Original Research Article

PLFA Profiling of soil microbial community structure and diversity in different dry tropical ecosystems of Jharkhand

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A B S T R A C T

Keywords

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Phospholipid fatty acid analysis (PLFA) is used as culture-independent tool in microbial ecology studies. Classification of PLFAs into chemically distinct subgroups will assess insights into microbial community structure involved in microbial processes, and will have direct impacts on soil fertility. The PLFAs are synthesized during microbial growth, degrade rapidly during cell death, and hence provide an accurate census of living microbial community. PLFA profile of FMS dominated with monounsaturated fatty acids suggesting the abundance of gram-negative bacteria, anaerobes, actinomycetes and methanobacter. Higher poly-unsaturated fatty acids in FS suggesting abundance of arbuscular mycorrhizal fungi and fungal populations. Shannon diversity index based on the distribution of 92 PLFAs varies from 2.4735 (FMS) to 2.9401 (PTS). Besides, higher Pielou's evenness index in AS (0.8034) suggesting greater diversity as compared to FMS (0.6118). Difference in fungal-to- bacterial ratio across the sites was found to be highest in FS (0.067). Principal component analysis can able to discriminate seven soil profiles with 52.3% cumulative percentage of variance. Further, cluster analysis can able to group the microbial community structure into six independent clusters. Thus, the PLFA analysis provides an approach for microbial diversity assessment, and ultimately the potential impacts of environmental processes on soil microbial community structure.

Introduction

Soil microbial community composition and activity determine the ecosystem processes such as biogeochemical cycles, organic matter decomposition, nutrient turnover (Zechmeister-Boltenstern *et al.,* 2000; Hackl *et al.,* 2005), bioremediation, and fertility (Zelles, 1999). Soil

microbiological characterization (number, respiration rate and enzyme activities) at the process level provide total pool size and gross activity measures, but are not treated as sensitive indicators because of the redundancy of functions and complex interactions within the communities. Soil

microbial community composition specifies clear relationship between microbial diversity, soil and plant quality, and ecosystem sustainability, and respond much faster to disturbances, stress/perturbation (Atlas, 1984), and long-term effects on processes caused by successions in microbial community. Changes in soil microbial communities resulting from ecosystem management can have significant impacts on ecosystem dynamics, and might provide a measure of the effects of land-use practices on soil health. Therefore, the relationship between soil microbial community composition and ecosystem function has attracted considerable research interest.

Microbial community structure is defined as the number and relative abundance of soil microbial populations. Most of the soil microorganisms can not be characterized by cultivation techniques. Therefore, a culture-independent approach is used to determine soil microbial community composition by phospholipid fatty acids analysis (PLFA) of microbial membranes (Frostegard *et al.,* 1991; Tunlid and White, 1992; Zelles *et al.,* 1992), which offer the most powerful approach to determine microbial biomass (Bardgett and McAlister, 1999), shift in microbial community structure and activities. Phospholipid fatty acids (PLFAs) are potentially useful signature molecules in microorganisms due to their presence in all living cells found exclusively in cell membranes. The cell membrane is rapidly degraded, and component phospholipid fatty acids are rapidly metabolized following cell death, and not found in storage lipid/anthropogenic contaminants and have high turnover rate (Tunlid and White, 1992). The fatty acid analysis measures three attributes of microbial communities: viable microbial biomass,

microbial community structure and nutritional/physiological status (White *et al.,* 1997). Besides, PLFAs have several features that reinforce their use as indicator of environmental stress, which allows them to respond both intracellular and extracellular environment conditions, and hence can be used as indicator of environmental monitoring and assessment (Heipieper *et al.,* 1996).

Phospholipid consists of a single molecule of glycerol (3C alcohol), with two OH groups being replaced by two fatty acids by ester or ether linked (hydrophobic tail) and third OH group by a phosphate group (hydrophilic head). Microbial fatty acids are typically 12-24 carbon long. The most common membrane fatty acids are 14-20 carbon long (Morgan and Winstanley, 1997). PLFA can be classified into ester-linked phospholipid fatty acids (EL-PLFAs, 60-90%) and non-ester linked phospholipid fatty acids (NEL-PLFAs, 10-40%). The ether-linked phospholipid fatty acid is rare, but has been found in *Archaea*. EL-PLFAs are further subdivided into ester-linked unsubstituted fatty acids (EL-UNFAs) and hydroxyl 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 hydroxyl substituted (NEL-HYFA) fatty acids (Zelles, 1999).

The PLFA profiles provide a broad diversity measurement of microbial community at the phenotypic level. The different subsets of the microbial community have various PLFA patterns with varying chain length, saturation and

branching, which can be used as 'microbial community fingerprint' (Vestal and White, 1989; White *et al.,* 1996; Zelles, 1999; Steer and Harris, 2000). The fatty acid extracted from sediments can able to classify distinct microbial groups: micro-eukaryotes (polyunsaturated fatty acids), aerobic prokaryotes (monounsaturated fatty acids), gram-positive and anaerobic bacteria (saturated and branched fatty acids; C_{14} to C_{16}), branched-chain fatty acids (*iso* and *anteiso*) are characteristic for gram-positive bacteria. Gram-negative bacteria contain unique hydroxyl fatty acids in the lipid portion of lipopolysaccharides in cell wall. LPS-OH fatty acids were used as an indicator of gram-negative bacteria in environmental samples (White, 1994) and sulfate-reducing bacteria including other anaerobic bacteria (saturated and branched fatty acids; C_{16} to C_{19}) (Morgan and Winstanley, 1997). Linoleic acid (18:2 ω6) is a good indicator of the number of fungi and fungal biomass (Frostegard and Baath, 1996). Besides, fungal biomarker (16:1 ω5c) is used for arbuscular mycorrhizal fungi (Dickens *et al.,* 2013). Therefore, the presence and abundance of these signature fatty acids revealed the presence and abundance of specific microbial community structure.

PLFA analyses have been considered as a robust tool that consistently discriminates between communities of different origin and land management strategies (Bardgett and McAlister, 1999; Steer and Harris, 2000). PLFAs provide a set of molecular markers for different microbial taxa, and indicators of microbial stress that can be used to track changes in soil microbial community composition and total viable microbial biomass (White *et al.,* 1996). The native forest soil was supported by specific microbial community with defined environmental requirements.

