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The 34th Dr. R.V. Tamhane Memorial Lecture*

Microbial Diversity, Soil Health and Sustainability

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I feel extremely honoured to have been asked to deliver the 34th R.V. Tamhane memorial lecture at the 72nd annual convention of the Indian Society of Soil Science at Birsa Agricultural University, Ranchi. Dr Tamhane was a greatly respected soil scientist and I feel privileged to speak on a topic of considerable current interest in his honor. Soil ecosystems remain firmly at the foundations of human life support systems, but are the least understood among the natural ecosystems, and increasingly among the most degraded. Soil erosion, loss of soil organic matter and nutrient depletion are among the leading contributors to impaired soil health, reduced crop yields and poverty in the developing world. Global warming will further exacerbate this problem. Not surprisingly therefore, soil health tops a list of priorities of UN Millennium Project's hunger task force. Given the current concerns about the *sustainability* of production systems and *soil biological health,* and the exciting developments in *microbial diversity*. I chose this theme to link this trinity and look at examples of how to manage agricultural production systems in ways that sustainability is maintained whilst not compromising on microbial diversity and soil health.

Microbial Diversity

The importance of soil microorganisms for sustenance of all other life forms needs no emphasis. Microbes are the basis of the biosphere; a staggering 5 x 10^{31} cells exist, weighing 50 quadrillion metric tones, constituting about 60% of the total biomass. Bacteria produce about half the oxygen on the planet. Of 41,000 Pg (10^{15} g) of carbon in the globe, soils contain 1550 Pg and biota 550 Pg. While the total C, N and P in the plant biomass globally is 560, 12-20 and 1-2 Pg respectively, in procrayotes the C, N and P content is 300-500, 70-120 and 7-12 Pg respectively. Soil organisms act as primary driving agents of nutrient cycling, regulating the dynamics of soil organic matter, soil carbon sequestration and green house gas emissions; modifying soil structure and water regimes; enhancing the amount of nutrient acquisition by vegetation; conferring stress tolerance, resisting pathogens and improving plant health. Microbes breakdown most of the 45,000 or so chemical compounds that humans use in daily life. They are the source of most antibiotics and of some other drugs and industrial enzymes. It is difficult to overstate their importance.

More than 90% of the planet's genetic biodiversity is resident in soils. With the advent of a molecular techniques, the incredible diversity of soil microorganisms is finally being unraveled. The genotypic diversity in all the Protists groups is far more than the combined diversity of plant, animal or fungal kingdom. The total estimated microbial species in the world habitat may be as high as 4 million. A gram of soil can contain as many as 10,000 different species. Current estimates for numbers of procaryotes, which include the Bacteria and Archaea, range from 300,000 to 1 million species or more but only about 5000 species have been described. Thus, less then 1% of the microorganisms have actually been isolated and identified. We have no idea of the function or the potential importance of the unculturable fraction.

Assessing Microbial Diversity

Traditional methods of studying microorganisms by enrichment culture is of very limited use in exploring diversity since a large majority the organisms cannot be cultured in the available media and will thus remain unknown. Some techniques are now available for getting a better appreciation of the soil microbial community. In community level substrate utilization (CLSU) fingerprints, soil suspensions are inoculated into multi-well plates e.g. BIOLOG, containing a selection of specific substrates (Garland

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1996), but is again limited for diversity studies due to its selectivity for culturable microorganisms and also because of the ability of multiple phylogenetic groups of organisms to use the same substrates. Soil bacterial diversity has been also been assessed by analysis of fatty acid methyl ester (FAME) composition of individual isolates as well as total FAME community profile of soils (Schutter and Dick 2000). Recently however recombinant DNA techniques have provided methods for characterizing natural microbial communities without the need to cultivate organisms. Total DNA community profile can be fingerprinted by extracting total soil microbial DNA and amplified by polymerase chain reaction (PCR) using bacterial oligonucleotide primers targeting conserved genes on operons (eg. 16 s RNA gene). The amplified products are separated by gel electrophoresis, extracted and sequenced. The sequences are then compared with known sequences of ribosomal RNA to allow for the determination of a 'phylotype'. Additionally hybridization probes complementary to specific regions of rRNA are synthesized and used to aid in screening other organisms (phylogenetic stains).

Exploitation of Bacterial Genes

A changed perception of soils from an exclusive focus as a substrate for food production to its ecological and biodiversity potential has emerged. Soil is a reservoir for mining useful genes for developing transgenics, improving productivity and quality, incorporating resistance to biotic/abiotic stresses in plants and livestock, genes for various antibiotics, and other pharmaceuticals and nutraceuticals. The most well known are *Cry* 1 group genes from *Bacillus thuringenis* for conferring insecticidal resistance and Cod A gene from *Arthobactor globiformis* for conferring salt tolerance in crops. Mining other useful genes requires a massive search for isolation, identification and characterization of the vast reservoir of hidden diversity present in many pristine environments as well as extreme habitats.

Determining the complete DNA sequence of a single microbial species has become almost commonplace. Now efforts are being directed towards sequencing "metagenomes", the DNA of entire ecosystems. DNA extracted from soil samples is sequenced (shot gun sequencing), sorted and aligned in the correct order to determine the complete sequence of each species for identification. Another approach is to create clone libraries of soil "metagenomes" in an organism like *E. coli* and then screen for production of antibiotics, enzymes and other useful compounds produced by microorganisms that are yet uncultivated. The pay-offs from such kinds of work could be vast, leading to design of compounds that inhibit antibiotic resistance mechanisms, thus extending the useful lifetime of currently available antibiotics as well as lead to the discovery of biosynthetic pathways encoding potentially novel antibiotics.

