The soil was deep, it absorbed and kept the water in the lumpy soil, and the water that soaked into the hills fed springs and running streams everywhere.

-Plato

Soil Biology and Biochemistry

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C1 The Soil Biota and its Processes

Soil organisms play a central role in soil formation, plant growth and the C cycle. Decomposition in turning CO₂ to the atmosphere effectively reverses the effects of photosynthesis, but is not 100% efficient, resulting in the production of soil organic matter (SOM) so important in nutrient release, soil physical attributes and ecosystem stability. The C cycle is normally tightly coupled. Global soil C storage is equivalent to 10 yr of photosynthesis (140 x 10⁹ g C yr⁻¹). Photosynthesis has in the past exceeded decomposition resulting in the present stocks of oil and gas that contain stored C equivalent to about 3 yr and coal that represents 35 yr of present day photosynthesis. Man is now acting as a decomposition agent in the use of fossil fuels and burning of forests, thus interfering with the C cycle and contributing to global climate change. The conversion of stored hydrocarbons to various toxic or recalcitrant chemicals that are new to the environment has resulted in the need to aid natural processes (bioremediation) in instances where the soil organisms are exposed to chemical structures with which they are unfamiliar.

Advances in molecular biology that make it possible to study the many organisms that do not grow on laboratory media should make it possible to better understand soil populations, and eventually, manage them for our benefit (Paul and Clark, 1996). Soil microbial communities are generally resistant to change and capable of persisting for many years. Those scientists that call soil a nutrient-starved, stressful situation do not understand the many safety factors that nature has built into soils during the billions of years of evolution. One should be most pleased that introduced organisms usually cannot persist in the highly competitive, diverse, multi-organism associations that exist within the many habitats and niches within soil. These are responsible for the soil self-cleansing that provides...
protection against the many plant and animal pathogens introduced to this milieu by both natural and anthropogenic means.

Ecosystem stability depends on the fact that soil C accumulates and is not easily degraded. The decomposition process results in a rapid degradation of most of the plant residues in a period of months. The remaining plant residues and associated biota, not easily separated from soil, constitute the small, active fraction of SOM that decomposes within one growing season (Paul et al., 1998). Microbial intermediates and plant residues, protected within soil aggregates, take years to decompose. These represent the slow fraction that accounts for up to half the soil C and constitutes the seat of soil fertility and ecosystem stability. The old, recalcitrant fraction can be identified with acid hydrolysis in conjunction with 14C dating. It represents the other half of the soil C and is on the average 1,200 years older than the total soil C, surface ages reach many thousands of years (Paul et al., 1997). Understanding the complex interaction of the soil biota and organic C is most important to our understanding of ecosystem stability and sustainable agriculture. It is equally important in our attempts to manage nature (Brussaard and Ferrera-Cerrato, 1997) by introducing organisms into soil.

C.1.1 The Commercialization of Soil Organisms

Commercialization is defined as the use of soil organisms for specific purposes not normally associated with their activity in that location (Alexander, 1994). This could include the use of yeasts in fermentation, streptomycines in antibiotic production or soil bacillus as a source of Bt genes for insect control, but these are not carried out in soil. The most successful soil commercialization that actually occurs in soils involves the inoculation of introduced legumes with N-fixing symb ions. Various forms of bioremediation and biocontrol constitute the major other forms of commercialization.

