Phospholipid fatty acid – A bioindicator of environment monitoring and assessment in soil ecosystem

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The key driving factor for sustainable agriculture is soil ecosystem, where pivotal services are provided by the soil biota, 'the biological engine of the earth', which can act as early warning signals of ecosystem health, and can be of use in environmental diagnosis. Phospholipid fatty acid (PLFA) profiles offer sensitive reproducible measurements for characterizing the numerically dominant portion of soil microbial communities without cultivating the organisms. The technique gives estimates of both microbial community composition and biomass size, and results represent *in situ* conditions in the soil. PLFA analysis has been used to detect various environmental stresses in the soil and was found to be more discriminatory than other methods. A set of certain specific PLFAs, viz. *trans/cis* ratio of monounsaturated fatty acids (16 : 1*w*7, 18 : 1*w*7), cyclopropyl (cy17 : 0 and cy19 : 0) fatty acids, and fungal PLFA (18 : 2w6) were able to distinguish the stressed environmental conditions such as heavy metal addition, tillage, organic compound toxicity, starvation and increased soil temperature. This set of PLFA needs further testing and validation in the wide spectrum of environmental stressed conditions under diverse ecosystems, before its implication as bioindicators of environment monitoring and assessment at the global scale.

Keywords: Assessment, environment monitoring, environmental stress, microbial community structure, phospholipid fatty acids, soil ecosytem.

THE earlier era of industrialization and technological revolution in the form of the Green Revolution in agriculture, although led to the overall progress and food security, underpinned the sustainable development which has started translating in stagnating/declining agricultural productivity, changing climate, increasing pollution, marching deserts, deteriorating condition of fragile ecosystems and loss of biodiversity. These issues have become subjects of serious global concern, as they have started putting a question mark on food security as well as the very existence of human beings. For risk analysis of the determinants of food security and to gauge the success of sustainability, environmental diagnosis of the ecosystem constitutes the first step.

One of the key driving factors for sustainability is soil ecosystem – the critical component of earth's biosphere, functioning not only in the production of food and fibre but also in the maintenance of local, regional and global environmental quality. These ecosystem services are provided by the soil biota – 'the biological engine of the earth' – by driving many fundamental nutrient cycling processes, soil structural dynamics, degradation of pollutants, etc. and microorganisms are key players in these services[1]. Thus, it is logical that the biological health of soil ecosystem has considerable potential as indicator of ecosystem health, which can be of use in environmental diagnosis. The indicators used for monitoring the state of the environment should be able to reflect the structure and function of ecosystem processes sensitive to variations in management and climate, reproducible, easily measurable and applicable from local to national scale[2].

Until recently, physico-chemical attributes (pH, CEC, available nutrient status, organic carbon) of the soil were used for monitoring the response of stress conditions on the soil environment. However, they are not sensitive enough to forecast early changes in the soil environmental status. On the other hand, the soil microbial properties respond much faster to disturbances and perturbations[3], and these changes need to be quantified for maintenance of an ecosystem. Traditionally, response to stress at the microbial scale has been studied at the process level, in terms of the number, respiration rates and enzyme activities[4], which provide total pool sizes and gross activity measures at a broader scale. However, these are not particularly sensitive indicators[5] because of redundancy of the functions and complex interactions within communities. Hence the microbial communities, which comprise diverse organisms should be studied as such and in terms of microbial diversity for assessing the real picture of an ecosystem, which may reflect the immediate displacement of organisms by stress/perturbation and long-term effects on processes caused by successions in the microbial community.

Bacterial diversity can be analysed at the genetic, phenotypic and functional scale of resolution. Monitoring entire

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microbial communities in the soil has conventionally been a time-consuming task. However, recent advances in molecular microbial ecological tools for direct extraction of total community DNA/RNA from environmental samples and their subsequent analysis by denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and terminal-restriction fragment length polymorphism analysis (T-RFLP) have made studies of microbial communities much easier[6,7]. Although DNA-based techniques have great potential, they also have problems, in the reproducibility of nucleic acid extraction and in the selectivity of the PCR step. Moreover, genetic analysis reveals the total, not expressed diversity and the genetic diversity is so great that the fine scale detection of impacts, which is required for the routine monitoring, and early warning may not be visible. This has been overcome by the determination of phospholipid fatty acid (PLFA) profiles[8], which provide a broad diversity measurement of microbial community at the phenotypic level. However, PLFA profiles do not give any information on species composition; rather, they divulge the fingerprint of community structure and have been widely used to study community structure in varied range of systems and are being considered as a robust tool that consistently discriminates between communities of different origin and land management strategies[9,10].

