MMG 301 Dr. Frank Dazzo
Microbial Ecology: Methodology & Soil Microbiology

Some methods used to study microbes in natural habitats:

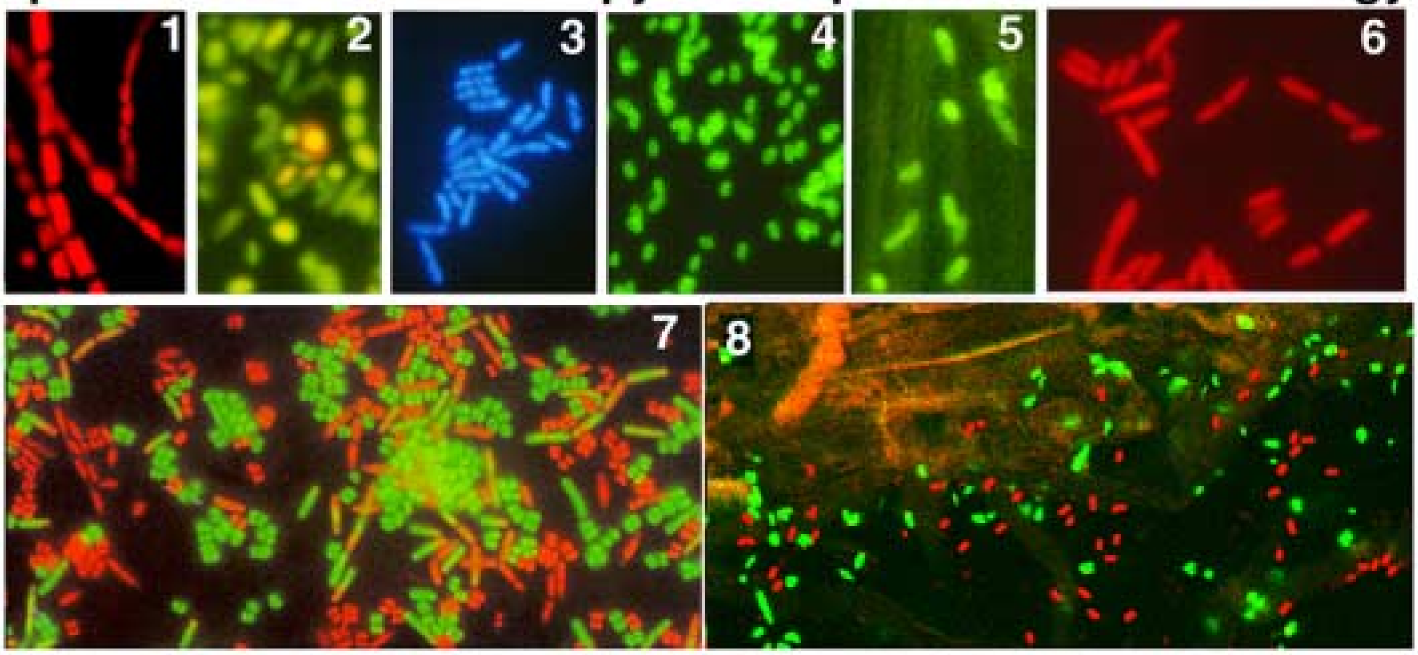
Microbial abundance:
- microscopy, computer-assisted image analysis
- measurement of cell constituents, e.g., ATP, muramic acid
- filtration / dry weight (aquatic habitats)
- viable enumeration techniques: plating, MPN, membrane filtration
- quantitative PCR of DNA/RNA using phylogenetic probes

Microbial viability
- Combination of fluorescence microscopy using vital stains (e.g., BacLite Live/Dead) and viable plating techniques

Microbial activity
- Microscopy, redox-sensitive dyes ($Cpd_{ox} + H^+ + e^- \rightarrow Cpd_{red}$)
- Microelectrodes
- Enzyme activity assays
- Gas exchange (e.g., uptake / production of O_2, CO_2, N_2, CH_4)
- Assess bacterial vs. fungal contributions by use of selective antibiotic inhibitors (e.g., chloramphenicol vs. cyclohexamide)
- Stable and radioactive isotope studies
- *In situ* rates of substrate utilization and product formation

Microbial Community Structure
- Computer-assisted microscopy and image analysis, e.g., CMEIAS
- Polyphasic taxonomy & physiological diversity of isolates
- Various types of 16S rRNA analysis, FISH, RDP-II bioinformatics
- DNA amplification, genomic fingerprinting, functional genomics

