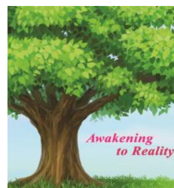

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A Review of Soil Enzyme, Humic Acid and Microbial Activities Methods of Assessment: The Pros and Cons

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ABSTRACT

In view of the place of biological parameters in defining soil health, many scientists of repute have developed methods of assessing environmental parameters one of which is soil health. Soil health is assessed from several angles: biological, chemical, physical, bearing in mind that the top and sub soils are the zones of plant growth and active flora and fauna activities. Biological parameters of the soil cannot do without reference to enzyme, humic acid and microbial activities. A lot of methods have been developed for their assessment and a lot of scientists have adopted and modified the methods with varying results. Each of them have additional benefits. There may be limitations but that is the basis of science. A big thank you to all the scientists living and dead who have shaped the scientific community and the world from their research and findings. All of them have been recognized globally directly as nobel laureates and indirectly through other honours by peer groups. This paper presents enzyme and microbial activities methods of assessment and their pros and cons.

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Introduction

Soil health has been defined as the capacity of soil to function as a vital living system, to sustain biological productivity, promote environmental quality and maintain plant and animal health (Girvan et al, 2003). Soil health has been reported as fundamental for agricultural sustainability It is the fundamental asset of any agricultural system (Pretty, 2002; Girvan et al, 2003; Pretty and Ball, 2002; RCEP, 1996). When soils are in poor health they cannot maintain productive agriculture. It is estimated that nearly 2 billion hectares of land worldwide are degraded. They suffer from a mix of physical degradation by water and wind erosion, crusting, sealing and waterlogging, chemical degradation by acidification, nutrient depletion, pollution from industrial wastes and excessive use of pesticides and fertilizers, biological degradation by organic depletion and loss of soil flora and fauna (Pretty, 2002).

Evidence exist that in Africa farmland is annually losing N, P, and K at a rate of at least 30 kg ha^{-1} with land in 23 countries losing more than 60 kg ha^{-1} (Pretty, 2002). The panacea to the above problems according to many workers lies in sustainable agriculture. Soil health can be improved through a variety of practices including the use of legumes, green manure and cover crops, the incorporation of plants with the capacity to release P from the soil and the use of composts and animal manures. In addition to the adoption of zero tillage, inorganic fertilizers may be used as a sustainable agricultural practice (Pretty, 2002; Briggs, 1989; Reijintjies, 1997; Vlaming et al, 1997; Bunch, 1997; Mullerriyawa and Wettasinha, 1997; Zakaria and Laban, 1997; Wanjau et al, 1997; Sharland, 1997)

Soil fertility management as a basis for sustainability has been described by Rejintjies (1997) as creating favourable conditions for soil life and plant growth through nutrient applications and soil conservation. The author describes soil fertility management as a complex issue involving many farming practices. In turn each farming system has its own unique way of soil fertility management which depends on a combination of factors: the condition of the natural resource base, the land available, labour and capital resources and their relative price, the history of local farming, farmers knowledge, their motivation, skills and degree of market orientation, the relative prices of inputs and of course agricultural policy. Today the ecological sustainability of agriculture and findings ways of increasing production are pressing issues (Pretty, 2002, Reijintjies, 1997). They should have been worse if novel scientists and researchers have not put all their talent, skill and resources to bear in solving ecological problems. One of them is the methods of first assessing soil health. The ingredient lies in biological, chemical and physical health. For this review soil enzyme, humic acid and microbial activities methods of assessment have been picked as they are vital for addressing the problems of soil infertility and unproductivity.

