

Microbes in composts – white hats and black hats

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Substrates that are high in organic matter, such as composts, will have high levels of microbial activity. These 'enrichment cultures' favour the development of specific microorganisms. Microflora is the general term applied to bacteria (including actinomycetes), fungi, algae and viruses. Sanitization is the elimination of pathogens, parasites and/or weed seeds from a medium. Stabilization is an EPA proscribed level of reduction or removal of pathogens from Biosolids (see Easton, 1999). Sterilization is removal of all biology.

Composting is a spontaneous and natural process in nature. Managed composting is the accelerated bio-oxidation of organic matter through an exothermic process by indigenous microbes that liberate heat, carbon dioxide and water to produce a stable organic compost. This process is often divided into three phases (Hoitink and Grebus, 1994):

- Establishment phase, first 24-48 hours, temperatures 40°C-50°C, sugars and other easily biodegradable substances destroyed.
- Turning and aeration phase, temperatures 40°C-65°C, cellulose and other intractable compounds reduced, thermophilic microbes predominate, pathogens and seeds are destroyed by the heat.
- Curing phase, biodegradable components reduce, temperatures reduce, mesophilic microbes recolonize, humic substances accumulate.

The heterogeneous organic material is transformed into a homogeneous and stabilized humus-like product consisting largely of lignins, dark humic substances and biomass. Vermicomposting uses earthworms to mix, fragment and aerate the source material making it more conducive to microbial activity and generally avoiding the exothermic stage (Domingues et al., 1997).

Vermicomposting offers the following advantages over thermophilic composting (Domingues et al., 1997):

- Less manual manipulation of the composting material.
- Greater rates of humification, the processing of organic wastes in 1-2 months.
- Pathogenic microbes are reduced and product can have suppressive effect on soilborne pathogens (as for compost).
- Greater reduction in bioavailable heavy metals than in composting.
- Product is microbially enhanced and has good crumb structure and nutrient status, also contains plant-hormonal agents.
- Potential by-product of earthworm biomass.

Vermicomposting can thus provide an organic product in less time with less handling when compared to thermophilic composting. However composting alone is simpler to manage for the purposes of stabilization and sanitization (i.e., removal of pathogens and weed seeds). Riggle and Holmes (1994: 60) report on a Canadian company's proprietary "Vermiconversion System" that combines thermophilic composting for 3-15 days to neutralize pathogens and weed seed, followed by vermicomposting for 30 days.

Microbial components of composts, including vermicomposts, generally comprise:

Yeasts/fungi – important for breaking down organic compounds; soil nutrient cycling; stabilizing soil aggregates; controlling plant parasites and diseases. Yeasts are single-celled fungi.

Actinomycetes – a non-taxonomic term applied to a group of gram + bacteria that have a superficial resemblance to types of fungi that form hyphal mycelia. They are important for breakdown and recycling of intractable compounds such as chitin and cellulose; involved in forming stable crumb structure; assist in reduction of plant pathogen suppression, including *Pythium* and *Fusarium* fungi, by producing antibiotics such as streptomycin and aureomycin; add dark colour to organic matter. Some actinomycetes, notably of the genus *Frankia*, form N-fixing nodules on roots of species of plants, including *Alnus* and *Casuarina*; a number of actinomycetes cause disease eg. scabs of potato or tuberculosis and leprosy in animals.

Mycorrhizae – fungi that form symbiotic associations with plant roots to greatly increase range and uptake of nutrients; some mycorrhizae are pathogens, eg. *Rhizoctonia solani* is an orchid mycorrhiza that is pathogenic to other plants. There are four main types of mycorrhizal fungi:

- Ectotrophic mycorrhizas (ectomycorrhizas) occur on the roots of most tree species, branch externally and produce fruiting bodies we call mushrooms and toadstools.
- Vascular-arbuscular mycorrhizas (endogone) form internal structures in the roots of most other plants producing vesicles and ‘tree-like’ arbuscules in the root cells.
- Ericoid and Arbutiod mycorrhizas are associated with heathland plants.
- Orchid mycorrhizas are required for these plants to germinate and grow.

Algae – tiny plants that contain chlorophyll, blue-green algae in moist soils fix nitrogen (eg. in paddy fields); provide food for other organisms.

Nanobes – very little is known about this group of minute organisms.

Viruses – pathogenic or benign, can infect other microbes; little is known of beneficial viruses.

Bacteria – single-celled organisms; range of functions and roles, some groups can transform metabolic pathways depending on micro-environmental conditions.

Protozoa – small single-celled animals, range of groups and functions, consumers and predators of some microbes, food source for other organisms. Predatory protozoa control bacteria in composts.

Microarthropods (mites etc.) – many different arthropods and acarids may be found in composts.

Nematodes - also called roundworms, have a variety of strategies. Some feed on bacteria, some feed on fungi, while others are predatory on other nematodes or protozoans. Only free-living forms are found in composts, as parasitic forms require a plant or animal hosts, however eggs of parasites may be present. Some fungi trap and consume nematodes.

Methods for determining levels of these microbes in samples are to use culture media for plate counts of colony forming units (cfu), or for viruses plaque forming units (pfu). In the case of mycorrhizae, root samples are cultured and the numbers of infections noted. Pathogens are usually assessed by extinction dilutions and expressed as ‘most probable number’ per gramme of sample material (MPN/g).

It is important to realize that ‘plate counts’ underestimate ‘true counts’ by orders of magnitude; this is known from microscopical comparison and is accounted for by not all microbes present forming cultures in certain media.

Six functional groups are used as standard analytical techniques for compost evaluation by BBC Laboratories in Tempe, Arizona (Bess, 1999). Required levels of these groups for mature composts are:

- heterotrophic (aerobic) bacteria – 10^8 or 10^{10} cfu /g dry wt compost
 - anaerobic bacteria – ratio of aerobic:anaerobic bacteria should be not less than 10 : 1
 - fungi – yeasts and moulds should be in the range of 10^3 or 10^4 cfu /g dry wt compost
 - actinomycetes – 10^6 or 10^8 cfu /g dry wt compost
 - pseudomonads – 10^3 or 10^6 cfu /g dry wt compost.
 - nitrogen-fixing bacteria – 10^3 or 10^6 cfu /g dry wt compost
- (cfu – colony forming unit).

Composts can be characterized into three main categories:

1. Bacterial dominated, substrates tend to have high organic content, neutral to alkaline pH.
2. Equal fungi-to-bacteria.
3. Fungal dominated, substrates tend to have higher mineral content and are acidic.

Composts that are bacterially dominated, especially with nitrifying-bacteria, are stimulatory for vegetables, turf, crops, and for weeds; row crops and gardens do best with equal fungal-to-bacteria media (Ingham, 1999). Trees and shrubs tend to prefer soils that are fungal dominated. Older composts tend to become fungi dominated.

The nature of the compost relates to the source material and method of management.

Characteristic microorganisms of sewage sludge.

Many types of bacteria, viruses and protozoa inhabit the human intestine and are shed in the feces. Fecal coliform bacteria have been used to indicate contamination by human sewage. Pathogenic microbes are passed by infected individuals, examples are *Salmonella* and *Shigella* bacteria; enteroviruses and rotaviruses; and protozoan cysts. Sewage treatment aims to contain and to eliminate or inactivate these microorganisms to prevent environmental contamination.

Suppression of soil-born pathogens by composts

One of the major benefits of composts relates to their potential to provide biological control of plant diseases (Hoitink and Grebus, 1994). Various composts may suppress soilborne plant pathogens, but the mechanisms for this are complex and in certain situations organically amended soil may be conducive to pathogens. Immature composts can inhibit seed germination and cause rapid nitrogen depletion. Conversely, over-aged composts can lose many of their beneficial characteristics.

Mechanisms of biological control of plant diseases by composts are believed to be due to combinations of:

- competitive exclusion
- antibiosis
- microbiostasis
- hyperparasitism
- induced systemic resistance in the plant

“General suppression” refers to the prevention of germination of pathogenic spores due to “general soil microflora”, while the targeting of pathogens with microbes known to be antagonistic is termed “specific suppression” (Hoitink et al., 1993). Tailoring the microbial composition to particular applications would produce most benefit. Predictable formulations of composts for suppression of some diseases have been determined, and specific fungal and bacterial inoculants have been developed (eg. Hoitink, 1990; Phae et al., 1990; Hoitink et al., 1993). Moreover, mycorrhizae are commercially available from many sources. However, we do not have sufficient knowledge to quantify these suppressive effects in every situation, and most composts are colonized by microbes adventitiously or by chance.

One of the first attempts to determine specific effects of vermicompost on some plant pathogens was by Szczech et al. (1993). These authors found that *Phytophthora* and *Fusarium* fungi were suppressed by vermicompost, but that parasitic nematodes were not affected. Inoculation of sorghum seeds with *Azospirillum brasiliense* and earthworm casts increased growth of sorghum (Savalgi and Savalgi, 1991).