Characteristics of PLFA

Fatty acids are the key component of cellular membrane of all living cells. Phospholipids consist of a single molecule of glycerol (3C alcohol), two OH groups of the glycerol are bound to the two fatty acid chains (hydrophobic tail) and one OH group is bonded to a phosphate group (hydrophilic head). Thus these lipids are asymmetric, having hydrophilic and hydrophobic regions and in the membrane they form a bilayer with hydrophilic ends towards the outer surface of the membrane and hydrophobic ends buried in the interior (Figure 1). PLFA can be classified into ester-linked phospholipid fatty acids (EL-PLFAs, 60–90% of the total) and non-ester linked phospholipid fatty acids (NEL-PLFAs, 10–40% of the total; Figure 2). EL-PLFAs are further subdivided into ester linked unsubstituted fatty acids (EL-UNFAs) and hydroxy substituted fatty acids (EL-HYFAs). EL-UNFA includes saturated (EL-SATFA), monounsaturated (EL-MUFA) and polyunsaturated fatty acids (EL-PUFA). EL-SATFA has two sub-groups, branched chain fatty acids (BRANCs) and straight chain fatty acids (STRAs). NEL-PLFAs are composed of unsubstituted (NEL-UNFA) and hydroxy substituted (NEL-HYFA) fatty acids. Hydroxy substituted fatty acids that are localized in lipopolysacchride portion of cell wall in Gram-negative bacteria are designated as LPS-HYFA[8].

Fatty acids most frequently used as biomarkers are 15 : 0 and 17 : 0 for bacteria in general[11,12], iso and anteiso isomers of 15 : 0 for Gram-positive bacteria, *b*-hydroxy fatty acids (*b* 10 : 0, 12 : 0) for Gram-negative bacteria which are present in lipid portion of the lipopolysaccharide (LPS) in the cell wall[13]; 10Me18 : 0 for actinomycetes[14,15], and 18 : 2*w*6 for fungi[14–16] (Table 1). Although PLFAs have great structural diversity coupled with high biological specificity, there are few fatty acids which can be applied to more than one group of organisms, e.g. branched fatty acids which are biomarkers for Gram-positive bacteria are also present in some anaerobic Gram-negative sulphate reducing bacteria and the genera *Cytophaga* and *Flavobacterium*[17]. Cyclopropyl fatty acids are generally common in Gram-negative bacteria, but are also present in some anaerobic strains of Gram-positive bacteria[13]. Similarly, LPS-HYFAs, which are the signature fatty acids of Gram-negative bacteria, has also been shown in fungi. Fatty acid 18 : 2*w*6, which has been considered as an indicator for fungi is regarded as one of the major fatty acids in the plant kingdom[18]. However, this fatty acid can be a good indicator of fungi when plant cells are not present[8].

Techniques for determination of fatty acids

Fatty acid extraction techniques that have been used so far for analysing the microbial community structure are: whole

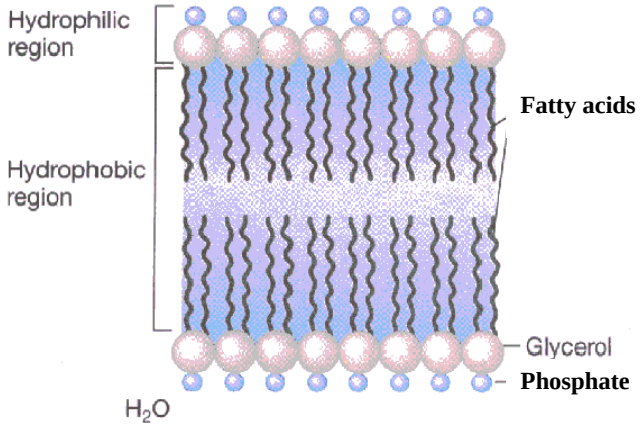

Figure 1. Arrangement of phospholipids in membrane of living cell.

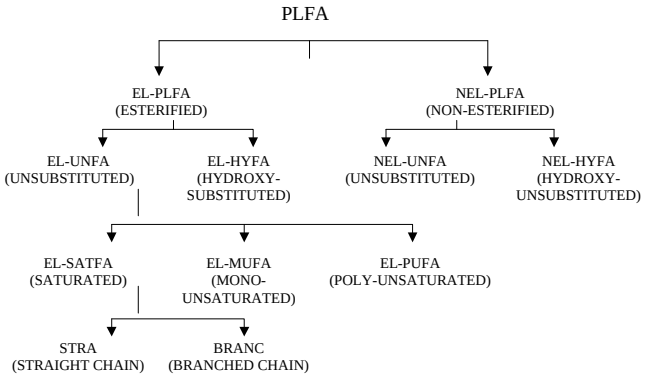

Figure 2. Classification of phospholipid fatty acids (Source: Zelles[8]).