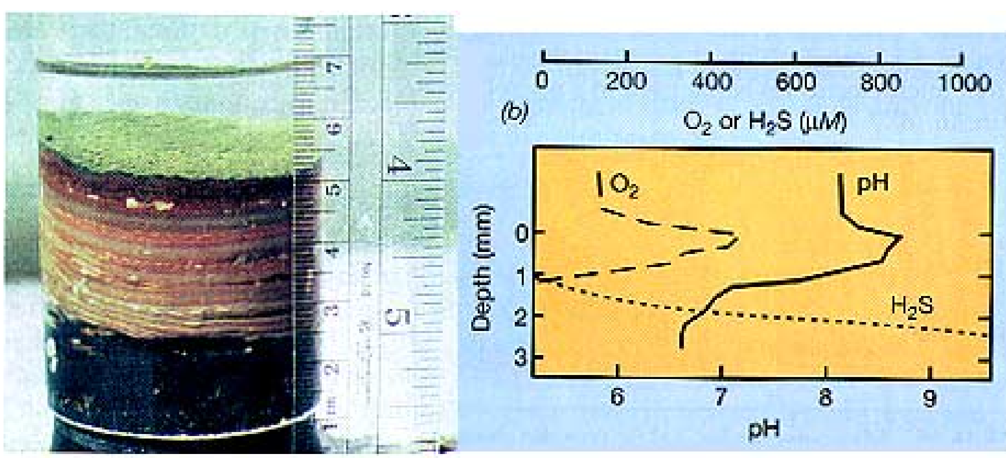
Epifluorescence microscopy techniques in microbial ecology



1. algal autofluorescence
2. direct count, acridine orange vital stain
3. direct count, DAPI stain for DNA

4 & 5: FITC-immunofluorescence using strain-specific anti-LPS antibody; 4- bacteria on slide; 5- bacteria on plant root, LSCM.

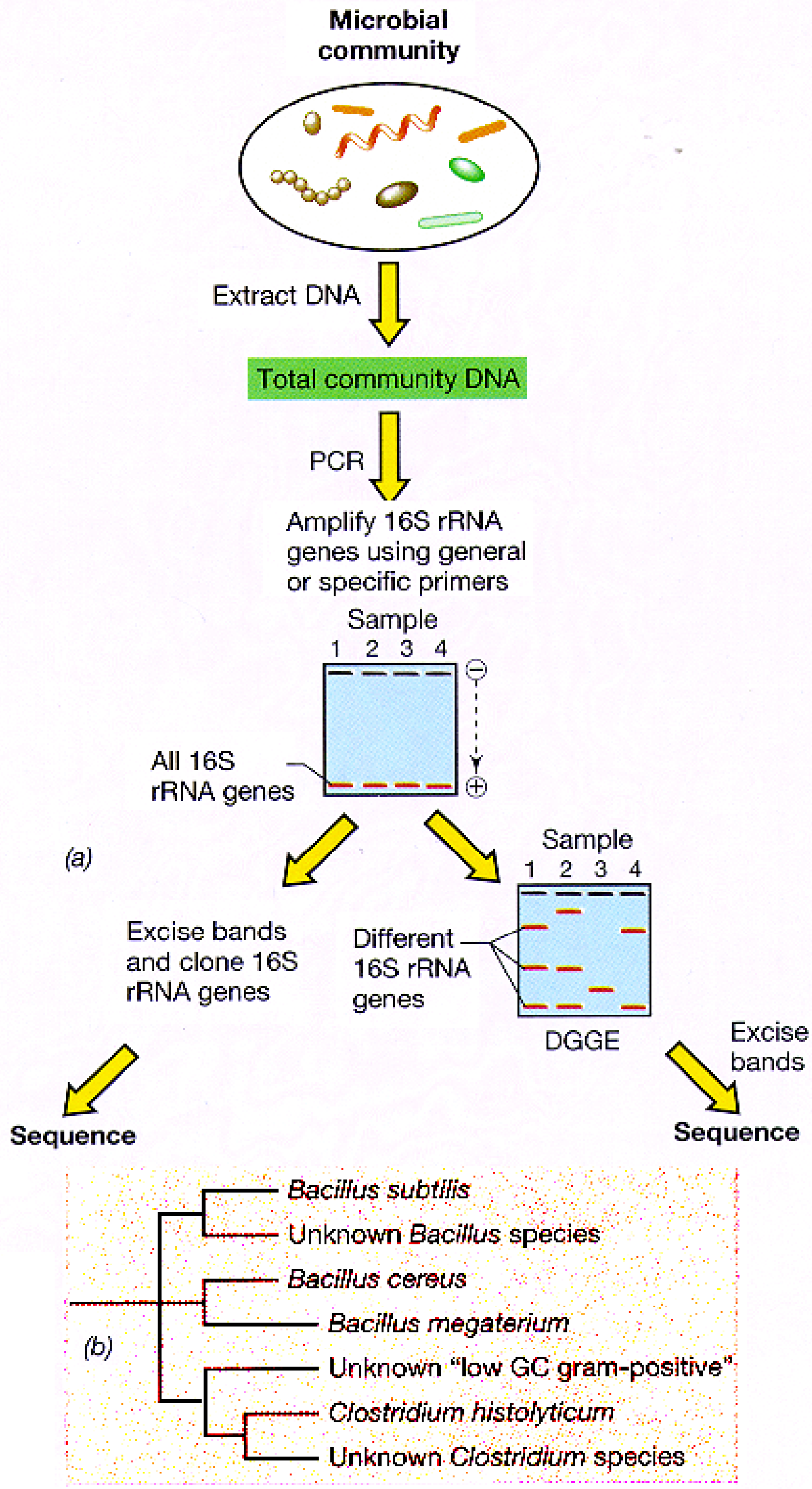
6. FISH: Fluorescence in situ hybridization (ritc-16S rRNA oligoprobe)
7. Live (green) / dead (red) viability stain (Molecular Probes BacLight)
8. Green Fluorescent Protein reporter strains for *in situ* sensing of N-acylhomoserine lactone quorum signal: red = source; green = sensor



