MEANING AND METHODS OF ASSESSMENT OF SOIL HEALTH

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Soil is a living system and has to be managed as such in agriculture to improve sustainability. Soil health refers to the biological, chemical and physical features of soil that are essential to long-term sustainable agricultural productivity with minimal environmental impact. Thus, soil health provides a overall picture of soil functionality. Soil provides the habitats for a wide range of living organisms (Lee and Foster, 1991). The soil health can be inferred by measuring specific soil properties (*eg.* organic matter) and by observing soil status (eg, fertility). Studying the soil microorganisms in their particular environments has generated interest as microbial diversity is intimately related to soil structure and function. The main objective in determining soil health is to acquire indicators that can be used to evaluate the soil's current status and hence to develop sustainable agricultural systems. In this regard, significant progress has been made over the last few years in the development of specific biomarkers and macro-molecular probes, enabling rapid and reliable measurements of soil microbial communities. In addition, the modern analytical techniques have provided data on soil chemistry. The combination of these two approaches offers promise in determining soil health status.

Soil health:

Soil health has been defined by Doran and Zeiss (2000) as 'the capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain plant and animal production, maintain or enhance water and air quality, and promote plant and animal health'. Healthy soils are the one which sustain biological productivity, store and cycle water and nutrients, decompose organic matter, inactivate toxic compounds, suppress pathogens, protect water quality and enhance catchment's health. Many agricultural practices increase the soil's vulnerability to degradation processes such as erosion, acidification, salinisation, soil structure decline and contamination. These degradation processes reduce the functional capacity of soils and, at a catchment level, can reduce the quality of water draining to streams and rivers. Hence, degradation of soil and water quality can be thought of as symptoms of poor soil health. The challenge for management of agricultural soils is to develop production systems that not only prevent soil degradation but also enhance soil health. The biological component of the soil components of the soil system has a high dependence on the chemical and physical soil components and hence tends to be a sensitive indicator to disturbance or degradation processes.

Properties of soil health:

Soil health integrates all components of the soil system and is assessed by indicators that describe or quantify biological, chemical and physical properties. Soil characteristics that contribute to a healthy soil include protected soil surface and low erosion rates, high soil organic matter, high biological activity and biological diversity, high available moisture storage capacity, favorable soil pH, deep root zone, balanced stores of available nutrients, resilient and stable soil structure, adequate internal drainage, favorable soil strength and aeration, favorable soil temperature, low levels of soil born pathogens and low levels of toxic substances.

Soil health is the net result of on-going conservation and degradation processes, depending highly on the biological component of the soil ecosystem, and influences plant health, environmental health, food safety and quality (Halvorson *et al*., 1997). Soil is a finite and non-renewable resource because regeneration of soil through chemical and biological weathering of underlying rock requires geological time (Huber *et al.*, 2001). Deterioration of soil, and thereby soil health, is of concern for human, animal and plant health because, air, groundwater and surface water consumed by humans can be adversely affected by mis-managed and contaminated soil (Singer *et al*, 2000).

Soil structure and biological processes

Soil is a heterogeneous environment. Many biological activities and processes in soils are modulated by soil structure, particularly *via* the interactions between soil structure and water. Soil is dominated by a solid phase consisting of particles of different size surrounded by water and gases, the amount and composition of which fluctuate markedly in time and space. Water is normally discontinuous, except when the soil is saturated with water. The pore space without water is filled with air and other gases and volatiles (Stotzky, 1997). There is continual interchange of molecules and ions between solid, liquid and gaseous phase, which are mediated by physical, chemical and biological processes (Doran *et al*., 1994). These processes represent a unique balance between physical, chemical and biological components (Doran *et al*., 1994) and maintaining this balance is of great importance to soil health.

The activity of different soil organisms and their survival ranges in terms of soil water potential. For instance, bacteria are most active in the soil moisture potential range of –0.01 and –0.03 MPa (Lavelle and Spain 2001). In the case of earthworms, activity in surface soil ceases when drier than –150 kPa (Baker *et al.*, 1993). Soil moisture characteristics control the mobility of many small soil animals such as nematodes, motile bacteria and aquatic phycomycetes, which are restricted to existing water-filled soil pores. The existence and activities of these organisms depend on the availability of sequences of water filled pores of the right sizes to permit their passage (Papendick and Campbell, 1985). This is pre determined by soil structure (pore size distribution) at particular soil water potential. The filamentous fungi are less restricted by such factors. Permeability affects the rate of soil water movement and solute

supply, and controls many biological activities such as wilting and germination of plants and hatching of nematode cysts and spores (Smiles, 1988). Further, soil is a complex inter-connected framework of pores and solids. At field scale and under particular soil water status, aerobic and anaerobic zones often exist in close proximity. This controls the distribution of the types of organisms and has direct impact on processes of nitrification, denitrification and decomposition processes and therefore nitrogen / carbon cycling.