C.1.1.1 Bioremediation

Bioremediation, in its broadest sense, includes the age-old composting techniques, as well as the modern applications that involve as much engineering as biology. Interactions and biotic controls in bioremediation are shown in Fig. C.1. Biodegradation is more narrowly defined as the biologically catalyzed reduction in the complexity of chemicals (Alexander, 1994). This can lead to mineralization, the conversion of much of the C, N, S, P and other elements to inorganic products. Microorganisms degrade substrates in environments as diverse as soils, groundwater, surface water, the vadose zone between soils and groundwater, sediments and geological structures as well as sewage facilities. This requires (1) the presence of an organism or organisms with the appropriate growth characteristics and enzymes, (2) accessibility of the substrate, and (3) appropriate abiotic and nutrient factors for microbial growth and activity. The degradation process usually involves an acclimation period that, if lengthy, can affect both distribution and toxicity of the chemical. Once a soil is acclimated, a second exposure can result in rapid degradation. This is desirable for detoxification, but can result in the too rapid breakdown of herbicides and insecticides before they have had time to act. Detoxification of a chemical involves changes in its structure to render it less harmful to or more susceptible organisms such as humans, plants, other animals and the soil biota. Conversely, microbial activity can produce more toxic intermediates by the process known as activation of other organisms or metals. The microbial methylation of Hg, Sn and As increases their toxicity and distribution.

Cometabolism is the transformation of an organic compound by an organism or organisms incapable of using that compound as a source of energy or cell constituent. While now being studied relative to detoxification of anthropogenic(s) (Pochin, 1993), perhaps, the most important cometabolism in nature is the degradation of the lignin molecule and soil humic constituents. Very little of the C of lignin added to soil enters the soil biomass; external energy supplies are required for its breakdown (Stevenson, 1994). Soil aromatics are most likely to behave similarly. The aromatics are decomposed to gain access to the associated carbohydrates and nitrogenous compounds.

Bioremediation in its simplest form involves the application of nutrients such as N and P to in situ spills and cultivation to provide aeration and mixing. Slightly more complex is land farming where the contaminant is removed and transported to another site for nutrient addition and mixing. The process is said to involve a bioreactor if it includes more complex engineering such as moisture and temperature control or the use of clay or plastic liners to control runoff and leaching. Contaminants at depth can be degraded by aeration via access wells and a vacuum system that can also collect volatiles in a bioventing system. Still more complex are systems that degrade groundwater contaminants via addition of substrate, electron acceptors and possibly added microorganisms (Bewley, 1996).
C.1.1.2 Composting

The controlled biological conversion of solid organics into a stable, humic-like substance involves two separate, but related processes, decomposition and humification. Normally an aerobic process, it decreases carbonaceous waste products into materials usable in gardening, field application and mushroom growing (Miller, 1993). It has potential for methane production and is used in the degradation of xenobiotics. The term composting, first utilized for mixtures of sewage sludge and other solid wastes also has been applied where xenobiotics are degraded. The composting process can occur in simple piles or in complex covered facilities with moisture, aeration, temperature and runoff control. In all cases, it is a complex, biological process (Fig. C.2) involving a sequence of numerous organisms and a temperature cycle that must go to 65°C if pathogens and pests are to be destroyed. The formation of a stable, high-nutrient end product with a N content > 2%, a C:N ratio < 20 and neutral pH requires a maturation period. The principles in composting are simple enough, but errors and mismanagement lead to odors, high nutrient runoff, a nonusable product and incomplete degradation of herbicides that can damage sensitive plants.

Mesophilic bacteria and fungi are important in early stages followed by thermophiles such as bacilli and actinomyces in the high temperature stage. Fungi predominate in the cooling, maturation period when soil animals often can appear. Composting can last for periods of weeks to years depending on the mixing management employed. Commercial inoculation is at times used but usually has not proven necessary. The composted material can have disease suppressive effects that are attributable to phenols and antibiotics produced under proper composting conditions (Hoitink et al., 1991).

![Diagram of composting process](Image)

Fig. C.2 Organisms and processes in composting [Reprinted from Paul and Clark, 1996, Soil microbiology and biochemistry. Copyright Academic Press, San Diego, CA with permission]

C.1.2 Ecology of Soil Microorganisms

Microorganisms, next to living plants, constitute the largest biomass on our planet. They also carry the greatest range of physiological processes ranging from decomposition to the many reactions in the N, S, P and other nutrient element cycles in a wide array of environments. Their small size and the difficulties in their isolation have inhibited both the study and the use of information on soil biota in the development of ecological theory (Coleman and Crossley, 1996). In turn, soil microbiologists have not been part of the mainstream in ecological thinking; concepts developed with larger plants and animals are difficult to test in the much more constructed, multifaceted, competitive soil environment (Wade and Giller, 1996).