Different soil profile would be associated with characteristics microbial community structure and function. Thus, the information on the microbial community composition in forest soil could then be used to facilitate interpretation of microbial community data derived from the degraded soils. Keeping the above facts into consideration, the aim of the study was to provide a comparative assessment of soil microbial community structure and diversity based on PLFA profiling in seven tropical ecosystems. Besides, the bacterial and fungal PLFAs within the microbial community were estimated in order to understand the relationship between the PLFAs and environmental characteristics.
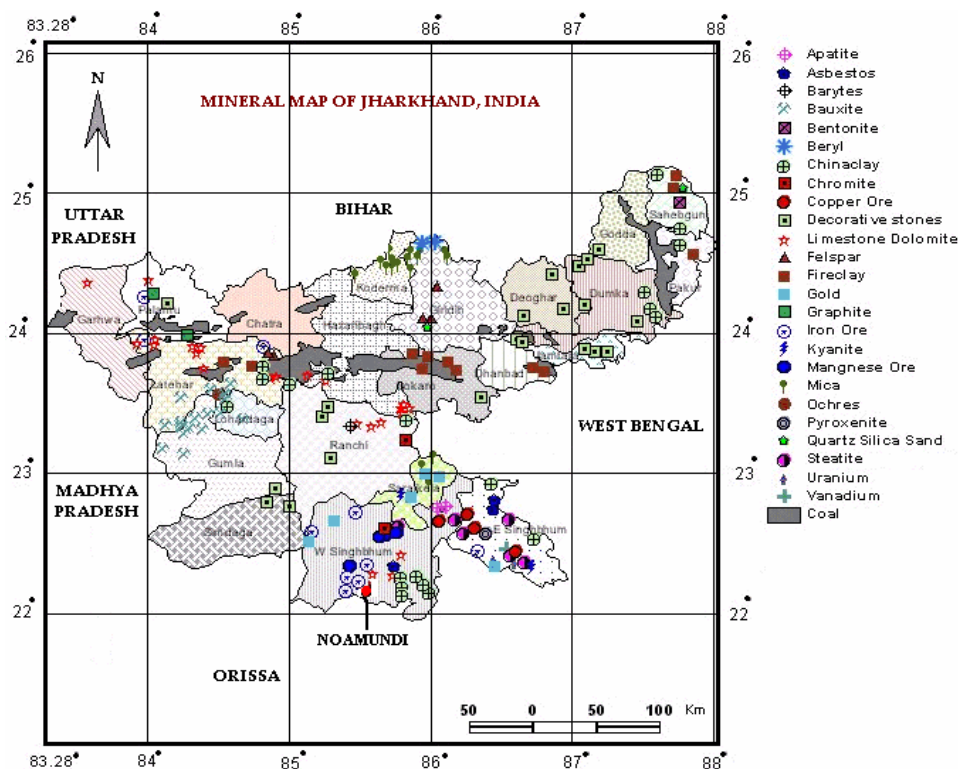
Materials and Methods

Study site

The study was carried out in nearby sponge mines area at Noamundi (85° 30' 33.61" east longitude and 22° 9' 49.96" north latitude), maintained by Tata Iron Steel Corporation limited, which is located in revenue district of West Singhbhum, Jharkhand, India (Figure 1). The study site is situated about 540m altitude from the mean sea level with mean annual temperature (19.67°C) and humidity (20%). The site is surrounded by a number of new, old and abandoned mines of iron ore overburden. Tropical dry deciduous forest is considered to be the natural vegetation of the area, but rapid development of industrialization led to decline of forest cover mainly due to the felling and biotic interferences.

Soil sampling

Sampling was done in accordance with general microbiological protocol from seven different sites [fresh mine spoil

Figure.1 Geographical location and the mineral map of study site, Jharkhand, India, Soil sampling

(FMS); 6yr old mine spoil (MS); degraded waste land soil (DWS); grassland soil (GS); pesticide-treated soil (PTS); agricultural soil (AS) and forest soil (FS)] near Noamundi within 10 km peripheral distance from the mining area. Each site was divided into 3 blocks, and five soil samples were collected randomly from 0-15 cm soil depth by digging pits of (15 x 15 x 15) cm^3 size from each block. The samples collected from each block were referred to as 'sub-samples', which were thoroughly mixed to form one 'composite sample'. During each sampling, three composite samples were obtained from each site, and were subjected to sieving (2mm mesh) for further characterization.

Phospholipid fatty acid (PLFA) analysis

Lipids were extracted from soil, fractionated and quantified using the procedure described by Bardgett *et al.* (1996), which is based on that of Bligh and Dyer (1959) as modified by White *et al.* (1979). Soil sample (2-25)g dry weight was shaken in a mixture of buffer solution, methanol and chloroform (2.5:5.0:2.5 v/v/v) for about 2h. Equal volumes of distilled water and chloroform were added, and subjected to incubation for 24hr for separation of two phases. The chloroform phase was reduced by evaporation, and stored at -20^{0}C. After extraction, the lipids were separated into neutral, glyco- and phospholipid on a silicic acid column by eluting with chloroform, acetone and methanol respectively. Following a mild, alkaline methanolysis of phospholipids, the resulting ester-linked fatty acid methyl esters, dissolved in isooctane or hexane, were separated and quantified by GC-MS. This method is simple, rapid and has been used for soil microbial community analysis including non-