Microbial mats and use of microelectrodes to study them. (a) A core sample through a hot spring microbial mat. Upper dark green layer contains cyanobacteria, beneath which are several layers of anoxigenic phototrophic bacteria (orange and yellow layers). (b) Microprofiles of Oxygen, sulfide, and pH in the hot spring microbial mat measured by microelectrodes.

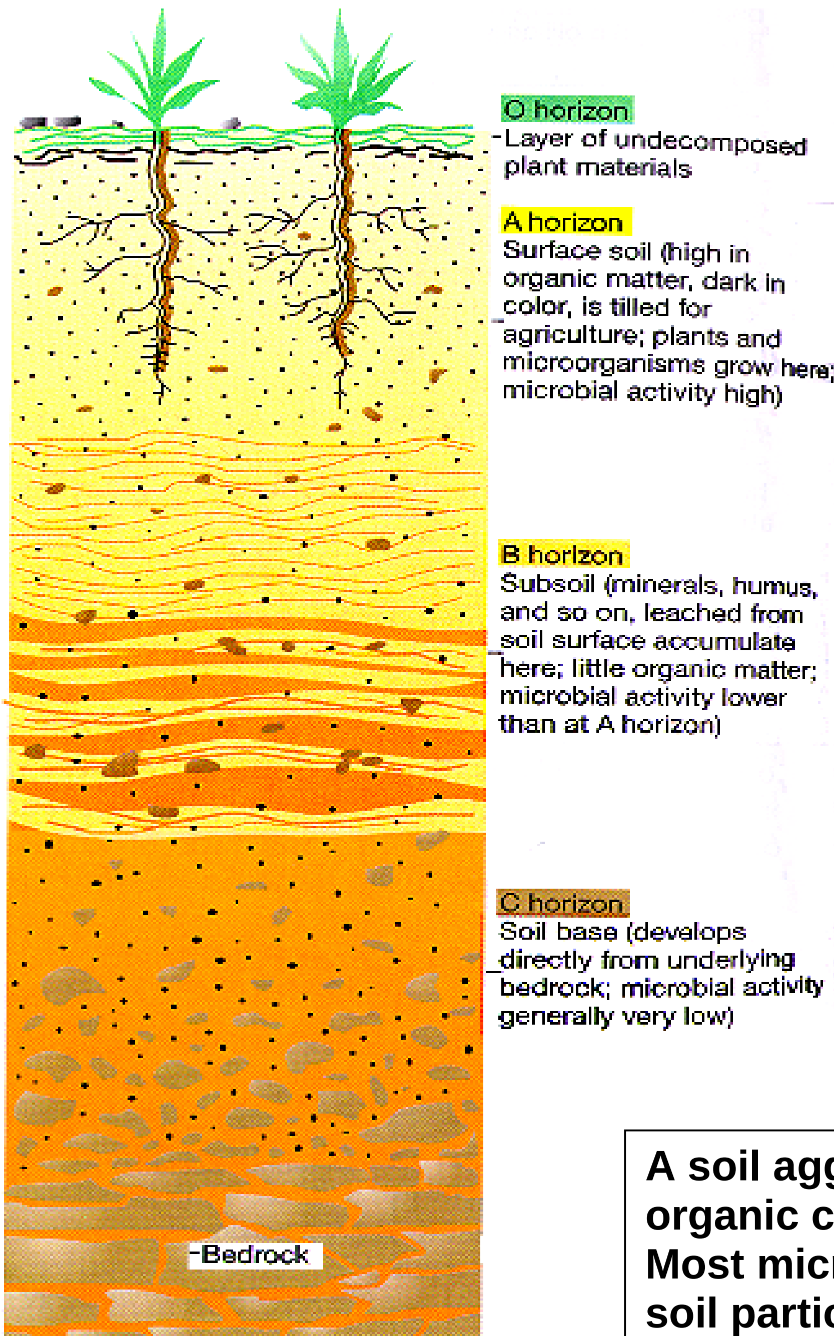
Microelectrodes are used to measure the *in situ* distribution of O_2, pH, and H_2S in hot spring microbial mats at sub-mm resolution.