It is reported that soil borne diseases are less prevalent on organic farms (Workneh et al., 1993 referred to in Hoitink and Grebus, 1994).

Beneficial organisms

Bacteria: *Bacillus* spp., these are “gram positives” that occur in soil; *Enterobacter* spp., *Flavobacterium balustinum*, *Pseudomonas* spp., “gram negatives” that also occur in soil, *Rhizobium* spp. are nitrogen fixing bacteria that form symbiotic associations in root nodules of legumes. Diazotrophs are bacteria that can reduce N₂ from the atmosphere to ammonia; *Acetobacter diazotrophicus* is endophytic of sugarcane and a few other cultivated plants. Recent research indicated that high levels of nitrogen fertilizers on crops like cane have reduced their colonisation by endophytic diazotrophs.

Biological nitrogen fixation (BNF) is the process in which atmospheric N is converted into substrates of nitrogen that plants can use. Worldwide about 155 million t/yr of nitrogen, valued at approximately US\$90 billion compared to commercial fertilizers, is fixed by microorganisms (Pimental, et al., 1997). Nitrogen fixation in leguminous plants is estimated to fix an average of 80 kg/ha/yr of nitrogen worldwide. In addition, recent discoveries indicate that obligate endophytic diazotroph bacteria add as much as 150 kg/ha/yr (Dobereiner, 1995 quoted in Pimental et al. 1997).

Pseudomonads are important in nutrient cycling, assisting plants with phosphorus availability; some have been linked to suppression of plant pathogens; for example, *Pseudomonas cepacia* repels and inhibits root pathogens, such as *Fusarium*.

Fungi: *Streptomyces* spp., have antibiotic characteristics and produce secondary metabolites as do *Trichoderma* spp. and *Gliocladium virens*. *Trichoderma* produces antibiotics that slow or arrest growth of take-all and *Rhizoctonia* fungi. Periera et al. (1998) have studied the survival and saprophytic ability of *Trichoderma harzianum* and *Bacillus subtilis* in vermicompost derived from by-products of sugarcane and rice.

Pathogenic and parasitic microbes

The major soilborne pathogens and compost microbes (from Hendreck and Black, 1994; Epstein, 1998; and other sources) are summarized in the tables below:

Damping off is a disease caused by *Pythium Rhizoctonia* and *Phytophthora*. Wilting is mainly by *Fusarium Sclerotinia* and *Verticillium*.

Fungi	Common names	Problem/ Characteristics	Control measures and refs.
<i>Rhizoctonia</i> spp.	wire stem, damping off, collar rot	Universally occurring pathogens which can infect a wide range of crops and soils. Damage to stems	
<i>Rhizoctonia solani</i>	damping-off	produces scleroctia	Hoitink and Grebus, 1994; Ghini, et al. 1998 – suppression by organic composts
<i>Phytophthora</i> spp.	dieback, collar rot, root rot	Major problem in many areas. Attack roots, leaves wilt and die back from tips	Hoitink and Grebus, 1994 – composts can decrease losses in nurseries
<i>Phytophthora nicotianae</i>		Tomatoes	Szczech et al., 1993 – vermicompost eliminated infection 100%f
<i>Fusarium</i> spp.	basal rot, fusarium wilt, fusarium yellows	Attack root or stem	Hoitink and Grebus, 1994, Szczech, 1999 – suppression by (vermi)composts
<i>Fusarium oxysporum</i>			Szczech et al., 1993 – vermicompost improved plant health
<i>Pythium</i> spp.	damping-off, water mould, root rot, collar rot back leg.	Attack seedlings	Hoitink and Grebus, 1994 – suppression by composts
<i>Scleroctinia</i> spp.	scleroctinia blight, blackrot	On stems and leaves, white cottony growth	
<i>Scleroctinium</i> spp.	stem rot	Stem at base	
<i>Scleroctinium cepivorum</i>	onion white rot	Occurs worldwide, persists for > 20 yrs, attacks Allium crops	
<i>Verticillium</i> spp.	wilt	Leaves wilt	

<i>Rosellinia necatrix</i>	white root rot	attacks apples trees	Stephens, et al. 1999, vermicompost reduced severity.
<i>Plasmodiophora brassicae</i>	clubroot of brassicas	truncates roots with clubs	Szczzech et al., 1993 – vermicompost prevented club development; Stephens, et al. 1999, vermicompost reduced severity
<i>Botrytis cinerea</i>	bunch rot in grapes		
<i>Aspergillus fumigatus</i>		respiratory disease, otomycosis	
<i>Candida albicans</i>	candidiasis		
<i>Cryptococcus neoformans</i>	subacute chronic meningitis		
<i>Epidemerothyton spp</i>			
<i>Trichophyton spp</i>			
<i>Trichosporon spp</i>	Infection of hair follicles		
<i>Phialophora spp</i>	Deep tissue infections		

Nematodes	Common name	Characteristics	Control measures
<i>Meloidogyne spp.</i>	root knot/root lesion	Stunts plants, root galls	
<i>Meloidogyne hapla</i>	root knot nematode		Szczzech et al., 1993 – vermicompost had no inhibitory effect
<i>Meloidogyne janatica</i>	root knot nematode		Zambolium et al. 1996 control using organic amendments on tomato
<i>Pratylenchus spp.</i>	root knot/root lesion	Stunts plants, root galls	
<i>Heterodera schachtii</i>	beet cyst nematode		Szczzech et al., 1993 – vermicompost had no effect
<i>Ascaris suum</i>	pig roundworm		
<i>Ascaris lumbricoides</i>	roundworm	ascariasis	
<i>Ancylostoma duodenale</i>	hook worm	anaemia	
<i>Necator americanus</i>	hook worm	anaemia	
<i>Enterobius vermicularis</i>	pinworm		
<i>Strongyloides stercoarlis</i>	threadworm	abdominal pain, nausea, diarrhoea	
<i>Toxocara canis</i>	dog roundworm	fever, abdominal pain	
<i>Trichuris trichiura</i>	whip worm	abdominal pain, diarrhoea	
<i>Taenia saginata</i>	tapeworm	taeniasis	

Bacteria	Common name	Characteristics	Control measures
<i>Agrobacterium tumefaciens</i>	crown gall	form large galls on the roots near trunk of trees	
<i>Erwinia spp.</i>	soft rot, bacterial stem rot	Soft slimy rot	

<i>Clostridium tetani</i>	Tetanus. Found in soils, animal pathogen, anaerobic		
<i>Bacillus anthrax</i>	Anthrax. Found in soils, animal pathogen, aerobic		
<i>Escherichia coli</i>	enteric bacterium	Gastroenteritis	
<i>Shingella spp.</i>	enteric bacteria (4 spp)	Bacillary dysentery	
<i>Salmonella spp.</i>	enteric bacteria (1,700 types)	Salmonellosis	
<i>Salmonella typhi</i>	Typhoid fever		
<i>Mycrobacterium tuberculosis</i>	Tuberculosis		
<i>Campylobacter jejuni</i>		Gastroenteritis	
<i>Yersinia sp.</i>	Yersinosis	Gastroenteritis	
<i>Leptospira</i>		Weil's disease	
<i>Vibro cholerae</i>	cholera	Cholera	

Pathogenic protozoa

Organism	Disease		
<i>Entamoeba histolytica</i>	dysentery	Amoebic dysentery, liver abscess, colonic ulceration.	
<i>Giardia lamblia</i>	giardiasis	Diarrhoea	
<i>Balantidium coli</i>	diarrhoea	Diarrhoea	
<i>Naegleria fowleri</i>			
<i>Cryptosporidium</i>	gastroenteritis	Diarrhoea	
<i>Toxoplasma gondii</i>	toxoplasmosis		

Enteroviruses potentially present in sewage sludge

Virus	Common name	Characteristics	Control measures
<i>Poliovirus</i>		Meningitis, paralysis, fever	
<i>Echovirus</i>		Meningitis, diarrhoea, rash	
<i>Coxsackievirus A & B</i>			
<i>New enteroviruses Types 68-71</i>			
<i>Hepatitis A (Enterovirus Type 72)</i>		Infective hepatitis	
<i>Norwalk virus</i>			
<i>Calicivirus</i>		Gastroenteritis	
<i>Astrovirus</i>		Gastroenteritis	
<i>Reovirus</i>			
<i>Rotavirus</i>			
<i>Adenovirus</i>			
<i>Pararotavirus</i>			
<i>Snow Mountain Agent (USA)</i>			
<i>Epidemic non-A non-B hepatitis</i>		Hepatitis	

Microbes in soils

Each soil has its own particular and dynamic microbial components, these vary with time due to seasonal or management changes. Usually bare soils have low microbial numbers on the surface due to excessive UV radiation. Most activity is just below the surface down to about 10 cm. Compared to soils, microbial composition of composts range thus:

