : N ratio of less than 17 : 1, and the woody tissues of macrophytes have much higher C : N ratios ($\sim 200 : 1$) than does phytoplankton ($\sim 20 : 1$). This is reflected in the relative extents of utilization by herbivores (table). Microbial decomposition of such recalcitrant materials is also less restricted by the type of nitrogen available and is thus essential to a balanced carbon cycle. An additional advantage accrues from microbial activity, in that microorganisms (C : N $\sim 6 : 1$–$12 : 1$) themselves become part of the detrital material, increasing the nutrient value for herbivores. The mammalian rumen (4.8) is a special example of this kind of effect.

Microorganisms are also vital to the anoxic decomposition of organic

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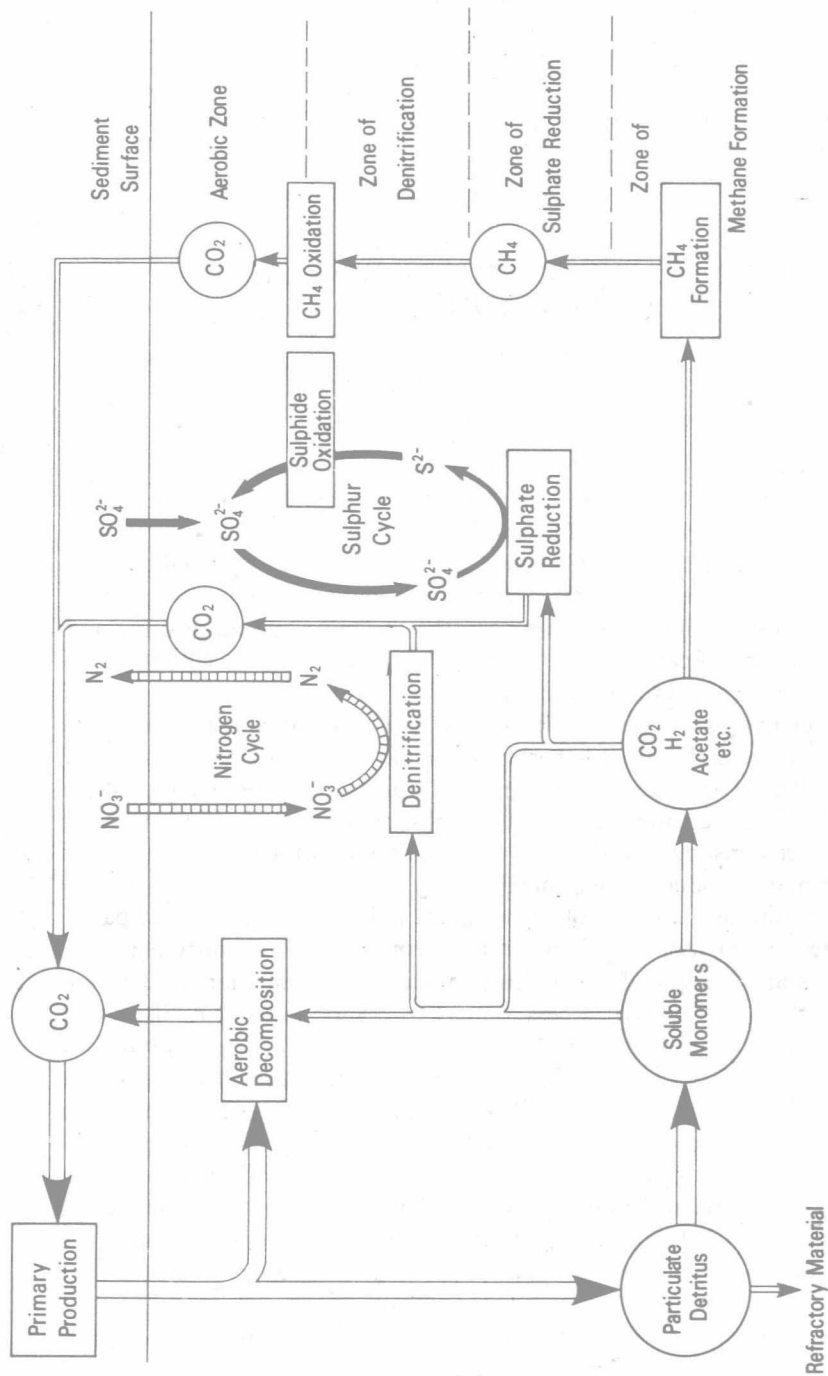
Pathways of carbon during anaerobic decomposition in an aquatic sediment, and their relationship with the sulphur and nitrogen cycles (modified from Fenchel and Jørgensen, 1978).