![Diagram of soil microbiology](Image)

Fig. C.3 Methods for studying the soil biota
C.1.2.1 Methodology

Techniques that include improved, automated microscopy, chemical analysis of signature molecules, the study of nucleic acids (molecular biology) and tracers (Fig. C.3) are providing rapid advances in the study of soil organisms and their processes. Microscopy while slow with a requirement for expensive equipment, provides vital information on morphological features such as size and shape, some characteristics useful in identification and biovolume (Bloom et al., 1995). The small size and greater density of microorganisms in soil than in pure culture (Paul et al., 1996) are related to their adaptation to the soil habitat. Chloroform fumigation with either incubation (CFI) or extraction (CFE) has made possible extensive comparisons of biomass to activity in many habitats and management conditions (Powelson, 1994). Phospholipid, ergosterol, and lipid measurements of isolates and of the soil directly give population abundance and community structure estimates (Zelles and Alfer, 1995).

Many of the recent advances in the ecology of the soil biota are centered around DNA and RNA analysis. There are probes for both phylogenetic characteristics and potential activity (Hartman et al., 1997) as well as the polymerase chain reaction (PCR)-related techniques for rRNA and tDNA. Analysis of the 5.16 and 23 S rRNA of bacteria or the 18 and 25 S rRNA (or their equivalent DNA) of fungi have the potential to characterize the soil population in situ. Restriction analysis of preserved regions, together with PCR, supplies information on major phylogenetic groups. Analysis of associated more variable regions can supply information at the species level. The separation techniques are continuously being expanded and now include restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE), etc. (Heuer and Smaali, 1997) for analysis of nucleotides from isolates as from plate counts and directly from soil. Probes that react with specific regions of RNA have worked well for measurement of both occurrence and activity in more active habitats such as sludge and bioreactors. The low rRNA content of soil bacteria and nitrification from autofluorescence, humics and clays are still restricting soil studies (Madsen, 1996).

Tracers combined with physiological measurements continue to be one of the best ways for following individual soil processes. The 14C signal provided by a shift in C4 and C3 plants in many parts of the world is especially useful for studies of SOM dynamics under different management conditions. The N cycle involves reactions that involve both organic and inorganic intermediates. This results in discrimination of the isotope involved leading to problems in the interpretation of natural abundance measurements. The use of added N, however, facilitates measurements of mineralization, immobilization, nitrification, denitrification, N fixation, leaching and crop uptake.

C.1.2.2 Interpretation of Data

Only 1% of the approximately 109 soil bacteria seen in the microscope grow on standard laboratory media. The majority of recognizable cells in soil are less than 0.3 m in diameter; less than 50% have a biovolume greater than 0.1 m3 (Bakken, 1997). Only 0.2% of the cells < 0.3 m in diameter grew on plates, but they retained their small size on the laboratory media. Those with a diameter > 0.8 m showed 38% growth. The DNA content of the small cell at 2 fg cell−1 was similar to that of the larger cells. Dwarf cells thus contain 50% of the soil bacterial DNA although they represent only 10% of the biovolume and probably much less of the physiological activity. Dwarf rhizobium cells isolated from soil (Bottomley and Dugirthi, 1989) failed to nodulate. The percentage of small cells increased with soil depth. Nucleic acid data could, therefore, represent a large population of cells that are responsible for only a very small proportion of the transformations carried out, and thus, also present problems in interpretation.

The soil fungi present special problems. Plate counts represent primarily spores if special washing techniques and hyphal separations are not conducted. Their DNA is difficult to isolate; most DNA extractions of soil probably represent primarily bacterial DNA. This is related to the observation that

most fungal cells have their cytoplasm concentrated in 2-5% of the hyphae at the growing tips. A comparison of fungal and bacterial DNA in one soil showed that, although the two had similar biomass, bacterial DNA was 10 times as prevalent as that of the fungi (Harris, 1994). Fungal nucleic acids may thus be more closely related to function in soil than are the more easily extractable, more common bacterial nucleic acids.