Methods of assessing soil enzyme activities

Research into soil enzymes has increased over the past 30 years. Various activities associated with biotic and abiotic components contribute to the overall activity of soil enzymes. Enzymes may be associated physically with proliferating animal, microbial and plant cells and it may be located in the cytoplasm in the periplasm of gram-negative bacteria or attached to the outer surface of cells. They can also be present in non-proliferating cells (for example, microbial spores or protozoan cysts), in entirely dead cells or in cell debris. Other enzymes are present as an extracellular soluble molecule. They can also be temporarily associated in enzyme-substrate complexes, adsorbed to clay minerals or associated with humic colloids. Some of these categories may represent various stages in the life of an enzyme. An intracellular enzyme may be released in the aqueous phase and may eventually be absorbed in an active form by soil colloids. Enzyme-clay and enzyme-organic polymer complexes show remarkable resistance to proteolytic and thermal denaturation (Burns, 1982, 1986; Alef and Nannipieri, 1998; Sarkar et al, 1980; Trasar-Cepeda and Gil-Sotres, 1987, 1988; Nannipieri, et al1988).

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Enzymes show extraordinary specificity in catalyzing biological reactions. A systematic classification of enzymes has been adopted on the recommendation of the International Enzyme Commission. The new system divides enzymes into six major classes, which are subdivided further into subclasses according to the type of reaction catalysed. For example a recommended name, a systematic name and a classification name for phosphodiesterase is phosphoric diester hydrolase while its classification number is EC 3.1.4 (Alef and Nannipieri, 1998).

Several methods exist for the measurement of enzyme activities (Igboji, 2015). These are summarized in Table 1. Many workers have cautioned about the interpretation of results arising from measurement of soil enzyme activities. The concentration of substrate when in excess and optimum values of pH and temperature are selected to permit the highest rate of enzyme activity. Also the volume of the reaction mix such that it allows free diffusion of substrate. Hence the interpretation of measured soil enzyme activity have often led to the conclusion that soil enzyme assays have no meaning in ecological and agricultural terms (Nannipieri, 1994; Nannipieri et al, 1990; Alef and Nannipieri, 1995; Burns, 1982).

Enzymes measurements do answer qualitative questions about specific metabolic processes and in combination with other measurements (ATP, AEC, CO_2 evolution) increase the understanding of the effect of agrochemicals, cultivation practices and environmental and climatic factors on the microbiological activity of soil (Skujins, 1978; Nannipieri, 1994; Alef and Nannipieri, 1995). Curci et al, (1997) cited by Girvan et al (2003) reported that enzyme activity was higher in the uppermost 20 cm of the soil in plots tilled by shallow ploughing. This was not the case in soils tilled by deep ploughing.

Humic matter in soils

Organic matter in soils and waters is conveniently categorized as humic and non-humic substances based on operational definitions. Humic substances are organic materials derived principally from decaying plant remains but with the normal plant components considerably altered by the soil animal and microbial populations. A biological chemical reactions are also possible. The entire process giving rise to a complex mixture of macromolecules whose composition is site-dependent, especially on vegetation and pedogenic processes (Anderson et al, 1984). Since Archards first extraction of humic substances in 1786, the use of different isolation and fractionation techniques has led to considerable confusion until in 1982, when the International Humic Substances Society published methodologies for the extraction and fractionation of humic substances from soils and water (Anderson et al, 1984). The percentage distribution of humic, fulvic and hydrophilic acids have been described by Anderson et al (1984).

Origins, composition and structure of humic substances

There is no evidence to suggest that there is genetic or biological control of the synthesis of humic substances. Hence, they lack regularity in the sequencing of the molecules which compose the macromolecules. Further intramolecular and intermolecular associations can take place within and between humic acid (and other) macromolecules to give a semblance of secondary and tertiary structure. However, there are no rigid regularities between such associations. Although associations can form between humic substances and other organic and inorganic materials such associations are invariably random, and are not necessarily a part of any biological function which humic substances may have. Hence, humic substances do not meet any of the criteria for structure as they apply for proteins (Hayes, 1991).

Two broadly based processes are considered to give rise to humic substances. The first of these, the "degradative process" involves the biological transformation of refractive organic macromolecules such as lignins, paraffines, substances, cutins, melanins, suberins, and other substances which give rise to humic substances (Nip et al, 1986; Hayes, 1991). Polysaccharides can undergo "browning" reactions to give rise to humic substances. Quinones from oxidized phenols are also known to give rise to humic-type substances. Such pathways are part of the second or synthetic process, for the genesis of humic substances (Hayes, 1991). Mailard (1912) as cited by Hayes (1985) introduced the "browning" reactions or the "melanoidin" theory when he observed that monomeric reducing sugars, such as glucose could condense with amino acids such as glycine to form brown macromolecules substances.