Soil Biological Quality

The steady decline in soil organic matter levels due to continuous cropping without recycling enough crop or animal residues, coupled with nutrient imbalances due to insufficient application of nutrients has led to negative nutrient balances in agriculture, impaired soil health and declining factor productivity. The effects of physical and chemical degradation of soils are quite obvious, but biological degradation due to the loss of specific soil organic matter fractions and the autochthonous microbial communities dependent upon them is insidious. Soil health has been defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health (Doran *et al* 1996). There is a surge of interest in soil quality assessments through measurement of various physical, chemical and biological parameters that are sensitive to soil management. Physical and chemical properties are shaped by biological activity, and biological activity is enhanced or limited by chemical and physical condition. Because soils' perform many simultaneous functions, the goal of relating indicator properties to specific functions or processes is very difficult.

Important physical indicators of soil quality include those related to water storage and movement e.g., soil infiltration rate, bulk density, water holding capacity, depth of soil and rooting, and texture. Practices like conservation tillage, no-till management, surface residue retention or incorporation are all known to increase the mean weight diameter of aggregates and water retention. Soil fertility, nutrient restoring and recycling, and environmental quality issues are directly related with chemical indicators. These include soil organic matter, pH, electrical conductivity, and extractable N, P and K.

Soil Organic Matter

Soil organic matter (SOM) is central to soil quality assessments since it influences many soil properties including infiltration rate, bulk density, aggregate stability, cation exchange capacity, and biological activity, all of which are related to a number

of key soil functions. SOM includes a number of fractions such as light fraction, microbial biomass, water soluble organics and humus. As a general rule, declining yield trends have been ascribed to reduction in SOM levels by many researchers. However, it is very difficult to state (a) what is a 'normal' SOM level, (b) what is the critical 'threshold'? (c) what level of SOM constitutes good or bad soil quality? The greatly increased crop yields during last 40 years in India have often been obtained despite decrease in SOM, to levels below those posed as critical. Similarly, generalized evidence for a threshold of SOM in relation to soil physical properties below which catastrophic failure of, for example, soil aggregation will occur, is lacking. However Bhattacharya *et al* (2007) have proposed a minimum threshold limit of 0.63% SOC with a corresponding maximum threshold limit of bulk density as 1.6 g/cc and a maximum threshold limit of 2.42% SOC with a corresponding minimum threshold limit of BD as 1.2 g/cc for the semi-arid tropical soils of India based on studies on carbon sequestration at 22 selected benchmark sites. These represent the quasi-equilibrium values of SOC for soils with similar parent material. The lower value is from agricultural systems while the higher value is from a forest system. Further Soil Taxonomy defines "mollic epipedon" as a diagnostic criterion to group those soils which are rich in organic matter. These soils known as Mollisols are characterised by a minimum threshold of organic carbon of 0.6% (1.0% organic matter).

Biological Indicators

A large, diverse, and active population of soil organisms may be the most important indicator of a healthy soil. A good ecological indicator should be one that is universal in distribution, reflect a crucial aspect of the functioning of the ecosystem, show prompt and accurate response to an external perturbation, and be readily and economically measurable. Such a stringent requirement coupled with a poor understanding of microbial diversity-function relationships has meant that we have made very little progress in identifying such indicators. Although this situation is likely to improve in the longer term, yet for the present for a working definition of soil biological quality and ease of analysis, biologically active fractions of SOM and biochemical attributes of soil have proven more useful.

Soil respiration and nitrogen mineralisation are widely used as indices of biological activity. Particulate organic matter (POM, >53mm) (Cambardella and Elliott, 1992), represents a significant proportion of the slow pool of SOM and is important in maintaining the stability of macroaggregates (>250mm). Microbial biomass represents the fraction of soil responsible for the energy and nutrient cycling and regulation of organic matter transformations. POM and microbial biomass are considered as biologically active fractions of SOM and are sensitive indicators of management-induced changes in the fate of crop residues and the turnover of SOM constituents. They predict the direction and rate of change of soil quality earlier and better than other indicators. Decrease of microbial biomass carbon as a fraction of total organic carbon implies a reduction in microbial transformation and intensity. The metabolic quotient or qCO_2 is a more sensitive indicator of soil microbial reaction to cropping systems, lower values implying more stable and mature systems where carbon utilization efficiency of the microbial population is higher due to shift from zymogenous (r-strategists) to autochthonous microflora (K-strategists). Dehydrogenase activity has been widely used as a generalized comparative index of microbial activity but it has not always been consistently correlated with microbial activity. There have been numerous attempts to correlate soil biology/ fertility with the activity of a number of extracellular soil enzymes, mostly hydrolases and esterases, but good correlations can be expected only in unmanaged ecosystems or low input agricultural systems. Increased activity of several enzymes have been shown with addition of organic amendments, green manure/crop residues, reclamation of alkali (Rao and Ghai 1985) or leaching of saline soils (Rao and Pathak, 1996).

Soil Fauna

Studies on below ground biological diversity must go beyond the current emphasis on only microbial activity indicators and also include soil biota. Soil microfauna (protozoa and nematodes) may reduce or increase microbial numbers and speed the turnover of microbial biomass and thus enhances nutrient availability. Mesofauna (mites, collembola) increase substrate surface by fragmentation, soil macrofauna (ants, termites, centipedes, millipedes, earthworms) communite and redistribute organic residues in soil profile which increase the surface area and substantially modify soil structure through formation of macropores and aggregates through burrowing activities and faecal pellets. Although soil fauna are present in many highly productive soils, it is difficult to make broad generalizations about earthworms or

soil fauna, in general, as indicators of high quality soils. Enumeration and identification of at least the burrowing soil fauna, and measurements of faecal deposits have been recommended to be a part of minimum data set for assessing soil quality.

