SOIL MICRO-ORGANISMS, PLANT GROWTH AND PLANT HEALTH

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Abstract: Knowledge of the biological processes in growing media is necessary where soils, whether synthetic potting mixes or natural soils, are to be used to produce vigorous, healthy plants. I describe some of these soil processes and also some electron microscope studies of soils which demonstrate the spatial distribution of micro-organisms in soil in relation to roots, clay, sand and organic matter. This paper deals also with soil-borne root diseases which can be devastating to plant health and are a part of the whole scene of soil biology. If soil is considered in this holistic way, control of root diseases through biological control, soil and root modifications, and bark composting will become a reality.

Soil contains a complex of micro-organisms made up of bacteria, fungi, actinomycetes, algae, and protozoa. These micro-organisms are essential for many soil processes involved in plant growth; some micro-organisms are beneficial to plant growth, some are detrimental, and some are neutral. The aim of this paper is to describe some aspects of the ecology of micro-organisms in soil and a number of the soil biological processes which are of interest to plant propagators working with natural soil, modified soils, or soil mixes.

DISTRIBUTION OF MICRO-ORGANISMS IN SOIL

Micro-organisms in soil depend on organic matter for the energy required for their growth and hence most bacteria and fungi are associated with living or dead plant material. As plant roots grow through soil they release sugars, amino acids, gummy polysaccharides and other compounds into the soil around the root making the root-soil interface a zone of intense activity where bacteria and fungi proliferate (28). Such an active population can influence plant health in many ways, e.g. release of nutrients from soil, production of plant growth hormones, biological control of plant pathogenic fungi, and stimulation and attraction of pathogens.

Electron microscope studies of ultra-thin sections of the root-soil interfaces (9) show colonies of different bacteria growing in contact with each other and in contact with the clay minerals, sand particles and particulate organic matter of the soil (Figure 1).

MICRO-ORGANISMS AND SOIL STRUCTURE

Soil micro-organisms play a major part in maintaining and improving soil structure, which is important in providing a