Diversity is an index of the number of different sequences or species in a habitat. A more useful index is the amount of community structure that includes quantitative information on numbers of different individuals or physiological groups. Extraction of bacterial DNA followed by denaturation/microassociation studies show 4,000 different genomes that could represent even more species in one soil (Torsvik et al., 1995). The available PCR analysis together with appropriate sequencing, RFLP and DGGE measurements (Liesack et al., 1997) show that the DNA isolated from nature often very seldom itself is similar to that obtained from isolated cultures. DNA representing whole new groups of organisms is often found, leading to conjectures on the number of new organisms yet to be found. This has led to questions concerning the relation of soil diversity to ecological fitness and possible redundancy relative to physiological function. Microorganisms appear to follow the concepts on diversity established with larger organisms. In macroecological systems, an increase in diversity appears to enhance productivity in low nutrient environments. High nutrient environments, such as those often found in agriculture, appear to increase dominance and reduce diversity (Odum, 1998).

Ammonia oxidizers in soil represent a select group of soil bacteria of importance in N cycling and the response to environmental stress (Prosser, 1989). Agricultural soils with high potential nitrification rates had only one genus (Nitrosospira). An adjacent grassland had very little nitrification capacity but contained Nitrospora, two Nitrosomonas, and a yet to be identified species (Brunts, 1998). One must ask, were the grassland species outcompeted in the disturbed soils by the strongly Nitrosospira that had a greater capacity to grow at variable NO3 concentrations, or did the uncultivated grassland provide more microhabitats for otherwise less competitive organisms? The data indicate that, in this case, diversity was highest in the grassland site with the most competition for the available mineral N. The agricultural soil displayed dominance. The activity of keytones and coniferous species (Wardle and Giller, 1996; OkTonen et al., 1997) must be further tested to microbial interactions within soils such as these.

The isolation of soil DNA has made possible the application of 16S rRNA gene amplification to measure soil bacteria: growth rates. Harris and Paul (1994) found bacterial turnover rates of 100 to 160 days. These were larger than those reported by Bakken (1997). The measured turnover rates in soil of the Harris and Paul (1994) study closely corresponded to calculation of growth potential based on rates of substrate incorporation and C02 evolution. They estimated 35% growth efficiency with an important portion of the available energy attributed to maintenance energy of the bacterial population.

No one approach answers all of today's questions. The best understanding comes from the application of a multiple, polyphyletic approach. In addition to the techniques discussed above, this could include the use of marker genes (Liesack et al., 1997) such as the fluorescing luc gene, the physiological gas genes, antibiotic resistance, green fluorescent proteins, fatty acids and related biochemical techniques as well as MPN-PCR (van Elsas et al., 1997).

C.13 Management and Inoculation of Soil Organisms

Specific crops, rotations, cultivation, fertilizers and pH control have in the past been the primary way to influence soil microbial activity. Dropping the pH of soils growing potatoes controls Streptomyces induced potato scab. Crop rotation deters the build up of both fungal pathogens and nematodes and has been said to control undesirable mycorrhizal dominance (Johnson and Pfister, 1992). Burning an
residue incorporation are regularly used for pathogen control. Leaving residues on the surface of other sites or environments allows a build-up of SOM necessary for aggregation and erosion control. The seedling of legumes in rotation or as cover crops makes it possible to selectively enhance symbiotic N fixation. Management of the soil system for agronomic or bioremediation sometimes involves inoculation of new organisms. The commercial use of genetically engineered soil biota has been slow because society is more willing to accept new genes incorporated in plants than in microorganisms where they can more readily escape to the environment. Placing a microbial gene into a plant also overcomes soil inoculation problems. Roundup-resistant crops and commercial crops containing the big gene from soil bacilli are now part of regular management.