Table Fraction of primary production consumed by herbivores (abbreviated from Fenchel and Blackburn, 1979)

Plant community	% production consumed
Phytoplankton	60–90
Grasslands	12–45
Kelp beds	10
Deciduous forests	1.5– 5

material. Sulphate respiration accounts for some 50% of the total oxidation of organic material in some marine environments (7.1), and the utilization of CO_2 as an electron acceptor by methanogenic bacteria has a profound effect on the overall oxidation of organic material in many anoxic environments (4.8, 9.4). Reduced electron acceptors in turn act as energy sources elsewhere in the environment. Accordingly, microbially-mediated transformations of various kinds enmesh in complicated and sometimes less than obvious ways in the maintenance of the environment. As an example, the intricate interrelationships of the carbon, nitrogen and sulphur cycle in an anoxic sediment are depicted opposite. It would be possible to draw up similar interrelationships for these and other elements in any environment, illustrating the important point that the planet owes its continued equability as an environment to microbial activity.

REFERENCES

Fenchel, T. and Blackburn, T. H. (1979) *Bacteria and Mineral Cycling*, Academic Press.

Fenchel, T. and Jørgensen, B. B. (1978). Detritus food chains of aquatic ecosystems: the role of bacteria. *Adv. Microbial Ecol.* **2**, 1–58.

Paul, E. A. and Voroney, R. P. (1980). Nutrient and energy flows through soil microbial biomass, in *Contemporary Microbial Ecology*, editors, D. C. Ellwood, J. N. Hedger, M. J. Latham, J. M. Lynch and J. H. Slater, Academic Press, 215–238.

PART 1—HABITATS

CHAPTER ONE

THE SOIL

1.1 Physical aspects of soil structure—the microhabitat concept

A fully developed soil is an intricate mixture of inorganic and organic components, a complex product of parent material, topography, climate, time and biological activity. In an average fertile loam (a good agricultural soil), mineral matter from weathered rock usually accounts for some 50% by volume, and organic matter from plants, animals and microorganisms for 10–15%. Air and water occupy most of the remaining volume in a complicated system of pores and channels where the amount of air and pore space is a function of the water content of the soil. In general, living organisms occupy considerably less than 1% of the total volume. Other types of soil may have much higher proportions of organic material (up to 30% v/v in peaty soils) or much lower proportions (as little as 1% v/v in tundra soils). It is convenient to consider the inorganic and organic components separately, although any soil is truly a composite entity, its properties depending on the exchange and interaction between organic and inorganic fractions.

Soil develops when rock is changed by a series of processes collectively known as *weathering*. The parent rock is broken down into small fragments by temperature changes, the action of water, extremes of acidity or alkalinity, and other chemical effects produced by biological activity. While the parent rock is disintegrating under a combination of these effects, it exchanges material with its immediate environment, and the final product consists of a surface layer of soil physically and chemically distinct from the underlying material. The most abundant elements in the mineral component of soil are Si, Al, Fe and O, the mineral component being mainly composed of silicates, exemplified by the simplest form silica (SiO_2)—the abundant mineral quartz for instance, is almost exclusively