FIGURE 18.14 Microbial community sampling using ribosomal RNA

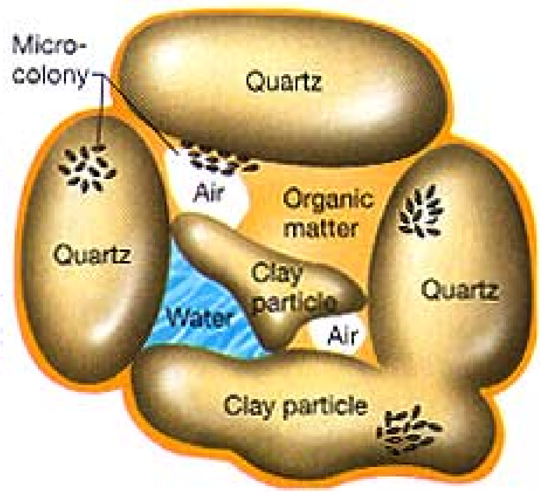


Microbial Ecology of Soil

- **3-phase (solids, liquids, gases) ecosystem dominated by the solid phase.**
- **Solids consist of <u>soil separates</u> (sand, silt, clay) & soil organic matter.**
- **<u>Clays</u> are the major inorganic soil separate affecting microbial activity in soil: their size ≤ bacteria, are the greatest surface area component, affect ion /nutrient mobility, buffer pH, water retention, porosity and gas exchange, they form bacterial clay envelopes**



Formation and movement of soil materials lead to discrete layers (horizons), producing a <u>mature vertical profile</u> as illustrated here. Microbial biomass and activity in this profile varies in proportion to the organic nutrient content: highest near the surface and decreases with depth.



A soil aggregate of solids (mineral and organic components), liquids & gases. Most microbes are in <u>microcolonies</u> on soil particles. They may escape predator activities by refuge in small pores.

Major groups of soil microorganisms and their significant activities:

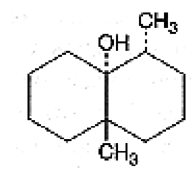
Bacteria:

- numerically abundant (10^9 cells / g soil) but most non-culturable
- along with fungi, most important decomposers of organic matter
- specialized groups participate in all biogeochemical cycles
- their extracellular polymers help bind soil particles into aggregates
- some form beneficial or pathogenic interactions with plants

Actinomycetes:

- specialized filamentous prokaryotes
- participate in decomposition of complex organic compounds
- produce many 2° metabolites, e.g., antibiotics, geosmins (earth odor) that give soil its characteristic distinctive aroma

Geosmin



Fungi:

- the major component of microbial biomass in soils
- major participants in decomposition of organic matter
- hyphal growth helps bind soil particles into stable aggregates
- some associate with plant roots: major plant pathogens, beneficial symbionts increase nutrient uptake and decrease disease incidence

Protozoa:

- major predators of soil bacteria, grazing activities accelerate decomposition of organic matter in soil

Cyanobacteria and algae (green algae, diatoms):