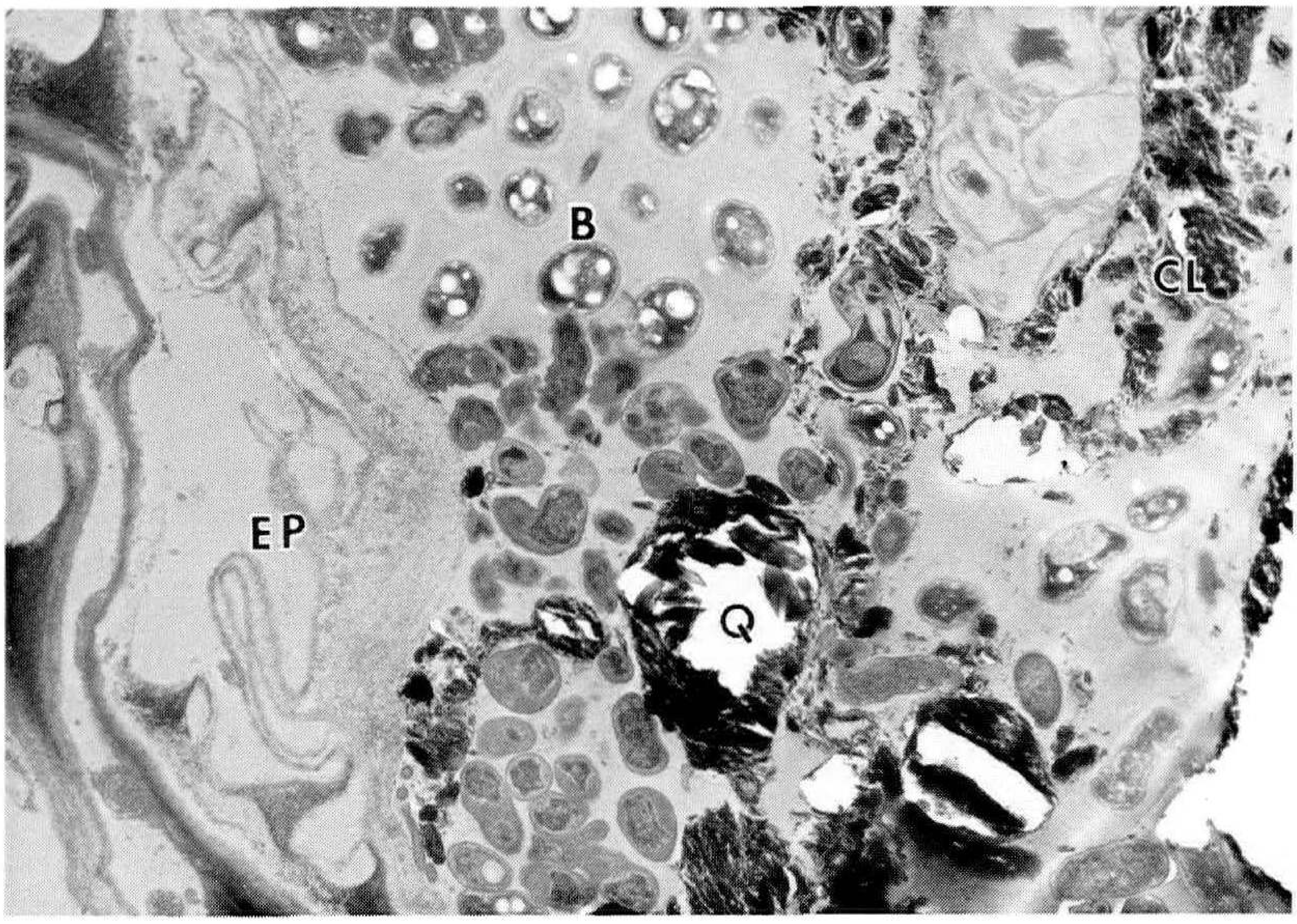

Figure 1. Transmission electron micrograph of a root-soil interface of clover. EP = epidermal cells which are distorted with lysed outer cell walls (cw). B = bacteria. CL = clay. Q = quartz grain. (x6000). Reprinted from: R. C. Foster and A. D. Rovira. The ultrastructure of the rhizosphere of *Trifolium subterraneum* L. in "Microbial Ecology" M. W. Loutiet and J. A. R. Miles, eds 1978. Springer-Verlag, Berlin, Heidelberg.