composed of SiO_2. The aluminosilicates constitute another large class of silicates where tetravalent Si is partially substituted by trivalent Al. Consequently these minerals often have a net negative charge.
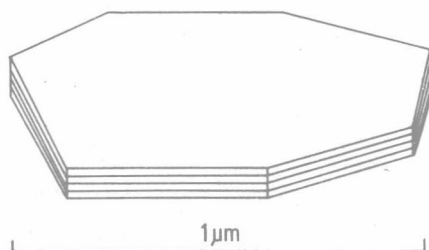
The atoms of many silicate minerals are arranged in negatively-charged pyramids, or silica tetrahedra, held together by other atoms, especially Ca(II) and Mg(II). There are various distinct weathering processes; an important one is hydrolytic attack, in which Mg(II) and Ca(II) are replaced by H^+, producing an unstable structure (7.5). Microorganisms quickly colonize freshly quarried stone surfaces, and a number of common soil fungi, bacteria and blue-green algae have been shown to contribute towards the weathering of rocks by mediating such effects. In addition, microbially-mediated weathering appears in part to be due to the production of organic acids, such as 2-ketogluconic acid, which remove divalent cations from silicates by chelation, producing an effect analogous to hydrolytic attack. Lichens are often colonizers of bare rock surfaces, and many lichens produce a variety of unusual phenolics and terpenes that have considerable chelation properties, and which over many years may decompose even the exceptionally stable mineral quartz—as exemplified by the etching of glass under lichen growth on old church windows.

The composition of the mineral fraction of a soil markedly influences the properties of the soil. Particularly significant is the relative proportion of primary minerals (those remaining unaltered from the original parent rock) and secondary minerals (those modified by the weathering process). One of the most important consequences of weathering is the production of *clays*. Clays are secondary minerals, and consist of minute aluminosilicate particles which constitute the smallest size class of the particles found in the soil mineral fraction (table 1.1). Of the four main types of clay found in soil, three (illite, montmorillonite and kaolinite) have a distinct crystalline structure, whereas the fourth type, amorphous clay,

Table 1.1 Classification of the sizes of soil particles

Texture class		International scale (mm)	American scale (mm)
Primary Minerals	Gravel	>2.0	>2.0
	Coarse sand	$2.0 \rightarrow 0.2$	$2.0 \rightarrow 0.25$
	Fine sand	$0.2 \rightarrow 0.02$	$0.25 \rightarrow 0.05$
	Silt	$0.02 \rightarrow 0.002$	$0.05 \rightarrow 0.002$
Secondary Mineral	Clay	less than 0.002	less than 0.002

A

1 μm

B

| SILICA LAYER |
| ALUMINIUM LAYER |
| O |
| H |
| O |
| SILICA LAYER |
| ALUMINIUM LAYER |
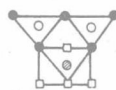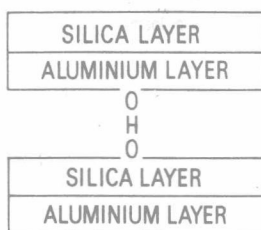
• Oxygen
○ Silicon
□ Hydroxyl
⊚ Aluminium

Figure 1.1 A. A clay micelle. Clay micelles are large. flat, leaf-like surfaces with silicon and aluminium atoms arranged alternately with oxygen atoms in a sandwich-like structure.
B. Diagrammatic representation of kaolinite showing a layer of silicon atoms joined to a layer of aluminium atoms (1:1 clay). This structure is then joined to the next leaf by a hydrogen–oxygen bond. The arrangement of atoms in one of the bilayers is shown on the right (redrawn from Courtney and Trudgill, 1976). Montmorillonite and illite have a layer of aluminium sandwiched between the two silica layers in each leaf (2:1 clays).

lacks any well defined structure. The crystalline types have a micellar structure composed of several layers or leaves of aluminosilicate material which expand or contract, depending on the degree of hydration bonding between the individual layers (figure 1.1). The overall electrical charge on clay particles is negative, due to replacement of Si(IV) by Al(III), and so cations are attracted to the particle surfaces, forming a layer of positive charge. Because sorbed cations include many plant nutrients, soils with high concentrations of clays are fertile, whereas soils having high concentrations of primary minerals with negligible ion exchange capacity are less fertile.