Table 1. Biomarker fatty acids (modified from Zelles[8])

Group	Biomarker
Bacteria	
Gram-positive	Branched chain fatty acids (br 17 : 0*, br 18 : 0, i17 : 0, a17 : 0, i16 : 0, i16 : 1, 10Me16 : 0, 10Me17 : 0), iso and anteiso isomers of 15 : 0, MUFA < 20% (16 : 1*w*9, 16 : 1*w*7c, 16 : 1*w*5, 18 : 1*w*7, 19 : 1)[13]
Gram-negative	Cyclopropane fatty acids (cy17 : 0, cy19 : 0)[46], *b*-hydroxy fatty acids present in cell wall[64,65], MUFA > 20% (16 : 1*w*9, 16 : 1*w*7c, 16 : 1*w*5, 18 : 1*w*7, 19 : 1).
Fungi	PUFA 18 : 2*w*6 (linoleic acid)[14]
Actinomycetes	10Me16 : 0, 10Me17 : 0 and 10Me18 : 0 (ref. 66)
Anaerobic bacteria	NEL-PLFA, e.g. Plasmalogens (*Clostridia*), sphingolipids (*Bacteroides*, *Flavobacterium*)
Sulphate reducing bacteria (*Desulfobacter* sp.)	cy17 : 0 and 10Me16 : 0 without high level of 10Me 18 : 0
Methanogens	
Type I	16 : 1*w*8
Type II	18 : 1*w*8 (refs 67–69)

*Nomenclature: Fatty acids are designated by the total number of carbon atoms and the number after the colon indicates the degree of unsaturation. The degree of unsaturation is followed by Δx, where x indicates the position of the double bond nearest to the carboxyl end, Δ or in a few cases nearest to the aliphatic end w. The prefixes a, i, C_y and d refers to anteiso, iso, cyclopropyl branching and dicarboxylic fatty acid respectively; br indicates that the type of branching is unknown, while a number followed by Me indicates position of methyl group. Prefixes a and b indicate that the OH groups of an OH fatty acid are located at positions 2 and 3 respectively. Numbers preceded by w indicate the position of OH groups from the aliphatic end of the fatty acids. MUFA; Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid.

cell or cellular fatty acid analysis by microbial identification system (MIDI), simple PLFA extraction method and extended PLFA extraction method (Figure 3).

Whole cell or cellular fatty acid analysis by MIDI

This procedure was originally developed by MIDI Inc. (Newark, DE) for analysis of fatty acids from laboratory-grown pure cultures. It is now also being employed for extraction of fatty acids from environmental samples such as soil and water. Extraction of fatty acids from the soil involves saponification of the soil at 100°C followed by acid methylation at 80°C for fatty acid methyl ester (FAME) formation, then an alkaline wash, and extraction of FAME into hexane[17] (Figure 3). FAME extracted is then analysed by gas chromatography (GC) using suitable software. Whole cell fatty acids have proven sufficient to distinguish microbial communities from soils under different management regimes[19,20]. However, this technique is laced with a few drawbacks despite the easy processing of samples. The main disadvantage of this software automated method lies in the fact that it determines only the reference fatty acid profiles, which are extracted from the single cultured organisms grown on solid medium and extraction of the fatty acids is not restricted only to membrane lipids but may also be derived from extracellular lipids. Moreover, acid methylation for FAME formation may disrupt cyclopropyl functional groups[21].

Simple PLFA extraction method

The most widely used extraction and separation method to obtain fatty acids derived from phospholipids is that proposed by Bligh and Dyer[22] and modified by White *et al.*[23]. Briefly, the soil sample is extracted with single-phase mixture of chloroform : methanol : buffer solution (1 : 2 : 0.8 v/v/v) for lipid extraction. After extraction, the lipids are separated into neutral, glyco and phospholipids on a silicic acid column. Phospholipids are methylated and resultant PL-FAME is separated and quantified by GC (Figure 3). This method is simple, rapid and has been used for a wide range of soil types for microbial community analysis[15,16], as well as the total microbial biomass determination. However, through this method only EL-PLFA can be analysed and not the NEL-PLFAs, which are key biomarkers of certain anaerobic bacteria (Table 1).

Extended PLFA extraction method

This extraction method is an extended version of the simple PLFA extraction procedure. In this method, the simple extraction procedure is followed by complex extraction technique[24] for fractionation of fatty acids into chemically relevant groups (Figure 3) before their final separation through GC. This method has been used successfully for evaluating the microbial community shift in different management regimes under diverse soil types[25,26] and yields accurate information about the chemical structure of fatty acids, which helps in accurate determination of these fatty acids as biomarkers for specific groups of organisms. Moreover, through this technique both the EL and NEL fatty acids can be detected. Furthermore, the low concentration of unusual fatty acids can be determined.