Microbes	Soil	Compost	Vermicompost
Total Bacterial CFU/g			5,300,000
Anaerobic count CFU/g			410,000
Bacteria	$10^6 - 10^9$ per g		
Actinomycetes			
Fungi	10^5		
Algae	10^4		
Protozoa	$10^2 - 10^5$ per g		
Microarthropods	$10^4 - 10^5$ per g		
Nematodes	10^2		

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Soil and Microbial Biodiversity

Introduction	Crops	Plants	Animals	Forests	Fish	Soil
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THOUGH SELDOM ACKNOWLEDGED in discussions of agricultural genetic resources, soils are "the critical life-support surface on which all terrestrial biodiversity depends" [1]. Soils are providers, storers and generators of biodiversity - but they are also one of the most undervalued and poorly researched habitats on earth [2]. At the very time soil ecologists are beginning to uncover the magnitude and importance of life in the soil, the resource itself is literally disappearing off the face of the earth. Human activities are the greatest threat to soil biodiversity:

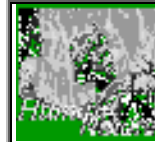
- Human-induced soil degradation by wind, water and pollution affects about 24% of the inhabited land area of the globe [3].
- A recent study by the UK's Royal Commission on Environmental Pollution concluded that some 10% of the world's soil has already been lost this century through deforestation, erosion, urban development and other abuses of the land [4].
- Approximately 30% of the world's arable crop land has been abandoned because of severe soil erosion in the last 40 years [5].
- Worldwide, soil is being lost at a rate 13 to 80 times faster than it is being formed. It takes about 500 years to form 25 mm of soil under agricultural conditions, and about 1,000 years to form the same amount in forest habitats [6].

The staggering diversity of soil biota may be orders of magnitude higher than above ground diversity of plants and animals, but no one has yet made an exhaustive census of even one natural habitat [7]. According to the Global Biodiversity Assessment, "a single gram of temperate forest soil could contain 10,000 million individual cells comprising 4,000-5,000 bacterial types, of which less than 10% have been isolated and are known to science;" more than 500 species of soil invertebrates (e.g. snails, earthworms, termites, mites, nematodes, etc.) have been recorded from a beech forest; over 2,500 species of fungi have been identified from a few hectares of land in southwest England [8]. Even moss tussock communities in the Antarctic Peninsula are home to over a hundred species of soil microorganisms and invertebrates [9]. Tropical soil biota, though perhaps richer than in temperate regions, is still relatively unknown and undocumented.

Microbial diversity encompasses a spectrum of microscopic organisms including bacteria, fungi, algae and protozoa. An estimated 50 percent of all living protoplasm on Earth is microbial [10]. There may be 1.5 million species of fungi yet only 5% are described; as many as one million species of bacteria may exist, but only about 5,000 have been described in the last century [11]. According to new estimates by the Center for Microbial Ecology at Michigan State University (USA) a gram of typical soil contains about 1 billion bacteria, but only 1 percent can be successfully grown (cultured) in the laboratory. Fewer than 5% of all microbial species have been discovered and named - and even less is known about the diversity within those species [12]. So little is known about most of the microbial world that no one has ever documented the extinction of a bacterium [13].

Life in the soil and life on Earth

Soil biodiversity influences a huge range of ecosystem processes that contribute to the sustainability of life on earth. For example, soil organisms maintain critical processes such as carbon storage, nutrient cycling and plant species diversity. Soil biodiversity plays a role in soil fertility, soil erosion, nutrient uptake by plants, formation of soil organic matter, nitrogen fixation, the biodegradation of dead plant and animal material, reducing hazardous waste, the production of organic acids that weather rocks, and control of plant and insect populations through natural biocontrol.



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Through production of food, fibre and renewable forms of energy, soil-based plant productivity supports the livelihood of every person on earth. Soil biota enhance crop productivity because they recycle the basic nutrients required for all ecosystems, including nitrogen, phosphorous, potassium and calcium. Soil organisms enhance the productivity of the soil by increasing water infiltration, thereby reducing surface water runoff and decreasing soil erosion.

Termites, earthworms and other burrow-building soil organisms enhance soil productivity by churning and mixing the upper soil, which redistributes nutrients, aerates the soil and increases surface water infiltration [14]. Earthworms and other invertebrates can bring to the surface from 10 to 500 tonnes/per hectare/per year of soil, and thus play a critical role in the formation of topsoil. Cornell University entomologist David Pimentel estimates that the value of soil biota to soil formation on agricultural land worldwide is US\$50,000 million per annum [15].

Nitrogen from natural and commercial sources is vital to plants and animals. It is the main nutrient required for growth in plants and for building proteins in animals. Biologically fixed nitrogen (primarily nitrogen-fixing microorganisms that live symbiotically on the roots of leguminous plants and trees) makes an enormous contribution to global agricultural productivity. In poor soils, where alternative sources of fertilizer are either unavailable or unaffordable, biological nitrogen-fixation is vital to crop production. Worldwide, an estimated 140 to 170 million tonnes of nitrogen, valued at approximately US\$90,000 million are fixed by microorganisms in agricultural and natural systems each year [16].

Soil biota play a major role in stabilizing and regulating the earth's climate. Global warming is the result of increasing levels of carbon dioxide and other greenhouse gases in the Earth's atmosphere - primarily caused by the burning of fossil fuels by humans. The rate of exchange of carbon between the earth's surface, the oceans and the atmosphere, known as "the carbon cycle", is the primary mediating force with regard to climate change. Through the process of photosynthesis, green plants absorb carbon dioxide from the atmosphere. It is well known that trees and forests store the absorbed carbon in woody biomass. But it is actually soil organic matter that is the major global storage reservoir for carbon. The living microbes, fungi and invertebrates found in the soil are responsible for decomposing carbon and nitrogen and making them available for plant growth, while at the same time contributing to the rate of production and consumption of carbon dioxide, methane and nitrogen.

Despite the importance of soil biodiversity to life-sustaining ecosystem processes, soils are one of the most neglected habitats on earth [17]. In most cases, soil biologists simply don't know which organisms or groups of organisms play the most important roles in ecological processes, they don't know which soil taxa are being lost, or what impact these losses will have in the future.

There is general consensus that we are losing soil biodiversity. Many microbes live symbiotically with higher organisms. Every plant and animal that becomes extinct is likely to take several species of microorganism with it. According to soil ecologist Diana Freckman of Colorado State University, knowledge of soil species remains a "black box" in our understanding of how soil systems function [18]. A study published by the US National Research Council in 1993 noted that "Our lack of knowledge of microorganisms and invertebrates, which are estimated to make up as much as 88% of all species, seriously hampers our ability to understand and manage ecosystems" [19]

Soil ecologists believe that it is essential and urgent to establish the cause and effect relationships between the loss of soil biodiversity and the impact on terrestrial and global ecosystem processes. Only by knowing and understanding life in the soil can we begin to conserve and better utilize its life-sustaining services. Industrial agriculture has contributed to the neglect of soil biodiversity because conventional soil science has generally relied on the use of purchased farm inputs to overcome constraints and modify the soil environment. (For example, if the soil is dry, irrigate; if soil fertility is low, buy synthetic fertilizer; if pests and weeds invade, spray chemicals). With growing awareness and need for low-input and sustainable agriculture, knowledge of soil biodiversity is increasingly important to future farming systems [20]. A better understanding of soil biota will enable farmers to depend less on modification of the natural environment and place greater emphasis on using biological processes to optimize nutrient cycling, minimize the use of purchased inputs, and maximize the efficiency of their use.

The value of microbial genetic resources

Microorganisms (or microbes) are tiny living things that are not visible except with a microscope. They include algae, bacteria, fungi (including yeasts), certain protists (one celled animals that are not bacteria) and viruses. Microbial biodiversity is a vast frontier and a potential goldmine for the biotechnology

industry because it offers countless new genes and biochemical pathways to probe for enzymes, antibiotics and other useful molecules [21].

Worldwide, the economic value of microorganisms is estimated to be "at least many tens of billions of US dollars" [22]. Pharmaceuticals of microbial origin account for sales of approximately \$35-50 billion per annum in the North [23]. It is the invisible world of microbes that has given us more than 3,222 antibiotics, for example, many derived from soil samples. In 1993, five of the pharmaceutical industry's top-selling drugs were derived from microbes; accounting for more than \$4,500 million in annual sales [24]. The commercial value of microbials extends beyond pharmaceuticals. The total world market for industrial enzymes, all produced by microorganisms, is \$1,300 million. Enzymes are natural catalysts that can speed up a chemical reaction. Because the process is biological, they are biodegradable and can be used instead of synthetic chemicals. For example, industrial enzymes are used to enhance detergents, as biological pesticides, to clean up toxic wastes, to replace chemicals in paper and pulp processing, and for oil extraction.