Organisms residing in pores of appropriate size are protected from predation by organisms of larger dimension since the latter are denied physical access to their prey. This has been demonstrated in the case of prey – predator relationship for *Pseudomonas flurorescens* and ciliate protozoan *Colpoda steinii* (Wright *et al*., 1993). When the bacteria are predominantly located in the smaller pores (<6µm), they better survive protozoa predation. Such interaction is influenced by pore size distribution and has impact on nutrient cycling, mineralization processes and disease prevalence via its effect on composition of the food web (Lee and Pankhurst 1992).

The soil structure also has a great significance as far as the substrate availability and carbon sequestration organic are concerned. The substrates existing in pores of appropriate size are protected from breakdown by soil microorganisms because of their physical inaccessibility. As a result, even labile forms of organic carbon can be sequestered in soil. The effect of tillage and other forms of soil disturbance is to expose these substrates to the microbial population and results in the commonly observed accelerated soil organic carbon decline under conventional cultivation practices.

Soil biota:

Soil biota can directly and indirectly influence soil structure. Directly, bacteria stabilize soil aggregates by their polysaccharides gel and, in the case of fungi, by the physical entanglement of their filamentous hyphae. Direct correlation between macro aggregate stability and hyphal length has been established (Tisdall and Oades 1980). Bacteria in colonies are often found within micro aggregates and in association with dispersed clay particles (Foster, 1994), and their carbohydrate gel surrounding them is important in micro aggregate formation (Emarson *et al*., 1986). Mesofauna such as protozoa and nematodes do not have direct effects on soil structure but can indirectly affect structure through their regulation of bacterial and fungal populations.

The biological component of soil is concentrated mainly in topsoil, occupying only a tiny fraction (<0.5%) of the total soil volume and making up less than 10% of total soil organic matter. A small part of this living component of soil consists of plant roots, with microorganisms and soil animals forming the bulk of the biomass.

Soil microorganisms:

Bacteria, fungi, protozoa and algae (mostly blue green algae or cyanobacteria) form the major part of the soil biomass. There is an enormous number of species in each of these groups, but our

knowledge of each of them is limited because many species have not been described taxonomically and most have not been cultured.

Bacteria play an important role in soil because their diverse metabolic capabilities enable them to exploit many sources of energy and carbon in the soil. They are the principal agents for the global cycling of inorganic compounds such as nitrogen, sulfur and phosphorus. They range between 10^6 and 10^9 per gram of soil. Certain bacteria also bring about biological fixation of nitrogen predominantly but not exclusively in symbiotic associations between plant roots and bacteria. The symbiotic bacterial genera most commonly involved are *Rhizobium* and *Bradyrhizobium*, which specifically infect leguminous plant roots. The actinomycetes are filamentous bacteria that are found in soil at populations of 10^5 to 10^8 per gram of soil. They a re involved in the decomposition of organic compounds including cellulose, chitin and recalcitrant substances such as humic acids. Some of them in the genus *Frankia* also fix atmospheric nitrogen in symbiosis similar to rhizobia but have a much wider host range that includes several non-legume plant groups. However, our knowledge of these organisms is still limited because they are difficult to culture. Among the free – living bacteria, *Azotobacter*, *Azospirillum* and *Bacillus* may also contribute relatively small amounts of nitrogen to soils. Blue green algae or cyanobacteria due to their nitrogen fixing capacity add valuable nitrogen inputs to the soil. They vary from 10 and 10^6 per gram of soil. Because of their photosynthetic capacity, they contribute to the organic carbon input of soil, and also produce extracellular polymers that may help to conserve soil structure.

The fungi contribute to about 70% of the microbial biomass, whose number varies from 10^4 to 10^6 per gram of soil. Fungi may be free living or have a mutually beneficial or parasitic relationship with the plant roots. They exploit a diversity of substrates because of their filamentous nature and are decomposers of large molecules such as cellulose and lignin produced by plants. Mycorrhizal fungi are involved in a mutually beneficial association with plant roots. They derive nutrients from roots and in turn aid the plant in uptake of relatively immobile nutrients such as phosphorus and zinc. By acting as agents of nutrient transport, they form a vital link between plants and soil and therefore play an important role in soil fertility.