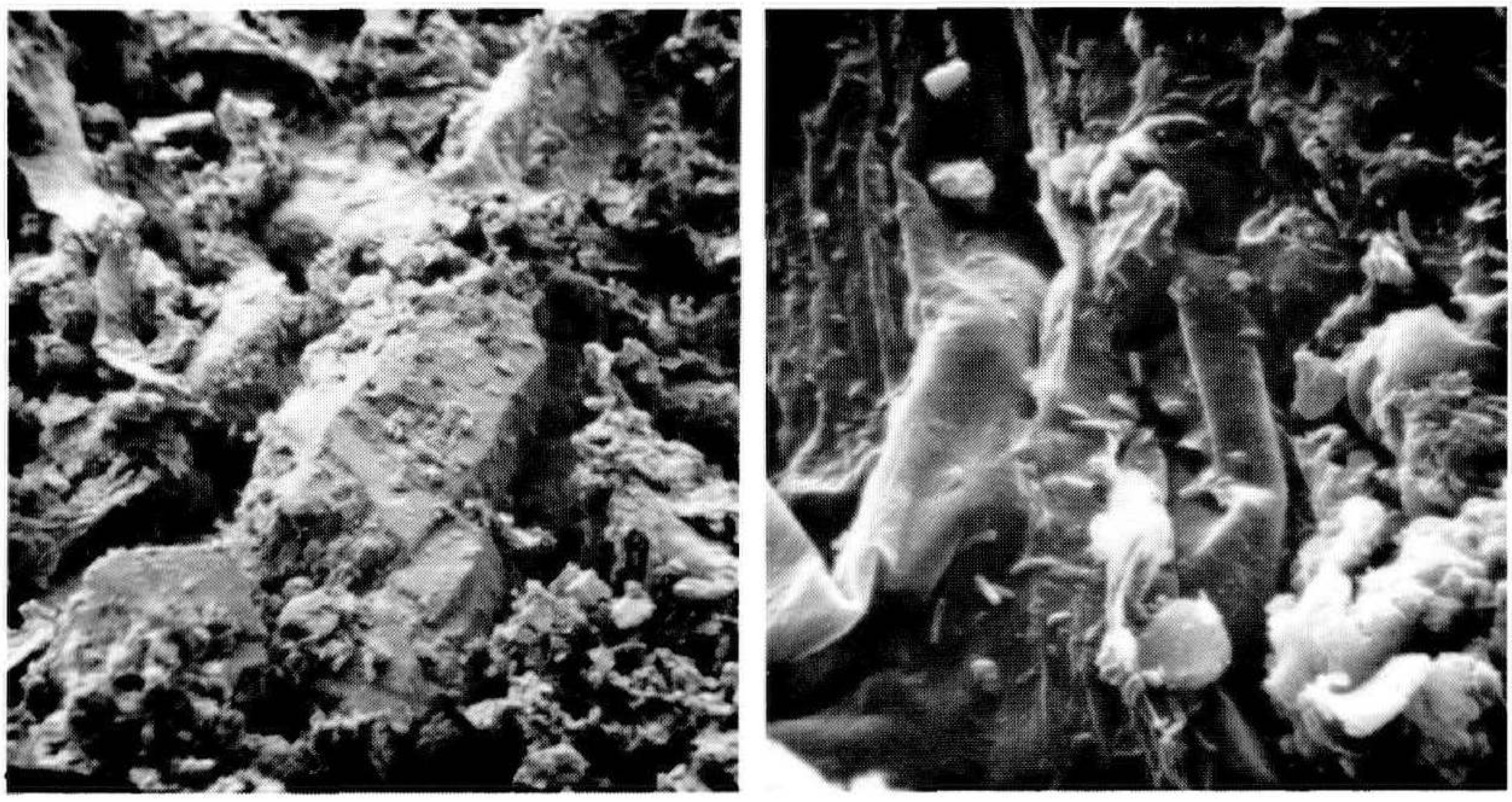

Figure 2. Scanning electron micrograph of roots. A (*Left*). Unwashed wheat root with quartz and clay held to root by the mucigel. (x1000). B (*Right*). Washed clover root surface showing bacteria in mucigel (centre), root hair (left) and clay (right) (x1600). Reprinted from: R. C. Foster, A. D. Rovira and T. W. Cock "Ultrastructure of the root-soil interface". *Amer. Phytopathol. Soc.* St. Paul, Minn. 1983.

favourable environment for plant roots and in reducing soil losses from erosion.

The gummy material, released by plant roots has been called "mucigel" (13). This mucigel plays a vital role in holding together soil aggregates and improving soil structure. Scanning electron micrographs of the root surface shows clay particles and a large quartz grain held together by the mucigel released from the root (Figure 2a) and the mucigel enveloping bacterial cells (Figure 2b).

It is generally found that soil structure is improved more by grass roots than by roots of legumes or crop plants. While much of this structure improvement can be attributed to the fibrous nature of the grass roots and also to the mucigel, recent work has shown that filaments of vesicular-arbuscular mycorrhizal fungi growing out from the roots of grasses play a major part in binding together larger soil aggregates (32). These fungal strands which hold the soil particles together can persist for several months after the grass host for the mycorrhizal fungi has been removed, but soil structure declines with cultivation, the death of these fungi and exposure of soil organic matter to microbial attack.

MICRO-ORGANISMS AND SOIL NITROGEN

Although the bulk of soil nitrogen is in the organic matter, it is mineralized forms, mainly nitrate (NO_3^-) and ammonium (NH_4^+), which are used by plants. The nitrogen cycle (Figure 3) is complex but an understanding of it can help manage the supply of nitrogen available to plants.

Organic nitrogen enters soil either through decomposing plant and animal residues or the fixation (conversion) of gaseous nitrogen to protein nitrogen by free living bacteria, such as *Azotobacter* and *Clostridium*, or through symbiotic associations between plants and bacteria, e.g. legumes and *Rhizobium* or casuarinas and *Frankia*.

The rate of release of mineralized or inorganic nitrogen (nitrate or ammonium) from added residues depends upon the nature and form of the residues. Grass residues are low in nitrogen, and the micro-organisms carrying out the decomposition of such material require extra nitrogen in the decomposition process; this is obtained from the mineral nitrogen in the soil. Legume residues, on the other hand, are high in nitrogen and as they decompose, inorganic nitrogen (which plants use) is released into the soil. However, the rate of release of inorganic nitrogen, i.e. available for uptake by plant roots, is influenced by the nature of the legume residues. Maximum release comes from incorporation of green material but if the residues stay on the soil over summer before incorporation, no mineral nitrogen is released for 60 days after incorporation and, after 110 days, these residues have released less than half the nitrogen released from green legume residues (17).