With the use of modern biotechnology, the potential applications of microorganisms is vast. Scientists are experimenting with genetically engineered bacteria that are capable of producing products such as biodegradable plastics, artificial skin, and fibres that are as strong as spider silk. Maize, rice, potato and cotton are among the crops that have been genetically engineered to produce insecticidal genes from a common soil bacterium, *Bacillus thuringiensis* (Bt). The Bt genes enable the crops to produce a toxic protein that kill insects which feed on the plant. Microbial diversity can play an important role in the decomposition of hazardous wastes. Molecular biologists are attempting to harness specific organisms, or groups of organisms, to clean-up toxic wastes in the environment, or reduce hazardous waste production in industrial processes.

Today, transnational microbe hunters are especially interested in exotic and hostile environments - including boiling hot springs, undersea hydrothermal vents, alkali lakes and the frozen tundra of Antarctica - as a source of unexplored microbial diversity. Bioprospecting for microbes goes, quite literally, to the ends of the earth. The following are just a few examples:

- A subsidiary of German agrochemical giant AgrEvo is conducting intensive soil sampling in India. According to German researcher Michael Flitner, the company has already screened over 90,000 Indian soil samples and is building a new, high-efficiency system in Frankfurt for screening plants and soil collected in India [25].
- When employees of Denmark's Novo Nordisk corporation go on holiday, they take soil-collection kits to gather exotic, enzyme-producing microbes. The father of one Novo Nordisk scientist collected a soil sample from an Indonesian temple which yielded an enzyme that is now widely used by soft-drink suppliers to change starch into sugar. In 1949, Filipino scientist Abelardo Aguilar sent his employer, Eli Lilly Co., samples of an antibiotic isolated from a soil sample that he collected in his home province of Iloilo. Three years later, Eli Lilly sent a congratulatory letter to Aguilar, promising to name the new antibiotic "Ilosone" after the Filipino province where the soil sample was found. The drug, erythromycin, sold under the brand name "Ilosone" has since earned Eli Lilly millions of dollars, but neither Aguilar nor the Philippines received any royalties, despite Aguilar's 40-year battle to be recognized and rewarded [26].
- Sponges growing on a coral reef off the coast of Papua New Guinea are the source of a powerful antifungal agent "Papuamine." Because the sponges yield only minute quantities of the antifungal agent, Myco Pharmaceuticals (USA) is now attempting to synthesize papuamine in the laboratory.
- Bacteria found in the whale gut from the last legal Eskimo whale hunt are capable of breaking down toxic petrochemicals. Scientist A. Morrie Craig of Oregon State University has applied for patents on some of the whale gut bacteria, and Pioneer Hi-bred has already secured rights over commercial products that may someday result from the bacteria.
- Bacteriologist Thomas D. Brock discovered a bacterium, *Thermus aquaticus* in the boiling hot springs of Yellowstone National Park (USA) in 1966. An enzyme isolated from *Thermus aquaticus* is the catalyst for the polymerase chain reaction, or PCR, a technique that is widely and routinely used for producing copies of any DNA sequence. Although Thomas Brock donated the bacterium he discovered to the scientific world, it was later patented and now brings royalties valued at hundreds of millions of dollars annually to Swiss pharmaceutical corporation Hoffman-LaRoche.

Microbial genetic resources in the international policy arena

Despite its growing economic importance, microbial genetic diversity has been under-valued and under-recognized in biodiversity debates. There is an obvious policy gap in the international arena, and it is poor farmers who will likely pay the greatest price for this oversight. The vast majority of microbial culture collections are located in the North, and there is a growing trend toward privatization and patenting of this material. Microbial genetic resources can no longer be disregarded as ubiquitous life forms outside of the mainstream of biodiversity policy debates. Today, the genetic resources of microorganisms are very much an issue in the international policy arena.

Microbial biodiversity - where's the political debate?

The Convention on Biological Diversity excludes from its scope all ex situ germplasm collected prior to the Convention coming into force at the end of 1993. This means that all microbial culture collections, the vast majority of which are located in the industrialized world, are the legal property of the depositor and not of the donor country, regardless of where the germplasm was collected. The U.S.-based American Type Culture Collection, the world's largest microbial culture collection, contains thousands of biological specimens from the South, dozens of which are the subject of patent claims by Northern pharmaceuticals such as Bristol-Myers, Pfizer and Eli Lilly.

Patent culture depositories are regulated internationally by the Budapest Treaty on International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure administered by the World Intellectual Property Organization in Geneva. Currently, 32 countries are signatories to the Budapest Treaty. An estimated 86% of global microbial collections is held in industrialized nations [27].

A network of microbial resource centres for the developing world (MIRCENs) was established in the early 1970s by UNEP and UNESCO. Today, there are no policies in place to protect these microbial genetic resources from privatization or to insure equitable exchange of microbial genetic resources in culture collections worldwide. Normally, MIRCENs have a policy of free exchange of microbial materials within the network, but each MIRCEN may decide on a case-by-case basis.

The Uruguay Round of the General Agreement on Tariff and Trade (GATT) incorporates an element called Trade Related Aspects of Intellectual Property Rights (TRIPs) which specifies that microorganisms may not be excluded from patent protection (Section 5, Article 27.2). All countries that are signatories to the World Trade Agreement are now obligated to adopt and implement patent laws for microorganisms and for biotechnology processes applied to living organisms. What is the definition of a microorganism? When is a microorganism patentable? For the purposes of patent protection, there is considerable uncertainty and controversy regarding the answer to these questions. In many countries, the term microorganism extends to cell lines and plasmids - including human genetic material.

The patenting of human genetic material is no longer a theoretical concern, but a shocking reality. On March 14, 1995 the US Patent and Trademark Office granted a patent to the US National Institutes of Health (NIH) for an unmodified human cell line drawn from a 20-year old Hagahai man from Papua New Guinea. It is the first time that an indigenous person's cells have ever been patented. Not only plants, animals and microorganisms from gene-rich ecosystems of the South, but also the genes and cells of indigenous peoples have become targets of Northern scientists and industrial bioprospectors. Private ownership of human biological materials raises many profound moral, ethical, and political issues. There is no international protocol to protect human subjects from patent claims and unjust commercial exploitation. And there is no mechanism to compensate individuals or communities from whom DNA samples are taken. Signatories to the World Trade Agreement must determine whether or not human genetic materials are included in its definition of microbial materials. At the Jakarta meeting of the Conference of Parties to the Convention on Biological Diversity held in November, 1995, delegates made it clear that they did not wish to regard human genetic materials as part of the Convention, despite the fact that the legally-binding Convention does not explicitly exclude human biodiversity from its mandate. The World Health Organization has yet to establish internationally-accepted medical ethics protocols covering the commercialization or patenting of human genetic material. There is a serious policy vacuum that some international body must fill.

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Introduction	Crops	Plants	Animals	Forests	Fish	Soil
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Note: The Rural Advancement Foundation International (RAFI) is an international, non-governmental organisation headquartered in Ottawa, Ontario (Canada) with an affiliate office in Pittsboro, North Carolina (USA). RAFI is dedicated to the conservation and sustainable improvement of agricultural biodiversity, and to the socially responsible development of technologies useful to rural societies. RAFI is concerned about the loss of genetic diversity - especially in agriculture - and about the impact of intellectual property rights on agriculture and world food security. RAFI encourages the wide dissemination of *Human Nature* by any means, including photocopying. It requests only that the author, RAFI, and the title of the work be cited if it is used. Visit the RAFI Web site at <http://www.rafi.ca>; e-mail: rafican@rafi.ca.

The Soil Foodweb: It's Importance in Ecosystem Health

Web-article by Elaine Ingham*.

*Dr. Elaine R. Ingham, President of Soil FoodWeb, Inc. Author USDA Soil Biology Primer.

www.soilfoodweb.com

Soil Foodweb Significance

The structure and function of the soil foodweb has been suggested as a prime indicator of ecosystem health (Coleman, et al. 1992; Klopatek, et al. 1993). Measurement of disrupted soil processes, decreased bacterial or fungal activity, decreased fungal or bacterial biomass, changes in the ratio of fungal to bacterial biomass relative to expected ratios for particular ecosystems, decreases in the number or diversity of protozoa, and a change in nematode numbers, nematode community structure or maturity index, can serve to indicate a problem long before the natural vegetation is lost or human health problems occur (Bongers, 1990; Klopatek et al. 1993).

Soil ecology has just begun to identify the importance of understanding soil foodweb structure and how it can control plant vegetation, and how, in turn, plant community structure affects soil organic matter quality, root exudates and therefore, alters soil foodweb structure. Since this field is relatively new, not all the relationships have been explored, nor is the fine-tuning within ecosystems well understood.

Regardless, some relationships between ecosystem productivity, soil organisms, soil foodweb structure and plant community structure and dynamics are known, and can be extremely important determinants of ecosystem processes (Ingham and Thies, 1995). Alteration of the soil foodweb structure can result in sites which cannot be regenerated to conifers, even with 20 years of regeneration efforts (Perry, 1988; Colinas et al, 1993). Work in intensely disturbed forested ecosystems suggests that alteration of soil foodweb structure can alter the direction of succession. By managing foodweb structure appropriately, early stages of succession can be prolonged, or deleted (Allen and Allen, 1993). Initial data indicates that replacement of grassland with forest in normal successional sequences requires alteration of soil foodweb structure from a bacterial-dominated foodweb in grasslands to a fungal-dominated foodweb in forests (Ingham, E. et al, 1986 a, b; 1991; Ingham and Thies, 1995).