Soil micro fauna:

The main components of soil micro fauna are the protozoa and nematodes. They feed on bacteria and fungi and are involved in nutrient recycling in soil. The protozoa are water dependent, unicellular organisms possessing a nucleus, and are classified as ciliates, flagellates and naked amoebae. They feed on soil bacteria and fungi and their populations range from 10 to 10^6 per gram of soil. Although, they constitute a small proportion in the soil, they are ecologically important because of their rapid turn over rates and the large grazing pressure they exert on the micro flora. Thus, microbial turnover is stimulated, resulting in increased rates of nutrient mineralization, especially nitrogen and phosphorus. While, nematodes are ubiquitous in soil and are the most abundant soil micro fauna. Some species are parasites

of plants and animals but the majority is beneficial, playing an important role in nutrient cycling processes. This latter group of nematodes, commonly known as 'free-living nematodes', feed on bacteria or fungi and prey on various soil micro-fauna. They, therefore obtain their nourishment from organisms that are associated with decaying organic matter.

Soil meso-fauna:

The soil meso-fauna comprise mites, collembola (springtails), enchytraeids (small worms from 1 mm to 5 cm in length), tardigrades (water bears) and small insects. They are a diverse group with a wide range of feeding habits, but collectively play a role in regulating microbial populations and disseminating microbial propogules. They also accelerate decomposition of plant residues by fragmenting large pieces of organic matter and reworking the faeces of larger fauna. The micro-arthropods (mainly collembola and mites) are usually the most obvious of the meso-fauna the population densities vary from 10 to 10^7/per square meter of soil. Populations are generally highest in the top five centimeter of soil and decline with increasing depth. Apart from fragmentation of residues, enchytraeids also affect soil porosity through their burrowing activities and influence soil aggregation *via* production of feacal pellets.

Soil macro-fauna:

These are the most conspicuous of the soil animals and because of their size, have the greatest potential for direct effects on soil functional properties. The member of this group includes ants, termites, millipedes, adult and larval insects, earthworms, snails and slugs. Through, their feeding habits and their movement through soils help to redistribute organic residues in the soil profile. This activity results in an increase in the surface area of organic substrates available for microbial activity. Certain groups, especially ants, termites and earthworms can greatly modify soil structure through the formation of macro-pores and aggregates. These effects may influence water infiltration and solute leaching through soil and hence the soil capacity to function as an environmental buffer.

Soil organic matter:

Soil organic matter has taken on a new significance because it correlates well with a number of important physical, chemical and microbiological properties of soil. Soil organic matter influences the physical and chemical properties of soil as well as the availability of nutrients for microbial and plant growth. It accumulates over long periods of time and its current distribution in a soil profile is the result of continuous reprocessing by microbes, recombination by chemical reactions, physical movement by soil animals, disturbances such as tree falls and movement of soil solution. Consequently, carbon cycling and its stabilization in soils are intimately associated with soil structure (Kogel-Knabner, 2000).

Contributions of soil biota towards soil health:

The soil biota has lot of significance in maintenance of soil structure. The binding substances that hold soil particles together have both mineral and organic origins. Some of the organic 'binding agents' are contributed by soil biota. Fungal hyphae, along with fibrous roots from plants, bind soil particles and

small aggregates together into larger units. Polysaccharides produced by microorganisms such as soil bacteria like *Bacillus, Pseudomonas, Agrobacterium, Azotobacter* and *Rhizobium* act as the gums that bind and stabilize aggregates. Plant residues are also broken down by soil biota to create soil aggregates. Mycorrhizal fungi (VAM) contribute significantly to soil aggregate formation and soil stability at both micro and macro levels by enmeshing mineral and organic debris in a network of external hyphae.

The soil health is also determined by its ability to supply nutrients in forms that can be taken by the plants. Most of the nutrients contained in soil organic matter are in complex organic forms that have to be mineralized to an inorganic form before they can be used by plants. Soil microorganisms play a dominant role in the decomposition of organic materials such as cellulose, hemicellulose, polysaccharides, hydrocarbons, and lignin, proteins and amino acids, and are also responsible for nearly all nitrogen and carbon transformations in soil. They are also important in transforming nutrients such as phosphorus, sulfur, iron, potassium, calcium, magnesium, manganese, aluminium and zinc into forms that can be used by plants.

The fore most important contribution of soil biota is in the suppression of plant pathogens. Soil borne disease problems are common in soils that have been intensively cropped for decades. Such soils are said to be conducive to disease. They have lost much of their microbial diversity and biological buffering capacity. The organisms involved in disease suppression act in many different ways. Fungi and bacteria are able to displace each other from specific ecological niches in soil by competing for nutrients. A group of bacteria known as the fluorescent pseudomonads (*Pseudomonas* spp), for example inhibit the growth of pathogens by limiting their access to soluble ferric iron. Other bacteria (e.g. *Bacillus* spp and some actinomycetes) produce antibiotics that are detrimental to pathogens. Fungi such as *Trichoderma* and *Gliocladium* are able to parasitise fungal pathogens and are therefore useful biological control agents. Other fungi can parasitise or prey on nematodes.