Soil classification systems often reflect the agricultural potential of a soil or its textural properties. Texture is an important classification feature
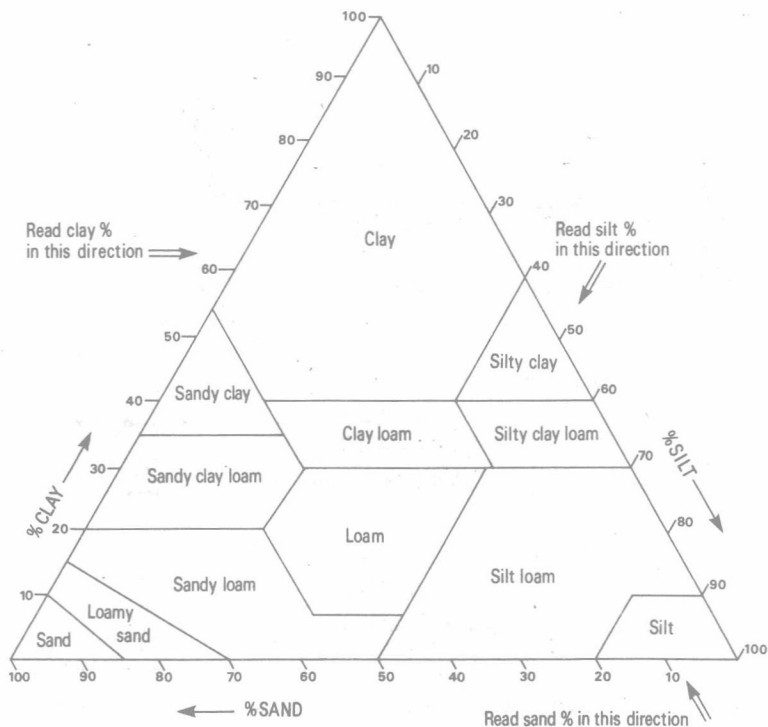
Figure 1.2   Soil texture graph.

related to particle size, and relative proportions of different size classes of particles derived by sedimentation or sieving procedures may be plotted on a triangular texture graph (figure 1.2). It can be seen that a sandy soil for instance is a coarse-textured soil with good drainage properties but is likely to be low in nutrients, due to the absence of significant amounts of clay. In contrast, a clay soil may be poorly drained, because of the preponderance of very small particles, but is high in plant nutrients. Loam soils (mixtures of sand, silt and clay) are clearly the most desirable agricultural soils, with intermediate drainage and nutrient properties.

Groups of mineral particles usually contribute to well-defined composite structures, sometimes called soil aggregates or *peds*. The type of structure found, which is governed by the texture of the soil, can be described by various terms—platy, blocky, spheroidal—and the tilth of a particular soil reflects the structure of the aggregates. Soil aggregates contain organic as well as inorganic material, and the major components