In addition to responses to disturbance, it is clear that species diversity, community diversity and foodweb complexity increases with increasing successional stage (Moore et al., 1991; Ingham, E. et al., 1989). Indeed, examination of foodweb interactions and ecosystem diversity, instead of community diversity, may result in new ecosystem measures which reflect this increased community diversity and increased connectivity in later successional stages.

The numbers, biomass, activity and community structure of the organisms which comprise the soil foodweb can be used as indicators of ecosystem health because these organisms perform critical processes and functions. Soil decomposers (bacteria, fungi and possibly certain arthropods) are responsible for nutrient retention in soil. If nutrients are not retained within an ecosystem, future productivity of the ecosystem will be reduced as well as cause problems for systems into which those nutrients move, especially aquatic portions of the landscape (Hendrix et al, 1986; Klopatek, et al. 1993).

As ecosystems become more productive, the total amount of nutrients retained within the system increases. As succession occurs, nutrients are increasingly immobilized in forms that are less available for plants and animals, such as phytates, lignins, tannins, humic and fulvic acids (Coleman et al, 1985, 1992). In order for nutrients to become available once again to plants and animals, they must be mineralized by the interaction of decomposers, i.e. bacteria and fungi, and their predators, i.e. protozoa, nematodes, microarthropods, and earthworms (if present). These predator populations and the rates at which they perform mineralization processes are important to ecosystem stability. The activity of these predator-prey interactions (which

determines the rate at which mineralization occurs) are in turn affected, and perhaps controlled by, higher level predators such as millipedes, centipedes, beetles, spiders, and small mammals. It is perhaps something of a conundrum that in healthy ecosystems, while nutrient cycling and productivity increases, nutrient loss is minimized. What makes this possible is the increasing complexity of the soil foodweb. As total ecosystem productivity increases, biodiversity below ground, i.e., the structure and function of the soil foodweb, also increases (Moore et al. 1991). The greater the foodweb complexity, i.e., the interaction of decomposers, their predators, and the predators of those predators responsible for nutrient cycling and the retention of nutrients within the soil (Coleman et al, 1985; 1992), the fewer the losses of nutrients from that system, the more tightly nutrients cycle from retained forms to plants, and back again. Without the soil foodweb, plants would not obtain the nutrients necessary for growth, and the above ground foodweb would not long continue (Nannipieri et al. 1990).

Interactions of decomposers with their predator groups (protozoa, nematodes and microarthropods) maintain normal nutrient cycling processes in all ecosystems (Coleman 1985, 1992). Plant growth is dependent on microbial nutrient immobilization and soil foodweb interactions to mineralize nutrients (Nannipieri et al. 1990). In undisturbed ecosystems, the processes of immobilization and mineralization are tightly coupled to plant growth. Following disturbance, this coupling is lost or reduced (Ingham et al. 1986a, b; Coleman et al., 1992).

By monitoring soil organism dynamics, we can detect detrimental ecosystem changes and possibly prevent further degradation (Lal and Stewart, 1992). The response of each group of soil organisms, i.e., soil saprophytic bacteria, symbiotic bacteria, saprophytic fungi, mycorrhizal fungi, protozoa, and nematodes, with respect to their total biomass and activity, can be used to indicate effects of contaminants on soil health. Instead of relying on an indirect measure of whether total biomass or activity is reduced (e.g., Paul and Clark, 1990; Nannipieri et al. 1991), active and total biomass of each organism group can be directly measured (Ingham et al. 1986).

Lal and Stewart (1992) reviewed the relationship between system health and soil organic matter, and suggested that soil organism losses correlate with detrimental ecosystem changes. Development of the relationship between soil foodweb structure and function and assessment of potential toxic impact could be extremely useful for assessing ecosystem health.

Two measures of ecosystem processes are discussed below: the ratio of fungal to bacterial biomass (Ingham and Horton, 1987) and the Maturity Index for nematodes (Bongers, 1985). Both appear to be useful predictors of ecosystem health, although they must be properly interpreted given the successional stage being examined. For example, recently disturbed systems have nematode community structures skewed towards opportunistic species and genera, while the less opportunistic, more K-selected species of nematodes return as time since-disturbance increases. Thus, healthier soils tend to have more mature nematode community structures. However, as systems mature, nutrients tend to be more sequestered in soil biomass and organic matter, and thus the maturity index reflects an optimal, intermediate disturbance period in which greatest ecosystem productivity is likely to occur.

Ratios of fungal to bacterial biomass also predict this type of response. Highly productive agricultural soils tends to have ratios near one, but as a system undergoes succession into a grassland, this ratio dips downwards, indicating that for a healthy grassland system, the ratio should be less than one. In other words, bacterial-biomass dominates in healthy grassland soils. However, as succession proceeds yet further, fungal biomass begins to dominate and healthy forest systems have fungal to bacterial biomass ratios of greater than one, usually greater than 10.

Piparian or deciduous forests appear to be intermediate within this range of values. Alder forest soils are dominated by bacterial biomass, while poplar forest soils are fungal-dominated. Clearly, further investigation is required.

The predators of bacteria and fungi tend to follow the dominance of the decomposer groups. Thus, bacterial-dominated soils have a majority of bacterial-predators (protozoa and bacterial-feeding nematodes), while fungal-dominated soils have a majority of fungal predators (fungal-feeding nematodes and fungal-feeding microarthropods).

Much work is still required at the bacterial and fungal species level. While the species of protozoa and nematodes have been researched in soils of this area of the west, publication of much of this information has yet to occur. Up-dates will be required as this information becomes available.

The Soil Foodweb: Function

[Detrital food web in shortgrass prairie](#)

Bacteria and fungi perform one of the major nutrient cycling processes, nutrient retention, in soil (Coleman et al. 1992). The amount of N, P, S and other nutrients immobilized in bacterial and fungal biomass can be considerable, from several micrograms to milligrams of biomass, comprising a significant portion of the stable nutrient pool (Ingham et al, 1986). When the bacterial or fungal component of the soil declines, more nutrients are lost into the ground and surface water (Hendrix et al, 1986; Coleman et al., 1992). A major means of retaining nutrients may also be arthropod fecal material (Rusek, 1983; Pawluk, 1983), depending on the ecosystem.

Soil bacteria are important in maintaining normal nutrient immobilization and decomposition processes in all ecosystems (Coleman et al. 1985; Ingham, et al. 1986a, b). Plants are strongly influenced by the presence of bacteria in the rhizosphere, especially with respect to microbial immobilization of nutrient, and mineralization of nutrients from bacterial biomass by predators. Disturbance of these soil processes may result in the un-coupling of mineralization and plant growth, with the resultant loss of nutrients from the system, causing problems for systems into which nutrients move (Ingham and Coleman, 1984).

As climate changes occur, bacterial populations in the soil could be significantly impacted (Coleman et al, 1992). As temperature increases, bacterial numbers could increase, resulting in greater immobilization of nutrients in their biomass, causing greater nitrogen limitation of plant growth. Alternatively, bacteria could be inhibited by increases in carbon dioxide, resulting in decreased decomposition of soil organic matter and plant litter, which ultimately would change soil structure and nutrient cycling. In addition, current work indicates that alterations in the fungal to bacterial biomass ratio strongly impacts vegetative community structure. If a forest soil, usually strongly dominated by fungi, loses the fungal component, reflected by a decrease in the ratio of fungi to bacteria, conifer species may be at risk of death. If the fungal to bacterial biomass ratio decreases past one, re-establishment of conifer species may be impossible.

Saprophytic fungi and bacteria form the base of the detrital foodweb, and as such are critically important for supporting the nutrient cycling sub-system of any ecosystem, landscape, or biome. Bacterial and fungal pathogens of plants, insects, rodents, and other organisms can control the population density of their hosts. Mutualist bacteria and fungi can be critically important for plants and animals alike, for example, nitrogen-fixing bacteria on legumes, or rumen bacteria in cows, deer or elk. Without their mutualists, these plants and animals are not capable of competing with other organisms and become locally extinct. While methods are not yet capable of distinguishing between saprophytic and pathogenic species of bacteria and fungi in soil, their total and active biomass, and effects of different disturbances on their distributions, can be estimated. However, work should continue on methods to differentiate bacterial and fungal community composition in soil.

Protozoa, comprised of the three groups; (1) flagellates, (2) amoebae (both naked and testate), and (3) ciliated, are important in maintaining plant-available N and mineralization processes (Coleman, 1985) and, as bacterial-feeders, are important in controlling bacterial numbers and community structure in the soil (Foissner 1986). The presence or absence of certain protozoa species is indicative of the presence of certain hazardous wastes and therefore may be highly useful indicator organisms of certain types of environmental impacts (Foissner 1986).

