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Microenvironments of soil microorganisms

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Summary. Ultrastructural studies of soil microorganisms and the microenvironments surrounding them are reviewed. Soil microfauna, and bacteria, actinomycetes and fungi, fixed and embedded in situ, were examined by electron microscopy (both transmission and scanning). In some cases ultrastructural histochemistry was used to detect and identify the organic matter with which microorganisms were associated and to examine the polymeric microbial materials (enzymes, extracellular polysaccharides) they produced. Although some small organisms (0.3 μm diameter) occurred singly in dense fabrics of clay or humified organic matter, larger bacteria occurred in rhizospheres, in small colonies in the larger micropores or associated with substantial deposits of organic matter (faecal pellets, carbohydrate-rich plant cell-wall debris). Whereas rhizospheres had mixed microbial populations, individual microvoids in the bulk soil usually contained only one type of microorganism. Following chloroform treatment, microorganisms were found only in mucigel deposits or deep in the interiors of micropores, suggesting that these constitute protected sites where microorganisms survive temporarily adverse conditions. Soil microfauna and fungi were mainly confined to the larger voids. Although some live hyphae occurred in the outer regions of aggregates, hyphae deep within soil fabrics were usually devoid of cytoplasmic organelles. Faecal pellets, plant tissues and cell-wall remnants comprised the most frequent, larger organic masses, while the most common micron- and submicron-sized organic matter consisted of fibrous or amorphous humified matter. Unequivocal detection of enzymes was limited to the surface of microorganisms.

Key words: Soil microorganisms – Microenvironment – Rhizobacteria – Spatial distribution – Soil enzymes – Plant debris – Carbohydrates

Traditional soil microbiology has relied on the extraction of microorganisms from soils and their isolation in laboratory media for identification and enumeration. While this has given consistent results worldwide for numbers of species present and for the populations of individual species in similar soils, it has provided no information on the microenvironments favoured by particular microorganisms.

Similarly, chemical analyses have provided extensive lists of organic compounds which can be extracted from soils (Flaig et al. 1975; Greenland and Oades 1975), as well as much data on the total amounts of organic matter and relative proportions of the major classes (carbohydrates, humic materials, lipids, nucleic acids, etc.) both before and after chemical or enzymatic hydrolysis. But the precise location of these complex materials and the spatial distribution of particular chemical groups in natural, undisturbed soils are still largely unknown.

This has left unanswered such questions as the precise location of organic remnants and microorganisms in soils, the location of soil enzymes (Burns 1982) and the sites of microbial weathering of soil minerals (Berthelin and Leyval 1982), why small concentrations of microbial polysaccharides stabilize soil structure so efficiently (Toogood and Lynch 1959), and where in the soil fabric particular chemical and biochemical transformations take place. Other questions include the reasons for the resistance of some soil polysaccharides to microbial decay (Ladd and Paul 1973; Oades and Ladd 1977) and why only 1% – 3% of soil N is mineralized each year (Ladd et al. 1981) even though similar materials are rapidly metabolized when added fresh to soils.

Microbial ecology using light microscopy of soil sections is limited by the fact that the most numerous soil microorganisms are less than 0.3 μm in diameter (Bae et al. 1972; Lundgren 1984; Foster 1985) which is

near the limit of easy light-microscopic resolution. Moreover, it has been estimated that conventional plating techniques detect less than 1% (sometimes as low as 0.1%) of the cells that can be observed by light microscopy (Skinner et al. 1952; Malajczuk 1979a, b; Ramsay 1984) and so give gross underestimates of microbial populations.

Electron optical (transmission electron microscopy) and scanning electron microscopy, scanning transmission electron microscopy and electron probe microanalysis and other advanced instruments (laser microprobe mass analysis, secondary ion mass spectroscopy, electron energy loss spectroscopy) are now beginning to provide answers to some of these ultrastructural and biochemical questions (Bisdom 1983). Laser microprobe analysis, secondary ion mass spectroscopy and electron energy loss spectroscopy are not generally available to soil scientists. Although electron probe microanalysis is useful for investigating the composition of soil minerals, it cannot be used, for example, to distinguish between the enantiomorphs and structural and optical isomers of complex organic compounds such as polysaccharides and proteins, distinctions which are matters of life and death to microorganisms.

Transmission electron microscopy can detect all the cells (and even viruses) in a soil section (Foster and Rovira 1978) and organic remnants down to particles of nanometre size can be located and identified (Foster and Martin 1981). This review discusses results from the use of transmission electron microscopy on ultrathin sections of natural soil fabrics that were obtained from a field in which particular materials of biological significance had been located and identified by using histochemical reactions. These techniques allow both the form (morphology) and function (biochemistry) of components of cells (e.g. microorganisms) and tissues (e.g. roots) to be investigated in situ, unextracted, uninhibited and it is hoped uncontaminated.

Methods

Smart (1974), Kilbertus and Reisinger (1975), Kilbertus (1980), Foster and Martin (1981) and Bisdom (1983) have described the techniques for preparing soils for electron optical investigations. In brief, soils may be physically stabilized (where necessary) by enclosure in agar or gelatine, chemically fixed with organic aldehydes (glutaraldehyde, formaldehyde and acrolein, alone or in various combinations), and particular organic components rendered electron-dense by using histochemical reactions involving heavy metal compounds. For example substituted carbohydrates are detected by using ruthenium red or alcian blue (Foster and Martin 1981). The soils are then dehydrated and examined directly (scanning electron microscopy, electron microscopy, electron probe microanalysis or embedded in plastic and sectioned using ultramicrotomy and diamond knives (transmission electron microscopy, electron probe microanalysis).

Further histochemical tests may be applied to the ultrathin sections. Thus, the periodic acid silver methenamine (Marinozzi 1961) and the periodic acid thiosemicarbazide-silver proteinate (Thiery 1967) techniques detect periodic acid Schiff's base reactive materials. In the case of carbohydrates, these tests depend on the oxidation of 1,2-diglycol groups by periodate and the attachment of heavy metal complexes to the aldehyde groups so generated.

In many plant, microbial, biomedical, and animal tissues these tests appear to be quite specific for neutral carbohydrates (Pickett-Heaps 1967; Swift and Saxton 1967; Thiéry 1967; Pears 1972; Luft 1976; Knight and Lewis 1977; Roland 1978; Foster 1981a, b; Frehel and Ryter 1982; Gianinazzi et al. 1983), steric hindrance preventing the reaction of substituted carbohydrates (Rybicka 1981). However, recent tests on *Pinus radiata* mycorrhizae, which contain specific polyphenols (Hillis et al. 1968), indicate that vacuolar polyphenolics also react with the periodic acid reagents, silver methenamine and thiosemicarbazide silver proteinate. Since vacuolar polyphenolics are released on cell death and polyphenolics are common constituents of plant cell walls, it is probable that in soils, humic substances as well as carbohydrates will give a positive reaction to these two reagents. In soils, it is likely that a large proportion of non-biomass soil polysaccharide is associated with humic substances, so that tests for one material locate the other also. In any case, deposits of carbohydrate can be distinguished from materials that contain polyphenolics by the use of suitable controls. These include sections which have not been oxidised by periodic acid or in which aldehydes have been blocked with dimedone (Pickett-Heaps 1967) or where the carbohydrate has been enzymatically removed before histochemistry. The examples of the periodic acid silver methenamine and the periodic acid thiosemicarbazide-silver proteinate tests for carbohydrates presented here are for soils fixed only in formalin with no OsO_4 postfixation (Knight and Lewis 1977).

Locations of soil microorganisms

The large numbers of microorganisms in soil [bacteria ca. 10^7, actinomycetes ca. 10^6, fungi ca. 10^5, algae ca.

10^4 colony-forming units g^{-1} dry weight of soil (Eckhardt 1985) and ca. 10^4-10^5 protozoa g^{-1} (Alexander 1961)] can constitute up to 50 kg dry weight ha^{-1} of biomass (Clarholm 1985). However, the biomass comprises only 1%–3% of the soil organic C, and may occupy only 0.001% of the soil volume (Sparling 1985). This is equivalent to only 0.17% of the surface of the soil organic matter and 0.02% of the soil mineral surface (Hissett and Gray 1976). Because the solid phase is composed of particles from less than 0.2 µm to greater than 2 mm diameter, soil contains a network of pores with a similar range of dimensions. Hence a soil is a composite of numerous small microbial communities, each circumscribed by its own immediate environment (Hattori and Hattori 1976). This is well illustrated by Fig. 1, an ultrathin section of a clay soil. This section also shows that soil microorganisms are neither randomly nor uniformly distributed through the soil fabric. First, microorganisms congregate in pores which are more than large enough to comfortably contain them. Thus Kilbertus (1980) showed that there was a consistent ratio of 3:1 between the diameter of pores and the diameter of the bacteria or colonies they contained. Pores occur both between and within aggregates, and may be completely closed, have one entrance (bottle-type pores; Hattori and Hattori 1976) or form irregularly shaped tubes with two or more entrances (Hattori and Hattori 1976; Kilbertus 1980). Kilbertus (1980) discusses the effect of the diameter of pores on their water-retaining capacity at different soil pF and its consequent effect on microbial growth and survival. Small pores retain their water longer in a drying soil and this may be why bacteria accumulate in narrow (less than 5 µm diameter) pores. Here they may also escape predation by soil protozoa, and the effects of fungal antibiotics.