Haack *et al.*[17] compared the MIDI-FAME and simple PLFA extraction method and concluded that the data acquired by MIDI-FAME appeared to be equivalent to those acquired by the more complex protocols. However, Petersen *et al.*[24] showed that MIDI-FAME included a significant background of nonmicrobial material and was less sensitive to soil environment conditions than the simple PLFA extraction method. The simple extraction method was found to be insensitive to microorganisms which comprise a small proportion in a community[27]. Thus, the extended version of the technique seems to be more appropriate in fingerprinting the entire microbial community structure. Furthermore, this technique has the capacity to detect the

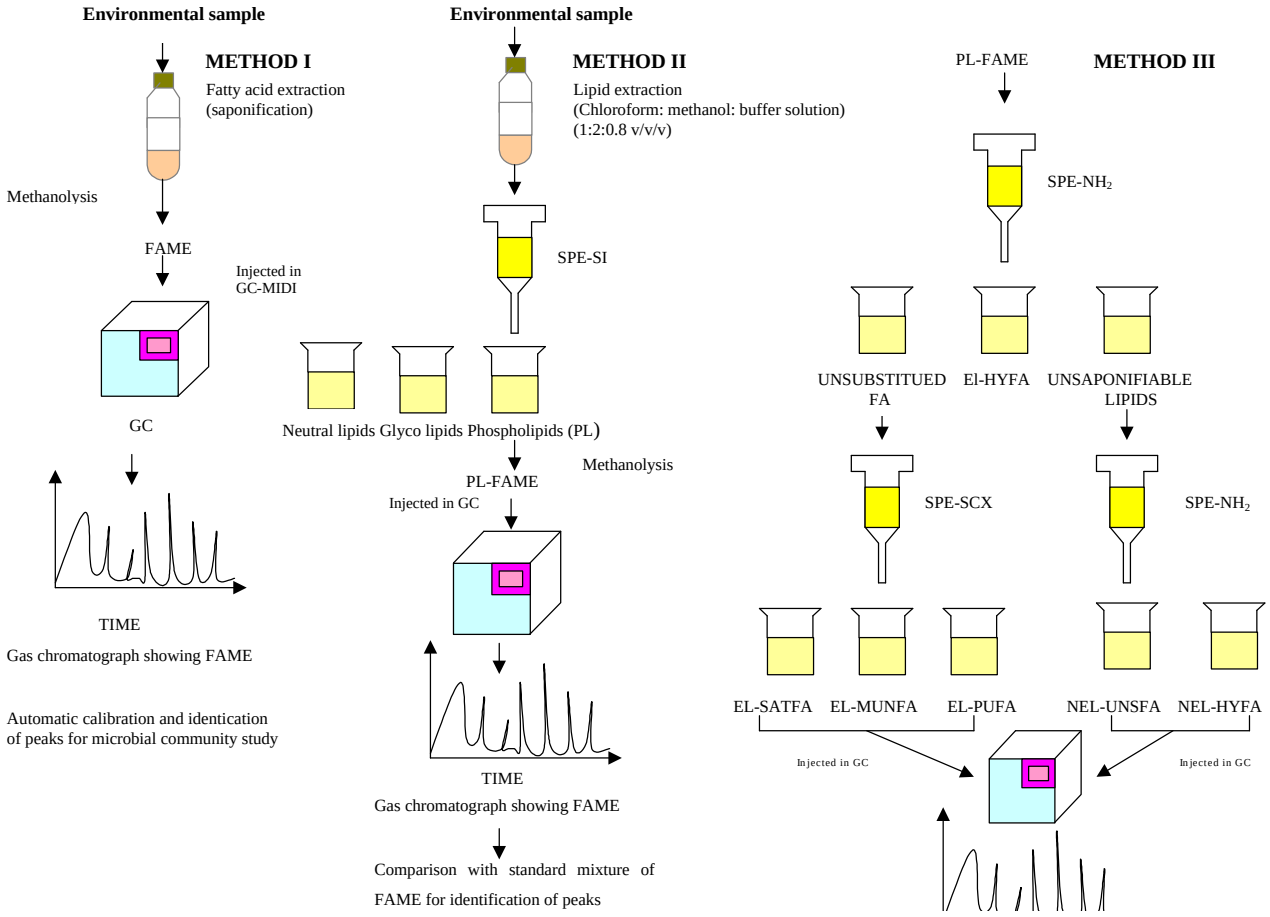

Figure 3. Flow chart for extraction and detection of phospholipid fatty acids using different methods. SPE-SI, Solid-phase-extraction silicic acid bonded phase column; SPE-NH_2, Aminopropyl bonded SPE column; SPE-SCX, Benzenesulphonic-acid bonded SPE column.

defined fatty acids in lower concentration in the soil extract and can also detect unusual fatty acids.