Nematodes are one of the most ecologically diverse groups of animals on earth, existing in nearly every habitat. Nematodes eat bacteria, fungi, algae, yeasts, diatoms and may be predators of several small invertebrate animals, including other nematodes. In addition, they may be parasites of invertebrates, vertebrates (including man) and all above and below ground portions of plants. Nematodes range in length from 82 μ m (marine) to 9 m (whale parasite) but most species in soil are between 0.25 and 5.5 mm long. Nematodes are recognized as a major consumer group in soils, generally grouped into four to five trophic categories based on the nature of their food, the structure of the stoma and esophagus and method of feeding (Yeates, 1971). Plant-feeding nematodes possess stylets with a wide diversity of size and structure and are the most extensively studied group of soil nematodes because of their ability to cause plant disease and reduce crop yield. Fungal-feeding nematodes have slender stylets but are often difficult to categorize and have been included with plant-feeders in many ecological studies. Bacterial-feeding nematodes are a diverse group and usually have a simple stoma in the form of a cylindrical or triangular tube, terminating in a teeth (Nicholas, 1975). valve-like apparatus which may bear minute nematodes (marine) to 9 m 0.25 and 5.5 mm

Predatory nematodes are usually large species possessing either a large stylet or a wide cup-shaped cuticular-lined stoma armed with powerful teeth (Nicholas, 1975). Omnivores are sometimes considered as a fifth trophic category of soil nematodes. These nematodes may fit into one of the categories above but also ingest other food sources. For example, some bacterial feeders may also eat protozoa and/or algae and

some stylet-bearing nematodes may pierce and suck algae as well as fungi and/or higher plants. Stages of animal-parasitic nematodes, such as hookworms, may also be found in soils but generally are not common in most soil samples.

Nematodes and protozoa function as regulators of mineralization processes in soil (Coleman, 1985).

Bacterial- and fungal-feeding nematodes release a large percent of N when feeding on their prey groups and are thus responsible for much of the plant available N in the majority of soils (Ingham, R. et al. 1985). Nematode-feeding also selects for certain species of bacteria, fungi and nematodes and thereby influences soil structure, carbon utilization rates, and the types of substrates present in soil (Ingham, R. 1992). Root-feeding nematodes are among the greatest pests in agricultural systems and, with the loss of many nematicides, are becoming a greater concern. Without doubt, plant establishment, survival and successional processes are influenced by these soil organisms

Soil processes are important for maintaining normal nutrient cycling in all ecosystems (Coleman et al., 1985; Dindal 1990; Ingham, E. et al. 1986a, b). Plant growth is dependent on the microbial immobilization and soil foodweb interactions to mineralize nutrients. In undisturbed ecosystems, the processes of immobilization and mineralization are tightly coupled to plant growth but following disturbance, this coupling may be lost or reduced. Nutrients may be no longer retained within the system, causing problems for systems into which nutrients move (Ingham and Coleman, 1984; Hendrix et al. 1986; Nannipieri et al. 1990). Measurement of disrupted processes may allow determination of a problem long before normal cycling processes are altered, before the natural vegetation is lost, or human health problems occur. By monitoring soil organism dynamics, we can perhaps detect detrimental ecosystem changes and possibly prevent further degradation.

Immobilization of nutrients in soil, i.e., retention of carbon, nitrogen, phosphorus, and many micronutrients in the horizons of soil from which plants obtain their nutrients, is a process performed by bacteria and fungi. Without these organisms present and functioning, nutrients are not retained by soil, and the ecosystem undergoes degradation. Thus, to assess the ability of an ecosystem to retain nutrients, the decomposed portion of the ecosystem, i.e., active and total fungal biomass, and active bacterial biomass must be assessed.

The Soil Foodweb: Structure

[Worksheet for 1 sq. m soil](#)

What is the soil foodweb? Per gram of healthy soil, which is about a teaspoon of soil plus organic matter, the following organisms are found: of which are mostly unknown to scientists. Bacteria break down easy to-use organic material, and retain the nutrients, like N, P and S, in the soil. About 60% of the carbon in those organic materials are respired as carbon dioxide, but 40% of that carbon is retained as bacterial biomass. The waste products bacteria produce become soil organic matter. This "waste" material is more recalcitrant than the original plant material, but can be used by a large number of other soil organisms, exemplifying the classic statement that "One man's garbage is another's treasure". Productive garden soil should contain more bacteria than any other kind of organism, although care must be taken to make sure beneficial bacteria, instead of disease-causing bacteria, are most prevalent. - S to 60 000 meters of fungal hyphae. Fungi break down the more recalcitrant, or difficult-to-decompose, organic matter, and retain those nutrients in the soil as fungal biomass. Just like bacteria, fungal waste products become soil organic matter, and these waste materials are used by other organisms. Gardens require some fungal biomass for greatest productivity, but in order for best crop growth, there should be an equal biomass of bacteria as compared to fungi. Most grasslands or pastures have less fungi than bacterial, while all conifer forests have much more fungal, as compared to bacterial, biomass. As with bacteria, some fungi cause disease and the soil must be managed to prevent these fungi from being a problem.

-100 to 100,000 protozoa. These organisms are one-celled, highly mobile organisms that feed on bacteria and on each other. Because protozoa require 5 to 10-fold less nitrogen than bacteria, N is released when a protozoan eats a bacterium. That released N is then available for plants to take up. Between 40 and 80% of the N in plants can come from the predator-prey interaction of protozoa with bacteria.

- 5 to 500 beneficial nematodes. Beneficial nematodes eat bacteria, fungi, and other nematodes. Nematodes need even less nitrogen than protozoa, between 10 and 100 times less than a bacterium contains, or between 5 and 50 times less than a fungal hyphae contains. Thus when bacterial- or fungal-feeding nematodes eat bacteria or fungi, nitrogen is released, making that N available for plant growth. However, plant-feeding nematode are pests because they eat plant roots. These "bad" nematodes can be controlled bacteria, fungi, and other nematodes. Nematodes need even less nitrogen than protozoa, between 10 and 100 times less

than a bacterium contains, or between 5 and 50 times less than a fungal hyphae contains. Thus when bacterial- or fungal-feeding nematodes eat bacteria or fungi, nitrogen is released, making that N available for plant growth. However, plant-feeding nematodes are pests because they eat plant roots. These "bad" nematodes can be controlled biologically, as they are in natural systems, by fungi that trap nematodes, by having fungi that colonize root systems and prevent nematode attack of roots, or by predation of nematodes by arthropods. In cases of extreme outbreaks, however, the only answer may be the use of chemicals to control these plant-feeding nematodes. However, once a chemical is used which kills the beneficial nematodes as well as the plant-feeding ones, the beneficial nematodes need to be replaced through inoculation.

- A few to several hundred thousand microarthropods. These organisms have several functions. They chew the plant leaf material, roots, stems and boles of trees into smaller pieces, making it easier for bacteria and fungi to find the food they like on the newly revealed surfaces. The "comminuting" arthropods can increase decomposition rates by 2- to 100- times, although if the bacteria or fungi are lacking, increased decomposition will not occur. In many cases, however, the arthropods carry around an inoculum of bacteria and fungi, making certain the food they want is inoculated onto the newly exposed surfaces! Arthropods then feed on bacteria and fungi, and because the C:N ratio of arthropods is 100 times greater than the bacteria and fungi, they release nitrogen which then is available for plant growth. Some arthropods eat pest insects, while others eat roots. Again, it's important to encourage the beneficial ones and discourage the ones that eat plants!

The Web of Life Can Be Degraded

The interactions between these organisms form a web of life, just like the web that biologists study above ground. What most people don't realize is that the above ground wouldn't exist without the below ground systems in place and functioning. Soil biology is understudied, compared to the above ground, yet it is important for the health of gardens, pastures, lawns, shrublands, and forests. If garden soil is healthy, there will be high numbers of bacteria and bacterial-feeding organisms. If the soil has received heavy treatments of pesticides, chemical fertilizers, soil fungicides or fumigants that kill these organisms, the tiny critters die, or the balance between the pathogens and beneficial organisms is upset, allowing the opportunist, disease-causing organisms to become problems.

Over-use of chemical fertilizers and pesticides have effects on soil organisms that are similar to over-using antibiotics. When we consider human use of antibiotics, these chemicals seemed a panacea at first, because they could control disease. But with continued use, resistant organisms developed, and other organisms that compete with the disease-causing organisms were lost. We found that antibiotics couldn't be used willy-nilly, that they must be used only when necessary, and that some effort must be made to replace the normal human-digestive system bacteria killed by the antibiotics.

Soils are similar, in that plants grown in soil where competing organisms have been knocked back with chemicals are more susceptible to disease-causing organisms. If the numbers of bacteria, fungi, protozoa, nematodes and arthropods are lower than they should be for a particular soil type, the soil's "digestive system" doesn't work properly. Decomposition will be low, nutrients will not be retained in the soil, and will not be cycled properly. Ultimately, nutrients will be lost through the groundwater or through erosion because organisms aren't present to hold the soil together.