culturable microorganism. Fatty acids designated in terms of total number of carbon atoms with the number of double bonds given after a colon. The position of the double bond is defined by a symbol ω followed by the number of carbons from the methyl end of the fatty acid molecule. *Cis* and *trans* configuration are indicated by c and t; i and a refer to *iso* and *anteiso* branching; br indicates an unknown branch position; cy refers to cyclopropyl fatty acids. Hydroxy groups are indicated by 'OH'. 10Me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule (Baath and Anderson, 2003; Steenwerth *et al.,* 2003). The total amount of PLFAs was used to indicate the total microbial biomass and the sum of PLFAs (14:0, 15:0, 16:0, 17:0, 18:0, 18:1ω9c, 20:0, 21:0 22:0, 24:0) was considered to be predominantly of bacterial origin (Vestal and White, 1989; Tunlid and White, 1992). The PLFAs (16:1ω7c, 17:0cy, 18:1ω7c) are the representatives of heterogeneous groups of soil microorganisms most prevalent in gram-negative bacteria (Lores *et al.,* 2010; Dicken *et al.,* 2013), the anteisobranched PLFAs (a15:0, i15:0, 16:0, a16:0, i16:0, a17:0, i17:0) typically represents gram-positive bacteria (Lores *et al.,* 2010; Dicken *et al.,* 2013), PLFAs (18:1ω9c, 18:2ω6c, 18:3ω6c) for common fungi (Myers *et al.,* 2001; Lores *et al.,* 2010; Dickens *et al.,* 2013), the unsaturated 16:1ω5c is typical for arbuscular mycorrhizal fungi (Dickens *et al.,* 2013), methylbranched PLFAs (10Me16:0, 10Me17:0, 10Me17:1ω7c, 10Me18:0, 10Me20:0) representing actinomycetes (Kroppenstedt, 1985, 1992; Zelles, 1999; Hill *et al.,* 2000), PLFAs (14:1ω7cDMA, i15:0DMA, 16:1ω7c DMA, 18:0DMA, 18:2DMA, 19:0cy9,10DMA) for anaerobes (Frostegard *et al.,* 1991; Zelles, 1997; Zelles, 1999); PLFA 16:1 ω8c for

methanobacter (Hill *et al.,* 2000), and PLFA 20:1, 21:0 for eukaryotes (Zelles, 1999).

Statistical analysis

The PLFA profiles were analyzed using a set of 92 fatty acids present in seven different soil profiles using Sherlock PLFA tool (Version 1.1). The Shannon's diversity index or Shannon-Weaver index (*H*) was calculated: ($-\Sigma p_i \ln p_i$), where p_i is the peak area of the *i*th peak over the area of all peaks. The Pielou's evenness index (*J*) was calculated as: (*H*/*Hmax*); where *H* is the number derived from the Shannon diversity index, and *Hmax* is the maximum value of *H* (*Hmax* = ln*R*; *R* = PLFA richness). PLFA data was analyzed with principal component analysis using SPSS 18.0 software. The distance matrix revealed the relatedness based on the relative distribution of 92 PLFAs across the sites through cluster analysis.

Results and Discussion

Community level microbial interactions are complex. The qualitative and quantitative changes in microbial community structure not only determine the microbial diversity, but also the function and nature of interactions among the existing species, the physiological state of ecosystem. Besides, the existence of different functional groups responds differently to prevailing environmental conditions in different ecosystems, which influence the microbial community composition. There are useful biomarkers or signatures for fingerprinting the existence of soil microbial community because of the relative abundance of certain PLFAs, which differ considerably among the specific groups of soil microorganisms (Zelles *et al.,* 1994; Hill

et al., 2000; Kaur *et al.,* 2005; Lores *et al.,* 2010; Dickens *et al.,* 2013).

PLFA profiles of soil samples

The relative contribution of individual PLFAs representing microbial community structure showed marked differences among seven different soil profiles (Table 1).

The FS was set apart from other soil profiles due to the higher relative abundance of three particular fungal PLFAs [18:1ω9c (oleic acid), 18:2ω6c (linoleic acid), 18:3ω6c (gamma-linoleic acid)] (Olsson, 1999; Hill *et al.,* 2000; Myers *et al.,* 2001; Lores *et al.,* 2010; Dickens *et al.,* 2013); and arbuscular mycorrhizal fungi PLFA 16:1ω5c (*cis*-11-palmitoleic acid) (Dickens *et al.,* 2013). The fungal fatty acids 18:1ω9c, 18:2ω6c, 18:3ω6c accounted for 4.86%, 2.09% and 0.88% respectively. Highest relative abundance of PLFA 16:1ω5c was exhibited by FS (6.1%) representing arbuscular mycorrhizal fungi. PLFA 18:1ω9c (oleic acid) is reported to be most common in fungal species (Zelles, 1999). High prevalence of fungal PLFA in FS across the sites may be attributed to the availability of high amounts of recalcitrant polymeric phenolic compounds (lignin and tannin), their ability and principally responsible for lignin degradation (Cairney and Meharg, 2002). Part of the fungal PLFA 16:1ω5c may be derived from arbuscular mycorrhizal fungi (Olsson, 1999), which are known to contribute substantially to the fungal biomass in FS. Further, PLFA 16:1ω5c may be indicative of soil microorganism, which responds to changes in easily available C (Frostegard *et al.,* 1996; Hackl *et al.,* 2005). Methyl branched PLFA 10Me17:1ω7c representing actinomycetes

populations, which accounted for 0.13% (FMS), 0.14% (GS) and 0.09% (PTS) respectively (Table 1). Besides, FMS also exhibited 0.35% of actinomycetes PLFA 10Me20:0. The methanobacter PLFA 16:1 ω8c (Hill *et al.,* 2000) accounted for 0.62% (FMS) and 0.52% (DWS) respectively (Table 1). Further, the distribution of gram-positive, gram-negative bacteria and anaerobes varies in accordance with the physiological and nutritional status of different soil profiles, environmental factors (temperature, pH, different hydrological regimes, activity, ions and chemicals), which can shift the lipid composition (Sajbidor, 1997; Denich *et al.,* 2003).