Biological indicators for determining soil health and standard analytical procedures:

The concept of soil health refers to the biological, chemical and physical features necessary for long-term, sustainable agricultural productivity with minimal environmental impact. Thus, soil health provides an overall picture of soil functionality. Healthy soils maintain a diverse community of soil organisms that help to (i) control plant diseases as well as insect and weed pests, (ii) form beneficial symbiotic associations with plant roots (eg. nitrogen –fixing bacteria and mycorrhizal fungi), (iii) recycle plant nutrients, (iv) improve soil structure with positive repercussions for its water and nutrient holding capacity and improve crop production.

One of the most important objectives in assessing the health of a soil is the establishment of indicators for evaluating its current status.

Microbial biomass:

Both direct and indirect methods have been used for the estimation of microbial biomass in the soil. Direct counting includes the use of staining techniques in conjunction with epifluorescence microscopy or automated image analysis (Bloem *et al*., 1995; Bloem and Breure, 2003). The most common indirect methods are chloroform fumigation and substrate induced respiration (SIR) (Carter *et al*., 1999). In chloroform fumigation, the chloroform vapors kill the microorganisms in the soil and the size of the killed biomass is estimated either by quantification or respired CO_2 or by direct extraction of the soil immediately after the fumigation, followed by quantification of extractable carbon. Soil microbial biomass represents the fraction of the soil responsible for the energy and nutrient cycling and the regulation of organic matter transformation (Gregorich *et al.* 1994; Turco *et al.* 1994). A number of studies have reported a close relationship between soil microbial biomass, decomposition rate and N-materialization (Jenkinson 1988; Smith *et al.*, 1990; Carter *et al,*. 1999). Microbial biomass has also been shown to correlate positively with grain yield in organic, but not in conventional farming (Mäder *et al.*, 2001). Finally, soil microbial biomass contributes to soil structure and soil stabilization (Fließbach *et al.*, 2000; Smith *et al.,* 1990). Soil microbial biomass has also been recommended as indicators of soil organic carbon (Carter *et al.*, 1999).

SIR measures the metabolically active portion of the microbial biomass by measuring the initial change in the soil respiration rate as a result of adding an easily decomposable substrate (*eg.* glucose) (Anderson and Domsch, 1978). Soil microbial biomass is subsequently calculated using a conversion factor (Kaiser *et al*., 1992).

Soil respiration is the biological oxidation of organic matter to CO_2 by aerobic organisms, notably microorganisms (Alef, 1995). It is positively correlated with soil organic matter content, and often with microbial biomass and microbial activity, and can be determined as CO_2 or O2 production using chemical titration, electrical conductivity, gas chromatography or infra red spectroscopy (Alef, 1995). The metabolic quotient, also called the specific respiratory rate, is defined as the microbial respiration rate per unit microbial biomass (Anderson and Domsch, 1990).

Phospholipid fatty acids:

Most soil microorganisms cannot be characterized by conventional cultivation techniques, indeed it has been estimated that 80-99% of all species have not yet been cultured. So, to overcome this limitation, currently the analysis of phospholipid fatty acids (PLFA), essential membrane components present in living organisms, can be used to overcome this limitation, thereby providing information on the trophic structure (at the phenotypic level) of microbial communities. In general, PLFA analysis is a fast, reliable method for the detection on changes in the structure of soil microbial communities (Frostegard and Baath, 1996) and the variations detected can be related to changes in the soil use and management.

Microbial Activity

Measurements of microbial activity at the community level include the quantification of bacterial DNA and protein synthesis. The amount of DNA synthesis can be determined by measuring the incorporation of ^{3}H or ^{14}C thymidine into bacterial DNA (Baath, 1998).

Similarly, the amount of incorporation of ^{3}H or ^{14}C leucine, an amino acid that is incorporated only into proteins, reflects the level of bacterial protein synthesis (Baath, 1998). There are a number of key indicators related to microbial activity and some can be used to estimate both biomass and activity. Indicators of carbon cycle measure activity at the ecosystem level. For example, organic matter decomposition can be estimated using litter bags (Verhoef, 1995), cotton strips or wood sticks (Harrison *et al*., 1988). In addition, well-documented assays are available for many soil enzymes activities (example cellulase, urease, phosphates, phenol oxidase (Dick *et al*., 1996). Measuring the activities of enzymes involved in various processes is an important aspect of determining overall microbial activity.

Soil physical and chemical indicators

Water infiltration rate:

Infiltration rates are subjected to significant changes with soil use, management and time. They are affected by the development of plant roots, earthworm burrows, and soil aggregation and overall increases in stable organic matter. Depending on the soil type, texture, structure and soil water content, the water infiltration rate may improve immediately after tillage due to the loosening of surface crust or compacted areas. Tillage also disrupts aggregates and soil structure, creating the potential for renewed compaction and surface crusting and leading to a loss of continuous surface connected pores.