The best way to manage for a healthy microbial ecosystem in a home garden is to routinely apply organic material, such as compost. To keep garden soil healthy, the amount of organic matter added must be equal to what the bacteria and fungi use each year.

Indiscriminate use of chemical fertilizers and pesticides should be avoided. If the soil is healthy for the type of vegetation desired, there should be no reason to use pesticides, or fertilizers. If a decision is made to change from grass to garden, or forest to lawn, a massive change in the soil foodweb structure is required and chemical use, along with judicious addition of the right kind of compost with the right kinds of organisms, may be necessary for a few years. But once the correct soil foodweb structure is in place, there should be no reason to apply chemicals.

If both bacteria and fungi are lost, then the soil degrades, than any other organism. If bacteria are killed through pesticide or chemical applications, and especially if certain extremely important bacteria like nitrogen-fixing bacteria or nitrifying bacteria are killed, fungi can take over and crop production can be harmed. For example, current research indicates that the reason moss takes over in lawn ecosystems is because the soil is converted from a bacterial dominated system to one dominated by fungi. nutrients are lost, erosion increases and plant yield is reduced. If inorganic fertilizers are used to replace the lost nitrogen,

the immediate effect may be to improve plant growth. However, as time goes on, it is clear that inorganic fertilizers can't replace the other kinds of food that bacteria and fungi need. After awhile, fertilizer additions are a waste of money, because there aren't enough soil organisms to hold on to the nutrients added. Surface and groundwater will become contaminated with the lost nutrients, causing problems.

Maintaining and Enhancing the Soil Foodweb

Bacterial dominance is maintained by mixing plant material into the soil. But the bacteria and fungi eat this material at an amazingly rapid pace and new inputs are required every year; Fungi can be maintained by letting litter accumulate on the soil's surface. Larger soil organisms like millipedes, centipedes, earthworms, and ants mix plant material into soil and open air channels, especially important in wet periods in heavy clay soils. To maintain a one-to-one ratio of bacteria and fungi needed for crop systems, a balance is needed between too much and too little mixing. Plant material needs to be mixed in enough to maintain bacterial dominance, but too much mixing results in soil degradation. Timing of mixing is important as well, but the optimal combination hasn't been determined for soil organisms in different types of soil. It's important to remember that grassland, garden and forest soils represent a gradient from bacterial to fungal dominance. Gardens require equal amounts of bacteria and fungi, while trees require fungi. There are a number of examples where the fungal component has been lost from forest soils and as a result, tree regeneration is impossible. If the soil foodweb was better understood, there would ways to fix the problem, but that research is yet to be done.

In order to determine the organisms in soil, the biomass and activity of bacteria and fungi, the numbers of protozoa and nematodes, the types (beneficial and root-feeding) of nematodes, and VA mycorrhizal colonization of roots need to be assessed. Reference information on the biomass, numbers and types of these organisms is being determined for soils all over the world. The goal is to determine what the healthy soil foodweb structure should be for every soil type, given vegetation and climate characteristics. If the foodweb structure is not at that healthy level, another goal is to determine what it will take to return it to a healthy level. Once a healthy foodweb structure is achieved, the only time testing would be needed is when some problem is detected, suggesting the foodweb has changed in an unproductive direction.

Soil Sampling

Soil sampling should result in three samples from any particular area, such as a meadow, crop field, forest stand or garden. Five samples per area, or more would be preferable, but time and cost of analysis must be a consideration. The idea is to take enough samples that the variability within that area can be assessed.

One possible approach is to mentally split the area to be sampled into three equal areas. From each of the three areas, between three and ten small soil cores should be mixed together in a plastic bag. The cores should be taken by pushing aside the litter (loose recognizable plant litter material) on the top of the soil and removing soil (may contain some unrecognizable plant material, but is mostly mineral soil material, or sand, silt and clay fractions) from the 0-5 cm depth. The core should be about 2.5 cm or 1 inch diameter, and all the soil from this small cylinder should be removed and placed in the plastic bag. If mycorrhizal colonization is to be performed, the roots in each core should be removed and placed in the plastic bag. Small scissors should be used to cut the small roots.

In fact, the foodweb structure in any kind of material, from lake sediment, to rumen material from cattle can be assessed, but most research has been performed on soil-related material.

Interpretation of Soil Foodweb Structure

Ratio of total fungal to total bacterial biomass

By examining the structure of the soil foodweb in a range of soils, all grassland and most agricultural soils have ratios of total fungal to total bacterial biomass less than one ($F/B < 1$). Another way to interpret this is that the bacterial biomass is greater than the fungal biomass in these soils.

In the most productive agricultural systems, however, the ratio of total fungal to total bacterial biomass equals one ($F/B = 1$) or the biomass of fungi and bacteria is even. When agricultural soils become fungal-dominated, productivity will be reduced, and in most cases, liming and mixing of the soil (plowing) is needed to return the system to a bacterial-dominated soil.

All conifer forest soils are fungal dominated, and the ratio in all forest soils in which seedling regeneration occurs is above 10. In general, productive forest soils have ratios greater than 100. This means that fungal biomass strongly outweighs the bacterial biomass in forest soils. In the case where forest soils lose this fungal-dominance, it is not possible to re-establish seedlings. When forest soil becomes bacterial-dominated, conifer seedlings are incapable of being re-established.

In the few studies of riparian forests that have been performed, some deciduous riparian forest soils are bacterial-dominated. In the case of riparian aspen and beech soils, the soils are bacterial dominated. But poplar, oak and maple soils are fungal-dominated, although not as strongly fungal-dominated as in conifer systems. No studies on establishment of seedlings in these systems have been performed.

The ratio of total fungal to total bacterial biomass has been related to ecosystem productivity, but numbers or length of active and total bacteria and fungi are also indicative of the health of soil. For different soils, vegetation and climate, the density of bacteria or fungi indicate the past degradation of the soil. As explained above, and again in the following sections, bacterial numbers should be greater than one million for all agricultural soils, preferably nearer 100 million for the most productive soils. For the most productive forest soils, for example, fungal length should be above 5000 meters of hyphae per gram soil.

Biomass of total fungi

Fungal biomass is extremely important in all soils as a means of retaining nutrients that plants need in the upper layers of the soil, i.e., in the root-zone. Without these organisms to take-up nutrients, and either retain those nutrients in their biomass, or to sequester those nutrients in soil organic matter, nutrients would wash through the soil and into ground or surface water. Plants would suffer from lack of nutrients cycling into forms that the roots can take-up, if these nutrients aren't first immobilized in the soil through the action of fungi or bacteria. For forest soils, fungi sequester most of the nutrients, although significant portions are immobilized by bacteria as well.

In soil in which only fungi are present, the soil will become more acidic, from secondary metabolites produced by fungi. Aggregates are larger in fungal-dominated soils than in bacterial-dominated soils, and the major form of N is ammonium, since fungi do not nitrify N. These conditions are more beneficial for certain shrubs, and most trees.

Total fungal biomass varies depending on soil type, vegetation, organic matter levels, recent pesticide use, soil disturbance and a variety of other factors, many of which have not been researched completely.

However, for normal grassland soils, total fungal biomass levels are usually around 50 to 500 meters per gram of soil. For agricultural soils, fungal biomass is around 1 to 50 meters per gram soil, while for forest soils, fungal biomass is between 1000 meters to 60 km per gram of soil. More work is necessary to establish what the optimal fungal biomass value should be for each type of crop, soil, organic matter, climate, etc. Very little information is available for tropical systems, but that small amount of data indicates that temperate systems perform very differently from tropical soils.

The average diameter of hyphae in most soils is about 2.5 micrometers, indicating typical mixtures of zygomycetes, ascomycetes and basidiomycetes species. On occasion the average diameter may be greater than 2.5 micrometers, indicating a greater than normal component of basidiomycete hyphae, while on other occasions, the average diameter of hyphae may be less than 2.5 micrometers, indicating a change in species composition of soil fungi to a greater proportion of lower fungi. Actinomycetes are not usually differentiated from fungi, since actinomycetes are hyphal in morphology and are rarely of significant biomass. In some agricultural soils, these narrow diameter "hyphae" are of considerable importance, as demonstrated by Dr. A. Van Bruggan.

Biomass of active fungi

Activity in all soil organisms follow a typical seasonal fluctuation. This cycle is related to optimal temperature and moisture, such that a peak in activity usually occurs in the spring as temperature and moisture become optimal after cold winter temperatures. In systems where snow accumulates on the soil surface, such that the soil does not actually freeze, fungal activity may continue at high levels throughout the winter in litter. Decomposition may continue at the highest rates through the winter under the snow in the litter. In systems where moisture becomes limiting in the summer, activity may reach levels even lower than in the winter. When temperatures remain warm in the fall and rain begins again after a summer drought, such as in Mediterranean climates, a second peak of activity may be observed in the fall. If these peaks are not observed, this suggests inadequate organic matter in the soil.