Advantages of PLFA analysis

The concentration of total PLFA provides quantitative insight into the soil viable/active microbial biomass because the phospholipids are rapidly degraded after cell death and are not found in the storage products. A significant correlation has been observed between total phospholipid content and other methods used for measuring microbial biomass, such as acridine orange direct counts of microorganism and also with ATP content[28].

They are useful biomarkers or signatures for fingerprinting the soil microbial community because of relative abundance of certain PLFAs, which differ considerably among the specific group of microorganisms[11,29].

PLFA profile analysis holds competitive advantage than the rest of the conventional methods (culturable technique) to study the soil microbial community structure, as it accounts for larger proportion of the soil microbial community[11].

Potential of PLFA as bioindicator of stress

PLFAs have several features that reinforce their use as indicator of environment stress. First, they are key components of microbial membrane, which allows them to respond both to the intracellular and extracellular environment conditions. Secondly, response to environmental disturbances is either with respect to changed PLFA composition of microbial membrane (phenotypic plasticity) or due to altered PLFAs profiles resulting in shift in the soil microbial community structure. This set of PLFAs, which are characteristic of environment stress can be used as indicator of environmental monitoring and assessment[30–32].

Response of trans/cis ratio of monounsaturated fatty acids (16 : 1w7, 18 : 1w7) to various stresses

Most of the environment stresses show their detrimental effect by increasing the fluidity of the cell membrane of microorganisms. The increase in fluidity may result in the formation of non-bilayer phases resulting in nonspecific permeability[33], which may affect the transport systems

leading to increase or inhibition of their activity. Alteration of PLFA composition to counteract the membrane fluidity is one of the adaptation mechanisms induced in microorganisms to compensate for such effects/stresses[34]. Isomerization of *cis* unsaturated fatty acids (16 : 1*w*7c, 18 : 1*w*7c) to *trans* unsaturated fatty acids (16 : 1*w*7t, 18 : 1*w*7t)[35] is one such adaptation mechanism that is induced due to environmental stress caused by increased membrane fluidity[32] (Figure 4 *a*). The *trans* fatty acids are formed by direct isomerization of the complementary *cis* configuration of the double bond without a shift in position[33]. Because of the steric differences between the two conformations of unsaturated fatty acids, conversion of *cis* into *trans* unsaturated fatty acids reduces membrane fluidity and counteracts against the stress. The *cis*/*trans* isomerization system is generally not activated in the presence of any kind of stress that reduces the ability of microorganisms to grow, but is associated with the stresses which specifically result in increase in membrane fluidity. The shift in the *trans*/*cis* ratio of monounsaturated fatty acids (16 : 1*w*7, 18 : 1*w*7) in response to stress conditions such as high temperature[32,36], organic compound toxicity[32], starvation[30], osmotic stress[32], low pH[37] and heavy metal toxicity[15] has been well documented.

High temperature: The increase in *trans*/*cis* ratio of monounsaturated fatty acids from 0.1 to 0.4 was evidenced with increase in incubation temperature from 25 to 45°C in pure culture of *Pseudomonas putida*[32] (Table 2). Similarly, in soil incubation studies, increase in the *trans*/*cis* ratio of 16 : 1*w*7 from 0.12 to 0.21 in severely heated humus (160°C) was observed by Pietikainen *et al.*[36]. A clear correlation between the decreased concentration of *cis* unsaturated fatty acids (16 : 1*w*7c, 18 : 1*w*7c) with increased soil incubation temperature from 4.5 to 25°C (Petersen and Klug[34]) and 5 to 30°C (Petersen and Baath[38]) has been reported.

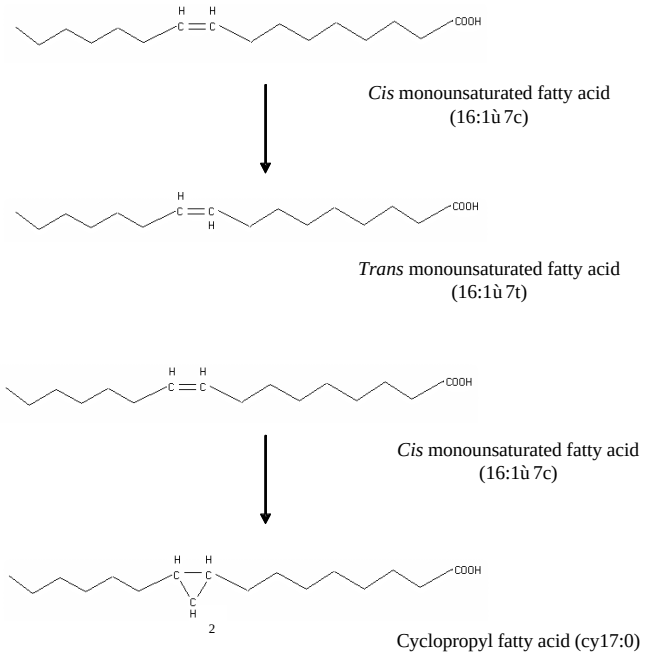

Figure 4. Structural changes in PLFA in response to stress conditions.