Bulk density:

It is defined as the ratio of oven dried soil (weight) to its bulk volume. Soil bulk density range from <1.0 (in organic soils) to 1.7g cm^{-3} and are dependent on the densities of the soil particles (sand, silt, clay and organic matter) and their packing arrangement. Compacted soil layer have high bulk densities, restrict root growth and inhibit the movement of air and water through the soil.

Soil pH

By estimating hydrogen ion activity in the soil solution, the acidity or alkalinity of a soil can be measured. Soil pH affects the solubility of soil minerals, the availability of plant nutrients, and activity of microorganisms. Acidity is generally associated with leached soils, whereas alkalinity generally occurs in drier regions. However, agricultural practices such as liming or the addition of ammonium fertilizers can alter the soil pH. In general pH values between 6.0 and 7.5 are optimal for crop growth.

Electrical conductivity

The electrical conductivity (EC) of a soil water mixture is an indication of the amount of ions (dissolved salts) present in the soil solution. Excess salt content seriously affects plant growth and soil water balance (Fitter and Hay, 1987). This may occur either naturally or as a result of inappropriate soil

use and management. In general, electrical conductivity values between 0 and 0.8 dSm^{-1} are acceptable for general crop growth.

Ion exchange capacity:

The soils ability to supply major plant nutrients mainly calcium, magnesium and potassium is reflected by its ion exchange capacity. The cation exchange capacity (CEC) is, to a large extent related to the amount of soil colloids, organic matter and clay, which are negatively charged and thus enable the soil to retain cat ions. Changes in pH and soil contents affect the CEC. For example, aluminum toxicity occurs in certain soils at pH <5, and soil dispersion with serious losses in structure may appear at high sodium concentrations (increasing salinity), both limiting factors for soil productivity and health.

Aggregate stability and soil slaking

An aggregate consists of several soil particles bound together and is usually formed by interaction of soil biota and plant community and their products with soil mineral components. Aggregates play a major role in several aspects of soil health, the movement and storage of water, soil aeration, physical protection of SOM, prevention of erosion, root development, and microbial community activity (Tate, 1995). Aggregates stability is a measure of the vulnerability of soil aggregates to external destructive forces. Soil aggregation can naturally develop and disintegrate and reform periodically (Hillel, 1982). Slaking is the process of fragmentation that occurs when aggregates are suddenly immersed in water (Chan and Mullins, 1994)) due to their inability to withstand the stresses of rapid water uptake. Soil slaking can be used as a measure of the ability of the soil to maintain its structure and is affected by water content, rate of wetting, texture, clay mineralogy, and organic matter content.

Topsoil depth, root growth and penetration resistance are also important indicators of soil health. Changes in top soil thickness and usually the result of erosion processes accelerated by ploughing, burning, overgrazing and other management practices that remove the protective vegetative cover. These changes result in loss of the most fertile soil layer and its water holding capacity as well as soil organic carbon content and productivity.

Molecular techniques to measure soil health:

A number of molecular and cellular techniques are currently being used in conjunction with biological and chemical indicators to increase our ability to evaluate soil health.

Fluorescence microscopy

The number of bacteria in soil, their cell volumes and the frequencies of dividing cells can be determined by fluorescence microscopy and computerized image analysis (Bloem *et al*., 1995). Soil microbial biomass can be estimated by staining with fluorescent dyes such as fluorescein isothiocyanate.

DNA measurement:

Quantification of DNA following its extraction from soil may provide a simple and practicable method for estimating the amount of microbial biomass (Girvan *et al*., 2004).

Fluorescence in situ hybridization

FISH is a direct; cultivation independent technique using r RNA-targeted oligo-nucleotide probes that is frequently used for the identification of microorganisms in soil. While this technique allows selective visualization of bacterial cells of different phylogenetic groups, it also has some limitations, particularly regarding quantitative analysis of complex samples (Moter and Gobel, 2000).

RNA measurement

The composition of soil microbial communities can be estimated by reverse transcriptase polymerase chain reaction (RT-PCR) followed by gel electrophoresis of the amplified cDNA fragments (Duineveld *et al*., 2001). The analysis of specific mRNA's reflects the expression of the corresponding gene in soil\. Such measurements can also be done by real time quantitative RT-PCR, which allows the detection and quantification of mRNA's present in low amount in environmental samples including soils (Pfaffl and Hageleit, 2001). However, this method requires previous knowledge of sequence of mRNA of interest.

Stable isotope probing

SIP is a culture independent technique that allows the identification of microorganisms directly involved in specific metabolic processes. In this method, labeled nucleic acids that were synthesized during assimilation of an isotopically enriched substrate are isolated and analyzed (Radagewski *et al*., 2000). The technique has been used to study forest soils (Radagewski *et al*., 2002) and to identify the active components of an ammonia oxidizing population in lake water (Whitby *et.al*., 2001).

