vailed during the growing season at Ennadai (the Arctic air mass extended south), so that the spruce forest retreated or became patchy, the peat grew slowly and oxidized, and the tundra spread around Ennadai Lake. The sand grains probably represent breakdown of the local plant cover, which exposed the nearby sandy eskers to attack by wind.

At Ennadai about 1500 years ago there is a peak of *Picea* and *Sphagnum*. The forest limit probably moved north for a time after this date as the Arctic front retreated poleward. An age of 1140 years (WIS 17) for a burned forest horizon at Dimma Lake (61°33′N, 100° 38′W) (*1*) provides evidence for the successful growth of spruce 40 km north of the Ennadai peat bank during this climatically favorable period.

The final peak in *Picea* representation is followed by a prolonged decline, and there is no increase of other arboreal pollen to take its place. Instead, numbers of nonarboreal pollen rise, particularly those of the Ericales, and macrofossils of *Ledum palustre* and *Vaccinium vitis-idaea* were recovered from the peat. Percentages of *Sphagnum* declined and the peat became very humified and stopped growing about 600 years ago. I suggest that this situation reflected a southward extension of the Arctic air mass in summer, which brought a colder, drier climate to Ennadai. The result was a retreat of the forest to the south of Ennadai peat bank, the establishment of tundra vegetation at the north end of Ennadai Lake, and the cessation of peat growth. This latest climatic deterioration recorded at Ennadai occurred at the same time as worsening climatic conditions in the North Atlantic inhibited the voyages of the Scandinavians between Vinland, Greenland, and Norway (*14*).

The vegetation changes registered in these pollen diagrams have dates which correspond to climatic changes experienced by northwestern Europe. This synchroneity and apparent climatic parallelism encourage the correlation of the late postglacial climatic histories of the two regions. It then becomes worthwhile to employ palynological evidence from central Canada (relatively undisturbed by human activity) to investigate climatic changes in northern Europe, where the fossil pollen record for the past 5000 years has been affected by anthropogenic factors.

The events described are not at variance with the generalized picture of climatic change in Canada (summarized by Terasmae, *15*) which resembles the familiar anathermal-hypsithermal-hypothermal sequence. However, no published Canadian pollen diagram has yet been as closely dated as that from Ennadai Lake. Radiocarbon determinations for the Canadian late Holocene period are particularly sparse. It is easier to compare the diagrams described here with the closely dated sequence of northern European climatic changes than with the imperfectly known history of neighboring parts of Canada (some of which are in different climatic zones). It is premature to correlate the changes in the Ennadai and Lynn Lake pollen diagrams with the palynological work of Ritchie (*16*) in adjoining southern Manitoba, since his diagrams have as yet no radiocarbon dates which relate to those of the above-mentioned pollen diagrams.

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## Stereoscan Electron Microscopy of Soil Microorganisms

Abstract. *Details of the surface structure of microorganisms growing in soil and the complex topography of individual soil particles were observed with the Stereoscan electron microscope. Because it was not necessary to remove the microorganisms from the soil particles, it was possible to establish their occurrence in different microhabitats. This technique can provide useful ecological information about the soil microflora.*

Much is known about the microorganisms that can be isolated from soil and studied in pure culture. Confirmation of the importance of these organisms in soil can be obtained by direct observation of their natural environments. The value of such observations is limited by the comparative lack of good techniques for making them.

Microscope slides (*1*) or glass capillaries (*2*) were buried in the soil and recovered from time to time. The rate and nature of their colonization was then assessed. While much important information has been obtained from these techniques, we cannot be certain whether colonization of the glass surfaces is similar to colonization of the mixture of humus and mineral particles occurring in undisturbed soil. To avoid this problem, soil smears (*3*), individual soil particles (*4*), and sections of soil impregnated with resins (*5*) were examined with the light microscope. Evidence of the size, nature, and distribution of different microorganisms was obtained, but the resolution of the light microscope is such that details of microbial and environmental structure cannot be seen. The value of these techniques was increased by use of fluorescent dyes coupled with incident ultraviolet illumination (*6*); in this way, organisms on opaque organic soil particles may be seen.