Polycondensation reactions between glycine and 2-oxopropanal (methyl glyoxal) were considered by Enders et al (1948) to provide plausible processes for the formation of humic substance. By regulating the ratios of the reactants, the macromolecular substances produced can have elemental contents and charge and other characteristics similar to soil humic acids (Schuffelen and Bolt, 1962). More recently there has been emphasis on the role of quinines from di and polyhydroxybenzene structures with –OH groups in the 1,2 and 1,4 ring positions on the synthesis of humic substances (Flaig, 1988). Lignins can give rise to the appropriate phenols and fungi are known also to synthesize phenols many of which are components of melanins, the coloured secondary metabolites formed during fungal degradation of saccharides (Martin et al, 1980).

Aiken et al (1985) considered humic substances to be a "general category of naturally occurring biogenic heterogenous organic substances that can generally be characterized as being yellow to black in colour, of high molecular weight and refractory". Hayes and Smith (1978) based on proposals by Kononova (1966) considered humic substances to be the amorphous, macromolecular, brown-coloured components of SOM which bear no morphological resemblances to plant or animal tissues from which they were derived and which can be differentiated into broad general classes on the basis of solubility differences in aqueous acids and bases. According to these authors humic acids are the components of humic substances precipitated when extracts in dilute aqueous alkali are acidified to pH 1. Fulvic acids are the components which are soluble in aqueous acids and base, humin is the term applied to the components which are insoluble in aqueous acids and bases. However, these three terms refer to gross mixtures and the elemental composition of these mixtures and their chemical and physicochemical properties can vary with their origins and with the environments in which they are formed (Hayes, 1991).

Isolation of humic substances from soil

Hayes (1985) reviewed the properties of solvents used for the isolation of humic substances from soil and the processes by which dissolution of the macromolecules take place in the Different solvent systems. At the pH of most agricultural soils, humic substances are significantly ionized and the negative charges of the conjugated bases in the acid groups are balanced mainly by divalent and polyvalent metal cations. Table 2 shows the data relating to the successive extraction of humic substances from the soil (Hayes et al, 1975; Swift, 1985).

Table 1. Methods of assaying soil enzyme activities

Enzyme	Activity	Method of assay	Remarks	References
Urease	Catalyses the hydrolysis of urea to CO_2 and NH_3, with the formation of carbamate as an intermediate product	1.Determination of NH_3 liberated on incubation of toulene-treated soil with buffered urea solution 2. Estimation of the rate of urea hydrolysis in soils by residual urea or $^{14}CO_2$ liberated after incubation 3. Use of buffer to control pH, or addition of toluene to inhibit microbial proliferation	1. Widely distributed in nature. 2.catalyses the hydrolysis of hydroxyurea, dihydroxyurea and semicarbazide 3.Denatured at 70°C 4. Incubation temperature of assay range from 15 – 42°C 5. Urease extracted from soil is resistant to thermal and proteolytic denaturation	Bremner and Mulvaney, 1978; Gosewinkle and Broadbent, 1984; Kandeler and Gerber, 1988; McCarty et al., 1989; Skujins and McLaren, 1969; Douglas and Bremner, 1970; Bremner and Mulvaney, 1978; Mulvaney and Bremner, 1979; Hoffman and Schmidt, 1953; Galstyan, 1965; Tabatabai and Bremner, 1972; Zantua and Bremner, 1975a; Frakenberger and Johanson, 1986; Kandeler and Gerber, 1988; Kissel and Cabrera, 1988; Moyo et al., 1989; Nannipieri *et al*., 1978a; Nannipieri *et al*., 1974; Burns *et al*., 1972.
Phosphatase	Catalyses the hydrolysis of phosphate esters		Enzymes with relatively broad specificity, capable of acting on a number of different structurally related substrates, but at widely different rates 2. Acid phosphatase is predominant in acid soils, alkaline phosphatase in alkaline soils 3. Assays carried out at neutral pH (6.5 – 7) 4. Optimum temperature for assays (40 – 60°C) 5. Activity affected also by OM content, soil moisture and anaerobiosis	Alef *et al*., 1998; Alef *et al*., 1995Florkin and Stotz, 1964; Beck, 1973; Burns, 1978; Chonkar and Tarafdar, 1981; Dick and Tabatabai, 1983; Tarafdar and Jungk, 1987; Alef *et al*., 1995; Speir and Ross, 1978; Eivazi and Tabatabai, 1977; Nannipieri *et al*., 1988
Cellulase	Catalyses the hydrolysis of cellulose to Ɗ-glucose	Based on the determination of either released sugars or evolved $^{14}CO_2$, using cotton strips, radio-isotope-labelled cellulose and carboxyl methyl cellulose (CMC)	Enzymatic hydrolysis of cellulose depends on degree of crystallinity, the nature of associated substances and surface area	Lee and Fan, 1980; Eriksson and Wood, 1985; Sinasabaugh and Linkins, 1989; Alef and Nannipieri, 1995; Hayano, 1986; Hunt, 1977; Schroder and Gewehr, 1977; Schroder and Urban, 1985; Sinsabaugh and Linkins, 1988; Tateno, 1988; Kshattriya *et al*., 1992; Speir and Ross, 1981; Benefield, 1971; Pancholy and Rice, 1973; Hope and Burns, 1987; Kiss *et al*., 1978; Clark and Stone, 1965; Yamana *et al*., 1970; Hayano, 1986; Rhee *et al*., 1987; Stutzenberger, 1972; Gottschalk et al., 1981; Joliff *et al*., 1989; Benefield, 1971; Latter and Howson, 1977; Ibister *et al*., 1980; Sato, 1981; Schinner and vonMersi, 1990