Distribution of PLFAs

The PLFAs can be classified into distinct microbial groups (Table 2) such as aerobic prokaryotes (monounsaturated fatty acids: MUFA), microeukaryotes (polyunsaturated fatty acids: PUFA), gram-positive and other anaerobic bacteria (saturated and branched fatty acids ranges from C14 to C16), anaerobic bacteria (saturated and branched fatty acids ranges from C16 to C19) (Morgan and Winstanley, 1997). The PUFAs are considered to be the signature acids for eukaryotes, which varies from 2.97% (FMS) to 7.49% (PTS). The MUFAs representing aerobic prokaryotes ranged from 11.66% (MS) to 26.94% (FMS). Although, the MUFAs can occur both in gram-negative and gram-positive bacteria, but their relative contribution to the total PLFA content in gram-positive bacteria is very small (*e.g.* < 20%). Thus, MUFAs can be used as general biomarkers for gram-negative bacteria (Ratledge and Wilkinson, 1988). Highest straight chain PLFAs was exhibited by MS (51.58%) as compared to other soils. Higher level of

unsaturated fatty acids with low levels of PUFAs supported the bacterial dominance. Branched-chain fatty acids have been used as biomarker for bacteria including anaerobic and sulfate-reducing bacteria. Branched-chain fatty acids (*iso* and *anteiso*) are characteristics of gram-positive bacteria, whereas *cyclopropyl* fatty acids are common in some gram-negative and anaerobic gram-positive bacteria (Ratledge and Wilkinson, 1988). Branched chain PLFAs varies from 8.2% (FMS) to 19.26% (AS). The differences in the relative distribution of branched and MUFAs have been used as a marker for the proportion of gram-positive and gram-negative bacteria (Morgan and Winstanley, 1997). The gram-negative bacteria contain unique hydroxyl fatty acids in lipid portion of lipopolysaccharides in cell wall, which is used as an indicator of gram-negative bacteria in environmental samples (Parker *et al.,* 1982; White, 1994). It is evident from the study that the soil microbial groups with hydroxyl fatty acids were confined to FMS (0.09%) and GS (0.95%). The methyl branching PLFAs was observed in FMS (0.56%), GS (0.15%) and PTS (0.09%) respectively (Table 2). The study indicated that the differences in PLFA profiles could be attributed to the variation in lipid contributing microbial communities and environmental conditions across the sites (Rajendran *et al.,* 1995).

Microbial community composition

PLFAs have several features that reinforce their use as indicator of environmental stress. They respond to environmental disturbances either by altering PLFA composition in microbial membrane (phenotypic plasticity) or shifting in soil microbial community structure (Kaur *et*

al., 2005). Marked differences in distribution of different microbial groups were observed across different soil profiles (Table 3).

FMS represents a disequllibriated geomorphic system, due to the altered physical and chemical structure and the resultant biotic deficiency, which disrupt the 'geology-soil-plant' stability and the pedogenic processes. Higher levels of MUFA (Parkes and Taylor, 1983; Rajendran *et al.,* 1995) with lower level of PUFA were reported as the biomarker for gram-negative bacteria (Ratledge and Wilkinson, 1988) that explained the abundant distribution of gram-negative bacteria in FMS (70.78%). Besides, the presence of hydroxy PLFAs in FMS revealed the occurrence of gram-negative bacteria (Parker *et al.,* 1982; White, 1994). Lower level of gram-positive bacteria (13.84%) and anaerobes (8.09%) were also estimated in FMS (Table 3), which may be due to the lower occurrence of branched chain fatty acids (Parkes and Taylor, 1983; Taylor and Parkes, 1983), and abundantly distributed in anaerobic bacteria and gram-positive bacteria (Guckert *et al.,* 1985). The study revealed higher relative dominance of gram-negative bacterial PLFAs in metal contaminated soil (FMS and MS) with concomitant decrease in gram-positive bacterial PLFAs (Frostegard *et al.,* 1993; Zelles, 1994; Liao *et al.,* 2005). Higher level of DMA PLFAs revealed the higher distribution of anaerobes (Frostegard *et al.,* 1991; Zelles, 1997; Zelles, 1999) in FMS. The methyl-branched PLFAs showed dominance of actinomycetes (Kroppenstedt, 1985, 1992; Zelles, 1999; Hill *et al.,* 2000) in FMS (0.94%), which may be due to their ability to withstand water stress (low water potential) by resisting plasmolysis and maintaining cell turgor by accumulating

compatible solutes (proline and glycerol). In addition, they are filamentous, enabling them to bridge air gaps between thin water films that occur in soil pore spaces during soil desiccation (Moore-Kucera and Dick, 2008). Lower fungal PLFAs (18:1ω9c, 18:2ω6,9c) suggested minimal fungal abundance in FMS (0.38%). Highest occurrence of PLFA 16:1ω8c reflects the distribution of methanobacter (Hill *et al.,* 2000) in FMS (0.99%). Lower longer chain fatty acids and PUFA indicated minimal input from microeukaryotes (Smith *et al.,* 1986) in FMS (5.02%). Further, the study indicated that the mode of action of heavy metals seems to interact with the microbial membrane proteins resulting in disturbances in the protein activities and conformations (Frostegard *et al.,* 1993; Liao *et al.,* 2005).

The ability of soil to maintain microbial community composition, nutrient concentration and functioning after a disturbance defines the resistance capacity of a system. Resilience refers to the response of the system impacted by a disturbance, and can be defined as the rate of recovery in the original versus restored state of system. In addition to the abiotic factors, soil microbial community composition is considered as one of the major components of soil resilience due to their key role in nutrient cycling. Therefore, the microbial community composition in MS should be compared with FMS. The lower level of distribution of gram-negative bacteria in MS (53.60%) was observed as compared to FMS (Table 3), which may be due to lower occurrence of MUFA and absence of hydroxy fatty acids in MS. Besides, higher level of gram-positive bacteria in MS (30.53%) may be due to the higher occurrence of branched chain fatty acids. Because of the minimal occurrence of DMA PLFAs in

MS, lower level of anaerobes (1.69%) was observed. Higher level of fungal PLFAs (18:1ω9c) revealed higher fungal dominance in MS (2.12%) as compared to FMS due to the gradual establishment of vegetation, plant inputs of litter and exudates (Potthoff *et al.,* 2006; Yu and Ehnerfeld, 2010). The distribution of actinomycetes and methanobacter was not observed in MS due to the absence of 10-methyl branched fatty acids and PLFA 16:1ω8c. Further, higher occurrence of long chain fatty acids and PUFA supported the higher level of distribution of microeukaryotes in MS (12.06%) as compared to FMS. Thus, the recovery of resource heterogeneity and pool sizes following restoration would indicate resilience of the system and variation in soil microbial community composition.