Integrated Measurements

The different metabolic activities of microorganisms are a crucial basis for the soil ecosystem function and determine soil biological quality (SBQ) but devising a single integrated index for measurement of SBQ is still a challenge. With respect to field measurements of SBQ and devising instrumentation for survey type work, the strong functional bridges between the physics and biology of soils needs to be factored in. Strong negative relationships have been found between activity of soil enzymes involved in carbon cycling and soil bulk density and positive relation with water infiltration in soils amended with crop residues and manures (Martens *et al* 1992). Further progress was achieved by Witty and Mytton (2001) who observed that in soils under clover plants, the soil particles are progressively aggregated into distinct crumbs leading to drastic physical changes that have a profound effect on soil function. As bulk density decreased, water percolation improved due to better aeration. They argued that although the soil system is complex, there are a small number of key physical and biological processes that when measured and integrated can be used to provide an index of overall function of soil function and soil health. These processes relate to soil permeability, respiration and production of a range of gaseous products. They developed a method for rapid measurement of soil porosity in the field and developed a small portable electrochemical sensor to measure O_2 uptake and production of CO_2, NO_2, H_2S and CH_4 by soils in the field which formed a basis for development of soil quality index for pasture soils.

Microbial Diversity and Soil Functions

Low soil microbial diversity indicates stressed conditions in soil while high diversity is an indicator of a healthy soil. Dehydrogenase and urease activities were low in alkali soils, which however improved greatly upon the addition of gypsum followed by cropping, growing trees or grasses (Rao and Ghai 1985). Amendment with a readily decomposable carbon source (*Sesbania)* removed the sodicity stress on microbes and improved the activity dramatically (Rao and Pathak, 1996). So, mineralization of added C was similar (~38%) throughout the pH range of 8.1 to 10.0 (ESP 2-89). This showed that autochthonous microflora of alkali soils are as effective as those in normal soils in decomposing added organic matter. In saline soils carbon amendment improved the activity in saline soils of low and intermediate salinity upto ECe 26 dS m^{-1} whereas at higher salinity the osmotic and ionic effects were predominant and added organic matter alone was not enough to improve activity. Desalinization immediately improved the activity, although it was not completely restored.

Maintenance of a diverse and functioning microbial community is important for soil sustainability. Loss of microbial diversity makes biological systems less able to adapt to environmental stresses. According to the theory of ecosystem functioning (Mikola and Setala, 1998) organisms can follow any of the three hypothesis- a) Redundant species hypothesis, b) Predictable change hypothesis or c) Idiosyncratic response hypothesis. According to the redundant species hypothesis the changes in community composition may not necessarily be linked to functional changes in a certain environment. In agricultural soils many typical functions such as the decomposition of organic matter seem to follow this, that is that loss of one species is compensated by the presence of others which can take on that function. According to b or c either predictable or unpredictable changes in soil functioning may result from changes in community composition. A central problem in drawing any rigorous conclusions on soil biodiversity-soil function relations is because of the poor understanding of the relation between genetic diversity and community structure on the one hand and between community structure and function on the other. Based on the published work of ours and others, I hypothesize that the degree to which a particular ecological hypothesis would apply will depend on the degree of soil degradation and propose this as the 'Stress Dependent Biodiversity-Functionality Relationship' (SDBFN). The main tenets of the hypothesis are as follows:

a) In undisturbed pristine soil ecosystems, with abundant biodiversity, the redundant species hypothesis would operate for most functions.
b) As the degree of stress on a soil ecosystem increases and biodiversity decreases (due to loss soil organic matter or high or low pH or salinity), while the redundant hypothesis would operate for the robust functions like carbon cycling, the more specialized functions e.g., ammonification, deamination or nitrification would follow the predictable change hypothesis and activity decreases

with increase in stress.

c) Under extreme stress as in drastically disturbed systems like complete removal of surface soil by strip mining and extreme acidification say by acid mine drainage waters (for example copper mining, lignite mine spoils etc.), or at extremely high salinity coupled by poor leaching, all of which result in considerable loss of soil microbial diversity, both the predictable and idiosyncratic hypothesis would operate for various functions. The restoration of the productivity and soil health of such sites would depend not only on application of specific soil amendments but also inoculation with mixed microbial communities including arbuscular mycorrhizae and plant growth promoting rhizobacteria.

The experimental evidence for the hypothesis 'b' comes from our earlier experiments (Rao and Pathak 1996) in which generalized microbial functions like carbon cycling were significantly reduced (due to reduced microbial diversity) only beyond pH 9.2, ESP 65 (Table 1). However due to reduced microbial activity there was reduction in soil microbial biomass carbon (SMBC) even at pH 8.7, ESP 18. This in turn affected specialized microbial transformations (urea hydrolysis, protein breakdown, deamination) which were thus affected at even lower levels of stress at pH 8.4, ESP 4. The evidence for the hypothesis 'c' comes from experiments of Liebich *et al* (2007) in which microbial diversity was destroyed in soils by ashing them for 48 hours at 600^0C and then followed by inoculation with soil suspension containing the full complement of the microbial diversity or with ten different artificial microbial consortia of increasing complexity. They showed that though reduced diversity still sustained microbial functionality at an acceptable level, not all mechanisms important for humification of added maize straw could be provided by communities with reduced diversity.

Soil Quality and Sustainability

Simple relationships between soil quality, soil functions and productivity are clearly discernible and interpretable only in undisturbed ecosystems, but precise relationships are difficult to arrive at or be meaningfully used in intensively managed agricultural production systems. Some management interventions show clearly discernible beneficial or detrimental effects.

Tillage

Tillage has a negative effect on soil organic matter. While moderate tillage may provide more favourable soil conditions for crop growth and development and weed control over the short term, intensive tillage of agricultural soils leads to substantial losses of soil carbon, ranging from 30 to 50%. Cultivation depresses enzymes activity; conservation tillage practices produce less soil disturbance and have higher levels of enzymes in surface soil. Higher organic C levels in no-tillage (NT) or reduced tillage (RT), along with higher organic C in various fractions like microbial biomass and POM, in comparison to conventional tillage (CT) have been shown in many studies (Beare *et al* 1994). Growing of cover crops led to accumulation of higher C, N, and P-SOM in soil than by organic manure treatment; and POM was the best index for biologically active SOM (Wander *et al* 1994).