Second, microorganisms congregate near suitable food sources (Fig. 1), such as cellular remnants, faecal materials and amorphous organic matter. Thus Hissett and Gray (1976) showed that in a sandy soil, 64% of the bacteria were associated with organic particles even though these comprised only 15% of the soil volume.

Soil microfauna

Normally, larger animals (here larger than 1 mm diameter!) escape soil-core sampling and subsequent subdivision into smaller blocks for electron microscopy. Soil animals in this treatment are generally limited to smaller arthropods, nematodes, enchytraeid worms and unicellular protozoans such as amoebae and ciliates.

In the rather arid soils of South Australia, amoebae and nematodes are usually associated with rhizospheres and roots (Foster 1986). Nematodes are found both free and within root tissues (Foster 1985). Occasionally, larger animals are found, some clearly soil-ingesting forms with clay particles in their guts (Foster and Martin 1981; Foster 1986).

Other microfauna are associated with populations of bacteria in rhizospheres and hyphaspheres. Amoebae are able to insinuate themselves both into the narrow space between the root surface and the nearby soil and between cast-off root-cap cells. Here, they conform their shape to whatever space is available (Fig. 2) and ingest rhizosphere bacteria and fungi. Feeding experiments have shown that protozoa prefer Pseudomonadaceae (Stout and Heal 1967), which dominate the rhizosphere. Because they browse the rhizosphere microflora, amoebae are important in rhizosphere ecology. Thus they have been implicated in the biological control of root pathogens, in reducing root colonization by mycorrhizal fungi (Chakraborty et al. 1983) and in N cycling in thizospheres with consequent increases in higher plant growth (Woods et al. 1982). Similarly, ciliates have been observed attached to mycelial strands from the litter layers of a pine forest (Foster 1986), again where there were abundant bacteria (Foster 1981c). Invertebrate grazing of rhizosphere microfloras may have significant consequences on both microorganism populations and plant growth, especially if particular species such as mycorrhizal fungi are selectively removed so that the beneficial effects of the mycorrhizae are negated (Visser 1985).

Soil microflora

Soil fungi. Fungi may constitute about 80% of the total microbial biomass in many soils (Shields et al. 1973). In bulk soils they tend to be restricted to the larger pores between aggregates, whereas bacteria tend to occur within aggregates (Kilbertus 1980) and on and within cell-wall debris (Foster 1985, 1986). So fungal hyphae appear abundant in scanning electron micrographs of whole-mounted fractured samples from surface layers of soils, whereas bacteria seem uncommon; in contrast, in transmission electron microscope sections of aggregates bacteria appear to be more common. This result occurs because the preparation for scanning electron microscopy tends to split soils along the surfaces of aggregates where the fungi are located, while soil sections cut through the aggregates reveal the bacteria within. Hence bacteria appear more frequent under transmission electron microscopy of soil samples.

Hyphae which grow in voids larger than their characteristic diameter assume their normal dimensions and cylindrical shape. In smaller voids they have

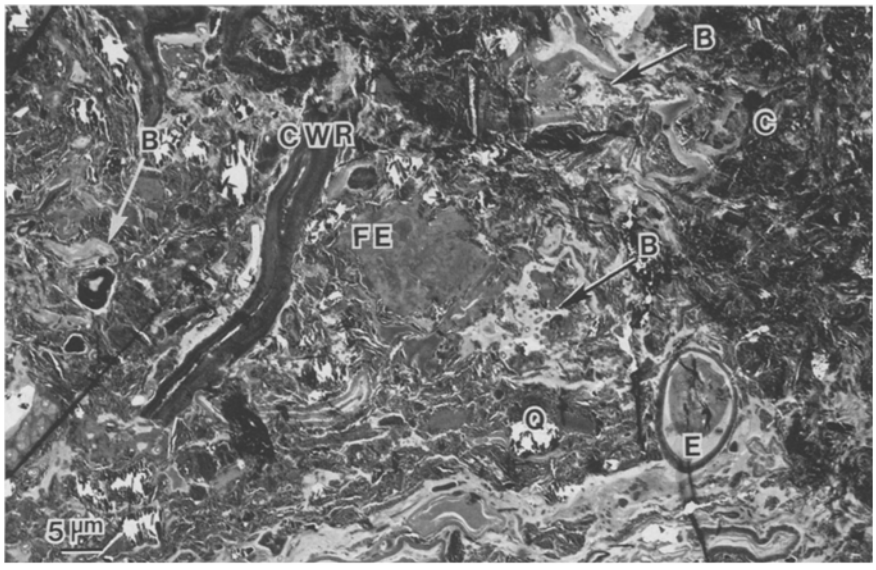


Fig. 1. General view of a clay. Small colonies of bacteria lie in small pores, and as isolated individuals throughout the fabric. G/Os/Pb. See footnote on p. 193 for explanation of symbols

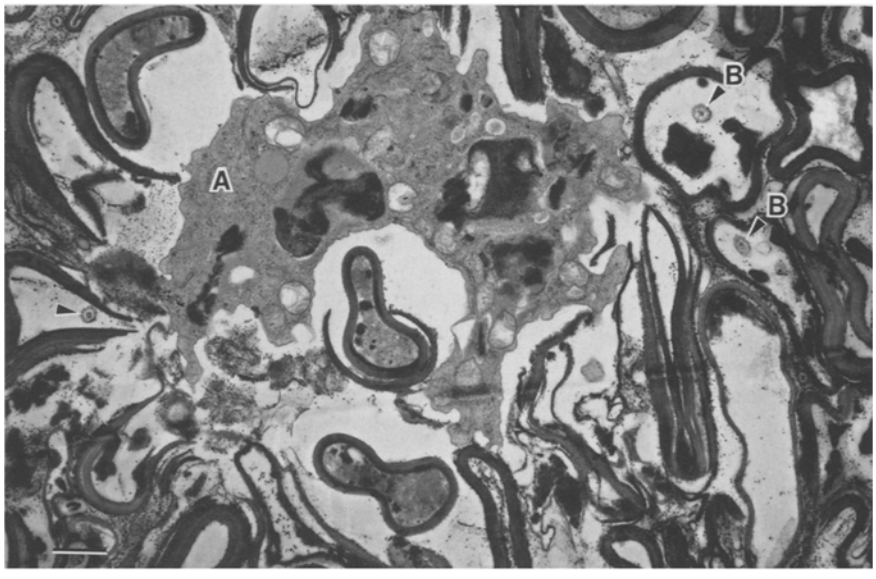


Fig. 2. An amoeba amongst cellular debris associated with rhizosphere of *Lepidosperma semiteres*, G/Os/Pb

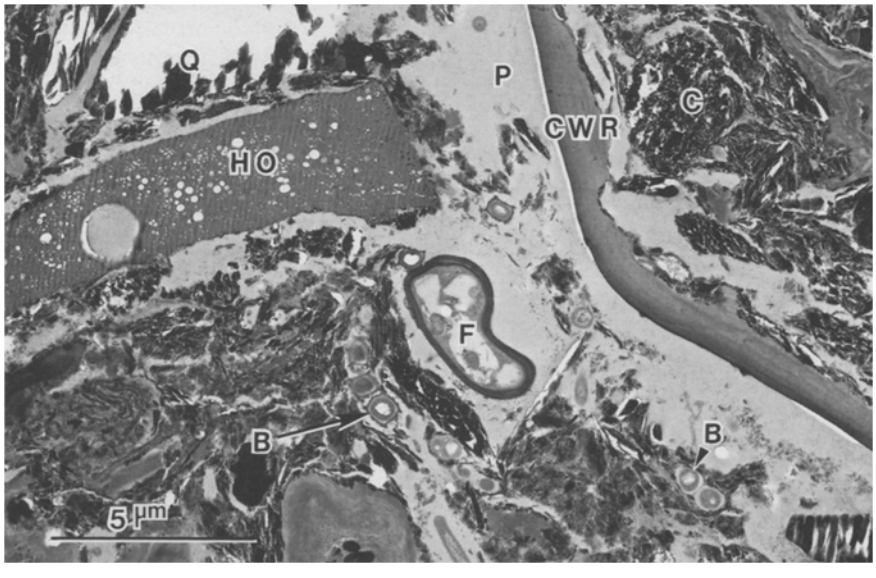


Fig. 3. Fungal hyphae, possibly vesicular-arbuscular mycorrhizal type, penetrating a clay fabric with occasional cell-wall remnants. G/Os/Pb

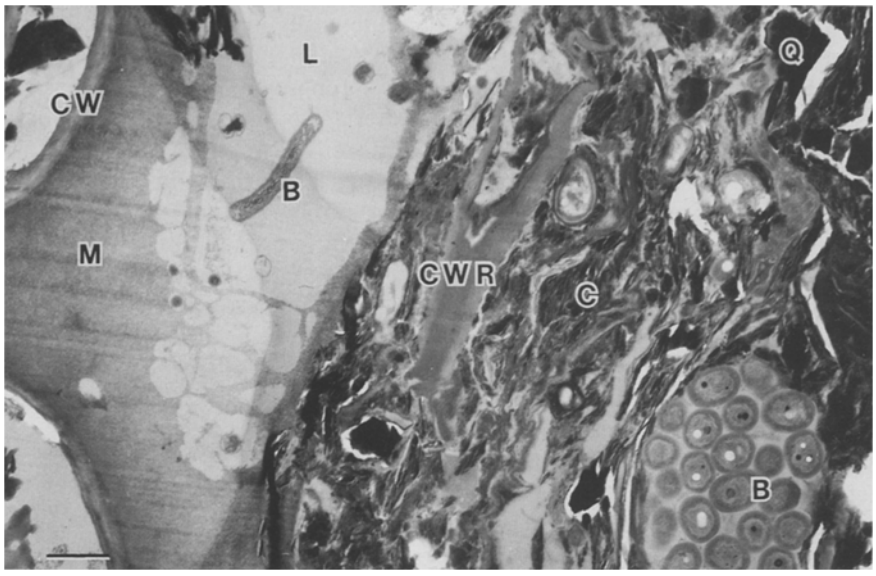


Fig. 4. Mucigel stained by lanthanum hydroxide and partly lysed by micro-organisms, lies between rhizodermal cells and clay fabric. A large colony of bacteria containing polyhydroxy-butyrate (*white*) and polyphosphate granules (*black*) lie nearby. G+La/Os/Pb