Genetic diversity is most commonly studied by analyzing the diversity of genes encoding 16s rRNA (18s rRNA for eucaryotes). These genes occur in all microorganisms and show species dependent variations in their base compositions. The three methods are commonly applied to examine the diversity of 16s (and 18s) r DNA sequences in total DNA extracted from the soil microbial communities.

Denaturing gradient gel electrophoresis

Differences in the melting behavior or small DNA fragment (200-700bp that differs in as little as a single base substitution can be detected by DGGE (Muyzer *et al*., 1993). The denaturants used are heat (Constant temperature of 60^0C) and a fixed ratio of formamide (ranging 0 –40%) and urea (0-7M). The position in the gradient were a domine of DNA fragment melts and thus nearly stops migrating and is dependent on the nucleotide sequence in the melted region. The benefit of this approach is that a molecular fingerprint of the community structure is generated from each soil. In fact each band in each lane of the gel theoretically represents a different bacterial species. In addition, this technique enables the exition and subsequent sequencing of bands, allowing species identification using existing data basis.

Temperature gradient gel electrophoresis:

In contrast to DGGE, the separation of DNA by TGGE (Heuer and Sumalla, 1997) does not depend on chemical gradient of urea but instead on a precisely defined and controllable temperature gradient. This highly reproducible technique has the same advantages as DGGE.

Terminal Restriction Fragment Length Polymorphism

In T-RFLP, the specific fingerprint of a community is revealed by analyzing the polymorphism of a certain gene. T-RFLP is a high –through out reproducible method that allows the quantitative analysis of the diversity of a particular gene in a community. It requires extraction of DNA from soil sample and its PCR amplification using fluorescently labeled primer. T-RFLP yields a mixture of amplicons of same or similar sizes with a fluorescent labeled at one end. After purification the amplicon mixture is digested with a restriction enzyme, which generates fragments of different sizes that are separated, by gel or capillary electrophoresis. The separated labeled fragments are then densitometrically detected and a profile based on fragment length is generated. Recently the potential of T-RFLP to discriminate soil bacterial communities in cultivated and non-cultivated soils was demonstrated (Buckley and Schmidt, 2001).

BIOLOG ™

Carbon utilization patterns can be measured by the BIOLOG ™ assay (Gardland and Mills, 1991). In this test, a soil extract is incubated with up to 95 different C sources in a microtitre plate, and the redox-dye tetrazolium blue is used to indicate microbial activity. Specific C sources have been selected for studies of soil microbial communities. The result of the assay is the qualitative physiological profile of the potential metabolic functions within the culturable portion of microbial community. Differences in the profiles can be then analyzed by multivariate statistics.

Conclusions

There is a need for a holistic consideration of soil health as well as transdisciplinary soil management approaches that integrate biological, chemical and physical strategies to achieve soil supporting sustainable agriculture. The environmental and economic benefits of sustainable soils are enormous- increased resource efficiency, decomposition and nutrient cycling, nitrogen fixation and water holding capacity, as well as prevention of pollution and land degradation. Current agricultural practices reduce soil bio-diversity, mainly as a result of the over use of chemicals, leading to compaction or other disturbances and hence irreversible adverse ecological alterations, resulting in loss of soil health. Therefore there is a need to asses soil health. But, determining soil health demands a holistic approach in terms of soil physical, chemical and biological properties. It is difficult to look into all aspects, however, any change in the adverse effects leading to loss in physical and biological attributes of soil is indicated by loss in soil biota activities, in particular soil microorganisms as they respond quickly to any changes. Therefore, determination of soil microbial activities will indicate the extent of soil health. The easiest

way of estimating soil microbial activities is through estimation of soil microbial biomass in terms of microbial carbon by soil fumigation –incubation and extraction methods. Hence, soil microbial biomass estimations the most convenient way to be carried out in the study area to asses soil health.

References

Alef, K, 1995. Soil respiration. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, New York pp. 214-218.

Anderson JPE, Domsch, K. H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.,* **10** : 215-221.

Anderson JPE, Domsch, K.H., 1990. Application of echc-physiological quotients (qCO_2 and qD) on microbial biomass from soils of different cropping histories. *Soil Biol. Biochem.,* **25** : 393-395.

Baath, E., 1998. Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. *Microbial Ecol*., **36** : 316-327.

Baker, G. H., Barret, V. J., Carter, P.J, Williams, P.M.L., Buckerfield, J. C., 1993. Seasonal changes in the abundance of earthworms (*Annelida*: *Lumzricidae* and *Acanthodrilidae*) in soils used for cereal and Lucerne production in South Australia. *Australian Journal of Agricultural Research,* **44** : 1291-1301.