Organic compounds toxicity and osmotic stress: Besides temperature, organic compound toxicity (toluene) and osmotic stress (created by sodium chloride and sucrose) in *Pseudomonas putida* resulted in increase in *trans*/*cis* ratio of unsaturated fatty acids[32]. For organic compounds, the concentration which resulted in the highest *trans*/*cis* ratio (> 0.1) was slightly higher than the Minimum Inhibitory Concentration (MIC; Table 2).

Starvation: This has also been shown to activate the *cis*/*trans* isomerization system resulting in enhanced *trans*/*cis* ratio of monounsaturated fatty acids[30] from 0.02 to 1.56 after 30 days in *Vibrio cholerae* (Table 2). Increase in *trans*/*cis* ratio of monounsaturated fatty acids has also been shown during short-term (< 24 h) starvation of a marine bacterial isolate[39]. During nutrient deprivation, there is utilization of *cis* monounsaturated fatty acids (16 : 1*w*7c) and corresponding increase in *trans* monounsaturated fatty acids (16 : 1*w*7t), which are more stable and are not easily metabolized by the bacteria than the former. This results in overall decrease in membrane permeability, which helps maintain a functional living membrane[30].

Low pH: Studies regarding the effect of pH on microbial consortium showed similar changes in PLFA profiles under pure culture[32] and field studies[37,40–42]. Increased concentration of *trans*/*cis* ratio for unsaturated fatty acids from 0.1 to 0.4 has been reported under low pH range in pure culture studies[32] (Table 2). Decrease in *trans*/*cis* ratio of monounsaturated fatty acids (16 : 1*w*7) from 0.3 to 0.07 (calculated from reference) was reported as the pH reaches the neutral side[37] (Table 2). The decreased stress with increased pH (towards neutral) could be related to increased nutrient availability at this pH[39].

Heavy metal: In pure culture studies, the *trans*/*cis* ratio of unsaturated fatty acids exhibited a strong increase at toxic concentrations of metal[31,32], and highest ratio was observed at concentrations higher than the MICs (Table 2). Similarly, in soil incubation studies, increase in *trans*/*cis* ratio of 16 : 1*w*7 with different metal toxicites (Zn, Cd, Cu, Ni, Pb) has been documented[15]; 64 mmol of Zn resulted in the highest *trans*/*cis* ratio (0.4) compared to 0.12 in control. The mode of action of heavy metals is still not understood, but they seem to interact with the microbial membrane proteins resulting in disturbances in the protein activities and conformations[15]. Initiation of *cis*/*trans* isomerization system in response to the heavy metal facilitates microorganisms to counteract stress as the *trans* unsaturated fatty acids are more stable than the *cis* counterpart.

Table 2. Response of *trans*/*cis* ratio of unsaturated fatty acids to environmental stresses

Environmental condition	*trans*/*cis* ratio of unsaturated fatty acids (control condition)	*trans*/*cis* ratio of unsaturated fatty acids (stressed condition)	Reference
High temperature			
(45°C)	0.1	0.4	32
(165°C)	0.12	0.21	36
Organic compound toxicity	< 0.1	> 0.1	32
Starvation	0.02	1.56	30
Osmotic stress	0.07	0.12	32
Low pH	0.1	0.16	70
	0.07	0.3**	37
Heavy metal			
Pure culture study			
Zn	< 0.1 (control)	0.67 (6.0 mM*)	32
Cd		0.76 (10.0 mM)	
Cr		0.63 (10.0 mM)	
Cu		0.28 (8.0 mM)	
Field studies			
Zn	0.12 (control)	0.4 (64 mmol)**	15

*Concentration of heavy metal at which maximum *trans*/*cis* ratio resulted.

**Calculated from reference.

Table 3. Response of cy17 : 0 and cy19 : 0 to various environmental stresses

Environmental condition	cy 17 : 0 (%)	cy 19 : 0 (%)	Reference
Increase in temperature			
(4.5 to 25°C)	66↑*	150↑	34
(45 to 165°C)	5↑	28.7↑	36
Starvation	100↑	390↑	30
Low pH	NS	33.3↑	37
Heavy metal			
Zn (64 mmol of Zn kg (dw) of soil^{-1})**	66.6↑	NS	15
Tillage	70↑	86↑	49
Pesticide	20↑	39.1↑	47
	30↑	25↑	48

*↑Increase in PLFA with respect to control (calculated from references).