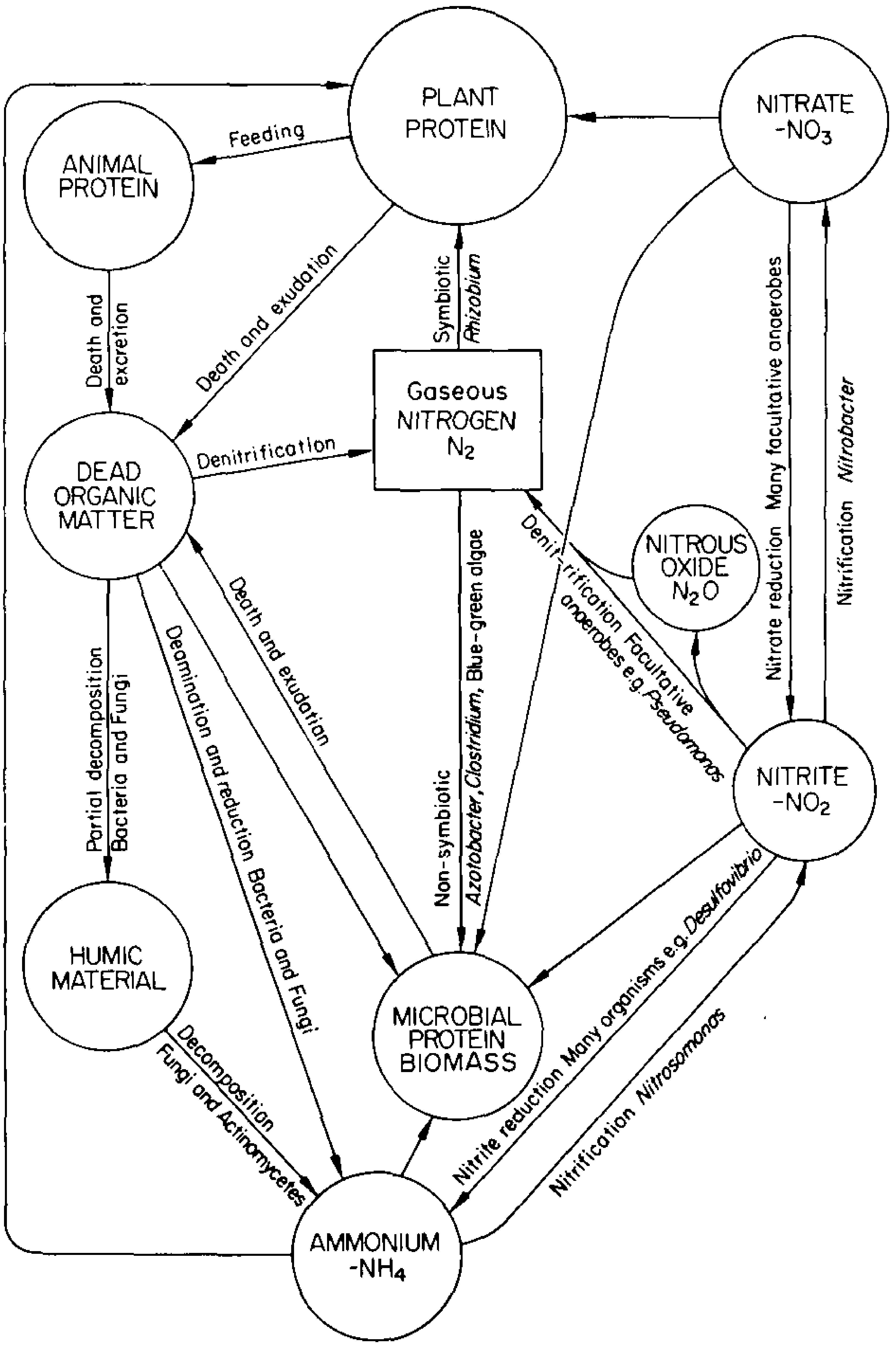

Figure 3. Nitrogen cycle in soil (4).