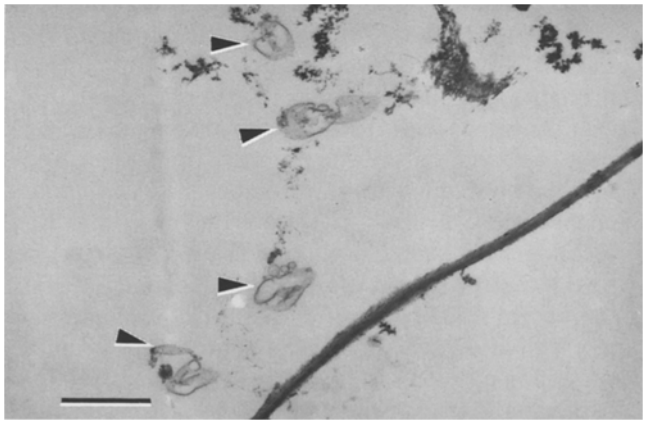


Fig. 5. Free rhizobacteria after chloroform treatment. Only cell-wall ghosts are present (*arrows*). G/Os/Pb. *Scale bar:* 1 micron

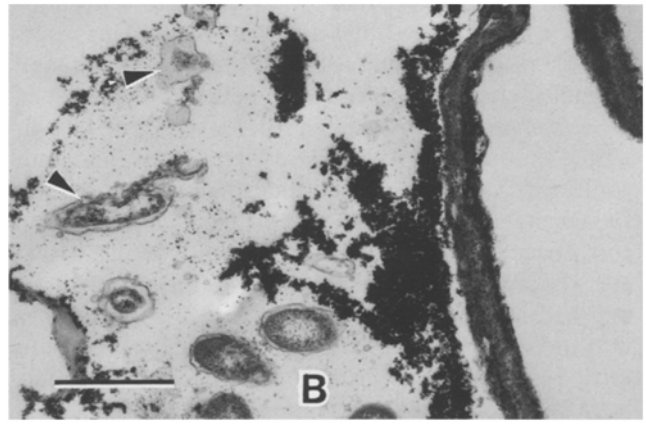


Fig. 6. Rhizobacteria embedded in gel after chloroform treatment. Bacteria deep in gel survive; those near gel surface are lysed. G/Os/Pb. *Scale bar:* 1 micron

Symbols used in figures

A, amoeba; *B*, bacteria; *C*, clay mineral(s); *CW*, cell wall; *CWR*, cell-wall remnant; *F*, fungal hypha (vesicular-arbuscular mycorrhizal?); *HO*, humified organic matter; *M*, mucigel; *PP*, polyphenols; *P*, pore; *Q*, quartz grain (site of); *E*, egg; *FE*, faecal pellet; *L*, lysis zone in mucigel.

Unless stated otherwise the figures are transmission electron micrographs of ultrathin sections of natural soils treated as follows: G/Os/Pb, soil fixed in 3% glutaraldehyde in 0.05 *M* sodium phosphate buffer pH 7.3 followed by 1% buffered OsO_4 overnight at 4 °C. Sections stained in lead acetate; G+La/Os/Pb, fixed 3 h in 3% glutaraldehyde in 0.1 *M* cacodylate buffer, 1% lanthanum hydroxide, and postfixation and staining as above; F+MS, fixed 1% formalin in 0.05 *M* phosphate buffer pH 7.3, 3 h, 4 °C. Sections treated with silver methenamine only (control). F+PAMS, fixation as F+MS but sections oxidised in 1% periodic acid for 30 min room temperature before silver methenamine treatment; F+PADMS, fixation as F+PAMS except 3 h treatment with saturated dimedone between the periodic acid and silver methenamine; APase, fixed 1% glutaraldehyde in 50 m*M* cacodylate buffer, 1 h, 4 °C, followed by incubation in acid phosphatase medium of Knight and Lewis (1977); ATPase, fixed as for APASE and then incubated in the ATPase medium of Knight and Lewis (1977). Unlabeled scale bars = 1 micron

a very irregular outline (Fig. 3). Like bacteria (Kilbertus 1980), fungi do not fill the whole lumen of the small pores they colonize, presumably to allow movement of water solutes and gases past their cells. Even where they occur in wide pores, living hyphae in soils are not associated with hyphasphere bacteria (Fig. 3). As in the rhizosphere, bacteria are associated with dead not living cells.

The hyphae of many soil fungi, especially mycorrhizae, are encrusted with tabloid or needle-shaped crystals (Foster and Marks 1967; Malajczuk and Cromack 1982), often of calcium oxalates. The crystals may constitute up to 23% of the dry weight of the mycorrhizal mat (Knutson et al. 1980) and represent 50% of the exchangeable soil Ca (Cromack et al. 1979). Oxalates secreted by fungi have been im-

plicated in the weathering of rocks and soil minerals (Sollins et al. 1981), and so may be involved in the release of essential nutrients, but since Binns (1980) showed that oxalate secreted by the mycelium of *Agaricus bisporus* limited feeding by sciarid larvae, oxalates may also have a protective function.

Because fungi are classified by their reproductive apparatus, which are seldom encountered in soil sections, it is not possible to closely identify hyphae in soils. However, their cytoplasmic structure, storage compounds and especially the structure of their septa are often characteristic enough to assign hyphae to one of the major classed, i.e. Basidiomycetes, Ascomycetes and Physomycetes.

Soil bacteria and actinomycetes. Bacteria and actinomycetes are the most numerous organisms in soils, but because of their small size they constitute only one-third of the soil biomass (Lyda 1981). The major sources of soil microbial substrates are living roots and rhizospheres and dead remnants of plant and animal tissues, including faecal pellets.

Rhizobacteria: Although Barley (1970) has suggested that under a well watered temperate grassland, roots are so densely packed that the whole of the soil fabric may be classified as rhizosphere, in general roots occupy less than 6% of the soil volume. However, because young roots themselves are so nutritious and because they secrete a wide range of metabolites into the soil (Rovira 1965), root surfaces are the main locations for soil organisms of all types. Rhizospheres have therefore been the main site of ultrastructural studies of soils and soil microorganisms (Jenny and Grossenbacher 1963; Darbyshire and Greaves 1967; Foster and Marks 1967; Marks and Foster 1973; Foster and Rovira 1978; Foster et al. 1983). More than one-third of the photosynthate reaching healthy roots is lost into the soil (Barber and Martin 1976; Martin 1977; Martin and Puckridge 1982) as sloughed cap cells, mucilages, soluble exudates and lysates, and decaying root hairs and outer cortical cells. Ultrastructural studies (Foster 1981b; Campbell and Porter 1982) have shown that dense mucilages may impregnate the soil fabric as far as 50 μm from the root surface, coating the nearby clay particles and becoming trapped in the fine pores between them (Foster 1981a, b; Fig. 4). Soil minerals may become sufficiently well trapped in root and root-hair gel to form persistent rhizosheaths (Buckley 1982).

The rhizosphere of fully differentiated rhizodermal cells is the site of large populations of microorganisms [$10^{10}-10^{12}\,cm^{-3}$, based on total cell counts in electron micrographs (Foster and Rovira 1978; Malajczuk 1979b)] including bacteria, actinomycetes fungi, amoebae, etc., many of which can be distinguished ultrastucturally on the basis of their size, cell-wall structure, cytology and storage granules, etc. Whereas fungi dominate the biomass in the bulk soil, bacteria dominate the rhizosphere biomass (van Vuurde and Schippers 1980). In the rhizosphere, bacteria are larger than those in the bulk soil and contain more storage granules (Foster 1985). However, since bacteria are so small, there is little difference between the biomass of the rhizosphere and that of the bulk soil (Sparling 1985). Bacteria occur in small colonies, separated from other colonies by layers of compacted clay minerals or by their own extracellular polysaccharides. Individual cells are attacked by bacteriophage and predatory bacteria such as *Bdellovibrio* (Foster and Rovira 1978; Foster et al. 1983) as well as by soil protozoa.