**Concentration of heavy metal at which maximum increase in cy 17 : 0 was observed.

NS, Non significant.

Moreover, it has been reported[43–46] that most of the cell cultures and environmental samples have *trans*/*cis* ratio of unsaturated fatty acids < 0.1. Thus a *trans*/*cis* ratio significantly greater than this can be used as an environment stress index[30].

Cyclopropyl (cy17 : 0 and cy19 : 0) fatty acids

The concomitant increase in cyclopropyl (cy17 : 0 and cy19 : 0) fatty acids along with increase in *trans*/*cis* ratio of unsaturated fatty acids was observed in almost all the aforementioned stress conditions such as increase in temperature[34], starvation[30], low pH[37] and heavy metal toxicity[15] (Table 3). Besides this, increase in production of cyclopropyl fatty acids has also been shown for pesticide application[47,48] and tillage[49]. Heat treatments from 45 to 160°C in the dry forest humus resulted in 5% increase in cy17 : 0 and 28.7% in cy19 : 0 (Table 3)[34]. Guckret *et al.*[30] reported enhanced production of cy17 : 0 (100%) and cy19 : 0 (390%) in response to starvation in *P. putida*. Tillage application has also been reported to increase the cy17 : 0 and cy19 : 0 productions by 70 and 86% respectively, compared to the control[49]. Cyclopropyl fatty acids (cy17 : 0, cy19 : 0) are formed by transmethylation of *cis* monounsaturated fatty acids (16 : 1*w*7c, 18 : 1*w*7c) as the cell enters the stationary phase (Figure 4 *b*). This modification of *cis* monounsaturated fatty acids to cyclo fatty acids (which are more stable and are not easily metabolized by the bacteria) helps in maintaining a functional living membrane by minimizing the membrane lipid losses or changes in membrane fluidity owing to cellular degradation during stress conditions[30]. The transformation of *cis* double bonds to cyclopropane ring restricts the overall mobility and resists ozonolysis and other mild oxidative treatments, as well as photochemical generation of singlet oxygen[50], which helps in reducing the impact of environment stress on membrane fluidity.

Fungal PLFA (18 : 2w6)

Fungi play an important role in ecosystem functioning and contribute significantly to soil quality by promoting soil aggregation[51] and release nutrients by decomposing a large proportion of plant residues. A lucid corelation between the increased intensity of physical disturbance and decrease in fungal biomarker has been reported in response to sieving[34,52], tillage[53], compaction[54], grazing[55] and fire treatment[40]. Physical

disturbances such as sieving and tillage lead to decrease in 18 : 2*w*6 by 14.5–42.8% and 47.6% respectively. Almost similar proportionate decrease in 18 : 2*w*6 by 29.5–43.3% was reported due to grazing (Table 4). This decrease in fungal biomarker could be attributed to the fact that filamentous fungi are more sensitive to physical disturbances than single-celled organisms[56,57], which could probably reinforce their use as indicators of environmental stress.

Although variations in the fungal biomarker (18 : 2*w*6) were significant with respect to chemical disturbances like heavy metal addition[15,58,59] and pesticide application[47,49,60] (Table 4), the response of this biomarker showed variation with respect to heavy metal toxicity. Frostegard *et al.*[15,16] found increase in fungal biomarker with different levels of metal contamination (except Cu) in laboratory experiments in arable soil. On the contrary, 9.5% decrease in fungal biomarker with respect to heavy metal pollution was shown in forest soil by Pennanen *et al.*[58] and Fritze *et al.*[59]. This decrease in abundance of 18 : 2*w*6 in the forest soil could be due to decline in ectomycorrhizal fungi because of damage to the fine roots of trees due to heavy metal pollution[58].

Fungal : bacterial biomass ratio

An index of fungal to bacterial ratio of the microbial biomass (calculated using PLFAs i15 : 0, a15 : 0, 15 : 0; 16 : 0, 16 : 1*w*9, 16 : 1*w*7t, i17 : 0, 17 : 0, cy17 : 0, 18 : 1*w*7 and cy19 : 0 for bacteria and 18 : 2*w*6 for fungi) has also been used to study the state of soil microbial community in response to different environmental stresses. The fungal : bacterial biomass ratio has been projected as a potential tool for discriminating the disturbed intensive agriculture system from the low input organic farming system[9]. It has been reported that the organically managed and unfertilized systems have increased fungal : bacterial ratio[61] than the conventional system, while under the disturbed ecosystems like grazing, burning and clear-cutting, decrease in fungal : bacterial ratio has been observed[55].