Table 1. Microbial biomass and enzymatic activities in sodic soils

Property	Soil ESP					LSD(p=0.05)
pH_s	8.1	8.4	8.7	9.2	10.0	
ECe	0.5	0.5	1.5	2.5	6.4	
ESP	2.0	3.8	17.7	65.1	88.8	
C min[a]	**166.3**	**167.5**	**135.9**	**115.5**	106.3	65.2
SMBC[b]	**19.6**	18.2	12.7	14.0	15.1	1.0
qCO_2[c]	8.5	9.2	10.7	8.3	7.0	-
Dehydrogenase[d]	**68.7**	37.6	40.3	25.4	25.8	14.8
Urease[e]	**1.9**	0.8	0.7	0.8	0.7	0.5
Asparaginase[f]	**8.18**	2.54	1.49	0.57	0.92	0.43
Glutaminase[f]	**50.8**	48.5	13.9	11.5	11.5	0.17
Deaminase[f]	**1.03**	**1.15**	0.46	0.34	0.23	0.23
Protease[f]	**0.075**	0.055	0.030	0.082	0.036	-

[a] cumulative mg CO_2 evolved 100 g^{-1} soil; [b] mg C 100g^{-1} soil; [c] mg CO_2 evolved mg^{-1} SMBC; [d] µg TPF g^{-1} soil in 24 h; [e] µ mol NH_3 g^{-1} soil h^{-1} ; [f] µ mol substrate hydrolysed g^{-1} soil in 48 h. Values in bold indicate stress levels beyond which there was a significant reduction. From Rao and Pathak (1996) and Pathak and Rao (1998)

Balanced Fertilization

In long term fertilizer experiments on pearl-millet-wheat rotation in a sandy loam soil at Hisar, Goyal et al (1992) showed that application of inorganic fertilizers increased the soil microbial biomass carbon and nitrogen, bacterial numbers and soil biological (dehydrogenase) activity at $N_{60}\,P_{30}\,K_{60}$ over $N_0\,P_0\,K_0$ even though organic carbon had not increased. At $N_{120}\,P_{60}\,K_{60,}$ all the soil quality indices-viz., organic carbon, microbial biomass carbon and nitrogen, bacteria and soil dehydrogenase activity increased. This was attributed to increased root growth, root exudates, mucigel and sloughed-off cells. Long-term application of recommended doses of chemical fertilizers in alfisols at Bangalore and Vertisols at Indore did not show any significant difference in numbers of bacteria, fungi or actinomycetes or culturable diversity of bacteria or fungi in comparison to the unfertilized treatments (Venkateswarlu 1998). Gunapala *et al* (1998) showed that although organically farmed soils had greater microbial abundance and activity, and higher number of bacterial-feeding nematodes, than those managed under conventional farming practices, but the ability of the microbial communities *per se* in the two soils (chemically fertilized or organic) to degrade added organic matter did not differ. In a long term experiment on rice grown in an inceptisol for 34 years at Cuttack, Orissa, Nayak *et al* (2007) reported that microbial biomass and enzyme activities in NPK plots were comparable to either fallow or to compost added treatments in most cases. The above studies are crucial and show that chemical fertilizers if applied in a balanced form did not impair the crucial functional abilities of soil microorganisms or biological soil quality.

Continuous cropping without application of adequate quantity of nutrients in balanced doses or without addition of organics leads to loss of soil quality and unsustainability of crop production systems. In long term fertilizer experiments, Manna *et al* (2007) showed that applying only nitrogen or nitrogen + phosphorous led to a decline in particulate organic matter (>53 m fraction) and soil respiration, microbial biomass C and N, which were however improved significantly on addition of NPK or NPK+ organics. Continuous cultivation with removal of residues or without addition of any organics year after year disrupted the aggregate stability, resulting in loss of physically protected organic matter such as POM thus resulting in loss of readily respirable substrates for microorganisms in the long run. Mandal (2005) computed the soil quality index (SQI) in several long term cropping rotations and concluded that SQI was negative in unfertilized control and positive with balanced NPK fertilization in most cases. Wherever organic matter was added as FYM or as green manure to substitute for 25% of chemical N, SQI improved dramatically over NPK. Organic carbon, microbial biomass, available P and K were most frequently identified as the master variables that contributed to soil quality index in a majority of the rotations.

In the context of chemical fertilization it is important to point out that while adding animal manures and sequestering carbon in passive fractions like humus with very low C: N ratio is crucial for sustaining the environmental soil quality functions like filtering and buffering, building up carbon in sand size fractions like POM is important for improving biological soil quality functions like transformation of added organics and nutrients which are very crucial for sustaining soil and plant productivity. Building up POM and microbial biomass would demand addition of crop residues and addition of more chemical fertilizers (in a balanced form and also to achieve intermediate C: N ratios) and not less since what is being built up (through biological mechanisms) is after all a reservoir of chemical nutrients.

Crop Rotations

Inclusion of legumes in crop rotations is known to improve sustainability. In an alkali soil in Haryana irrigated with good quality or sodic water, higher organic C and N, soil respiration, metabolic quotient and microbial biomass nitrogen were observed under rice- clover than under other cropping sequences like rice- mustard, rice- wheat and sorghum-wheat (Batra and Rao, unpublished). Rao and Gill (2000) showed that in sequential agroforestry with perennial nitrogen fixing *Sesbania sesban* for 4 years followed by rice-wheat for 6 years, the residual effects of legume growth resulted in a permanent improvement in soil quality resulting in additional yield of 1.2 t of rice and 0.5 t ha^{-1} of wheat each year on a sustained basis, that was attributed to non-symbiotic nitrogen fixation of 30.8 kg ha^{-1} yr^{-1}.

In contrast, allelopathic or deleterious soil microorganisms have been known to evolve where a single crop is grown continuously causing yield declines which cannot be restored by application of mineral fertilizers (Oz and Friedman 2001). Soils with high levels of organic matter and soil organisms activity, or a specific group of antagonistic microorganisms seem to prevent more aggressive pathogens from taking hold. They are called ‘suppressive’ soils.