Bezdicek, D. F., and Stewart, B. A. (eds) *Soil Science Society of America*, Inc., Madison, pp. 3-21.

Bloem J, Bolhuis P. R, Veninga M. R., Wieringa J., 1995. Microscopic methods for counting bacterial and fungi in soil. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, New York, pp 162-172.

Bloem J., Breure A.M., 2003. Microbial indicators. In: Breure A.M, Markert B, Zechmeister HG (eds) bio-indicators and bio-monitors, Principles, assessment, concepts. Elsevier, Amsterdam, pp. 259-282.

Buckley DH, Schmidt TM. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microb. Ecol., **42:** 11-21.

Cater, M.R., Gregorich, E.H., Angers, D.A., Beare, M.H., Sparling, G.P., Wardle, D.A., Voroney, R.P., 1999. Interpretation of microbial biomass measurements for soil quality assessment in humid temperate regions. *Can. J. soil Sci*., **79**: 507-520.

Chan, K.Y., Mullins, C.E., 1994. Slaking characteristics of some Australian and British soils. *Eur. J. Soil Sci*., **45**: 73-283.

Dick, R.P., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity measurements as integrated microbial indicators. In: Doran JW, Jones AJ (eds) Methods for assessing soil quality. *Soil Sci. soc. Am*., pp 107-121.

Doran, J. W. and Parkin, T. B., 1994. Defining and assessing soil quality. In: *Defining soil quality for a sustainable environment*. (eds.) Doran, J. W., Coleman, D. C.

Doran, J. W. and Zeiss, M. R. 2000. Soil health and sustainability: managing the biotic component of soil quality. *Appl. Soil Ecol.*, **15**, 3-11.

Duineveld, B.M., Kowalchuk, G.A., Keijzer, A., van Elsas, J.D., van Veen, J.A., 2001. Analysis of bacterial communities in the rhizosphere of chrysanthemum *via* denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. *Appl. Environ. Microbiol.*, **67** : 172-178.

Emerson, W. W., Foster. R. C., Oades, J. M., 1986. Organic- mineral complexes in relation to soil aggregation and structure. In: Interactions of soil minerals with natural organics and microbes. *Soil Science Society of America*, Special Publication. **17 :** 521-548.

Felske, A., Wolterink, A., Van Lis, R., de Vos, W. M., and Akkermans, A. D. Carter, M. R., Gregorich, E. G., Angers, D. A., Beare, M. H., Sparling, G. P., Wardle, D. A., and Voroney, R. P., 1999. Interpretation of microbial biomass measurements for soil quality assessment in humid temperate regions. Canadian

Fitter, A.H., Hay, R.K.M., 1987. Environmental physiology of plants. Academic Press, London, UK.

Fliebach, L. (2000). Response of a soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. Applied and Environmental Microbiology, 66:3998-4003.

Foster, R.C., 1994. Microorganisms and soil aggregates. In: soil biota: management in sustainable farming systems. (eds.) Pankhurst, C. E., Double, B. M., Gupta., VVSR,. Grace, P.R., pp. 144-155, CSIRO Australia.

Frostegad, A., Baath, E., 1996. The use of phospolipid fatty acids analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soil*, **22** : 59-65.

Garland, J.L., Mills, A.L., 1991. Classification and Characterization of heterotrophic microbial communities on the basis of community level sole-carbon source utilization. *Appl. Environ. Microbiol.*, **57** : 2351-2359.

Girvan, M.S., Bullimore, J., Ball, A.S., Pretty, J.N., Osborn, A.M., 2004. Responses of active bacterial and fungal communities in soil under winter wheat to different fertilizer and pesticide regiments. *Appl. Environ. Microbiol.*, **69** : 1800-1809.

Gregorich, E. G., Carter, M. R., Angers, D. A., MonrealL, C. M., and Ellert, B.H., 1994. Towards a minimum data set to assess soil organic-matter quality in agricultural soils. Canadian Journal of Soil Science, 74:367-385.

Halvorson, J.J., Smith, J. L. and Papendick, R. I., 1997. Issues of scale for evaluating soil quality. Journal of Soil and Water Conservation, January-February: 26-30.

Harrison, A.F., Latter, T.M., Walton, D.W.H., 1988. The cotton strip assay: an index of decomposition in soil. In: Institute of Terrestrial Ecology Symposium No. 24, Institute of Terrestrial Ecology, Grange- Over- Sand, UK.

Heuer, H., Sumlla, K., 1997. Application of denaturing gradient gel electrophoresis and temperature gel elecrophoresis for studying soil microbial communities. In: van Elsas JD, Trevors JT, Wellington EMH (eds.) Modern soil Microbiology. Marcel Dekker, New Yark, pp 353-373.