Table 2. Methodologies for successive extraction of humic substances from a soil (yield in % of total OM)

Extractant	Humic Acid	Fulvic Acid	Cumulative Total	PH value of Extractant
Water	0.0	2.8	2.8	-
DMF	15.0	2.2	20.0	6.8
Sulfolane	4.1	1.0	25.1	3.7
DMSO	0.7	0.2	26.0	5.9
Pyridine	14.8	0.6	41.4	11.6
EDA	23.2	6.3	70.9	13.0

Table 3. Methods of estimating whole ecosystem and soil respiration

Condition of measurement	Methods of determination	Remarks	References
Soil respiration in the laboratory	1. Incubation of soils in jars, closed petri dishes or different types of flask	CO_2 is usually adsorbed in NaOH and determined by HCl titration	Isermeyer, 1952; Pochon and Tardieux, 1962; Jaggi, 1976; Rowell, 1994.
	2. Electrical conductivity of the NaOH solution following incubation of soil samples		Anderson and DOMSCH, 1978a; Cheng and Coleman, 1989
	3. Use of gas chromatography		Brookes and Paul, 1987; Trevors, 1985
	4. Infrared spectroscopy		Heinemeyer *et al*. 1989
	5. Use of labelled CO_2 ($^{14}CO_2$)	This is following the decomposition of specific organic compounds in the soil	Naklas and Klein, 1981
	6. Use of Warburg apparatus	Measures the O_2 consumption in incubated soils	Domsch, 1962; Stotzky, 1965
	7. Use of electrorespirometer		Birch and Melville, 1969; Krockel and Stolp, 1986; Alef *et al*. 1998
Soil respiration in the field	1. Placing of NaOH solution in an open glass jar above the soil surface and covering the area to be measured with a metal cylinder closed at the upper end.	Used for determining CO_2 evolved from undisturbed soils	Anderson, 1982
	2. Use of gas chromatography	Measures CO_2 and O_2 concentrations at various soil depths	Richter, 1972; Anderson, 1982.
	3. Automated monitoring of biological trace gas production and consumption	Estimates gas concentration under field conditions in a covered soil	Brumme and Beese, 1995.
Whole ecosystem and soil respiration in the field	1. Micrometeorological technique		Franzluebbers et al. 2002; Verma, 1990; Norman *et al.* 1992
	2. Static chamber with alkali absorption method	Nocturnal trial	Franzluebbers et al., 2002; Zibilske, 1994
	3. Portable environmental gas monitor linked to soil respiration chamber		Sowerby *et al.*, 2000; Ball et al., 1999.