PLFA profiling was able to differentiate between ungrazed DWS and grazed GS based on the relative abundance of PLFAs (14:0, i15:0, i17:0, 18:0 and 18:1ω7c) in GS (Bardgett *et al.,* 1997). The grasses differ from native shrub species in phenology, rooting structure and depth (Wolkovich *et al.,* 2009), and tissue nutrient composition, which cause significant changes in soil microbial community composition. PLFAs of DWS and GS with variation in their vegetation pattern exhibited a clear distinction (Ibekwe and Kennedy, 1988). In addition, the reduction of available moisture at deeper soil profile in DWS (Wolkovich *et al.,* 2009), homogenous distribution of resources in GS (Partel and Helm, 2007) was reported to alter microbial community (Dickens *et al.,* 2013). The distribution of gram-negative bacteria in DWS and GS was found to be 63.35% and 68.87% respectively (Table 3). Similarly, the occurrence of gram-positive bacteria was estimated to be 18.98% (DWS) and 17.40% (GS). Besides, higher level of

distribution of anaerobes was observed in DWS (6.0%) as compared to GS (4.54%), which may be due to the higher prevalence of DMA PLFAs in DWS (21.82%) as compared to GS (20.92%) (Table 2). Comparative analysis of PLFAs suggested the microbial distribution of methanobacter in DWS (0.78%), where as the distribution of actinomycetes is confined to GS (0.23%), which may be attributed to the variation in distribution of PLFA 16:1ω8c in DWS, and 10-methyl branched fatty acids in GS. However, higher level of fungal and microeukaryotes were exhibited by DWS as compared to GS. The increased intensity of physical disturbance and decrease in fungal biomarker (18:2ω6c) in GS as compared to DWS may be due to grazing (Bardgett *et al.,* 1997).

PLFA profiles suggested higher level of gram-negative bacteria in PTS (55.92%) than AS (47.64%), which may be due to the higher level of MUFA in PTS (Parkes and Taylor, 1983; Ratledge and Wilkinson, 1988; Rajendran *et al.,* 1995). Pesticide application leading to increased abundance of PLFAs as biomarker for gram-negative bacteria in PTS as compared to gram-positive bacteria was reported (Zelles, 1994). However, higher distribution of gram-positive bacteria was observed in AS (37.35%) as compared to PTS (23.74%) due to the higher occurrence of branched-chain PLFAs in AS (19.26%) (Zelles, 1999). Several investigations have suggested that the level of the PLFAs (15:0, i15:0, a15:0, 16:1ω7c, i17:0, a17:0 and 18:1ω9c) decreased in PTS relative to their level in unpolluted AS (Baath *et al.,* 1992; Zelles, 1994). In addition, higher level of anaerobes was found in PTS (6.29%) due to higher occurrence of PLFAs (14:1 ω7cDMA, 16:1ω7cDMA, 18:0DMA and

19:0cy9,10DMA) in PTS as compared to AS (Table 1). Further, PTS exhibited a lower level of actinomycetes (0.16%). Higher relative abundance arbuscular mycorrhizal fungi in AS (1.88%) than PTS (0.84%) may be due to the higher occurrence of PLFA 16:1ω5c in AS (Olsson, 1999; Dickens *et al.,* 2013). Higher fungal dominance was exhibited in AS (2.55%) as compared to PTS (0.66%), which may be attributed to the fungicide induced effect on soil microbial community composition (Zelles, 1999; Yang *et al.,* 2011; Kalia and Gosal, 2013; Mall *et al.,* 2013). Besides this, increase in production of cyclopropyl fatty acids (19:0cy 9,10DMA) was exhibited by PTS as compared to AS, which may be due to pesticide application (Macalady *et al.,* 1988; Widenfalk *et al.,* 2008). Further, higher level of distribution of microeukaryotes was observed in PTS (12.4%) than AS (10.5%).

The level of distribution of gram-positive, gram-negative bacteria and anaerobes in FS was found to be 27.34%, 41.34% and 1.46% respectively (Table 3). Higher relative abundance of gram-negative as compared to gram-positive bacteria in FS indicating the profound effects of plants have on soil development and lipid profiles. Highest abundance of arbuscular mycorrhizal fungi (12.11%), fungi (4.51%) and microeukaryotes (13.35%) were observed in FS, which may be due to the greater litter inputs and root turnover, and symbiotic nitrogen fixation contributed to the formation of highly localized soil resources characterized by higher concentrations of C and N that are believed to support more diverse population of heterotrophic soil microorganisms. Further, fungi are uniquely adapted to degrade substrate (lignin), and formation of organic matter

(Cairney and Meharg, 2002). Comparative analysis of the level of distribution of PLFAs across the sites suggested that the heavy metal contamination in FMS and MS (Frostegard *et al.,* 1993; Pennanen *et al.,* 1996; Zelles, 1999; Liao *et al.,* 2005) and lower pH (Bath and Anderson, 2003) resulted a decrease in PLFAs (i15:0, i17:0, 16:1 ω5c, 16:2ω6c) in FMS as compared to undisturbed FS. The decreased stress with gradual increase in pH towards neutral in FS (Kujur and Patel, 2012) could be related to increase nutrient availability leading the shifting in microbial community structure across the sites. The combined effects of changes in both aboveground and belowground inputs would influence soil microbial community by affecting the C inputs from root exudates and litter (Myers *et al.,* 2001).

Shannon-Weaver diversity index

The ability of an ecosystem to withstand extreme disturbances may contribute to microbial community structure and hence microbial diversity. Diversity index is a quantitative measure, which not only accounts for the existence of different PLFAs richness (*R*), but also accounts how evenly they are distributed (evenness). The Shannon-Weaver index (*H*) has been a popular diversity index frequently used in microbial ecology studies. The bacterial and fungal PLFAs are used as a measure of the relative distribution of different microbial groups (fingerprints of soil microbial community) because of the relative abundance of certain PLFAs (Bardgett *et al.,* 1997), which differ considerably among different microbial groups (Zelles, 1994). The study revealed a significant variation in PLFA richness, Shannon diversity index, and evenness across the sites (Table 4). Greater PLFA richness (*R*) was attributed by FMS (57) as

compared to other soil profiles. The Shannon diversity index (*H*) across the sites varies from 2.4735 (FMS) to 2.9401 (PTS).