With electron microscopes having great resolving power, detailed pictures of soil organisms were obtained (*7*). Unfortunately, the organisms had to be removed from the particles they were colonizing since it was not possible to examine thick specimens. Ultrathin sections of the root surface and its environment were prepared (*8*), and bacteria were seen clearly; but only in such regions of dense microbial colonization are sectioning techniques applicable. Elsewhere, ultrathin sections would rarely include microorganisms because only a very small percentage of the sur-

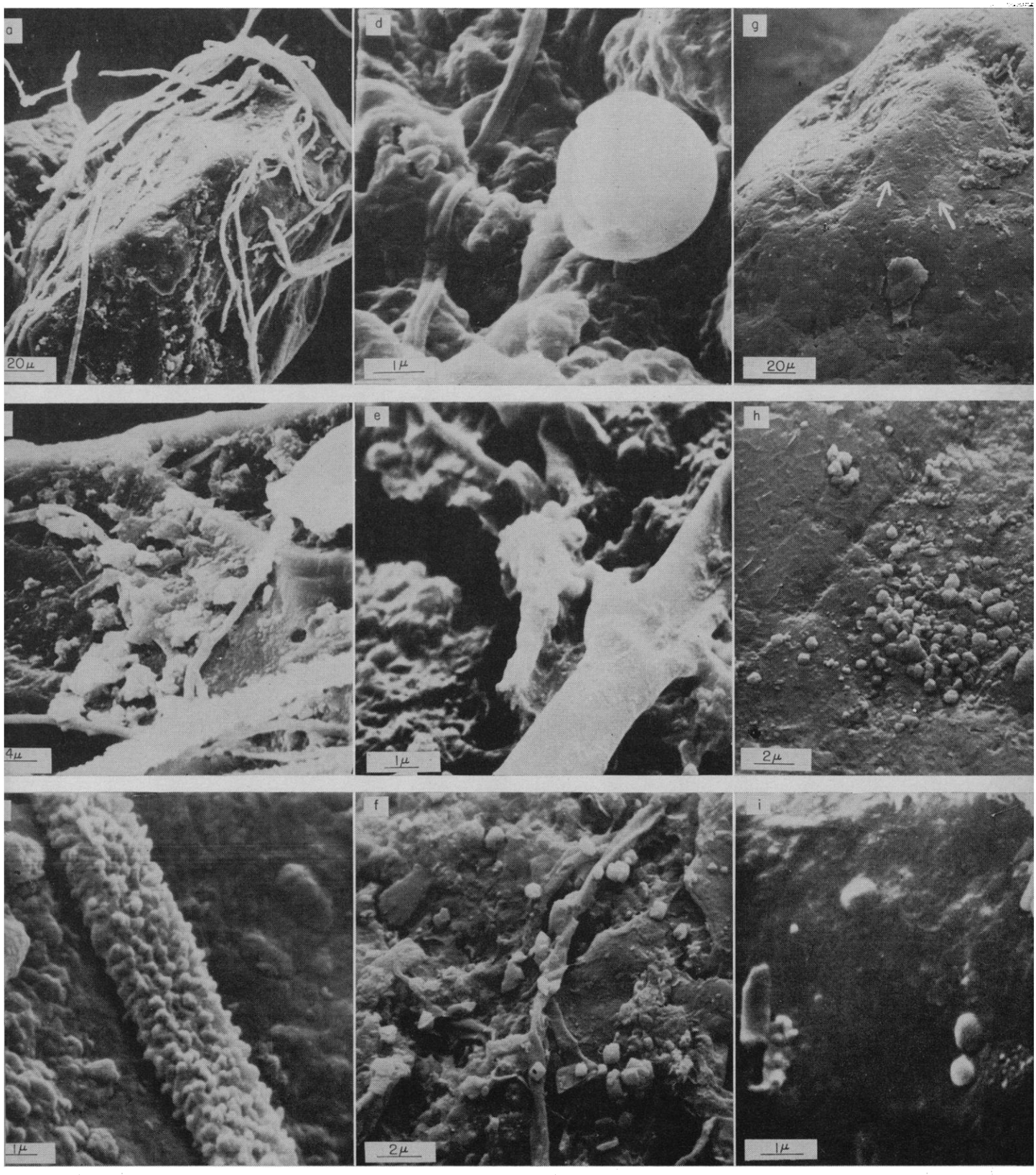


Fig. 1. (a) Fungal hyphae enveloping a sand grain. (b) Different mycelial types and organic debris on a sand grain. (c) Mycelium with protuberances colonizing a humus particle. (d) Fine mycelium and a sporelike body on decaying humus. (e) Mycelium with a clamp connection (?) on decaying humus. (f) Fine mycelium associated with bacteria on decaying humus. (g) Sand grain with isolated bacterial colonies indicated with arrows. (h) Bacterial cells in a colony on a sand grain. (i) Bacteria on the root surface of *Trifolium repens*.

face of soil particles is colonized (see *4*).