determining aggregate stabilities are clays, certain organic materials including humic substances (1.2), and exopolysaccharides produced by microorganisms. The importance of exopolysaccharides prod·ced by common soil bacteria such as *Bacillus* spp. has been demonstiated by showing that soils subject to periodate treatment (which destroys polysaccharides) often lose their aggregate structure. Fungal hyphae and other filamentous structures may also contribute towards binding mineral particles together. It is also thought that humic substances may act together with clays as a cement in binding groups of smaller particles. An idealized representation of a soil aggregate is depicted in figure 1.3. Microorganisms are present in larger pores, but there are also pores too small to permit the access of microorganisms, and which contain humic material and domains or packs of clay micelles or platelets. Such a model has considerable microbiological implications. Since there is no microbial access to the smaller pores, the organic material in them will be protected, and this may account in part for the longevity of humic substances in soil (1.2). Further consideration of the structure of the soil aggregate depicted in figure 1.3 leads to the conclusion that microbial life must exist in many discrete microhabitats that may undergo quite independent environmental fluctuations. In the absence of appropriate techniques with which to
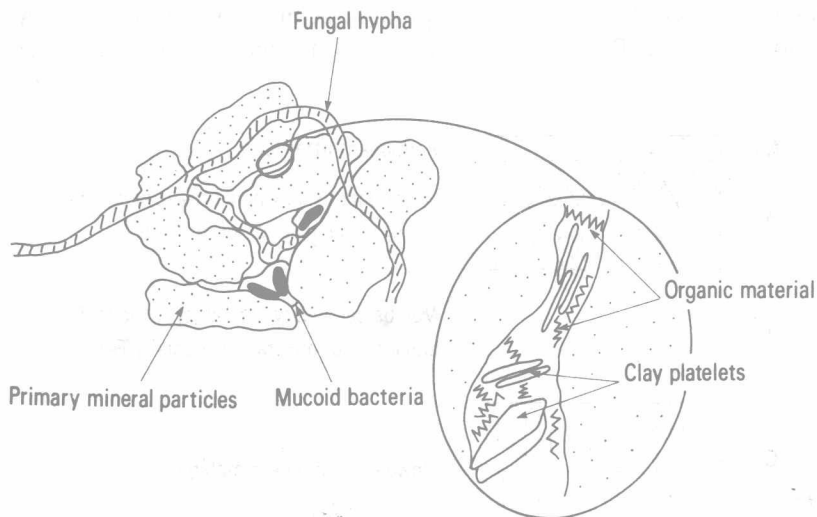

Figure 1.3   Diagrammatic representation of a soil aggregate.

approach the study of individual microhabitats, it is clear that assessments of the activity of soil microorganisms represent, at best, the net result of a large number of presumably rapid and independent fluctuations in microhabitat conditions, and so must provide an over-simplified and generalized view of microbial behaviour.

Natural soil-forming processes do not produce a uniform homogeneous soil throughout the depth of the soil layer. If a vertical section of uncultivated soil is examined, it is usually possible to see several distinct layers or *horizons* which collectively form the soil profile. These horizons are a consequence of weathering, leaching, and the mobilization and accumulation of clays, chelates and humic material at different depths. The general term *eluviation* is often used for the washing out or removal of materials, and the term *illuviation* for the washing in and immobilization of materials in a horizon.

Horizons are usually differentiated on the basis of colour, texture, mineral content and organic content. There are many subdivisions depending on the differentiation scheme used, but the three major horizons recognized are the ·A, B and C horizons (figure 1.4). The uppermost, or A horizon, is. where humic material accumulates derived from plant and animal litter (if a permanent litter layer exists it is sometimes referred to as the A_0 horizon), and the layer is characterized as a zone of maximum eluviation; it is usually subdivided into an upper humus-rich layer, and a lower layer which is an area of maximum leaching of silicates, Fe and Al. Below this, the B horizon is a zone of maximum illuviation, where
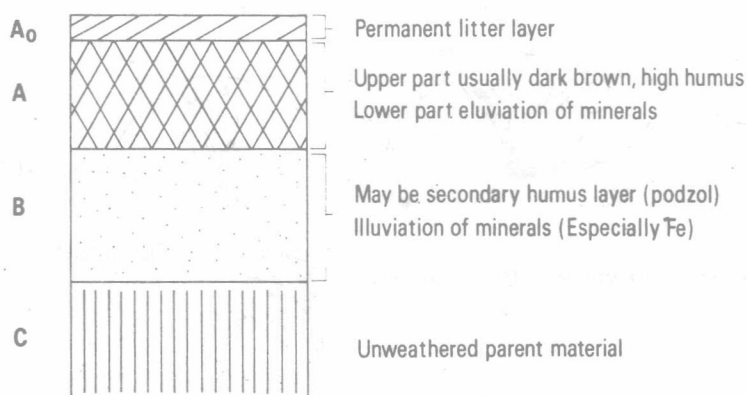

A_0	Permanent litter layer
A	Upper part usually dark brown, high humus
	Lower part eluviation of minerals
B	May be secondary humus layer (podzol)
	Illuviation of minerals (Especially Fe)
C	Unweathered parent material

Figure 1.4 Diagrammatic representation of a soil profile.