Besides, the evenness is defined as a measure of diversity index, which quantifies how equal the community is numerically. The evenness of a community represented by Pielou's evenness index (*J*) is constrained between 0 and 1. The evenness of PLFA reflects the broad-scale changes in terms of the relative dominance of certain microbial groups (Kaur *et al.,* 2005). The Pielou's evenness index (*J*) based on the distribution of 92 PLFAs exhibited an increasing trend from FMS (0.6118), and found to be maximum in AS (0.8034) (Table 4). The different fertilizer practices in AS have an impact on microbial community structure suggesting greater diversity. The more even the distribution of PLFAs or less variation in community between microbial groups, greater is the diversity. Thus, the value of diversity index increases when both the number of types of PLFAs and evenness increases.

Further, the Shannon diversity index based on the distribution of different microbial groups across the sites was calculated. Higher Shannon-Weaver index in FS (1.4451) suggesting higher population diversity as compared to other soil profiles. In addition, higher level of microbial diversity was exhibited by MS (1.1022) as compared to FMS (0.4719). Further, GS (0.9610) exhibited lower microbial diversity than DWS (1.0963), which indicated that the microbial communities in less disturbed ecosystems like GS may be dynamic in terms of functional responses to a perturbation but more resistance to changes in community composition (Steenwerth *et al.,* 2003).

Similarly, greater diversity in PTS (1.1828) as compared to AS (1.1261) may be attributed to the pesticides induced effect on soil microbial community. Besides, the differences in microbial community structure and associated diversity across may be attributed to the variation in microbial biomass nutrient to soil nutrients ratio (MB-C:OC), which represents the quantum of soil nutrients reflected in the microbial biomass (Anderson and Domsch, 1980; Kujur and Patel, 2012), and functional index of the soil subsystem (Insam and Domsch, 1988). Besides, the decline in soil pH from FS to FMS (Kujur and Patel, 2012) may be one of the major constraints/stress shifting microbial community structure under low soil pH (Anderson and Domsch, 1993; Hackl *et al.,* 2005). Furthermore, the gradual increase in moisture content from FMS to FS (Kujur and Patel, 2012) may also affect soil microbial community through its effect on osmotic potential, transport of nutrients and energy, and cellular metabolism as well as on the competitive interactions between microbial species (Williams and Rice, 2007; Meimei *et al.,* 2008).

Fungal:bacterial biomass ratio

An index of fungal to bacterial (F/B) ratio of the microbial biomass (PLFAs i15:0, a15:0, 15:0, 16:0, 16:1 ω9, 16:1 ω7, i17:0, 17:0, cy17:0, 18:1 ω7 and cy19:0 for bacteria, and 18:2ω6 for fungi) was used to study the state of soil microbial community in response to different environmental stresses (Kaur *et al.,* 2005). The F:B ratio was reported to be a potential tool to discriminate the disturbed from undisturbed soil system (Bradgett and McAlister, 1999; Bailey *et al.,* 2002; Moore-Kucera and Dick, 2008). Highest F:B ratio was observed in FS (0.067),

which may be due to higher prevalence of fungal PLFAs exhibiting higher C:N ratio and low bulk density (Kujur and Patel, 2012). The capacity of fungi for translocation N to C sources is thought to be important in FS with high C:N ratio (Clarholm, 1994). Besides, FS appeared to be set apart from other soil profiles by a higher abundance of arbuscular mycorrhizal fungi, which may be better able to cope with available N and organic matter. Comparatively lower F:B ratio was estimated in PTS (0.016) due to fungicide induced decline in fungal populations as compared to AS (0.039). Higher F:B ratio in DWS (0.060) can be explained on the basis of the existence of higher fungal PLFAs associated with low water content (Hakcl *et al.,* 2005) as compared to GS (0.026). In addition, AS and GS support distinct microbial communities that are correlated with factors that defines the land-use history and the associated soil quality influence microbial community composition (Calderon *et al.,* 2000; Steenwerth *et al.,* 2003). However, the difference in F:B ratio in disturbed ecosystems was less pronounced *i.e.* FMS (0.008) and MS (0.028) due to more extreme environmental conditions. The study indicated that the disturbed ecosystems have lower F:B ratio (Bradgett *et al.,* 2001), whereas the organically managed soil systems have increased F:B ratio than conventional system (Bradgett *et al.,* 1997).

Cluster analysis

Relative distributions of 92 PLFAs among seven soil profiles were subjected to cluster analysis based on the distance matrix revealed the existence of six clusters (I – VI) in the dendrogram (Figure 2). The analysis revealed highest similarity level (71.0774) between FS and AS (cluster-VI).