Populations of collembola and mites were found to be higher in soils under long term pasture (70,000 m^{-2}) compared to soils under wheat-wheat (20,000 m^{-2}) or wheat-lupin rotations. Soils with high levels of mycophagous (fungal-feeding) amobae have been associated with disease suppression of *Verticillium* wilt, 'take all' and *Rhizoctonia*. *Brassica* crops have been found to be better 'take-all' break-crops than legumes. Decomposing *Brassica* roots release isothiocyanates. These compounds act as a fungicide and a process of bio-fumigation is thought to occur.

Pesticides

Most of the pesticides in common use in agriculture have been widely tested and there is a plethora of literature which conclusively proves that at recommended doses of application, they have no adverse influence on microbial activity. Hart and Brooks (1996) found in U.K. that 19 years of cumulative annual field application of five pesticides (benomyl, chlorfenvinphos, aldicarb, triadimefon and glyphosate) used continuously either singly or in combination applied at or slightly above the recommended rate had no measurable harmful effects on the soil microbial biomass or its activity. More studies are needed to assess the effects of pesticides on microbial diversity since there are reports of both lack of effects of fungicides and herbicides as well as measurable effects on diversity (Johnsen *et al* 2001).

Heavy Metals

There is increasing concern about land application of bio-solid wastes, laden with heavy metals. The effects of heavy metals on microbial processes and possible mitigation options are receiving increasing attention. None of the heavy metals studied by Chander *et al* (1995) had any adverse effect on soil microbial biomass at the currently permitted EU levels. Zinc, Copper or Cadmium at twice the EU limits decreased the biomass by 20%, whereas nickel at 4 times decreased the biomass by 15%. Toxicity towards nitrogen mineralization was as follows: Zn > Ni > Cu > Cd, providing evidence that zinc is much more toxic to microorganisms than commonly believed. Twenty months after addition of ~500t/ha of fly ash and cropping with a fallow-corn-wheat rotation or continuous fescue, no detrimental effect on soil microbial community was observed (Schutter and Fuhrman, 2001); *Arthrobacter* was the dominant population and fly ash amendment benefited fungi and gram-negative bacteria.

Organic Farming

In a long term experiment at ICRISAT, Rupela *et al* (2005) evaluated two low cost systems involving no tillage, addition of biomass mulch, microbial inoculants and biopesticides with a conventional tillage system with NP fertilizers and chemical pesticides and an integrated system involving chemicals and biomass mulch. At the end of five years organic matter, microbial biomass and soil respiration were higher in the first two systems as compared to conventional and were highest in integrated system. Microbial activity *per se* did not differ in the organic and conventional plots. The values of respiratory quotient (qCO_2), dehydrogenase activity, microbial quotient and C/N ratio of microbial biomass was similar in all the four systems. This supports the findings of Gunapala *et al* (1998) that the ability of soil microorganisms to decompose added organic matter was the same in organic or conventional systems and that microbial diversity was not compromised by chemical farming. Integrated system were the best and shifted the equilibrium to higher side.

Increased microbial populations have been measured by a number of workers in cultivated organically managed soils. Shannon *et al* (2002) found that differences in microbial communities in soils under different management practices (conventional, organic, integrated etc.) were subtle, rather than dramatic. Greater amount of readily-extractable ATP, increased numbers of viable but non-culturable bacteria, total and vital fungal biovolumes in soil in organically managed soils pointed to greater physiological diversity of microorganisms in such soils. Microbial biomass was higher in integrated farming systems whereas fungal biomass was higher in organic soils. Organically managed soils maintain higher biodiversity and have been shown to have lesser incidence of soil borne diseases compared to conventional farming. Higher incidence of mycorrhiza in organically managed soils has been known since long. In a long term experiment at Frick, Switzerland, soil microbial biomass and enzymes activities were higher in organic than in conventional soils, mycorrhiza were 40% higher, biomass and abundance of earthworms were higher by a factor of 1.3 to 3.2, average density of carabids, staphylinids and spiders in the organic plots were almost twice those in conventional plots (Mader *et al* 2002). In a soybean-wheat rotation in a vertisol in Madhya Pradesh we recently showed (Rao *et al* 2007 unpublished) that the microbial numbers and enzymatic activities during wheat growth were much higher in integrated nutrient man-

agement as compared to chemical farming or farming without addition of fertilizers. Thus the best option for longer term biodiversity conservation is to use integrated farming systems (IFS) involving practices like no-till, residue mulching, integrated nutrient management and integrated pest management.

Microbial Inoculation for Plant and Soil Health Promotion

There is increasing interest in seed and soil inoculation with a group of bacteria called plant growth promoting rhizobacteria (PGPR) or plant health promoting rhizobacteria most of which have several polyfunctional abilities like fixing atmospheric nitrogen, solubilizing phosphorus from insoluble sources, producing growth hormones and suppressing the activity of pathogens through production of antibiotics, ammonia, siderophores, HCN etc. Cyanobacteria or blue green algae are well known for their effect on rice growth through biological nitrogen fixation but also exert many other beneficial effects. Rao and Burns (1990) showed that inoculation with cyanobacteria in submerged soils improves the general health of soils through building up organic matter, stimulating the bacterial and fungal populations, enzyme activities, polysaccharide production and soil aggregation (Table 2).

Emerging Issues

It has been hypothesized that in a future scenario of global warming, the litter produced under elevated CO_2, having a high C:N ratio would be difficult to decompose, but experiments by van Ginkel and Gorissen (1998) showed that microorganisms were found to adopt to changing soil C input under elevated CO_2 and there was no effect on their turnover and behaviour. There were no differences between microbial communities from field plots that contained harvested transgenic canola plants and control plants indicating that the changes in the microbial community structure associated with genetically modified plants were temporary and did not persist into the next season (Dunfield and Germida, 2001).