These results on the release of inorganic nitrogen from legume residues were obtained under the winter rainfall conditions of southern Australia; patterns of nitrogen release differ in different environments, e.g. the rate of breakdown of plant residues in soil doubles for each 8 to 9°C increase in mean annual air temperature (16).

SYMBIOTIC ASSOCIATIONS BETWEEN PLANTS AND SOIL MICRO-ORGANISMS

Mycorrhizal Fungi. These fungi form beneficial symbiotic associations with plant roots and improve plant growth by acting as extensions of the root systems and increasing the uptake of nutrients such as phosphate and trace elements. The nature of these associations in nursery-grown plants have been reviewed recently (7,19). Mycorrhizal associations form best under low nutrient conditions and their formation may be inhibited by high levels of fertilizer. If such plants with poor mycorrhizas are transplanted to the field with low nutrient levels poor plant growth would be expected. One strategy to prevent this inhibition of mycorrhizal formation in nursery stock is to use slow-release fertilizers (21). The benefits of introducing spores of mycorrhizal fungi into nursery beds of *Pinus radiata* are considerable (Figure 4), e.g. 48 percent and 36 percent increases in growth in fumigated and unfumigated soil, respectively (31).

Figure 4. Response by *Pinus radiata* in fumigated soil to coating of seeds with spores of *Rhizopogon luteolus Left.* Uninoculated. *Right.* Inoculated. Photograph from C. Theodorou, CSIRO Division of Soils.

NITROGEN FIXING MICRO-ORGANISMS

Legume-Rhizobium Association. The benefits of legumes through their nodule formation with the bacterium *Rhizobium* in building up soil nitrogen and thus improving growth of following crops are well known. However, it should be remembered that there are different species of *Rhizobium* for different groups of legumes and that, without the appropriate *Rhizobium,* there is no value in growing legumes for improving soil fertility. Acacias make up an important component of both native forests and ornamental plants in Australia and, as members of the Leguminacae family, form nodules and fix nitrogen with *Rhizobium,* but little research has been done on this association.

Casuarina-Frankia Association. Members of the genus *Casuarina* (common name: she oak) are widespread throughout Australia from foreshore dunes to understories in forests to the arid interior. A feature of these native Australian trees is that they often grow in extremely poor soils. This is made possible by the associations formed between their roots and two groups of beneficial micro-organisms. The first group is the mycorrhizal fungi which extract phosphate, trace elements, and possibly other nutrients from soil and transfer these to the host plant; the second group is the actinomycetes, known as *Frankia*, which form nodules on the roots and convert atmospheric nitrogen to protein as does *Rhizobium* with legumes.

The importance of these two groups of micro-organisms has been demonstrated by growing *Casuarina* in sterilized soil with low nutrient levels, with and without Frankia and mycorrhizal fungi; when applied separately neither organism increased seedling growth but when applied together growth was increased by 100 percent over the uninoculated control (P. Reddell, CSIRO, pers. comm.). One problem with applying *Frankia* is that, unlike *Rhizobium*, it has proved difficult to isolate and grow in laboratory media and, at present, for most *Casuarina* species crushed nodules taken from the same or a compatible species must be used as inoculum.

Figure 5 illustrates the responses of a single species of *Casuarina* to several frankias. Other studies have shown considerable specificity between different casuarinas and different frankias (27).

Figure 5. Responses by Casuarina *equisetifolia* ssp. *incana* to inoculation with different strains of *Frankia*. C = uninoculated control. Inset—Nodule of *Frankia* nodule from *Casuarina*, 1.5 cm in diameter. Photograph from P. Reddell, CSIRO Division of Soils.

SOIL-BORNE ROOT DISEASES

There is little doubt that soil-borne root diseases caused by bacteria, fungi, and nematodes impose a major constraint on plant production from the favourable environment of plant propagation nurseries to field crops to forests. A brief review of the major soil-borne fungal diseases of ornamentals and standard control methods was presented in these Proceedings in 1980 (18). Knowledge of the biology, ecology, and epidemiology of these root-attacking organisms can help in developing adequate control measures.