Table 4. Methods used in assaying soil microbial biomass

Parameter	Method of determination	Remarks	References
Soil microbial biomass	1. Staining and counting of microbial biomass		Babiuk and Paul, 1970; Trolldenier, 1973; Anderson and Slinger, 1975; Paul and Johnson, 1977; Soderstrom, 1977; Torsvik and Goksoyr, 1978; Lundgren, 1981
	2. Use of physiological parameters such as ATP content, respiration, and heat output		Anderson and Domsch, 1978; Sparling et al., 1981; van de Werf, 1989/1990; Sparling and West, 1990; Jenkinson, 1988; Sparling, 1981; Kaiser et al., 1992, van de Werf and Verstraete, 1987a
	3. The fumigation -incubation technique	1. Soil fumigation only kills the microbial biomass and does not affect non-living OM 2. The flush in respiration exclusively derives from the microbial biomass 3. The number of organisms killed in the unfumigated soil is negligible compared with that in fumigated soil 4. The fraction of dead microbial biomass carbon mineralised over a given period does not differ in different soils. 5.The method is not recommended for acidic soils ($H_2O < 4.5$) because soil inoculation can be difficult 6. The method is unsuitable for soils recently treated with OM, because the large microflora of the unfumigated soil decomposes the substrate more effectively, than the smaller microflora of the fumigated soil. 7. This method cannot be used when fresh roots are present in soil because cell membranes of young living roots are affected by $CHCl_3$	Shen et al., 1984; Jenkinson and Powlsen, 1976a, 1976b; Brookes et al., 1985; Vance *et al.*, 1987a, 1987b, 1987c; Joergensen et al., 1990; Jenkinson and Ladd, 1981; Martens, 1985; Mueller *et al*., 1992.
	4. Modified fumigation incubation procedure	1. Suitable for determining microbial biomass N for waterlogged soil 2. In calcareous soils, low in OM, errors can occur due to the decomposition of HCO_3^-. This is reduced by placing beakers with soda lime in desiccators holding fumigated and unfumigated soils	Inubushi et al., 1984; Jenkinson and Powlson, 1976b.
	5.Colourimetric		Chaussod *et al*., 1986
	6. Gas chromatography		Martens, 1985; Anderson and Domsch, 1978; Sparling, 1981; Chaussod and Nicolardot, 1982.
	7. Fumigation-extraction		Vance et al., 1987c; Kaiser *et al*., 1992
	8. Dichromate oxidation	OM is oxidised and Cr(+VI) reduced to Cr(+III). Amount of dichromate left is back titrated	Kalembasa and Jenkinson, 1973; Vance *et al.,* 1987c
	9.Ultraviolet persulphate oxidation	Extracted OC is oxidised by UV light to CO_2, which can be measured by IR or photospectrometry	Wu et al., 1990
	10. Ninhydrin nitrogen reaction	Ninhydrin forms a purple complex with molecules containing AA, peptides, and proteins	Moore and Stein, 1948; Lamonthe and McCormick, 1973; Amato and Ladd, 1988
	11. SIR	O_2 uptake or CO_2 evolution immediately after the amendment with quantities of glucose	Sparling, 1995
	12. Respiration-simulation method	Based on continuous monitoring of O_2 uptake by soil supplied with readily degradable OM in a respirator	Van der Werf et al. 1995; van de Werf and Verstraete, 1987a

Purification and fractionation of extracts

Extracts from soil in organic and in aqueous solvents are mixtures containing humic and non-humic substances. Water scientists have developed a resin treatment process to separate hydrophilic substances from the humic substances in waters. The poly (methylmethacrylate) resin, XAD-8 for example binds the H^+-exchanged humic substances but it allows salts and small molecules and macromolecules organic substances such as polysaccharides to pass through the resin column. The humic substances are recovered by raising the pH causing the acidic groups to ionize and the macromolecules to desorb from the resins (Hayes, 1991)