It is becoming increasingly clear that cortical cell death is a normal, genetically controlled process, often unrelated to disease (van Vuurde et al. 1979; Henry and Deacon 1981; Deacon and Mitchell 1985), though adverse environmental conditions such as drought, and disease organisms may hasten the process. Together with the cap cells, mucilages and dead root hairs, the cells of the primary cortex of the roots are quickly removed by nematodes, *Collembola* and enchytraeid worms, etc. (Head 1967), so that until secondary thickening occurs, the root may be reduced to the central stele protected by the endodermis and the tannin-filled cells of the pericycle and inner cortex. This allows a massive annual turnover of root tissues in many ecosystems. Thus, in some semipermanent grasslands, where 53% – 90% of the standing crop is below ground, some grasses show a 100% root turnover each year (Dickinson 1982), amounting to ca. 5000 kg dry matter $ha^{-1}\,year^{-1}$ (Whitehead et al. 1979). This represents a major energy input into the soil ecosystem. Under waterlogged conditions, breakdown of root tissues may be inhibited, and cell-wall remnants may accumulate near the root (Foster 1978; Foster et al. 1983). Many rhizobacteria secrete organic materials which are able to weather soil minerals (micas, apatite, etc.) and release elements which promote plant growth (Berthelin and Leyval 1982; Berthelin 1984). Following rhizodermal differentiation the cortex lyses, exudate release ceases, and the microbial cells lyse; the lytic products and the excretory products of browsing protozoans return to the root in the transpiration stream. The rhizosphere microflora and microfauna can therefore be regarded as an external rumen. Clarholm (1985) elegantly summarised these changes in the rhizosphere.

The microenvironment in the rhizosphere is therefore very different from that in the bulk soil. Compared with the generally oligotrophic conditions of the bulk soil, organic substrates are abundant, al-

though the supply of other nutrients such as N, P, Mn and Fe may be limit growth. Rhizosphere bacteria are generally larger than those in the bulk soil, ranging in diameter from 0.2 (*Bdellovibrio*) to 2.3 μm (sulphur bacteria in rice rhizospheres), with a mean width of 0.47 μm (n = 1200). Rhizosphere bacteria also contain larger reserves of C, as poly-hydroxy butyrate or glycogen granules, and P, as polyphosphate (Foster and Rovira 1978).

There are some disadvantages, however, in rhizosphere life. Microorganisms embedded in the root gel may suffer large fluctuations in pH as different ions are removed from the soil by roots and replaced by balancing ions; they may experience very low water potentials as water is removed by high transpiration rates. High osmotic potentials may occur as a result of the accumulation of ions (e.g. Ca^{2+}) carried by the transpiration stream to the root surface but not taken up by the roots: In some cases calcium salts may be precipitated on the rhizoplane. High respiration rates by the root and by rhizosphere organisms may form local transient or even semipermanent microaerophilic or anaerobic sites (Foster and Bowen 1982; Foster 1986 for review). However, the gel secreted by roots and the extracellular polysaccharides of microorganisms may protect them from unfavourable environmental changes. Thus, when treated with chloroform (method of Jenkinson and Powlson 1976), naked bacteria and those at the surface of the mucigel were lysed (only cell-wall remnants remained; Fig. 5) but bacteria deep in the gel (2 – 3 μm inside) survived (Fig. 6; Martin and Foster 1985).

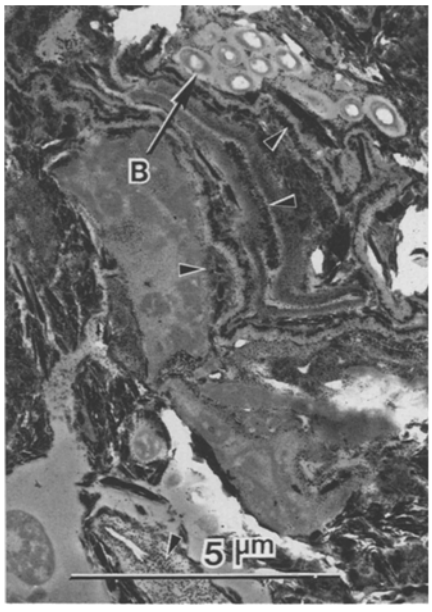
Microorganisms associated with plant residues: As well as living roots, soils contain dead root tissues and materials such as leaves, twigs, and bark derived from the aerial parts of plants. In some forest ecosystems, flower parts (bud scales, petals, and even pollen) may make an important contribution to soil C reserves. Organic C levels in many bulk soils indicate that most soil microorganisms live under rather oligotrophic conditions (Poindexter 1981). Even so, bulk soils may contain 10^9 microorganisms g^{-1}, and Clarholm and Rosswall (1980) showed that in forest soils the normal metabolism of this population is more than sufficient to account for all the organic C accession each year.

Leaves are often well colonised by microorganisms before they fall onto the soil surface, and the cells are soon broken down by removal of the cellulose- and hemicellulose-rich cell-wall layers, leaving the rather resistant, lignin-impregnated remnants such as the middle and terminal lamellae of cell walls (Kilbertus and Reisinger 1975; Martinez et al. 1980; Foster 1986). Waterlogged soils may contain large amounts of these more stable remnants (Foster 1981 d).

Transmission electron micrographs of soils show that isolated microorganisms occur in both organic and compact clay fabrics, but that most occur in small colonies, usually composed of one type of cell (Fig. 1). Although amorphous osmiophillic materials rich in humic substances contain low populations of small bacteria (Foster 1986), most colonies of bacteria in soils are associated with cells, wall remnants especially those still containing non-lignified, carbohydrate-rich lamellae (Foster 1981 d, 1986). Colonies of different types of bacteria are distinguished by differences in their size, cell-wall fine structure (Gram positive, Gram negative), cytoplasmic ultrastructure, and the nature of the extracellular polysaccharides. The bacterial extracellular polysaccharides in soils may be granular or fibrillar (Foster 1981 b, 1986). They may be largely composed of substituted carbohydrates that stain with lanthanum, ruthenium red or OsO_4-alcian blue complexes, or contain neutral carbohydrates which can be detected with the periodic acid silver methenamine and thiosemicarbazide silver proteinate reagents. In some colonies the walls of the individual cells stain, in other only the extracellular polysaccharides (Fig. 7). In other colonies the extracellular polysaccharides enclosing the whole colony stain, but not those surrounding the individual cells (Fig. 8). These staining patterns may be characteristic of different species of microorganisms; alternatively, the various microenvironments occurring in different microsites may induce the secretion of chemically different extracellular polysaccharides by the same species.

As in individual aggregates (Kilbertus 1980), bacteria in the bulk soil may occur in pores which have multiple or only single entrances. Sometimes bacteria appear to be completely enclosed in a clay fabric, perhaps trapped there as the clay swelled or by mechanical compaction through growth of nearby roots or the movement of soil animals. As in aggregates, bacteria in pores in the bulk soil do not fill the pore void. There is always a space around individual bacteria presumably to allow the free diffusion of water, and soluble nutrients and to allow gas exchange. Sections of voids in bulk soils often have highly irregular outlines and are connected with narrow, convoluted channels (Figs. 1,3,4,9). Comparison with nearby deposits of plant tissues suggests that some of these voids are formed by the decay of cell-wall remnants. Transmission electron microscopy projects all the mineral particles in a section onto one plane: In scanning electron microscopy the three-dimensional arrangement of the particles is seen more clearly (Fig. 10).

Treatment with chloroform, as in the Jenkinson biomass method (Jenkinson and Powlson 1976),



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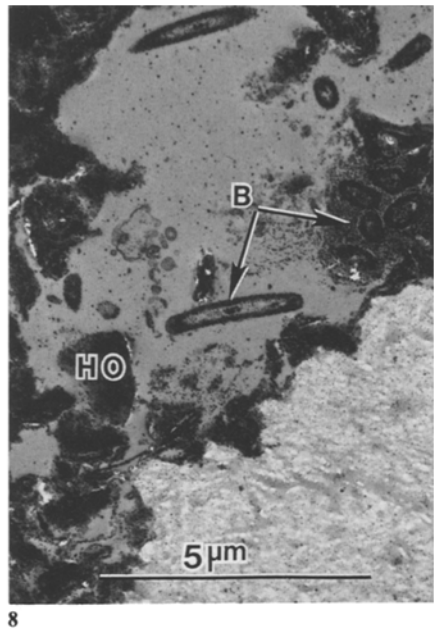

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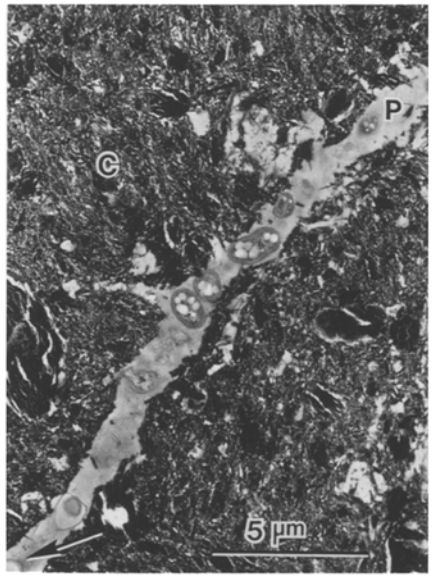
Fig. 7. Soil section. Some much-folded cell-wall lamellae stain intensely (*arrows*). Scattered low-intensity staining throughout the organic matter. F+MS only

Fig. 8. Soil section. Most of the organic matter reacts, especially bacterial cell walls and cell-wall remnants. F+PAMS



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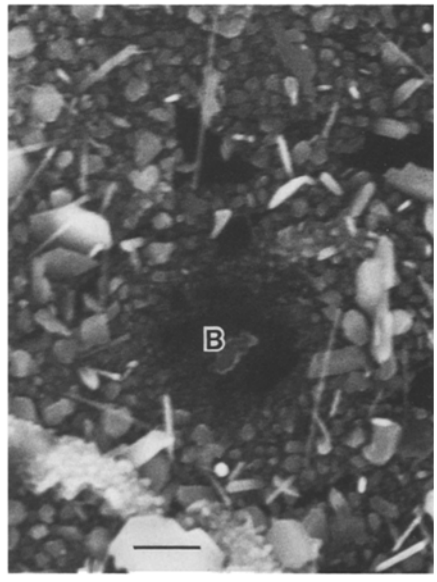