Huber, S., Syed, B Freudenschuss, A., Ernstsen, V. and Loveland, P., 2001. Proposal for a European soil monitoring and assessment framework. Technical report no. 61, European Environment Agency, Copenhagen, Denmark.

Jenkinson, D. S., 1988. Determination of microbial biomass carbon and nitrogen in soil. In: Advances in Nitrogen Cycling in Agricultural Ecosystems. Wilson, J. R. (eds.). CAB International, pp. 368-386.Journal of Soil Science, 79:507-520.

Kaiser, E-A., Muller, T., Jorgensen, R.G., Insam, H., Heinemeyer, O., 1992. Evaluation of methods to estimate the soil microbial biomass and the relationships with soil texture and organic matter. *Soil Biol. Biochem*., **24** : 675-683.

Kogel-Knabner, I., 2000. Analytical approaches for characterizing soil matter. *Org Geochem*., **31**: 609-625.

Lavelle, P. and Spain, A.V., 2001. Soil Ecology, pp 426-529. Kluwer Academic Publishers, Dordrecht.

Lee, K. E., Foster, R.C., 1991. Soil fauna and soil structure. *Australian Journal of Soil Research,* **29**: 745-775.

Lee, K. E., Pankhurst, C. E., 1992. Soil organisms and sustainable productivity. *Australian Journal of Soil Research,* **30** : 855-892.

Moter, A., Gobel, U.B., 2000. Fluorescence *insitu* Hybridization (FISH) for direct visualization of microorganisms. *J. Microbiol. Methods,* 41: 85-112.

Muyzer, G., de waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal-RNA. *Appl. Environ. Microbiol.,* **59** : 695-700.

Papendick, R. I., Campbell, G. S., 1985. Theory and measurement of water potential. In: Water potential relations in soil microbiology. (eds.) Parr, J. F, Gardner, W. R, Elliot, L. F., Soil Science Society of America, Madison, WI, pp 1-12.

Pfaffl, M.W., Hageleit, M., 2001. Validities of mRNA quantification using recombinant RNA and recombinant DNA external calibration curves in real-time RT-PCR. *Biotechnol. Lett*., **23** : 275-282.

Radajewski, S., Ineson, P., Parekh, N.R., Murrel, J.C., 2000. Stable-isotope probing as a tool in microbial ecology. *Nature,* **403** : 646-649.

Radajewski, S., Webster, G., Reay, D.S., Morris, S.A., Ineson, P., Nedwell, D.B., Prosser J.I., Murrell, J.C., 2002. Identification of active methylotroph population in an acidic forest soil by stable isotope probing. *Microbiology*, **148**: 331-2342.

Singer, M. J. and Ewing, S., 2000. soil quality. In: handbook of soil Science. Sumner, M. E. (eds.). CRC Press, Boca Raton, pp. G271-G298.

Smiles, D. E., 1988. Aspects of the physical environment of soil organisms. Biology and fertility of soils. **6** : 204-215.

Smith, J. L. and Paul, E. A., 1990. The significance of soil microbial biomass estimations. In: Soil Biochemistry 6. Bollag, J.-M. and Stotzky, G. (eds.). Marcel Dekker, Inc., New York, pp. 357-396.

Stotzky, G., 1997. Soil as an environment for microbial life. In: Modern Microbiology. Van Elsas, J.D., Trevors, J.T., and Wellington, E.M.H (eds.) Marcel Dekker, Inc., New York, pp. 1-20.

Tate, R.L., 1995. Soil Microbiology. John Wiley, New York.

Tisdall, J. M., Oades, J. M., 1980. The effect of crop rotation on aggregation in a red-brown earth. *Australian Journal of Soil Research,* **18** : 423-434.

Turco, R. F., Kennedy, A. C., and Jawson, M. D., 1994. Microbial indicators of soil quality. In: Defining Soil Quality for a Sustainable Environment. Doran, J. W., Coleman, D. C., Bezdicek, D. F., and Stewart, B. A. (eds.). Soil Science Society of America, Inc., Madison, pp. 73-90.

Verhoef, H.A., 1995. Litterbag method. In: Alef K, Nannipieri P (eds.) Methods in applied soil microbiology and biochemistry. Academic Press, New York, pp 485-487.

Whitby, C.B., Hall, G., Pickup, R., Saunders, JR., Ineson, P., Parekh, N.R., Mc Carthy, A., 2001. C^{13} incorporation into DNA as a means of identifying the active components of ammonia-oxidizer populations. *Lett. Appl. Microbiol*., **32** : 398-401.

Wright, D. A., Killham, K. Glover, L. A, Prosser, J. I., 1993. The effect of location in soil on protozoan grazing of a genetically modified inoculum. *Geoderma,* **56**: 633-640.