Table.1 Percentage composition of 92 PLFAs in seven different soil profiles

PLFAs	Different soil types						
	FMS	MS	DWS	GS	PTS	AS	FS
10	0.380	-	-	-	-	-	-
10:0 2OH	0.13	-	-	1.29	-	-	-
a11:0	-	1.45	-	-	-	-	0.70
12:0	-	13.06	16.43	9.74	12.73	16.34	13.49
a13:0	2.30	4.36	3.67	3.12	4.66	4.49	3.36
13:1 ω4c	1.15	0.82	1.12	0.82	0.86	1.78	1.27
13:0	-	-	-	0.19	0.10	-	-
i14:0	-	-	-	0.35	0.19	-	-
a14:0	0.04	-	-	0.19	0.14	-	-
14:1 ω5c	-	0.57	-	-	-	-	0.42
14: 1 ω8c	-	-	-	-	0.40	-	-
14:1 ω9c	0.04	-	-	-	-	-	-
14:0	2.05	5.03	1.75	2.77	1.64	2.26	1.67
14:1 ω7c DMA	4.67	-	3.10	2.79	2.93	-	-
i15:1 ω6c	0.15	-	0.43	0.39	0.40	0.71	0.52
15:4 ω3c	0.07	-	-	-	0.09	-	-
a15:1 ω9c	0.07	-	-	-	-	-	-
i15:0	0.13	0.53	-	0.45	0.35	0.81	1.02
15:3 ω3c	0.29	0.97	0.90	0.50	0.90	1.16	0.84
a15:0	1.71	4.70	4.21	2.93	4.00	5.53	4.26
15:1 ω6c	-	-	-	0.31	-	-	-
15:1 ω5c	-	-	-	-	-	0.23	-
15:1 ω7c	0.22	-	-	-	-	-	-
16:0 aldehyde	_	-	0.66	-	0.27	-	-
16:1 ω9c aldehyde	0.18	-	-	0.79	0.22	-	-
15:0	0.40	1.16	-	-	0.74	0.83	0.97
i15:0 DMA	0.08	0.29	-	0.15	0.16	-	0.30
16:0 N alcohol	0.75	1.76	1.70	1.00	1.85	2.95	1.41
a16:0	-	-	0.25	0.17	0.14	-	-
i16:0	0.05	0.34	0.11	0.29	0.23	0.46	0.54
16:1 ω7c	-	1.58	-	0.58	0.74	2.33	1.25
16:1 ω5c	-	-	-	-	0.51	0.94	6.10
16: 1 ω8c	0.62	-	0.52	-	-	-	-
16:1 ω4c	0.08	-	-	-	-	-	-
16:0	24.68	23.23	10.82	16.02	15.75	19.36	22.86
16:1 ω7c DMA	23.10	4.75	18.36	16.61	18.37	7.89	7.67
i17:1 ω9c	2.19	0.51	1.18	1.01	0.89	1.09	0.75
a17:1 ω9c	-	-	0.33	-	-	-	-
i17:0	-	0.47	-	0.33	0.75	0.96	0.40
a17:0	1.84	3.19	3.17	1.92	2.79	3.83	2.56
17:1 ω9c	-	-	-	-	-	0.14	-
17:1 ω5c	0.10	-	-	-	-		-
17:1 ω4c	-	-	-	-	-	0.62	-
17:1 ω8c	-	0.33	-	-	0.14	0.42	-
17:1 ω3c	-	-	-	0.08	-	-	-
17:0	0.22	0.58	0.30	0.29	0.32	0.78	0.49
10Me 17:1 ω7c	0.13	-	-	0.14	0.09	-	-
16:0 2OH	-	-	-	0.14	0.14	-	-
18:3 ω6c	0.58	0.64	1.43	1.11	1.04	1.73	0.88

i18:0	0.08	-	-	-	-	0.30	-
18:2 ω6c	0.22	0.92	0.92	0.54	0.36	1.16	2.09
18:1 ω9c	2.37	12.72	2.77	2.49	1.44	3.93	4.86
18:1 ω7c	0.14	1.60	-	0.47	0.44	0.85	1.00
18:1 ω5c	0.32	0.52	0.43	2.56	1.28	1.40	1.62
18:0	12.16	9.33	4.71	7.02	6.90	7.64	6.93
18:0 DMA	-	0.44	0.57	-	0.47	-	-
18:2 DMA	0.34	-	0.17	-	-	-	-
19:3 ω3c	0.13	0.78	0.69	0.51	0.71	0.80	0.60
19:1 ω6c	0.04	-	-	-	-	-	-
19:3 ω6c	-	-	0.47	-	-	-	-
19:4 ω6c	-	-	0.16	0.21	0.24	-	-
19:0 cy ω7c	-	-	-	0.21	-	-	0.52
19:0 cy 9,10 DMA	11.28	-	14.71	15.11	6.72	1.95	0.33
20:1 ω8c	-	-	0.36	-	0.32	-	-
20:4 ω6c	-	-	-	-	-	-	0.91
20:5 ω3c	-	-	-	-	-	-	0.35
20:3 ω6c	0.33	0.53	-	0.76	-	-	-
20:1 ω9c	0.39	-	-	-	-	0.75	0.36
20:1 ω6c	0.10	-	-	-	-	-	-
20:0	0.44	0.19	0.39	-	0.66	1.01	0.86
10 Me 20:0	0.35	-	-	-	-	-	-
21:3 ω3c	-	-	0.41	0.29	0.53	0.45	0.45
21:0	-	-	-	-	0.22	-	-
21:1 ω4c	-	-	0.12	-	-	-	-
21:1 ω6c	0.03	-	-	-	-	-	-
22:4 ω6c	0.04	-	-	-	0.08	-	-
i22:0	0.22	-	0.21	0.68	0.43	0.84	0.39
22:2 ω6c	0.16	-	-	-	-	-	-
22:1 ω3c	0.37	-	-	0.14	-	-	0.35
22:0	0.19	-	0.21	0.34	0.60	0.71	1.77
22:1 ω9c	-	0.28	0.17	0.32	0.25	-	-
22:1 ω6c	-	0.45	-	-	-	-	-
23:3 ω6c	0.36	0.37	-	-	-	-	-
23:3 ω3c	0.22	-	-	-	0.89	-	0.17
23:4 ω6c	-	1.57	0.63	0.25	1.14	-	-
23:1 ω5c	-	-	0.30	-	-	-	-
23:1 ω4c	-	-	0.28	-	-	-	-
23:0	0.28	-	0.34	0.16	-	-	-
24:3 ω3c	0.41	-	0.20	0.89	0.63	0.60	-
24:1 ω9c	0.19	-	-	-	-	-	-
24:1 ω3c	-	-	-	-	-	-	0.59
24:0	0.47	-	0.36	0.56	1.16	-	1.05

Table.2 Distribution of different groups of PLFA (%) in seven different soil types

Soil sample	Straight	Branched	Hydroxy	MUFA	PUFA	DMA	18:1 w9c	18:2 w6,9c	10-methyl
FMS	40.74	8.20	0.09	26.94	2.97	17.79	2.48	0.22	0.56
MS	51.58	14.78	nd	11.66	5.84	0.82	14.29	1.03	nd
DWS	32.71	13.05	nd	22.09	6.29	21.82	3.05	1.00	nd
GS	35.35	11.29	0.95	22.84	5.23	20.92	2.69	0.58	0.15
PTS	39.60	14.34	nd	24.57	7.49	11.93	1.58	0.40	0.09
AS	48.44	19.26	nd	18.59	5.46	2.45	4.49	1.32	nd
FS	49.56	13.95	nd	20.83	6.81	1.16	5.40	2.30	nd

nd: not detected.