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Fig. 9. Bacteria in pore in clay aggregate after chloroform treatment. Bacteria deep in pore survive; those near entrance are lysed (*arrow*). G/Os/Pb

Fig. 10. Backscatter scanning electron micrograph of ultrathin section of soil, showing arrangement of minerals around pore containing a microorganism. G/Os

showed that whereas the bacteria near the entrances of deep pores were lysed, so that in electron micrographs of sections they were represented by cell-wall remnants (ghosts), those in the interiors of the pores survived (Fig. 9). This suggests that deep pores like mucilages (Martin and Foster 1985) are safe havens for bacteria and may be sites where non-sporulating Gram-negative species survive transient and even long-term unfavourable conditions. Individual bacteria and whole colonies are commonly coated with clay platelets (Kilbertus and Reisinger 1975; Foster and Rovira 1978; Foster et al. 1983). These are usually attached face-on, but occasionally they assume a radial orientation. With colonies, it often appears that an increase in the colony size by cell multiplication or by the secretion of extracellular polysaccharides causes mechanical displacement, compaction and reorientation of nearby clay minerals, to form a dense layer of face-on, overlapping platelets, like tiles on a roof. It has been suggested that as the soil dries, surface tension may draw these clay layers closer and closer together to produce a very convoluted pathway to the exterior. This may further reduce water loss from the colony and, by restricting the diffusion of O_2 and CO_2, may so slow the metabolism of the colony within, that energy

resources are conserved until the return of more favourable conditions.

Bacteria in the bulk soil are generally smaller than those in the rhizosphere. Thus Bae et al. (1972) showed that 63% of soil bacteria are less than 0.3 µm in diameter, but Foster (1985) found that only 20% of more than 1100 rhizosphere bacteria fell into this size range. Again, Bae et al. found that only 6% of the cells were greater than 0.5 µm in diameter whereas 30% of the rhizosphere bacteria were more than 0.5 µm wide. This may reflect the generally oligotrophic conditions in the bulk soil or the fact that the bulk soil is dominated by *Bacillus* and *Arthrobacter* spp. (Poindexter 1981) while pseudomonads are more common in the rhizosphere.

Animal residues and their microorganisms. Microarthropods are common constituents of soils, and their cast-off exuviae are commonly found in soil sections, recognisable by their shape, size and lamellate ultrastructure (Foster and Martin 1981). Occasional eggs are encountered (Fig. 1) and, rarely, whole animals with ingested soil in their guts (Foster 1986). In surface soils faecal pellets are commonly encountered (Fig. 1; Foster and Martin 1981). These stain densely with OsO_4, presumably because of the enzymes secreted into the gut, and may contain plant-cell debris in which subcellular organelles, e.g. chloroplasts, are still recognisable (Foster and Martin 1981; Foster 1986). Other faecal pellets are filled with masses of bacteria (Fig. 1) forming local populations as dense as or even denser than those in the rhizosphere. Usually, however, there appears to be a smaller range of size and form than in the rhizosphere. Faecal pellets are often accompanied by satellite colonies of microorganisms in the nearby soil, which presumably depend on materials leaching out of the faecal mass (Foster and Martin 1981).

Location of substrates

Humified plant debris

Humified materials are here defined for histochemical convencience as those materials which are wholly or partially composed of OsO_4-reactive materials, such as proteins and polyphenolics (Bland et al. 1971). They also stain with silver methenamine and silver proteinate in the absence of periodate oxidation (cf. Figs. 11 and 12), and after treatment with aldehyde-blocking agents or agents which destroy sulphhydryl groups. They fall into three classes:

1. Morphologically recognisable cell-wall remnants;
2. fibrous materials linking aggregate components;
3. granular and amorphous materials within aggregates and clay fabrics.

Cell-wall remnants. Most plant-cell walls are multilamellate, with the lignin-rich middle lamella and terminal lamella separated by several wall layers rich in carbohydrate, the microfibrils of which pass at different directions to the cell axis, giving the layers their characteristic mechanical and optical properties. Wall remnants may adsorb polyphenols from the surrounding soil, since they often appear more electron-dense in soil sections than walls with similar dimensions and the fine structure still within tissues. As carbohydrates are removed by microbial enzymes, polyphenolic groups are released, so that after OsO_4 staining the cell wall becomes more and more electron-dense. In waterlogged soils a major part of the fabric may comprise highly convoluted, electron-dense cell wall-remnants (Foster 1981 d; Foster et al. 1983). Eventually only the resistant terminal and middle lamellae may be left, to give complex multilayered structures.

Most aggregates contain some cell-wall remnants that are recognisable on morphological, cytological, or histochemical grounds (Kilbertus 1980; Foster et al. 1983).

Fibrous materials. Within the secondary wall layers, lignin is deposited between the cellulose fibrils to form a continuous, fibrous, three-dimensional network. With the enzymatic removal of the carbohydrate, these networks may be left in the soil, and in OsO_4-treated aggregates the various mineral and organic constituents, including soil microorganisms, may be linked by similar fibrous networks (Foster 1981 a; Foster et al. 1983).

Granular and amorphous materials. In other places granular, or almost amorphous, electron-dense materials are observed after OsO_4 staining, and some have a few small bacteria embedded in them. These granular deposits are especially common in organic-rich soils such as those in compost heaps. The individual granules may be only 5 nm in diameter and since carbohydrates do not stain with OsO_4, whereas polyphenols and proteins do, these materials are probably similar to those derived from fulvic and humic acids by Chen and Schnitzer (1976).

Soil carbohydrates

Between 50% and 80% of the dry weight of plants consists of polysaccharides, so that carbohydrate is the most common class of organic matter entering soils. Although most polysaccharides are rapidly metabolised by soil microorganisms, even so, carbohydrates constitute between 5% and 10% of soil organic matter (Jenkinson and Ladd 1981). Besides

providing a readily available energy source for heterotrophic soil microorganisms, carbohydrates are important to aggregate stability (Sparling and Cheshire 1985) and to the ion-exchange properties of soils. The extracellular polysaccharides secreted by roots and microorganisms may help to protect them from the deleterious effects of excesses of metal ions such as Al (Mugwira and Elgawhary 1979) and Pb (Tyler 1981). Finch et al. (1971) and Griffin (1981) believe that the gels secreted by roots and microorganisms act as reservoirs of water.

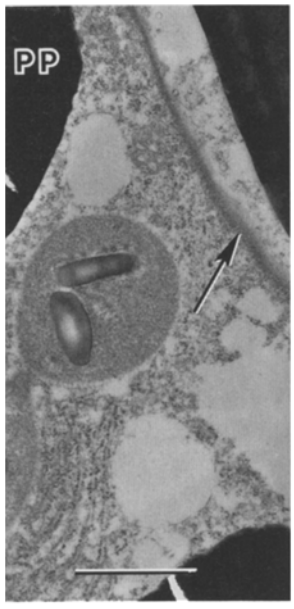
Since simple carbohydrates are readily extracted from soils and the mechanical disturbance of a soil can increase soil respiration (Rovira and Greacen 1957; Powlson 1980), it is evident that normally some soil polysaccharides are protected from microbial attack. Jenkinson and Powlson (1976) have suggested that the polysaccharides may be both physically protected by the simple spatial separation of organisms from suitable substrates and chemically protected through the induration of the carbohydrates by materials that inhibit enzyme action, e.g. polyphenolics or metal ions. Where are these protected polysaccharides? What form do they take?

Substituted carbohydrates (Ru/Os, Alcian/La, La/Os, colloidal Fe-reactive). These are characteristic of middle lamellae, and of the extracellular polysaccharides secreted by roots and soil bacteria and fungi. They are generally electron-transparent and therefore invisible unless stained with one of the above reagents, though in a compact clay their presence may be deduced from the otherwise unaccountable voids between the root or microorganisms and the surrounding clay fabric (Foster 1981 a; Foster and Martin 1981). After staining, the extracellular polysaccharides are revealed as electron-dense fibrils or granules, depending on the type of microorganism or species of root. In some cases the gel secreted by a soil microorganism exceeds the cell diameter (Foster et al. 1983). Thick layers of gel are secreted by cap and epidermal cells and root hairs of most species. Soil minerals, especially clays, become adsorbed face-on to these gels, and in a compact soil the gel penetrates between and often completely surrounds the nearby soil particles (Foster 1981 b; Foster and Martin 1981; Foster et al. 1983). Isolated pockets of granular gels, some less than 0.5 μm in diameter, can be located in soils completely enclosed by clay (Foster 1981 a). These probably constitute part of the physically protected soil organic matter, since soil bacteria are unable to penetrate such small voids. How far exogenous microbial enzymes can penetrate the clay fabric without becoming adsorbed onto mineral surfaces is unknown: The surface of these deposits may be amenable to erosion by the enzymes.