The suppression of one organism by another can be of use in biocontrol of pests and pathogens (Killham, 1994). Soil bacteria operate naturally to maintain our soils in a relatively pathogen-free soil situation considering all the human and plant pathogens that are routinely dumped on them. Certain soils show suppressiveness to one disease, but are conducive to another. Principles involved include those of antagonism and stimulation where one organism detaches or enhances the growth of another. The competitive inhibition of newly inoculated rhizobia in many legume systems may be an outcome from the viewpoint of a soil microbiologist, but not from that of the long time survival of the indigenous rhizobia.

Antibiotics are strong antagonistic agents in vitro and are active in vivo, but have not proved amenable to management in the multi-organism, multi-habitat, soil system in which many organisms have an active demand for a limiting resource. This includes not only organic substrates, but also competition for space as in the competition for root entry sites in mycorrhizal-root infection interactions that help prevent plant diseases. Parasitism and predation in the rhizosphere and the normal food web of soil; soil structure (Ladd et al., 1996) plays a predominant role in protecting organisms from predation. Attempts to alter populations in such an environment require a large inoculum and conditions that give a nutrient advantage and protective habitat to the introduced organism.

Inoculation has achieved its widest and most successful application with rhizobia for symbiotic nitrogen fixation in the nodulation and effective fixation by legumes where there is not excessive competition from indigenous rhizobia. The soybeans in their native habitat in China were initially inoculated with strains good enough for growth without N fertilization, but not for maximum fixation. Attempts to add more efficient fixers have largely failed because of the competitive ability of the original introductions. These persist in soil for up to 12 yr in the absence of soybeans by developing rhizosphere of other plants and may even have weak fixation capacities in the free-living state. The introduction of new symbionts to such situations must await the selection and engineering of plants that give the new introductions a competitive edge in the soil. Similarly, the use of inoculants in composting and bioremediation often produces results that are no different than amelioration of the soil by fertilizers, aeration, etc. and development of the indigenous flora.

The stability of the SOM is as equally important as the resistance to change and stability of the soil biota in sustainable ecosystem functioning and long-term agriculture. The active and slow pool can be increased with additional inputs and restricted cultivation. The large resistant pool that supplies physical stability must generally remain intact. The consequences must be carefully considered before one dratically attempts to alter either the soil biota or SOM.

C.2 References

1.1 Viruses

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Viruses are the most numerous of all organisms in soil. Populations may reach as high as $10^{14}$ g

To say that viruses are living organisms, however, and thus comparable to higher organisms

somewhat of a misnomer. Most viruses contain a core of DNA or RNA surrounded by a protein particle (Fig. 1.1). Because they contain little if any metabolic machinery, they are unable to autonomously replicate and must first invade a living host cell. The nucleic acid from the virus then directs the host cell to replicate new viral particles. Furthermore, once produced by the host, virions, or complete particles, do not grow in size. Since the only characteristic suggesting that viruses are living is their ability to maintain genetic continuity, few scientists consider viruses to be living organisms.

The overall importance of viruses is not easy to determine since so little is known about their exact role. However, the fact that they can significantly affect bacterial growth in soil (Basit et al., 1992). When the population of the bacterial species declines to a low level, the virus no longer can maintain its population size, which subsequently also declines in number. The low viral population then allows for increased number of the bacterial host.

Viruses are also routinely used for biocontrol of insects. Since viruses are specific to individual genera and species, they can be applied to a field and infect only the desired target species of the host. The nuclear polyhedrosis viruses are most often used for biocontrol. Although still under development, these viruses may one day also be used to control fungal, bacterial, nematode and even other viral pathogens with the use of specific viral antagonists.

1.1.1 Taxonomy

Viruses are classified according to the hosts they infect but are not grouped into a distinct taxonomic group. Viruses are generally not included in any of the three domains of living organisms. To a large extent, viruses can be considered as parasites of animal, plant, fungal or bacterial parasites. However, these relationships become blurred when individual species are able to infect hosts in different domains.

Bacterial viruses are also called bacteriophages or simply phages. Bacteriophages, which have been isolated for nearly every known species of soil bacteria, are classified according to their host range.