Soil pasteurization. Soil pasteurization by fumigation or heat, followed by proper hygiene, is the most effective method of reducing losses from root diseases. In view of the finding in nurseries that *Pythium* spp. and *Rhizoctonia solani* have been isolated from the dust which can be wind-borne and reinfest pasteurized soil in containers (30), the introduction of a form of biological buffering into pasteurized soil appears desirable. This is achieved by heating soil to 60°C for 30 minutes with aerated steam, which destroys pathogens but leaves behind sporing bacteria of the genus *Bacillus,* some of which are antagonistic to fungi and can prevent the rampant growth of chance contaminants (1). Further protection of plants can be achieved if, after pasteurization, the soil is inoculated with bacteria, such as *Bacillus subtilis,* known to suppress pathogens (3).

An alternative method of partially pasteurizing soil to leave behind a residual "buffering" microflora and/or introduce suppressive organisms has been achieved by "solarization"—the process whereby moist soil is covered with transparent polyethlene and exposed to the sun during summer (14). It has been demonstrated that build up of the pathogens, *Verticillium dahliae* and *Fusarium oxysporum* f.sp. *dianthi,* in soil treated by solarization is considerably less than in soil fumigated with methyl bromide (19). The effectivess of this method of partial soil pasteurization has been further improved by introducing the fungus, *Trichoderma harzianum,* which is capable of controlling diseases caused by *Sclerotium rolfsii* and *Rhizoctonia solani* (5).

This form of integrated control of root disease, viz. partial pasteurization, followed by inoculation with a bacterial or fungal culture capable of biological control of chance infestations by pathogens has yet to be used by the Australian nursery and plant propagation industries, despite the fact that much of the pioneering research has been conducted in Australia.

Biological Control of Root Diseases. This topic is far too large for comprehensive treatment in this paper, so readers wishing to gain an insight into many examples of biological control are referred to the excellent treatise on this subject by Cook and Baker (6).

Here I shall describe several examples with particular relevance to the horticultural and nursery industries.

Introduction of Biocontrol Organisms. A major problem on ornamental and tree rootstocks until recently was crown gall caused by *Agrobacterium radiobacter* pv. *tumefasciens*. However, the discovery in South Australia that treatment of seed and roots with a non-pathogenic form of *Agrobacterium radiobacter* (strain K84) protects roots from the pathogen is one of the outstanding successes of biological control (15). The non-pathogenic strain K84 is closely related to the pathogen and occupies wounds on roots and the lower stem, which are the usual entry points for the pathogens. Strain K84 also produces Agrocin 84, an antibiotic to which the crown gall strain is sensitive, and this antibiotic plays a major part is excluding the pathogen. It is now standard practice in most nurseries throughout the world to treat rootstocks of susceptible species with Strain K84. Crown gall control provides an example of how a root pathogen can be controlled by a closely related organism.

The control of *Pythium ultimum*, which is responsible for damping off and seedling blight in nursery plants, has been achieved in greenhouse trials by pelleting seed with a related species, *Pythium oligandrum*, which is parasitic on its pathogenic relative (20).

Biological control of Pythium spp. and *Rhizoctonia solani* has been achieved experimentally by bacteria belonging to the fluorescent psuedomonads. These bacteria protect plants by producing antibiotics toxic to the pathogens—a different antibiotic was found to be responsible for the activity against each pathogen (11,12).

The report (33) that the growth of carnation, stock, zinnia, and sunflower was improved when soil was treated with a plant growth promoting strain of *Pseudomonas fluorescens* (possibly through controlling low grade (minor) root pathogens) shows that such manipulation of the soil microflora offers considerable promise to the nursery industry.

Protection. Cross pollination against *Fusarium* wilt of sweet potato has been achieved by treating tubers with non-pathogenic strains of *Fusarium oxysporum*. This preliminary treatment with the non-pathogenic strain probably causes biochemical changes in the host plant; these changes produce resistance products which are translocated to other plant parts, giving protection against the pathogen (25).

A further example of induced protection was reported recently in California when scientists investigated the mechanism by which a particular cultural practice developed by avocado growers protected trees from *Phytophthora cinnamomi*. This cultural practice consists of interplanting citrus (lime) trees in orchards heavily infested with *P. cinnamomi*. Local growers maintain that this interplanting protects the avocado from *P. cinnamomi* and improves production. The research has shown that infection of avocado with *Phytophthora parasitica* (a very mild root pathogen of avocado) which would have been introduced into the orchards with the citrus interplanting, induced within the avocado a systemic protection against *P. cinnamomi* (8). The precise factors responsible for such induced resistance are not known, but with further study the application of such a phenomenon to protect trees from certain root diseases may become a nursery practice.