The development of the Stereoscan scanning electron microscope (*9*) provides a method for resolving most of these difficulties. Microorganisms can be examined in place on the surfaces of soil particles because an image is formed by electrons liberated from the surfaces examined. The degree of resolution (always better than 500 Å and sometimes better than 200 Å) and the depth of focus are superior to those obtained with the light microscope. As a result, an almost three-dimensional effect is produced. The method of specimen preparation is simple (*9*) and can be readily adapted for examining soil particles. A specimen holder, a small metal stub (diameter, 12.5 mm), is coated with an adhesive. While still sticky, the holder is pressed gently onto a freshly exposed soil surface. Upon removal, the soil particles remain fixed to the holder in approximately the same relation to one another that they had in the soil. Alternatively, soil particles may be smeared on the surface of the holder. The specimens are coated with a layer of gold-palladium alloy (about 800 Å thick) by means of a vacuum evaporation technique; this minimizes charging effects. The holder is then placed in the microscope. After the instrument is evacuated, the specimen is scanned by an electron beam. The electrons liberated from the surface of the specimen are detected by a scintillator-photomultiplier system and induce the formation of an image on a cathode-ray tube. This image may be photographed on either Polaroid or 35-mm film.

This technique was used to examine microorganisms on sand grains and humus particles taken from a sand-dune soil planted with *Pinus nigra* var. *laricio* (*10*) and bacteria on the root surface of *Trifolium repens*. Some sand grains were enmeshed entirely by fungal mycelium (Fig. 1a), although most particles were not so densely colonized. Different types of mycelium could be distinguished at higher magnifications. One commonly observed type was covered with small protuberances (Fig. 1, b and c) that presumably increased the surface area of the hypha, over which secretion of enzymes and absorption of nutrients could take place. Other mycelium was smooth and often much narrower in diameter; it frequently showed signs of collapse, probably due to desiccation during preparation (Fig. 1, d and f). It is impossible to say whether this finer mycelium was fungal or actinomycete in origin. Fungal spores were observed only rarely. Figure 1d shows such a spore which is about 3.0 $\mu$ in diameter. Other fungal structures observed included probable clamp connections, characteristic of basidiomycete mycelium (Fig. 1e). Sometimes, bacteria were seen to be associated with mycelium (Fig. 1f), usually in the form of coccoid rods. Bacteria were also observed as discrete colonies. Figure 1g shows part of a sand grain at a low magnification, with barely visible colonies at the points indicated. A few other cells were detectable, but the overall impression was one of sparse colonization. At higher magnifications (Fig. 1h), the colonies were seen to consist of up to 100 cells, although most colonies were smaller and single cells were frequent. The cells were almost spherical, although a few rods were visible. Definitely rod-shaped cells were seen on the root surface of *Trifolium repens* (Fig. 1i). One of the cells (bottom left) had projections on its surface, reminiscent of pictures of ectoparasitic bacteria (*11*).

The nature of the soil-particle surfaces was also visible. Mineral particles were often cratered (Fig. 1, b and g), but were comparatively smooth at high magnifications (Fig. 1h). Humus particles had irregular surfaces (Fig. 1, c–f). These irregularities make it clear that techniques which aim to remove dormant propagules from soil by washing (*12*) may not be successful because spores may become lodged in minute crevices that also provide niches for the growth of microorganisms.

Identification of microbial structures in the soil was based on a comparison of their size with organisms seen with the light microscope; comparisons with pure cultures were also made. The observation of surface mycelial structures should make possible a closer identification of mycelium than light microscopy provides. This will only be achieved when a collection of pure reference cultures is examined with the scanning electron microscope.

The technique has certain limitations. Dehydration and distortion of the organisms, especially of bacteria, occur. The degree of resolution is still insufficient for the examination of many of the surface structures of bacteria. However, this instrument has provided us with pictures of the hitherto invisible and ill-defined microhabitats in a relatively simple soil, indicating that it will help to solve a number of problems concerning the relation of cells to their environment in more complex soils. It should also enable us to determine whether the types of colony seen on contact slides are representative of those which occur in undisturbed soil.

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## Disaccharidase: Localization in Hamster Intestine Brush Borders

Abstract. *Electron microscopy of isolated brush borders from epithelial cells of hamster intestine demonstrates 60-angstrom knobs attached to the lumen surface of the plasma membrane. Digestion with activated papain removes these knobs. Separation and recovery of the knobs and the plasma membrane are possible. The activities of the disaccharidases invertase and maltase reside in the knobs and are not found with the plasma membrane.*

The site of activity of invertase and maltase and their localization in the small intestine remain controversial. Therefore, the details of absorption of the associated sugars remain unclear. If the enzymes act predominantly extracellularly in the succus entericus, then hydrolysis of the disaccharides to monosaccharides probably occurs before transport into the cell. On the other hand, if the enzymes are pre-