Table.3 Relative distribution of microbial community (%) across seven soil profiles
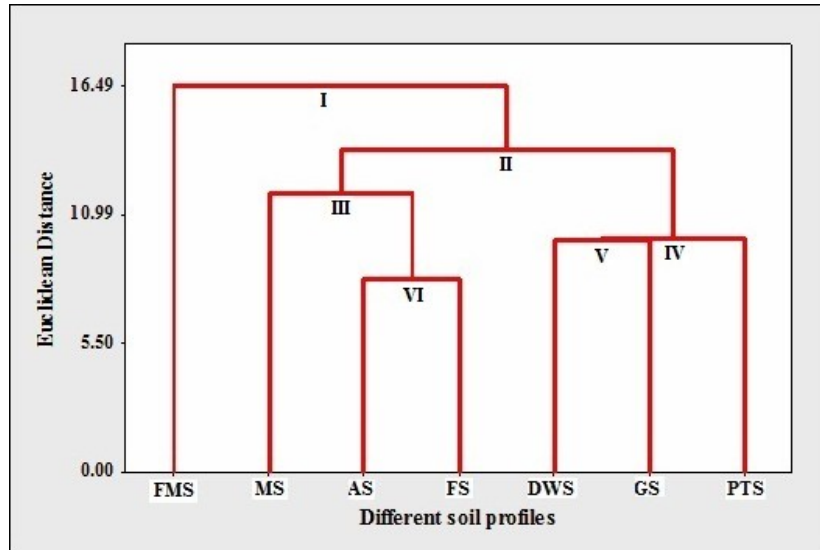
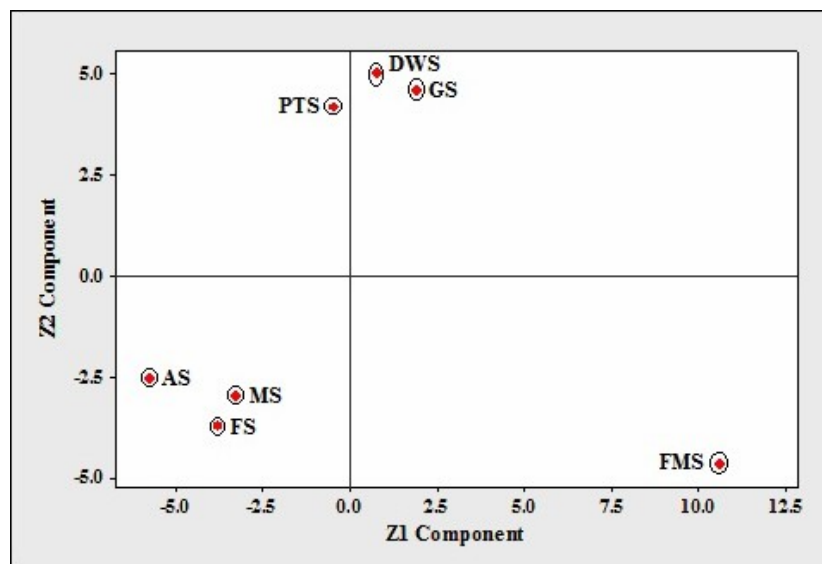| Soil sample	Gram positive	Gram negative	Anaerobes	Actinomycetes	A M Fungi	Fungi	Methanobacter	Eukaryote
FMS	13.84	70.78	8.09	0.94	nd	0.38	0.99	5.02
MS	30.53	53.60	1.69	nd	nd	2.12	nd	12.06
DWS	18.98	63.35	6.00	nd	nd	1.50	0.78	9.39
GS	17.40	68.87	4.54	0.23	nd	0.90	nd	8.07
PTS	23.74	55.92	6.29	0.16	0.84	0.66	nd	12.40
AS	37.35	47.64	nd	nd	1.88	2.55	nd	10.50
FS	27.34	41.34	1.46	nd	12.11	4.51	nd	13.35

nd: not detected.

Table.4 Shannon diversity index and Pielou's evenness index based on the distribution of 92 PLFAs in seven different soil profiles

Site	PLFA richness (*R*)	Shannon diversity index (*H*)	Pielou's evenness index (*J*)
FMS	57	2.4735	0.6118
MS	35	2.6782	0.7533
DWS	43	2.7374	0.7278
GS	49	2.8381	0.7292
PTS	44	2.9401	0.7769
AS	38	2.9226	0.8034
FS	42	2.9181	0.7807

Figure.2 Cluster analysis illustrating the relatedness based on the relative distribution of 92 PLFAs among seven different soil profiles

Figure.3 Principal component analysis based on the relative abundance of different PLFAs among the microbial communities in different soil profiles

The relatedness between DWS and GS (cluster-V), and DWS and PTS (cluster-IV) exhibited similarity level 65.4047 and 65.1881 respectively. The similarity level

level (42.2744) was observed between FMS and MS (cluster-I). Since the six clusters exhibited similarity level above 50%, the tree likeness of original (unrandomized) tree was statistically well resolved.

Further, in order to discriminate seven different soil profiles, principal component analysis was performed (Ludwig and Reynolds, 1988) on the basis of the relative distribution of 92 PLFAs among the microbial community. The eigen vectors determine the direction of maximum variability, and the eigen values specify the variances. The principal component analysis suggested that the Z1 and Z2 components explained the maximum variance with their cumulative percentage of variance estimated to be 52.3%. The contribution of 92 PLFAs from different microbial groups suggested that the seven different soil profiles have differential microbial community composition and were well segregated (Figure 3).

The study suggested that PLFA offers a powerful approach to demonstrate the change in soil microbial community structure across the sites. Unlike nucleic acid profiling, where there is high species specificity, PLFAs largely profile functional groups and can be used as biomarkers for specific microbial populations. Besides, PLFA analyses are quantitative and allow relatively high throughput, which is an advantage over nucleic acid profiling for field-based microbial ecology studies well suited for ecosystem management. The study

(58.3376) between MS and AS was estimated (cluster-III). MS and DWS exhibited similarity level (51.6891) representing cluster-II. Minimal similarity indicated strong evidence that PLFA analyses coupled with the microclimatic condition may have profound impact on whole soil lipid profile, and hence can be used to assess microbial dynamics for given landscape, as well as the shift in soil microbial community structure across the sites at an ecosystem level.

Acknowledgement

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