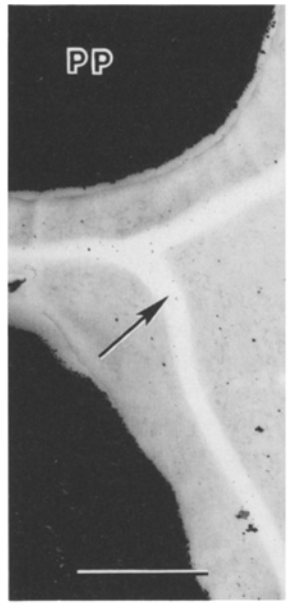
In aqueous environments, fibrous gels are often produced by microorganisms and become attached to the substrate (Fletcher and Floodgate 1973), and the same may be true in soils (Barclay and Lewin 1985). Bacterial extracellular polysaccharides often fill the spaces surrounding the cells, and fibrils can be seen linking the components of aggregates after Ru/Os treatment. Even after the cells of a bacterial colony have autolysed their extracellular polysaccharides may continue to hold the components of an aggregate together (Foster and Martin 1981; Foster 1985). Because the fibrils are so fine (7 – 10 nm diameter), a small mass of polysaccharide will comprise a large length of fibre, and this may explain why such small quantities of microbial polysaccharide (0.02% – 0.2%; Toogood and Lynch 1959) are so effective in stabilising clay fabrics (Foster 1981 c).

Neutral (structural) carbohydrates (periodic acid silver methenamine and thiosemicarbazide silver-proteinate reactive). Neutral polysaccharides are the main structural components of cell walls of both plants and soil microornaisms, but they are also present in the extracellular polysaccharides of many roots, bacteria, and fungi. Traditionally, neutral carbohydrates have been detected histochemically by the periodic acid silver methenamine and thiosemicarbazide silver-proteinate reagents, but unfortunately both these agents also react with polyphenols. Since polyphenolics are secreted into carbohydrate-rich cell walls, the interference by polyphenolics is not easily detected. However, in differentiating *Pinus radiata* roots, polyphenols are secreted into the vacuoles (Fig. 11). These vacuolar deposits give a dense reaction with silver methenamine, whether the sections have been pretreated with periodic acid or not and whether they have been treated with the aldehyde-blocking agent dimedone or not. Carbohydrates in cell walls and amyloplasts only react after periodate and do not react after dimedone (Figs. 11 and 12).

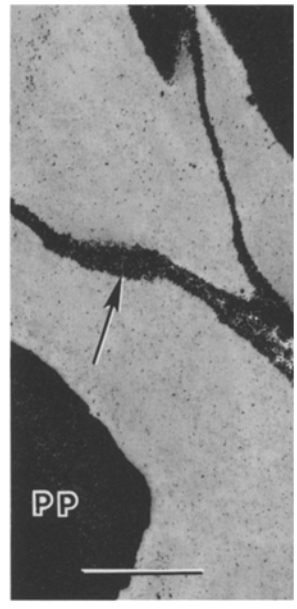
Treatment of ultrathin sections of soil fabrics by either periodic acid silver methenamine or periodic acid thiosemicarbazide silver-proteinate gives a remarkable transformation in the appearance of the soil fabric. First, carbohydrate-rich layers in cell-wall remnants, which are only faintly stained by OsO_4, now appear intensely stained (Fig. 13). Thus the walls of bacteria and fungi are intensely electron-dense, whereas they are faint after OsO_4, silver methenamine, or silver proteinate alone. The individual granules composing some extracellular polysaccharides also stain densely, so that lysis zones caused



11



12



13

Fig. 11. *Pinus radiata* root tissue. Conventional G/Os/Pb treatment showing polyphenol deposits in vacuoles. Cell wall slightly stained (*arrow*). *Scale bar:* 1 micron

Fig. 12. *Pinus radiata* root tissue. Only the polyphenols in the vacuoles react. Cell wall unstained (*arrow*). F + MS. *Scale bar:* 1 micron

Fig. 13. *Pinus radiata* root tissue. Carbohydrates in the cell walls react as well as the vacuolar polyphenols (*arrow*). F + PAMS. *Scale bar:* 1 micron

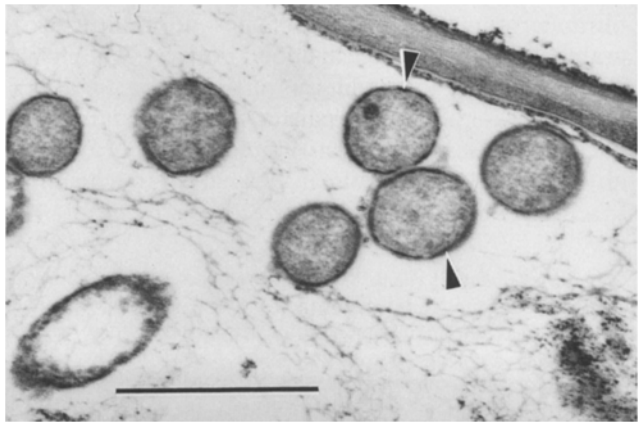


Fig. 14. Soil section tested for ATPase. Electron-dense deposit in periplasmic space of soil bacteria (*arrow*). Not all cells react. ATPase. *Scale bar:* 1 micron

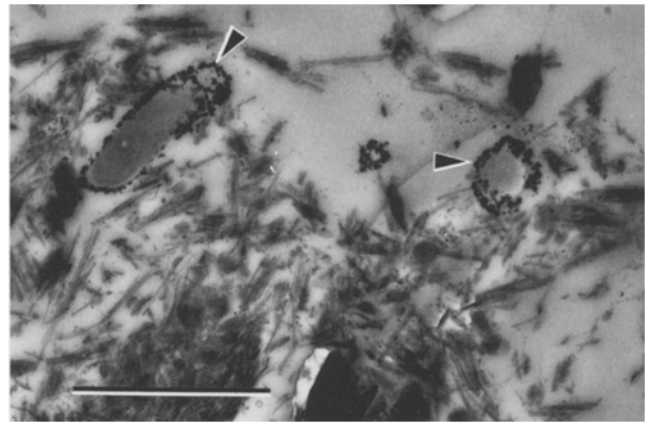


Fig. 15. Soil section tested for acid phosphatase. Deposit of lead phosphate associated with bacteria (*arrows*). APase. *Scale bar:* 1 micron

by microbial enzymes become clearly visible (Foster and Martin 1981). Many small voids 0.5 – 1 µm in diameter, which appear to be empty after conventional staining, are now seen to be filled with electron-dense materials (Foster 1981 b; Foster et al. 1983). Most important, almost all the clay particles are found to be coated with a fine layer of electron-dense material, both without oxidation (polyphenols; Fig. 7) and after oxidation (polyphenols and carbohydrates; Fig. 8; Foster 1981 a, b; Foster et al. 1983).

The fact that most clay particles are coated with carbohydrate has been confirmed by treating clays with periodic acid silver methenamine, periodic acid thiosemicarbazide silver-proteinate, and gold-labelled lectins (Foster 1986). Since, as in sectioned material, not all the clay platelets react, it is not possible that the observed result is due to a non-specific reaction between the clay surface and the heavy-metal tag.

Soil enzymes

Twenty years ago Martinez and McLaren (1966) remarked that although hundreds of reports dealing with soil enzymes had been published, their origin and location in soils was still as obscure as at the turn of the century. Despite 20 years' more work, the same is still true today. Burns (1982) has suggested at least four possible locations for enzymes in soil, viz. in the living cells, inside or adsorbed onto cell-wall fragments, associated with clay minerals, and adsorbed onto or forming co-polymers with polyphenolics in humic materials. Ultrahistochemical methods are still

largely dependent on precipitation of electron-dense, heavy-metal complexes. Unfortunately the soluble heavy-metal components in the enzyme-specific media tend to complex with humified organic matter, whether an enzyme is present or not. Enzymes such as phosphatases (e.g. ATPase, Fig. 14, and acid phosphatase, Fig. 15), peroxidase, catalase, succinic dehydrogenase, etc. have been located in soil microorganisms and root cells by heavy-metal precipitation techniques (Gianinazzi et al. 1979; Foster 1981 d; Foster and Martin 1981; Gianinazzi et al. 1983; Heritage and Foster 1984; Foster 1985). Moreover, the absence of precipitates in substrate-free controls, or in controls in which specific inhibitors have been added (e.g. NaF for phosphatase, KCN for peroxidases), have demonstrated that in cells and tissues these reactions are enzyme-specific. However, non-specific heavy metal adsorption has led to false-positive results in soil organisms in the control treatment.

Future possibilities

The capacity to locate microorganisms, substrates, and enzymes at high resolution and with high specificity should theoretically make it possible to locate the sites where specific soil processes take place and, conversely, sites where such reactions are unlikely. Unfortunately, as discussed above, both substrate and enzyme localization in soils has largely been restricted to rhizospheres (Foster 1981 a; Heritage and Foster 1984; Foster 1986) and to mycorrhizas (Gianinazzi et al. 1979, 1983) and mycelial strands (Foster 1981 b). Moreover, studies have largely been limited to a very few enzymes (phosphatase, peroxidases) and to very few substrates (carbohydrates, polyphenolics).

Three technical difficulties limit the specific location of enzymes and substrates in soils by conventional histochemical methods. First, most of the ultracytological stains presently used require heavy metals to confer electron density on the reaction products, and heavy metals are often non-specifically adsorbed onto both mineral and organic soil components (Tyler 1981; discussion in Foster and Martin 1981) giving false-positive results in the substrate-free or enzyme-inhibited controls. Unequivocal results are limited to cells (living or dead) where there is evidence *on other grounds* that the enzyme could occur, and where non-specific reactions can be detected on cytological grounds.