Table 2. Changes in bacterial and fungal numbers and soil microbial biomass after 21 weeks of growth of inoculated BGA or a native moss in surface cm soil of a brown earth

Treatment Property	Dark Control	Inoculated Cyano-bacteria	Native Cyanobacteria + Moss
Bacteria (x 10^7)	17.5	48.4	50.7
Fungi (x 10^4)	1.7	2.7	2.4
SMBC[a] (mg C 100 g^{-1})	59.2	118.6	129.4
Dehydrogenase[b]	50.9	107.5	139.1
Urease[c]	1.31	3.61	5.60
Phosphatase[d]	0.44	1.40	2.47
Polysaccharides[e]	3.94	6.67	7.45
Stability index[f]	0.787	0.818	0.812

* at 13 weeks; Rao and Burns (1990); [a] mg C 100g^{-1} soil; [b]µg TPF g^{-1} soil in 24 h; [c] µ mol NH_3 g^{-1} soil h^{-1} ; [d] µg PNP g^{-1} h^{-1} , [e] mg glucose g^{-1} , [f]indirect measure of POM.

Minimum Data Set for BSQ

It will be an expensive and needless to devote the time and effort involved in measuring dozens of biological soil quality (BSQ) indices. Based on above review, measurement of microbial biomass carbon, active (particulate) organic matter, soil respiration and N mineralisation would suffice to give a reliable picture of soil biological quality. This could be complimented with soil pH and organic matter and integrated measurement of soil aeration of the type described by Witty and Mytton (2001) along with to give a complete picture of soil biological quality.

Soil Genomics and Soil Quality

While the standard suite of biological soil quality parameters mentioned above are sufficiently discriminatory, yet they cannot provide information on differences in the structure of the microbial community or its potential functional significance. The DNA extracted from soils can be analyzed for specific marker gene pools. Combinations of specific polymerase chain reaction (PCR) amplification of target gene pools and genetic fingerprinting techniques, such as restriction fragment length polymorphism (RFLP), denaturant/temperature gradient gel electrophoresis (DGGE/TGGE), single strand conformation polymorphism (SSCP), ribosomal intergenic spacer analysis (RISA), terminal restriction fragment length polymorphism (T-RFLP) are applied (Nannipieri *et al* 2003, Entry *et al* 2007). Soil microarrays, fluorescent *in situ* hybridization (FISH) and stable isotope probing have been developed to identify and track specific soil microorganisms. Many investigations focused on the amplification of specific marker genes from total soil DNA by using specific probes like nif H (for diazotrophs), nirK/nirS (denitrifiers), amo A (nitrifiers), nod C (rhizobia), as well as genes involved in P and S cycling. These analyses yield DNA banding patterns (genetic fingerprints), which can be quantified and compared among different soil samples. Although such data represents an image of the soil microbial community but an accepted defini-

tion of the taxonomic unit, which can be used for defining soil microbial diversity, is still lacking at this point. The difficulties encountered with this inaccessibility of soil microbial diversity has been described as 'counting the uncountable'. But there is hope that scientific advancements in the future would lead to more robust assessment of biological condition and devising of soil health kits based on DNA chips.

Environmental Genomics

The industrial and urban pollution in agricultural fields covering the peri-urban interface has become a major environmental problem which can be tackled through bioremediation using plants and microorganisms**.** Naturally occurring organisms or genes from hitherto unexploded sources can be isolated and deployed for cleaning up of heavy metals like arsenic, lead and chromium and pesticides in water bodies, soils and land fills. Lal *et al* (2006) isolated several bacteria from the family Sphingomonadaceae that degrade Hexachlorocyclohexane (HCH) from contaminated sites, and studies the organization and diversity of *lin* genes (responsible for HCH degradation), which are plasmid borne and appear to undergo significant horizontal transfer in soil. This knowledge can now be used for developing bioremediation techniques for the decontamination of HCH contaminated sites.

Building Capacity

"*When it was first proposed to establish laboratories at Cambridge, Todhunter the mathematician objected that it was unnecessary for students to see experiments performed, since the results could be vouched for by their teachers, all of them men of the highest character, and many of them clergymen of the church of England*." (The Scientific Outlook, Bertrand Russell, 1931).

It is unthinkable today that only about 150 years ago there could be opponents in England to what is the very bedrock of the scientific method. Progress in science and other human endeavors has always been achieved by overcoming scepticism and venturing into uncharted territories.

Soil Science and Soil Scientists have thrived because of such scientific enterprise and inter-disciplinary co-operation, and shown how soils frequently play roles as the keystone of environmental systems, both natural and managed, and contributed significantly to local, regional and global environmental management. Now they are required to play a greater role in addressing the complex nature of land use, climate change impacts on soils and agriculture, environmental, and biodiversity challenges. To meet this challenge, it is a virtual imperative to re--fashion soil science education by greater re-integration of the basic disciplines (Rao 2006). We need a two-tier upgradation of facilities and skills. At state level, a few soil testing laboratories in each state would need to be upgraded to measure the minimum data set of biological soil quality parameters proposed. At national level, we need create at least five world class 'Soil Microbiological Resource Centres' (SOILMIRCEN) in different geographic regions of the country for upstream research in soil biotechnology and soil genomics. This can be initiated through re-training and partnerships as a short-term strategy but for sustainable scientific development, we need to induct new experts in molecular biology, biochemistry, biophysics and nanotechnology. This is an urgent priority if we are not to be left behind in the revolution sweeping the environmental and biotechnological facets of soil science and in the quest to exploit the uncommon opportunities now available. The need of the hour is to build more capacity, create more first class laboratories, induct new expertise and build partnerships.

References

Beare, M.H., Hendrix, P.F. and Coleman, D.C. (1994) Water-stable aggregates and organic matter fractions in conventional and no-tillage soils. *Soil Science Society of America Journal* **58***,* 777-786.

Bhattacharya, T., Chandran, P., Ray, S.K., Pal, D.K., Venugopalan, M.V., Mandal, C., Wani, S.P., Manna, M.C. and Ramesh, V. (2007) Carbon sequestration in red and black soils. Agropedology **17**, 26-34.