Gram-negative bacterial PLFA

Different stress conditions such as pesticide application, heavy metal toxicity and tillage reported increase in abundance of Gram-negative bacterial PLFA, with concomitant decrease in Gram-positive bacterial PLFA[15,29,48]. Greater survival of Gram-negative bacteria under stress conditions could be attributed to the presence of the cyclo fatty acids in their membrane[30] and the outer lipopolysacchride layer which can better counteract with the stress. It is usually thought that Gram-negative bacteria dominate in metal contaminated soils compared to Gram-positive bacteria. However, contradictory views are also reported[58], leading to variable response of bacterial community to heavy metal stress.

Table 4. Fungal biomarker (18 : 2*w*6) response to various environmental stresses

Environmental stress	Fungal biomarker (%)	Reference
Sieving	42.8*↓	34
	14.5↓	52
Tillage	47.6↓	49
Grazing	29.5–43.3↓	55
Heavy metal	20↓	15
	9.5↓	58
	6–18.3↓	59
Pesticide	20↓	48

*↓Increase, ↓ decrease (calculated from references).

Shannon–Weaver diversity index

The ability of an ecosystem to withstand extreme disturbance may depend in part on the diversity of the system. Diversity is a function of two main components: species richness or species abundance and species evenness or species equitability[62]. The evenness index that is mostly used is the Shannon–Weaver evenness[63]. Recently, this index has also been used on the bacterial and fungal PLFAs as a measure of relative distribution, or degree of dominance of microbial groups[57,61]. The more even the distribution of PLFA, greater is the diversity. It is calculated as $(\sum p_i \ln p_i)$, where p_i is the peak area of the ith peak over the area of all peaks. Bardgett *et al.*[61] reported a decrease in index values from 0.91 to 0.84 as the intensity of grazing increases. The evenness of PLFA can provide information regarding broad-scale changes in relative abundances, or dominance, of certain microbial groups. However, it cannot be used to measure species or genetic diversity.

Conclusion

The recent advances in molecular microbial ecology tools, which can describe functional and physiological diversity, are expanding our understanding of microbial community structure and ability to track changes in dynamic whole communities. As PLFAs – the quantitative measurable biomolecules in soil microorganisms are rapidly turned over and represent the current living community, both qualitatively and quantitatively, they seem to have high indicative value to assess and monitor the microbial community structure, physiological and stress state. The induction of certain changes in PLFA components, viz. ratio of *trans* to *cis*-monoenoic unsaturated fatty acids, proportion of cyclopropyl fatty acids and fungal biomarkers in response to various stresses (Figure 5), specific sensitivity to heavy metal contamination in soils and measurement of species richness, and evenness for biodiversity assessment makes them potential indicators for monitoring the soil environment and its assessment.

However, there are few limitations and pitfalls associated with PLFA biomarker analysis, which may limit its use at the

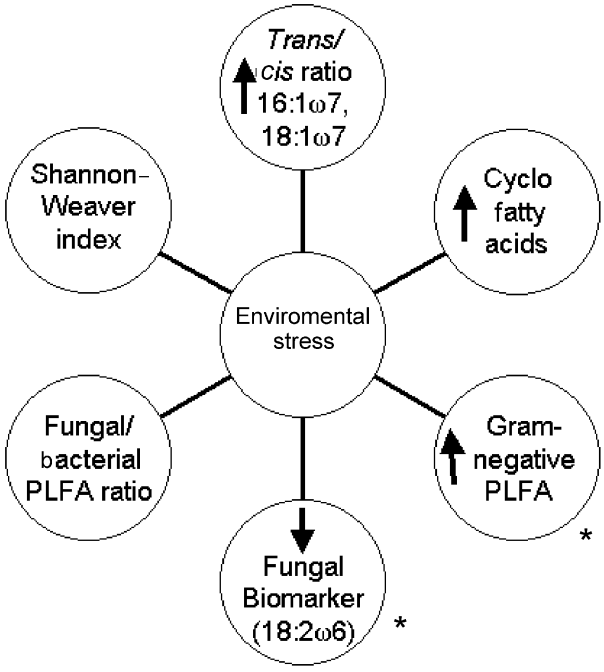

Figure 5. Response of PLFA biomarkers to environment stress. *Response is variable for fungal biomarker and Gram-negative PLFA.

regional and global scale. It does not reveal any information at the species-level, archae bacteria cannot be determined using this method and databases for interpretation of biomarkers are centred on fatty acids from microorganisms from pure cultures. Nevertheless, the full potential of PLFA as a bioindicator of environment monitoring and assessment at higher scales of resolution is certainly growing as databases and novel methods focusing on functions are being developed. Furthermore, calibration of changes in stress biomarkers under diverse ecosystems, soil type and climate, linking of PLFA profiles with functions of ecosystems, and automation of the technique need to be strengthened for implementation of this bioindicator in the regional assessment of environmental impact of agriculture and its incorporation in soil quality indices.

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