A second difficulty arises from the necessity of using different specimens for the full enzyme treatment and the control treatments. In tissues, comparisons between full and control treatments are easily made, because tissues are organised so that cytologically similar sites are easy to locate and identify in different samples. This is not the case with soil samples where the various components are more or less randomly distributed. Similar difficulties with controls also apply to the identification of non-enzyme components, such as carbohydrates and polyphenols.

However, a combination of transmission electron microscopy and electron probe microanalysis will allow the use of elements in enzyme reactions which are not electron-dense and/or which are not so prone to react non-specifically with soil minerals and organics. Thus, in tissues, the use of cerium as a trapping agent for phosphatases has proved more specific than the use of lead (Robinson and Karnovsky 1983). Gold-tagged lectin-labelling for carbohydrates and glycoproteins (Foster 1986) and the use of gold-labelled antibody techniques should overcome some of these difficulties.

A third major difficulty arises from the fact that ultramicrotomy is limited to sites devoid of sand grains, which damage diamond knives. The most promising way to avoid this difficulty is to apply histochemical tests to polished soil blocks and to use back scatter scanning electron microscopy (Taylor et al. 1984) or electron probe microanalysis to detect the specific products of reactions.

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References

Alexander M (1961) Introduction to soil microbiology. Wiley, New York

Bae HC, Cota-Robles EH, Casida LE (1972) Microflora of soil as viewed by transmission electron microscopy. Appl Microbiol 23:637 – 648

Barber DA, Martin JK (1976) The release of organic substances by cereal roots into soils. New Phytol 76:69 – 80

Barclay WR, Lewin RA (1985) Microalgal polysaccharide production for the conditioning of agricultural soils. Plant Soil 88:159 – 169

Barley KP (1970) The configuration of the root system in relation to nutrient uptake. Adv Agron 22:159 – 201

Berthelin J (1984) Microbial weathering. In: Krumbein WE (ed) Microbial geochemistry. Blackwell Oxford, pp 223 – 262

Berthelin J, Leyval C (1982) Ability of symbiotic and non-symbiotic rhizospheric microflora of maize (*Zea mays*) to weather micas and to promote plant growth and plant nutrition. Plant Soil 68:369 – 377

Binns ES (1980) Field and laboratory observations on the substrates of the mushroom fungus gnat *Lycoriella auripila* (Diptera: Sciaridae). Ann Appl Biol 96:143 – 152

Bisdom EBA (1983) Submicroscopic examination of soils. Adv Agron 36:55 – 96

Bland DE, Foster RC, Logan AF (1971) The mechanism of permanganate and osmium tetroxide fixation and the distribution of lignin in the wall of *Pinus radiata*. Holzforschung 25:137 – 143

Buckley R (1982) Sand rhizosheath of an arid zone grass. Plant Soil 66:417 – 421

Burns RG (1982) Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol Biochem 14:423 – 428

Campbell R, Porter R (1982) Low temperature scanning electron microscopy of microorganisms on soil. Soil Biol Biochem 14:241 – 245

Chakraborty S, Old KM, Warcup JH (1983) Amoebae from a take-all suppressive soil which feed on *Gaeumannnomyces graminis* var *tritici* and other soil fungi. Soil Biol Biochem 15:17 – 24

Chen Y, Schnitzer M (1976) Scanning electron microscopy of humic acid and of a fulvic acid and its metal and clay complexes. Soil Sci Soc Am Proc 40:682 – 686

Clarholm M (1985) Possible roles for roots bacteria, protozoa and fungi in supplying nitrogen to plants. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil: Plants, microbes and animals. Blackwell, Oxford, pp 355 – 365

Clarholm M, Rosswall T (1980) Biomass and turnover of bacteria in a forest soil and a peat. Soil Biol Biochem 12:49 – 57

Cromack K, Sollins P, Graustein WC, Speidel K, Todd AW, Spycher G, Li CY, Todd RL (1979) Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biol Biochem 11:463 – 468

Darbyshire JF, Greaves MP (1967) Protozoa and bacteria in the rhizosphere of *Sinapsis alba* L., *Trifolium repens* L. and *Lolium perenne* L. Can J Microbiol 13:1057 – 1068

Deacon JW, Mitchell RT (1985) Comparison of rates of natural senescence of the root cortex of wheat (with and without mildew infection), barley, oats and rye. Plant Soil 84:129 – 131

Dickinson NM (1982) Investigations and measurement of root turnover in semi-permanent grassland. Rev Ecol Biol Sol 19:307 – 314

Eckhardt FEW (1985) Solubilization, transport and deposition of mineral cations by microorganisms – efficient rock weathering agents. In: Drever JL (ed) The chemistry of weathering. Reidal, pp 161 – 173

Finch P, Hayes MHB, Stacey M (1971) The biochemistry of soil polysaccharides. In: McLaren AD, Skujins J (eds) Soil biochemistry, vol 2. Dekker, New York, pp 257 – 319

Flaig W, Beutelspacher H, Rietz E (1975) Chemical composition and physical properties of humic substances. In: Gieseking JE (ed) Soil components, vol 1, Organic components. Springer, Berlin Heidelberg New York, pp 1 – 211

Fletcher M, Floodgate GD (1973) An electron-microscopic demonstration of the acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J Gen Microbiol 74: 325 – 334

Foster RC (1978) Ultramicromorphology of some South Australian soils. In: Emerson WW, Bond RD, Dexter AR (eds) Modification of soil structure. Wiley, New York, pp 103 – 109

Foster RC (1981 a) Polysaccharides in soil fabrics. Science 214: 665 – 667

Foster RC (1981 b) The ultrastructure and histochemistry of the rhizosphere. New Phytol 89:263 – 273

Foster RC (1981 c) Mycelial strands of *Pinus radiata* D Don: Ultrastructure and histochemistry. New Phytol 88:705 – 712

Foster RC (1981 d) Localization of organic materials in situ in ultrathin sections of natural soil fabrics by using cytochemical techniques. In: Bisdom EBA (ed) Submicroscopic techniques as applied to thin sections of undisturbed soil materials. Pudoc Press, Wageningen, Holland, pp 309 – 317

Foster RC (1985) The biology of the rhizosphere. In: Parker CA, Rovira AD, Moore KJ, Wong PTW, Kollmorgen JF (eds) Ecology and management of soil borne plant pathogens. Am Phytopath Soc, St Paul, Minnesota, pp 75 – 79

Foster RC (1986) In situ identification of organic materials in soils. Questiones Entomologicae 21:609 – 633

Foster RC, Bowen GD (1982) Plant surfaces and bacterial growth: The rhizosphere and rhizoplane. In: Mount MS, Lacy GH (eds) Phytopathogenic prokaryotes, vol 1. Academic Press, New York, pp 159 – 185

Foster RC, Marks GC (1967) Observations on the mycorrhizas of forest trees. II. The rhizosphere of *Pinus radiata* D. Don. Aust J Biol Sci 20:915 – 926

Foster RC, Martin JK (1981) *In situ* analysis of soil components of biological origin. In: Paul EA, Ladd JN (eds) Soil biochemistry, vol 5. Dekker, New York, pp 75 – 110

Foster RC, Rovira AD (1978) The ultrastructure of the rhizosphere of *Trifolium subterraneum* L. In: Loutit MW, Miles JAR (eds) Microbial ecology. Springer, Berlin Heidelberg New York, pp 278 – 290

Foster RC, Rovira AD, Cock TW (1983) Ultrastructure of the root-soil interface. Am Phytopath Soc, St Paul, Minn, USA

Frehel C, Ryter A (1982) Electron microscopic cytochemical study of cell-wall polysaccharides in *Bacillus subtilis* and two strains of *Bacillus megaterium*. J Ultrastruct Res 81:66 – 77

Gianinazzi S, Gianinazzi-Pearson V, Dexheimer J (1979) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected by *Glomus mosseae* (Nicol & Gerd). New Phytol 82:127 – 132

Gianinazzi S, Dexheimer J, Gianinazzi-Pearson V, Marx C (1983) Role of the host – arbuscule interface in the VA mycorrhizal symbiosis: Ultracytological studies of the processes involved in phosphate and carbohydrate exchange. Plant Soil 71: 211 – 215

Greenland DJ, Oades JM (1975) Saccharides. In: Gieseking JE (ed) Soil components, vol 1, Organic components. Springer, Berlin Heidelberg New York, pp 213 – 261

Griffin DM (1981) Water and microbial stress. In: Alexander M (ed) Advances in microbial ecology, vol 5. Plenum, New York, pp 91 – 136

Hattori T, Hattori R (1976) The physical environment in soil microbiology: An attempt to extend the principles of microbiology to soil microorganisms. Crit Rev Microbiol 4:423 – 461

Head GC (1967) Studies of growing roots by time-lapse cinematography. Trans 9th Int Congr Soil Sci, vol 1, pp 751 – 758

Henry CM, Deacon JW (1981) Natural (non-pathogenic) death of the cortex of wheat and barley seminal roots, as evidenced by nuclear staining with acridine orange. Plant Soil 60:255 – 274

Heritage AD, Foster RC (1984) Catalase and sulfur in the rice rhizosphere: An ultrastructural histochemical demonstration of a symbiotic relationship. Microbiol Ecol 10:115 – 121

Hillis WE, Ishikura N, Foster RC (1968) The role of extractives in the formation of ectrotropic mycorrhizae. Phytochemistry 7: 409 – 410