Cambardella, C.A. and Elliott, E.T. (1992) Particulate soil organic matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal* **56**, 777-783.

Chander, K., Brookes, P.C. and Harding, S.A. (1995) Microbial biomass dynamics following addition of metal-enriched sewage sludges to a sandy loam. *Soil Biology and Biochemistry* **27**, 1409-1421.

Doran, J.W., Sarrantonio, M. and Liebig, M.A. (1996) Soil health and sustainability. *Advances in Agronomy* **56**, 1-54.

Dunfield, K.E. and Germida, J.J. (2001) Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microbiology Ecology* **38**, 1-9.

Garland, J. L. (1996) Analytical approaches to the characterization of samples of microbial communities us-

ing patterns of potential C source utilization. *Soil Biology and Biochemistry* **28**, 213-221.

Goyal, S., Mishra, M.M., Hooda, I.S. and Singh, R. (1992) Organic matter-microbial biomass relationships in field experiments under tropical conditions: Effects of inorganic fertilization and organic amendments. *Soil Biology and Biochemistry* **24**, 1081-1084.

van Ginkel, J.H. and Gorissen, A. (1998) *In situ* decomposition of grass roots as affected by elevated atmospheric carbon dioxide. *Soil Science Society of America Journal* **62***,* 951-958.

Gunapala, N., Venette R.C., Ferris, H. and Scow, K.M. (1998) Effect of soil management history on the rate of organic matter decomposition. *Soil Biology and Biochemistry* **30**, 1917-1927.

Entry, J.A., Mills, DeEtta., Jayachandran, K. and Moorman, T.B. (2007) Symposium: Molecular based approaches to soil microbiology. *Soil Science Society of America Journal* **71,** 561.

Hart, M.R. and Brookes, P.C. (1996) Soil microbial biomass and mineralization of soil organic matter after 19 years of cumulative field applications of pesticides. *Soil Biology and Biochemistry* **28**, 1641-1649.

Johnsen, K., Jacobsen, C.S., Torsvik, V. and Sorensen, J. (2001) Pesticide effects on bacterial diversity in agricultural soils- a review. *Biology and Fertility of Soils* **33**, 443-453.

Lal, R., Dogra, C., Malhotra, S., Sharma, P. and Pal, R. (2006) Diversity, distribution and divergence of *lin* genes in hexachlorocyclohexane-degrading sphingomonads. *Trends in Biotechnology* **24**, 121-130.

Liebich, J., Schloter, M., Schaffer, A., Verecken, H. and Burauel, P. (2007) Degradation and humification of maize straw in soil microcosms inoculated with simple and complex microbial communities. *European Journal of Soil Science* **57**, 446-455.

Mader, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U. (2002) Soil fertility and biodiversity in organic farming. *Science* **296,** 1694-1697.

Manna, M. C., Swarup, A., Wanjari, R. H., Ravankar, H. N., Mishra, B., Saha, M. N., Singh, Y. V., Sahi, D. K. and Sarap, P. A. (2005) Long term effect of fertilizer and manure application on soil organic carbon storage, soil quality and yield sustainability under subhumid and semi-arid tropical India. *Field Crops Research* **93**, 264-280.

Mandal, B. (2005) Assessment and improvement of soil quality and resilience for rainfed production system. Completion Report. National Agricultural Technology Project, Indian Council of Agricultural Research, New Delhi, pp 30.

Martens, D.A., Johanson, J.B. and Frankenberger, W.T. Jr (1992) Production and persistence of soil enzymes with repeated addition of organic residues. *Soil Science* **153**, 53-61.

Mikola, J. and Setala, H. (1998) Relating species diversity to ecosystem functioning: mechanistic background and experimental approach with a decomposer food web. *Oikos* **83**, 180-194.

Nayak, D.R., Jagdeesh Babu, J. and Adhya, T.K. (2007). Long-term application of compost influences microbial biomass and enzyme activities in a tropical aeric endoaquept planted to rice under flooded condition. *Soil Biology and Biochemistry* 39, 1897-1906.

Pathak, H and Rao, D.L.N. (1998) Carbon and nitrogen mineralization from added

organic matter in saline and alkali soils. *Soil Biology and Biochemistry* **30,** 695-702

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. and Renella, G. (2003). Microbial diversity and soil functions. *European Journal of Soil Science* **54***,* 655-670

Oz, B. and Friedman, J (2001) Allelopathic bacteria and their impact on higher plants. *Critical Reviews in Microbiology* **27**, 41-55.

Rao, D.L.N. (2006) Maintaining the Soil Ecosystems of the Future. In: *The Future of Soil Science* Ed: A. E. Hartemink, International Union of Soil Sciences, Wageningen, pp. 116-118.

Rao, D.L.N. and Burns, R.G. (1990) The effect of surface growth of blue green algae and bryophytes on some microbiological, biochemical and physical soil properties. *Biology and Fertility of Soils* **9,** 239-244.

Rao, D.L.N. and Ghai, S.K. (1985) Urease and dehydrogenase activity of alkali and reclaimed soils. *Australian Journal of Soil Research* **23,** 661-665.

Rao, D.L.N., and Pathak, H. (1996) Ameliorative influence of organic matter on biological activity of salt affected soils. *Arid Soil Research and Rehabilitation* **10**, 311-319.

Rao, D.L.N. and Gill, H.S. (2000). Residual effects of *Sebania sesban* forestry on yield and nitrogen uptake by rice and wheat in a reclaimed alkali Soil in Haryana. In: *Long-term soil fertility experiments in rice-wheat cropping systems* (I.P. Abrol, K.E. Bronson, J.M. Duxbury and R.K. Gupta, Eds). Rice-Wheat Consortium Paper series 6, Rice-wheat Consortium for the Indo-Gangetic Plains, New Delhi, pp 139-148.