Modification of the Root Environment. Phytophthora cinnamomi is a devastating disease of avocado and damage by this fungus in the wet year of 1974 destroyed 12,000 of the 40,000 avocado trees in Queensland. In 1969, a 30-year-old plantation of healthy avocado trees was found surrounded by heavily diseased plantations. The practice on this healthy plantation was to grow continuous legume-maize cover crops, plus added poultry manure each year together with applications of dolomite limestone to maintain soil pH above 6.0. An investigation of this "Ashburner system" demonstrated that soil from the plantation was highly suppressive against *P. cinnamomi,* whereas soil from surrounding diseased groves were not suppressive (2,26). The suppressive activity survived 60°C for 30 minutes but not 100°C for 30 minutes, indicating that heat-resistance actinomycetes or spore-forming bacteria were responsible but, so far, no single organism isolated from the soil has reproduced the suppressive effect of the whole soil. Two further factors involved in the control of *P. cinnamomi* in this soil are the high levels of calcium and ammonium which result from the green manuring, fowl manure, and dolomite. Such soil treatments are effective on many avocado plantations, especially when combined with the planting of disease-free trees to minimize initial infection.

Root rot and heart rot of pineapples caused by *P. cinnamomi* were serious problems in Queensland, but are now kept under control by applying sulphur to reduce the pH below 3.9. At this low pH zoosporangium formation by the pathogen is reduced and growth of *Trichoderma viride,* which parasitises *P. cinnamomi,* stimulated (26).

The finding in Western Australia that floristically attractive banksias (which are extremely susceptible to *P. cinnamomi*) could be grafted on to a rootstock of *Banksia integrifolia*, resistant to the pathogen (22), offers an alternative strategy for controlling this root disease which is so devastating to the Australian native flower industry.

Bark Composts and Biological Control. Some composted hardwood bark has been demonstrated to suppress *Rhizoctonia solani, Pythium ultimum,* and *Phytophthora cinnamomi* in container media; composted pine wood bark, on the other hand, was not suppressive to R. solani (23,24). This suppression in composted hardwood bark has been shown to be biological rather than chemical and members of the genus *Trichoderma* are implicated as the major biocontrol agent (24).

A study on barks of Australian trees done in Western Australia demonstrated that composted bark of marri (*Eucalyptus calophylla*) and karri (*E. diversicolor*) reduced root rot in *Banksia grandis* caused by *P. cinnamomi* but did not eliminate the disease (29).

CONCLUSIONS

The aim of this paper has been to demonstrate that growing media such as soils and potting mixes are living systems, with a balance between beneficial and detrimental micro-organisms. Rotation, cultivation, soil treatment, and hygiene are all important in maintaining a desirable balance. Creation of a "biological vacuum" in soil by fumigation or heat sterilization produces an environment in which the chance introduction of a pathogen can create havoc. This can be avoided by treating soils to retain a population which can suppress the chance contaminant. Further inprovement could be obtained by introducing micro-organisms with biocontrol activity.

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A FOGGING SYSTEM FROM SOUND WAVES FOR PLANT PROPAGATION

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When evaluating methods to improve cutting propagation, the need to overcome the following problems associated with conventional mist propagation became obvious:

(a) Nutrient leaching from the leaves
(b) Cutting media becoming saturated, resulting in decay of cuttings below the surface of the medium
(c) Wide fluctuations in humidity level, especially when *misting is done in conjunction with evaporative cooling or* fans
(d) High volume of water used

One method of overcoming these problems is to create a fog which will remain suspended in the air. This maintains a very high humidity and reduces transpiration loss from the cutting.

We have found Sonicore nozzles a cost-efficient method of producing fog which produces particles between 3 and 5 microns in size. These nozzles are air-driven acoustic oscillators for atomising water, by passing sound waves through a convergent/divergent section into a resonator cap where it is reflected back to compliment and amplify the primary shockwave.

The result is an intense field of sonic energy focused between the nozzle body and the resonator cap. The liquid pumped into the shock wave is vigorously sheared into minute droplets by the acoustic field.