Hissett R, Gray TRG (1976) Microsites and time changes in soil microbe ecology. In: Anderson JM, MacFadyen A (eds) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell, Oxford, pp 23 – 39

Jenkinson DS, Ladd JN (1981) Microbial biomass in soil: Measurement and turnover. In: Paul EA, Ladd JN (eds) Soil biochemistry, vol 5. Dekker, New York, pp 415 – 471

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol Biochem 8:209 – 213

Jenny H, Grossenbacher KA (1963) Root-soil boundary zones as

seen in the electron microscope. Soil Sci Soc Am Proc 27: 273 – 277
Kilbertus G (1980) Etude des microhabitats contenus dans les aggrégats du sol, leur relation avec la biomasse bactériene et la taille des procaryotes présents. Rev Ecol Biol Sol 17:543 – 557
Kilbertus G, Reisinger O (1975) Dégradation du matériel végétal activité in vitro et in situ de quelques microorganismes. Rev Ecol Biol Sol 12:363 – 374
Knight DP, Lewis PR (1977) General cytochemical methods. In: Lewis PR, Knight DP (eds) Staining methods for sectioned material. Elsevier/North Holland, Amsterdam, pp 77 – 135
Knutson DM, Hutchins AS, Cromack K (1980) The association of calcium oxalate-utilizing *Streptomyces* with conifer ectomycorrhizae. Antonie van Leeuwenhoek; J Microbiol Serol 46: 611 – 619
Ladd JN, Paul EA (1973) Changes in enzymatic activity and distribution of acid-soluble, amino acid-nitrogen in soil during nitrogen immobilization and mineralization. Soil Biol Biochem 5:825 – 840
Ladd JN, Oades JM, Amato M (1981) Microbial biomass formed from ^{14}C, ^{15}N-labelled plant material decomposing in soils in the field. Soil Biol Biochem 13:119 – 126
Lyda SD (1981) Alleviating pathogen stress. In: Arkin GF, Taylor HM (eds) Modifying the root environment to reduce crop stress. ASAE monograph No 4. Am Soc Agric Eng, Michigan, pp 195 – 214
Luft JH (1976) The structure and properties of the cell surface coat. Int Rev Cytol 45:291 – 382
Lundgren B (1984) Size classification of soil bacteria: Effects on microscopically estimated biovolumes. Soil Biol Biochem 16: 283 – 284
Malajczuk N (1979a) Biological suppression of *Phytophthora cinnamomi* in eucalypts and avocados in Australia. In: Schippers B, Gams W (eds) Soil-borne plant pathogens. Academic Press, London, pp 635 – 652
Malajczuk N (1979b) The microflora of unsuberized roots of *Eucalyptus calophylla* R. Br and *E. marginata* Donn ex Sm. Seedlings grown in soils suppressive and conducive to *Phytophthora cinnamomi*: II. Mycorrhizal roots and associated microflora. Aust J Bot 27:255 – 272
Malajczuk N, Cromack K (1982) Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. New Phytol 92:527 – 531
Marinozzi V (1961) Silver impregnation of ultrathin sections for electron microscopy. J Biophys Biochem Cytol 9:121 – 133
Marks GC, Foster RC (1973) Structure, morphogenesis and ultrastructure of ectomycorrhizae. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae: Their ecology and physiology. Academic Press, New York, pp 1 – 41
Martin JK (1977) Factors influencing the loss of organic carbon from wheat roots. Soil Biol Biochem 9:1 – 9
Martin JK, Foster RC (1985) A model system for studying the biochemistry and biology of the root-soil interface. Soil Biol Biochem 17:261 – 269
Martin JK, Puckridge DW (1982) Carbon flow through the rhizosphere of wheat crops in South Australia. In: Galbally IE, Freney JR (eds) The cycling of carbon, nitrogen, sulfur and phosphorus in terrestrial and aquatic ecosystems. Austr Acad Sci, Canberra, Australia, pp 77 – 82
Martinez R, McLaren AD (1966) Some factors influencing the determination of phosphatase activity in native soils and in soils sterilized by irradiation. Enzymologia 31:22 – 38
Martinez AT, Montez C, Toutain F, Mangenot F (1980) Influence d'épaisseur de la litière et du type sol sur les processus de biodégradation de feuilles de hêtre. Rev Ecol Biol Sol 17:307 – 325
Mugwira LM, Elgawhary SM (1979) Aluminum accumulation and tolerance of triticale and wheat in relation to root cation exchange capacity. Soil Sci Soc Am Proc 43:736 – 740
Oades JM, Ladd JN (1977) Biochemical properties: Carbon and nitrogen metabolism. In: Russel JS, Greacen EL (eds) Soil factors in crop production in a semi-arid environment. University of Queensland Press, Australia, pp 127 – 160
Pears AGE (1972) Histochemistry: Theoretical and applied, vol 1, 3rd edn. Churchill, London
Pickett-Heaps JD (1967) Preliminary attempts at ultrastructural polysaccharide localization in root tip cells. J Histochem Cytochem 15:442 – 455
Poindexter JS (1981) Oligotrophy: Fast and famine existence. In: Alexander M (ed) Advances in microbial ecology, vol 5. Plenum, New York, pp 63 – 89
Powlson DS (1980) The effects of grinding on microbial and non-microbial organic matter in soil. J Soil Sci 31:77 – 85
Ramsay AJ (1984) Extraction of bacteria from soil: Efficiency of shaking or ultrasonication as indicated by direct counts and autoradiography. Soil Biol Biochem 16:475 – 481
Robinson JM, Karnovsky MJ (1983) Ultrastructural localization of several phosphatases with cerium. J Histochem Cytochem 31:1197 – 1208
Roland JC (1978) General preparation and staining of thin sections. In: Hall JL (ed) Electron microscopy and cytochemistry of plant cells. Elsevier/North Holland, Amsterdam, pp 1 – 62
Rovira AD (1965) Plant root exudates and their influence upon soil microorganisms. In: Baker KF, Snyder WC (eds) Ecology of soil-borne plant pathogens. University of California Press, Berkeley, Los Angeles, pp 170 – 184
Rovira AD, Greacen EL (1957) The effect of aggregate disruption on the activity of microorganisms in soil. Austr J Agric Res 8: 659 – 673
Rybicka K (1981) Simultaneous demonstration of glycogen and protein in glycosomes of cardiac tissue. J Histochem Cytochem 29:4 – 8
Shields JA, Paul EA, Lowe WE, Parkinson D (1973) Turnover of microbial tissue in soil under field conditions. Soil Biol Biochem 5:753 – 764
Skinner FA, Jones PCT, Mollison JE (1952) A comparison of a direct and a plate counting technique for the quantitative estimation of soil microorganisms. J Gen Microbiol 6:261 – 271
Smart P (1974) Electron microscope methods in soil micromorphology. In: Rutherford GK (ed) Soil microscopy. Limestone Press, Kingston, Ontario, pp 190 – 206
Sollins P, Fogel R, Ching YL (1981) Role of low molecular weight organic acids in the inorganic nutrition of fungi and higher plants. In: Wicklow DT, Carrol GC (eds) The fungal community. Dekker, New York, pp 607 – 619
Sparling GP (1985) The soil biomass. In: Vaughan D, Malcolm RE (eds) Soil organic matter and biological activity. Martinus Nijhoff Junk, Dordrecht, pp 223 – 262
Sparling GP, Cheshire MV (1985) Effect of periodate oxidation on the polysaccharide content and microaggregate stability of rhizosphere and non rhizosphere soils. Plant Soil 88:113 – 122
Stout JD, Heal OW (1967) Protozoa. In: Burges A, Raw F (eds) Soil biology. Academic Press, London, pp 149 – 195
Swift JA, Saxton CA (1967) The ultrastructural location of the periodate-Schiffs reactive basement membrane at the dermo-epidermal junctions of human scalp and monkey gingiva. J Ultra Res 17:23 – 33
Taylor JSH, Fawcett JW, Hirst L (1984) The use of backscattered electrons to examine selectively stained nerve fibres in the scanning electron microscope. Stain Technol 59:335 – 341
Thiéry JP (1967) Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. J Microsc (Paris) 6:987 – 1018
Toogood JA, Lynch DL (1959) Effect of cropping systems and fertilizers on mean weight-diameter of aggregates of Breton plot

soil. Can J Soil Sci 83:151 – 156
Tyler G (1981) Heavy metals in soil biology and biochemistry. In: Paul EA, Ladd JN (eds) Soil biochemistry, vol 5. Dekker, New York, pp 371 – 414
Van Vuurde JWL, Schippers B (1980) Bacterial colonization of seminal wheat roots. Soil Biol Biochem 12:559 – 565
Van Vuurde JWL, Kruyswyk CJ, Schippers B (1979) Bacterial colonization of wheat roots in a root-soil model system. In: Schippers B, Gams W (eds) Soil-borne plant pathogens. Academic Press, London, pp 229 – 234
Visser S (1985) Role of the soil invertebrates in determining the composition of soil microbial communities. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil: Plants, microbes and animals. Blackwell, Oxford, pp 297 – 317
Whitehead DC, Buchan H, Hartley RD (1979) Composition and decomposition of roots of ryegrass and red clover. Soil Biol Biochem 11:619 – 628
Woods LE, Cole CV, Elliot ET, Anderson RV, Coleman DC (1982) Nitrogen transformations in soil as affected by bacterial-microfaunal interactions. Soil Biol Biochem 14:93 – 98

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