Rupela, O.P., Gowda, C.L.L., Wani, S.P. and Bee, H (2005). Evaluation of crop production systems based on locally-available biological inputs. In: *Biological Approaches to Sustainable Soil Systems* (N. Uphoff ed.) CRC Press, Boca Raton, Florida, USA, pp 501-515.

Schutter, M.E. and Dick, R.P. (2000) Comparison of fatty acid methyl ester (FAME)

methods for characterizing microbial communities. *Soil Science Society of America Journal* **64***,* 1659-1668.

Schutter, M.E. and Fuhrman, J.J. (2001) Soil microbial community responses to fly ash

amendment as revealed by analysis of whole soils and bacterial isolates. *Soil Biology and Biochemistry* **33**, 1947-1958.

Shannon, D., Sen A.M. and Johnson D.B. (2002) A comparative study of the microbiology of soils managed under organic and conventional regimes. *Soil Use and Management* **18,** 274-283.

Soil Survey Staff (1999) Soil Taxonomy 2nd edition, USDA, NRCS, Washington DC.

Venkateswarlu, B. (1998) Final technical report, ICAR adhoc scheme "*Assessment of soil biodiversity in rainfed environment as influenced by degradative and restorative processes"*, CRIDA, Hyderabad, p 57.

Wander, M.M., Traina, S.J., Stinner, B.R. and Peters, S.E. (1994) Organic and conventional management effects on biologically active soil organic matter pools. *Soil Science Society of America Journal* **58***,* 1130-1139.

Witty, J.F. and Mytton, L.R. (2001) Soil Quality: manifestations, mechanisms and measurment. *IGER Innovations* **5**, 54-57.

About the Memorial Lecture

This Memorial Lecture was instituted in 1973 by the Indian Society of Soil Science out of a donation received from Dr. Tamhane Memorial Committee in memory of Dr. R.V. Tamhane who passed away on January 23, 1973.

After a short period of service in the Department of Agriculture of the eastwhile Bombay Province, Dr. Tamhane joined the IARI, New Delhi in 1945 as Assistant Soil Survey Officer; in 1958, he became the Head of the Division of Soil Science and Agricultural Chemistry; in 1961, he joined the Ministry of Food and Agriculture, Government of India as Advisor (Soil Conservation) and held that position until his retirement in 1970. Soon after, he was invited by the Reserve Bank of India to take over the responsibilities of the Director (Technical), Agricultural Refinance Corporation at its Bombay (now Mumbai) office.

His main scientific contributions have been in the field of soil survey, soil genesis, soil conservation and soil testing. The credit for organizing the Soil Testing Service and a chain of Soil Testing Laboratories in different states of the country goes to him. On several occasions, he was assigned important responsibilities of representing India at national and international conferences.

Dr. Tamhane had a very long association with the Indian Society of Soil Science starting as a Member, Joint Secretary and finally as the President. Whosoever had occasion to come in contact with him, whatever be his walk of life would remember his sweet, straight-forward and cordial nature. All his colleagues found in him the qualities of an able administrator and a true sportsman in every sense.

Since 1974, Dr. R.V. Tamhane Memorial Lecture is being delivered annually by an eminent scientist on any topic in Soil Science and Agricultural Chemistry. The lecture in this series during 2007 was delivered by Dr. D.L.N. Rao whose brief biographical sketch follows.This is for the information of the Readers that all the Dr. R.V. Tamhane memorial lectures delivered up to year 2002 have been published as the ISSS Bulletin No. 20.

About the Speaker

Dr. D.L.N. Rao graduated from the University of Delhi in 1973 with a first class first and took his M.Sc and Ph.D degrees in Microbiology from Indian Agricultural Research Institute, New Delhi in 1975 and 1979 respectively.

Dr. Rao began his career in the Agricultural Resarch Service of the ICAR at the Central Soil Salinity Research Institute, Karnal in 1978 as Scientist S-1 and worked as a senior scientist during 1984-1998. He held a Commonwealth Post-doctoral fellowship in Soil Microbial Ecology at the University of Kent at Canterbury, U.K. during 1986-87. During

1995-96, he was a Visiting Research Fellow at the Imperial College at Wye, University of London and at the University of Sussex, U.K working on Biological Nitrogen Fixation in chickpea . In 1998, Dr. Rao moved to the Indian Institute of Soil Science, Bhopal as Project Coordinator of the All India Coordinated Research Project on Biological Nitrogen Fixation (1998-2003), and now All India Network Project on Biofertilizers (2004- present). He officiated as Director, Indian Institute of Soil Science during 2003 and Head of Division of Soil Biology during 2006.

Dr. Rao has made original and well recognized contributions on Non-symbiotic nitrogen fixation by bacteria and blue green algae, Nitrogen and Organic matter transformations, and Symbiotic nitrogen fixation in pulses and tree legumes in salt affected soils, creating a well known school of thought. His current research interests are on microbial diversity in vertisols and diversity of soybean rhizobia. Dr. Rao has travelled and lectured widely in many countries and published about 82 research articles in national and international journals which include 12 book chapters and 3 edited books.

In recognition of his professional contribution, Dr. Rao was awarded 12th International Congress of Soil Science Commemoration award and Gold Medal for the year 2000 by the Indian Society of Soil Science He was also awarded Fertilizer Association of India Silver Jubilee Award for 'Excellence in Biofertilizer Use Research-2002 and Gold medal.

Dr. Rao is a Fellow of the National Academy of Agricultural Sciences of India (2004) and the Indian Society of Soil Science (2003). He was the Vice-President of the Indian Society of Agricultural Resource Management during 2003-04. He has been the Editor of the Journal of the Indian Society of Soil Science for more than six years and is currently the Editor, Indian Journal of Microbiology and several other journals.

Dr Rao is on the Institute Management Committee and Research Advisory Committee of several ICAR institutes, and is member of several ICAR committees. In addition to his research interests in Soil Science and Microbiology, he has special interest in the history and philosophy of Science, and Science Policy.