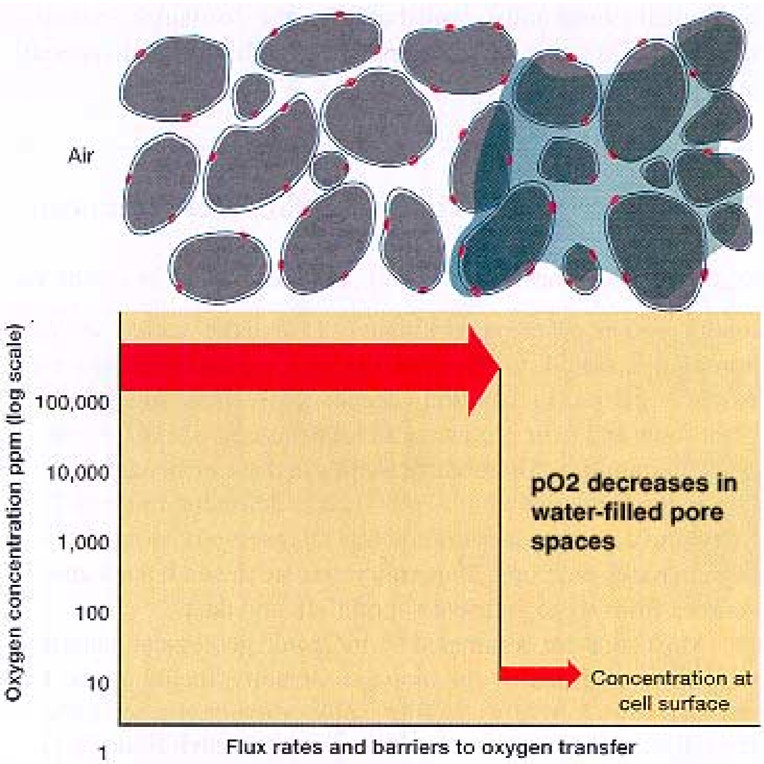
- photoautotrophs, form surface algal crusts important in H_2O retention
- some cyanobacteria carry out free-living and symbiotic N_2-fixation

Viruses:

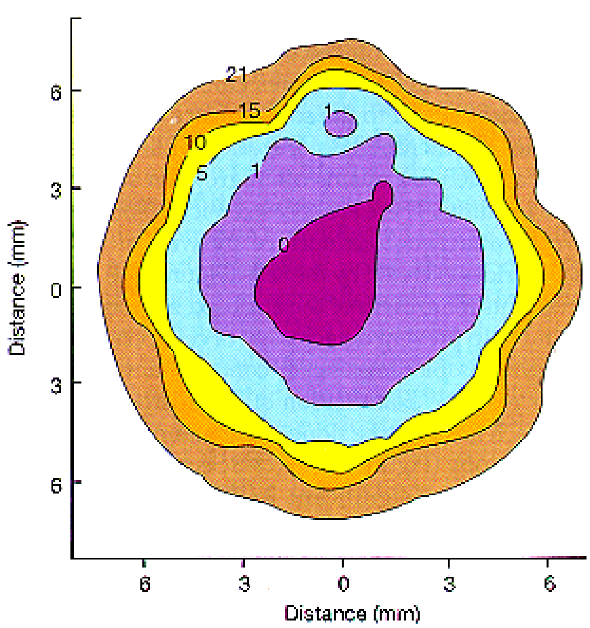
- Numerically abundant, ecology not well defined
- Both lytic and lysogenic bacteriophage (latter very common)
- Persistance and migration of human enteroviruses pose serious health issues with land disposal of sewage and fecal wastes

Special considerations for gas/liquid relationships affecting microbial activity in the soil environment:

- **Soil atmosphere/water occupy the pore spaces in the soil matrix**
- **Oxygen flux controls the type of metabolism (aerobic vs. anaerobic) accomplished by the microbes**
- **Oxygen diffusion is 10^4 – fold faster in gas than through water, hence “water-logged” soils quickly become anaerobic**



Microbes in <u>discontinuous</u> water films on the surface of soil particles have good access to O_2. In contrast, microbes in <u>continuous</u> water-filled pores have limited O_2 fluxes, creating anoxic microenvironments. Bacterial movement through soil can occur when water-filled pore spaces are continuous but ceases when they are discontinuous.



(Q?): How can one explain the detection of fermentative endproducts of microbial anaerobic metabolism even in sandy, well-drained soils?

(A): Sandy soils still contain soil aggregates where radial O_2 diffusion is restricted, so anoxic microenvironments develop within their interior where microbes are still actively conducting anaerobic fermentative metabolism.

In highly productive regions, waterlogged soils (bogs, swamps) accumulate high levels of organic matter since microbial decomposition processes are slow under anaerobic conditions. When drained, the anaerobic $\rightarrow$ aerobic conversion accelerates the microbial mineralization of the accumulated soil organic matter (S.O.M. $\rightarrow H_2O + CO_2\uparrow$), resulting in soil subsidence. E.g., Some areas of Florida everglades drained for intensive sugarcane agriculture; ~1" soil loss/yr. Some areas already reached bedrock.

- **Soil subsidence in Belle Glade, FL, mediated by aerobic microbial mineralization of bog soil high in organic matter.**
- **Profound example of how soil microorganisms can effect physical properties of soil.**