This procedure is applicable to the fulvic acid fraction contained in the supernatants when aqueous alkaline extracts from soils are precipitated at pH 1. Thus, the substances retained when the fraction is applied under acidic conditions to XAD-8 resins and subsequently eluted when the pH of the solvent is raised are true fulvic acids (Hayes, 1991). According to Hayes humic acid precipitates cannot be applied directly to resins. However, these acids can be dissolved in DMSO and passed into XAD-8 resin columns. Elution with acidified water (pH 1 – 2) removes the DMSO and polar substances and the humic acids are then eluted as the pH is raised. This procedure allows fractionation of the humic acids using a pH gradient system. It also allows humic substances to be recovered from the acidified DMSO extracts of soils. These extracts are applied to XAD-8 columns, the DMSO is removed and the humic substances are recovered as described (Hayes, 1991)

Fractionation of the humic substances on the basis of molecular size differences are most frequently used to further analyse the humic substances. The procedures generally involve uses of gel permeation chromatography (Swift, 1989; DeNobili et al, 1989), ultrafiltration or centrifugation (Hayes, 1991). For gel permeation and ultrafiltration procedures to be effective it is important that the humic substances should not interact, either adsorb to or be rejected by the gel or membrane with the media used (Hayes, 1991).

Appelqvist (1990) fractionated sodium humate preparations using ultrfiltration with Sartorius membranes of pore sizes with nominal molecular size exclusion values of 5,000, 20,000 and 100,000 daltons and gel chromatography using Sephacryl S-200 gel, a cross-linked dextrant from Pharmacia). Differences were observed between the samples excluded by the gel (MW>150,000) and those retained materials of 100,000 MW and above (>100,000), 20,000 and above (100,000 – 20,000 MW) fraction and 5,000 and above (20,000 – 5,000). As the molecular sizes decreased the carbon contents also decreased (52.5, 51.6; 49.2; 47.4%) and the E_4/E_6 ratios increased. The E_4 (solvent mixture absorbance at 465_{nm}) and E6 (solvent mixture absorbance at 665_{nm}) are indicators of differences in solution conformations. It aids comparison of humic substances in different solvent systems. Such data support the concept of greater aromaticity for the higher molecular weight materials (Chen et al, 1977). This is in keeping with the concept of greater numbers of aromatic carboxylic acids, in the higher molecular weight substances. Fourier transform infrared spectroscopy (FTIR) indicated increased aliphatic characteristics for the lower molecular weight components. Nip et al (1986) showed that H-NMR spectra for the high molecular weight materials confirmed that phenols were present in the aromatic structures. This substantiates the data from the potentiometric titrations.

There were also differences between the >150,000 MW obtained by gel filtration and the >100,000 MW obtained by ultrfiltration fractions. Some of these differences can be deduced from examinations of the titration data. Other differences were evident in the amino acid content of the high molecular weight materials isolated by ultrfiltration and gel filtration (43.24 and 38.25 nmol mg^{-1}. Prior to all analyses the humic acids which were isolated in 0.1 M NaOH from a sapric histosol) were dissolved in DMSO-HCl (1% v/v) and adsorbed on and recovered by back elution with 0.1 N NaOH from XAD-8 resin. The resin treatments had separated from the humic acid fraction sugar and amino acid containing residues which were not covalently bonded to the humic acid “core” or “backbone” structures (Hayes, et al, 1989).

The most useful of the physicochemical procedures for studying the structures of humic substances has focused on molecular size, shape and charge characteristics (Hayes, 1991). For such studies it has been desirable to work with molecules that are relatively homogenous with respect to sizes and shapes. The application to the study of humic substances of liquid and solid state NMR have been discussed by Wershaw (1985); Malcolm (1989); Wilson (1989) and Steelink et al (1989). Progress in the use of liquid and solid state NMR analyses now make it possible to obtain reasonably quantitative and well resolved spectra for humic substances. The availability of cross polarization magic angle spinning (CPMAS) ^{13}CNMR has done much to improve resolution and application of this procedure to the study of humic substances in the solid state (Igboji, 2015; Hayes, 1991)

Microbial activities

Whole ecosystem and soil respiration

Living cells need a constant supply of energy which for heterotrophic microflora is derived from the transformation of organic matter such as cellulose, proteins, nucleotides and humifields compounds. Energy supplying reactions in the cell are redox reactions based on the transfer of electrons from a donor to an acceptor. Through aerobic respiration that is the oxidation of organic matter by aerobic micro-organisms oxygen functions as the terminal acceptor of the electrons. This metabolic activities of soil micro-organisms can therefore be quantified by measuring the CO_2 production or O_2 consumption (Alef, 1995; Nannipieri et al, 1990).