Numbers of total bacteria

Just as fungi are the most important players in retaining nutrients in forest soil, bacteria are the important players in agricultural and grassland soils. Bacteria retain nutrients first in their biomass, and second, in their metabolic by-products. In soil in which only bacteria are inoculated, the soil will become more alkaline, will have small aggregates, and generally will have nitrate/nitrite as the dominant form of N. These conditions are beneficial for grasses and row crop plants.

Numbers of total bacteria generally remain the same regardless of soil type or vegetation. Total bacterial numbers range between 1 million and 100 million per gram soil in agricultural soils, and between 10 million and 1,000 million in forest soils. Bacterial numbers can be above 100 million in decomposing logs, in anaerobic soils, in soil amended with sewage sludge or in soil with high amounts of composted material. In some instances following pesticide treatment, bacterial numbers can fall below 1 million, and this has been correlated with signs of severe nitrogen deficiency in plants. Bacterial numbers can drop to extremely low levels, below 100,000 per gram of soil, in degraded soils where nutrient retention is a problem.

Biomass of active bacteria

As with active fungal biomass, bacterial activity usually peaks in the spring and decreases during the summer with drought. If the temperature remains warm in the fall and fall rains begin, a second peak of activity usually occurs. The ratio of active fungal to active bacterial biomass, even in forests, shows that bacterial biomass is usually more active than fungal biomass. If these peaks with temperature and moisture are not seen, then lack of appropriate food in the soil to support bacterial and fungal biomass is suspected. If bacterial and fungal activity does not respond to seasonal fluctuations, then subsequent impacts on the predators in the soil will be observed.

Protozoan numbers

Protozoa feed on bacteria, and as they feed on their prey, N is released. It's unclear just how much N is released per individual feeding event, since it depends on whether the bacterium was actively growing, thus containing more N. or whether the bacterium was in a resting starving phase, and containing much less N. Several studies have shown that a major portion (40-80%) of the nitrogen that cycles through in certain agricultural soils is cycled by protozoa. Without these organisms in soil, plants suffer a significant reduction in available N. However the optimal relationship between the number of bacteria and the number of protozoa has not been quantified.

There appears to be a great range in protozoan numbers from soil to soil, and even from field to field. Some of the observations that have been made, when dealing with agricultural soil (i.e., bacterial dominated) is that when protozoan numbers are high, bacterial-feeding nematode numbers will be low, and vice versa. Thus there appears to be significant competition between bacterial-feeding predators for the bacterial prey. Whether this is indicative of the type of bacteria present in the soil, and whether this has any relationship to productivity in agricultural situations is not known.

Testate amoebae are only found in significant and constant numbers in forest soils, and are never found in temperate agricultural soils. Why this is the case is not known, but continues to be observed.

Nematode numbers, community structure

Nematode handout

There are four major types of nematodes, which includes bacterial-feeding, fungal-feeding, root-feeding and predatory nematodes. All nematodes are predators, and thus reflect to some extent the availability of their prey groups. However, other organisms prey upon these nematodes as well, and nematode numbers can also reflect the balance between the availability of nematode prey, as well as feeding by nematode predators.

Both bacterial-feeding and fungal-feeding nematodes mineralize N from their prey groups. Bacterial-feeding nematodes are more important in bacterial-dominated soils (agriculture and grassland systems), while fungal-feeding nematodes are more important in fungal dominated soils (conifer and most deciduous forests). Between 70 and 80% of the nitrogen in rapidly-growing trees has been shown to come from interactions between nematode predators and their prey. Between 30 and 50% of the N in crop plants appears to come from the interactions of bacterial-feeding nematodes and bacteria. Thus, the presence and numbers of bacterial- and fungal-feeding nematodes is extremely important for productive soils.

Root-feeding nematodes are detrimental to plant growth. As few as one endo-parasitic nematodes per plant may be enough to result in decreased productivity or death, while plants may tolerate several hundred ecto-parasitic nematodes per root system without reduction in production. Compensatory plant production has been observed with a little root-feeding, in that plant production is greater with a few herbivores munching on the plant than without feeding taking place.

Root-feeding nematode numbers can be reduced by competition for root space. VA mycorrhizal fungi may prevent root-feeding nematodes from reaching the roots through a variety of mechanisms. Nematode trapping fungi trap and kill many root-feeding nematodes. Other fungi and bacteria may be active inhibitors of nematode presence in the rhizosphere. Effective biocontrol of these plant-feeders is being worked on and may be possible in the near future.

T. Bongers, in the Netherlands, suggested the use of a Maturity Index for nematodes in soil. Certain species of nematodes are more commonly found following disturbance, while other species are more typical inhabitants of less-disturbed soils. The numbers of the four different trophic groups, or of different genera or species of matodes can be interpreted in several ways. First, significant differences in the number of individuals in a trophic group, or in nematode species in any treatment, compared to the control or reference treatment indicates an impact on that particular group or species. Several studies have recently shown that changes in numbers of even a single species of nematode in soil can significantly alter nutrient cycling within a soil. However, until more work is done to determine whether the same (or similar) nematode species control that same process in a similar way in other soils, extrapolation of results from a study performed in a different soil, with different plant species, different bacterial and fungal species, and different numbers of competing predators remains fraught with difficulty. However, it clearly suggests that alteration in nematode species composition could have negative impacts on plant growth, plant species composition, and thus ecosystem productivity.

A second way of interpreting nematode data is to assess changes in process rates. Since a bacterial-feeding nematode consumes 106 bacteria per day, reductions in this trophic group should result in an increase in bacterial biomass, with concomitant increase in net nutrient immobilization, and a decrease in nutrient mineralization with a concomitant effect on availability to plants. A fungal-feeding nematode consumes the cytoplasm in 10-50 meters of hyphal length per day, with similar effects on nutrient cycling as decreases in bacterial-feeding nematodes.

When root-feeding nematodes numbers are decreased, the ecosystem impact is positive, since root-feeding nematodes reduce plant growth/yield. However, root-feeding nematodes are highly opportunistic organisms, and are among the first organisms to invade after disturbance. Thus, one result of any disturbance which seriously affects ecosystem stability is reduction in the number of organisms which displace root-feeding nematodes. These competitors of root-feeding nematodes are mycorrhizal fungi, nematode-feeding nematodes, nematode-feeding microarthropods which frequent the rhizosphere, and fungal-feeding nematodes which apparently interfere with the ability of root-feeding nematodes to find roots. A second result of disturbance is a reduction in the ability of plants to resist nematode feeding. Thus, following a disturbance which disrupts ecosystem stability, root-feeding nematode numbers increase more rapidly than other groups, to the detriment of ecosystem productivity. Thus, as a general indicator of ecosystem health, increases in root feeding nematode numbers suggest serious negative impacts on ecosystem stability.

A decrease in nematode-feeding nematode numbers initiates a trophic cascade effect. For example, nematode-feeding nematodes can control the populations of bacterial-, fungal- or root-feeding nematodes and reduction in nematode-feeders results in an immediate increase in their prey group - i.e., bacterial-fungal- or root feeding nematodes. This in turn results in a decrease in the prey of these three nematode groups; a reduction in bacteria, fungi or roots, each with it's detrimental effect on nutrient cycling Indoor plant growth, as outlined above.

VAM spore numbers

Vesicular-arbuscular mycorrhizal (VAM) fungi are critically important for all crop plants, except species of the brassica family (e.g., mustards, kale). A number of researchers have shown that the lack of VAM inoculum, or the lack of the appropriate inoculum can result in poor plant growth, in poor competition with other plants or inability to reproduce or survive under certain extreme conditions. However, most crop fields have adequate VAM spores present, especially if crop residue is placed back into the field. Only in a few situations where soil degradation has been severe, such as with intensive pesticide use, fumigation, or intense fertilizer amendment, will VAM inoculum become so low that plant growth will be in jeopardy.

In restoration studies, the lack of appropriate inoculum is more likely to be a problem than in other situations where sources of appropriate VAM spores are near-by. Thus, the presence of at least 1 to 5 spores per gram of soil is adequate for most crop fields. When the number of spores falls below one per gram, then addition of compost containing high numbers of VAM spores (for example from an alfalfa field, or other legume), or inoculation of VAM spores from a commercial source generally results in positive effects.

Percent VAM colonization

At least 12% of the root system of grasses, (i.e., most crop plants), should be colonized by VAM in order to obtain the minimum required benefits from this symbiotic relationship. Colonization upwards of 40% is usually seen in healthy soils. VAM colonization can limit root-feeding nematode attack of root systems, if the nematode burden is not too high. A great deal of knowledge of the relationship between plant species, VAM species and soil type, including fertility, is needed in order to fully predict the optimal relationship between crop plant, VAM species and soil.

For more information about the Soil Microbial Biomass Service and how to submit a soil sample, write Dr. Elaine Ingham, Department of Botany and Plant Pathology, Cordley Hall 2082, Oregon State University, Corvallis, OR 97331-2902.

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