Soil respiration is one of the oldest and still most frequently used parameters for quantifying microbial activities in soils (Kieft and Rosacker, 1991). Basal respiration is respiration without the addition of organic substance to soil. Substrate-induced respiration (SIR) is the soil respiration measured in the presence of an added substrate such as glucose. Respiration is not only restricted to micro-organisms but is also carried out by other organisms inhabiting the soils. Like other metabolic activities it depends on the physiological state of the cells and is influenced by different factors in the soil (Alef, 1995).

Respiration is influenced by soil moisture, temperature, the availability of nutrients, soil structure and tillage. Air drying significantly reduces it. Remoistened soils show very high initial activities as a result of the high concentrations of easily degradable organic compounds such as amino and organic acids caused by chemical and physical processes at the moistening of dry soils (Igboji, 2015; Clark and Kemper, 1967; Anderson, 1975; Wilson and Griffin, 1975a; 1975b; Kowalenko et al, 1978; Krockel and Stolp, 1986; Kieft et al, 1987).

The re-moistening of air dry soils containing carbonate also causes the release of abiotic CO_2.In this case it is recommended that the O_2 consumption is measured (Anderson, 1982; Kieft et al, 1987). Soil respiration decreases with the depth of soil and

correlates significantly with SOM (C_{org}) and most microbial parameters (Stotzky, 1965; Thalmann, 1968; Parkinson et al, 1971; Alexander, 1977; Gray and Williams, 1977; Anderson and Domsch, 1978a, 1978b; Domsch et al, 1979; Sparling, 1981a, 1981b; Sparling and Elland, 1983; Beck, 1984a; Alef and Kleiner, 1987; Alef et al, 1988; Suttner and Alef, 1988; van de werf, 1989; Alef, 1990)

Soil respiration changes according to soil treatment and cultivation methods and has been used as a measure of the effects of chemicals such as pesticides and heavy metals (Jaggi, 1976; Anderson et al, 1984; Beck, 1984b, 1985; Malkolmes, 1985; Carlisle and Trevores, 1986; Domsch and Schroder, 1986; Wilke, 1986; Somerville and Greaves, 1987; Alef et al, 1998; Schlosser, 1988; Schuster, 1988)

Basal soil respiration can be followed for long periods of time. However, changes in the composition of aerobic microflora can occur during long term incubation. In the case of the SIR method, a change in population is expected when the incubation period is longer than 4 – 6 h (Anderson and Domsch, 1978b). The incubation temperature used varies between 20 and 30°C and the water holding capacity between 50 and 70%. The pH value of the measurement is usually that of the soil in water (Alef, 1995). A summary of the methods used in estimating whole ecosystem and soil respiration (Igboji et al, 2015) is presented in table 3.

Methods of assaying microbial biomass

Microbial biomass has been part of the OM in soil that constitutes living micro-organisms smaller than 5 – 10 μm^3 and is generally expressed in mg C kg-1 soil or µg C g-1 dry weight and ranges from 1 – 5% of SOM (Jenkinson and Ladd, 1981; Sparling, 1985; Smith and Paul, 1990). Table 4 summarizes the various methods used in its determination.

Conclusion

All methods for assaying soil enzyme, humic acid and microbial activities listed in this review are very coherent and reliable. Even though most laboratories especially in developing world cannot afford most of the instrumentation and high-tech required for routine environmental monitoring they can still rely on basics titrimetric and portable systems to drive the research. For the developed world that have gone nuclear the love in the air is molecular methods for the assay of these parameters. That is helping drive the genesis and revelation of biological life. By the time their methods come to developing world they must have starting planting trees in the stars, moon and sun.

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