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Microbial community structures of degraded and undegraded humid tropical forest soils as measured by phospholipid fatty acid [PLFA] profiles

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ABSTRACT

Phospholipid fatty acid [PLFA] profile analysis was used as culture-independent biochemical technique for measurement of microbial community structures in a degraded [seven year old shifting cultivated fallow] and a moderately degraded [selectively logged forest] site as compared to an undegraded natural forest stand at two soil depths. There was no significant variation in total PLFA contents of the three study sites at surface soil layer though the subsurface soil layer displayed significant variation among the soil samples. Altogether 133 fatty acids of ester-linked [EL-PLFA] and non-ester-linked [NEL-PLFA] phospholipid fatty acids were recorded from all soil samples under investigation. Saturated phospholipid fatty acid fraction was the largest EL-PLFA fraction in terms of their contribution to total PLFA content in all soil samples while the hydroxy substituted phospholipid fatty acid fraction formed maximum number of 57 fatty acids. The hydroxy substituted unsaponifiable fatty acid [NEL-PLFA] fraction was smallest subgroup of PLFA with only two fatty acids recorded from all the sites except the surface layer of degraded site. Two unusual PLFAs [10-15:0 and 15:1, 4] were recorded in the present study with different distribution pattern among the soil samples. The fungal biomarker [18:2, c6] was recorded only from the surface layers of the three study sites but these fatty acids were not detected in any of the subsurface soil samples. Microbial community structures of the soil as measured by selective PLFA biomarkers revealed that fungal and bacterial communities are dominant in the surface soil layer. The actinomycetes community exhibited as a sensitive microbial group with clear variation in distribution of their biomass among the three soil samples at surface soil layer. The surface layer of the selectively logged forest site with continuous timber extraction, forest floor clearing and burning caused significant decline in the biomass content of this microbial group as compared to the undegraded forest stand. The present study demonstrated the successful application of PLFA profile analysis as a bias-free and culture independent biochemical approach for measuring microbial community structures of degraded and undegraded tropical forest soils.

Keywords: *Actinomycetes, degraded soil, forest, microbial community, PLFA.*

INTRODUCTION

Phospholipid fatty acid [PLFA] or fatty acid methyl ester [FAME] profile analysis has become a culture-independent, modern biochemical approach, for studying the community structure, diversity and biomass of microorganisms in environmental samples. PLFA profiles of soil samples offer a rapid, inexpensive and reproducible means for characterizing diversity, numerically dominant portions of soil microbial communities including those organisms not culturable and for microbial biomass estimations [Cavigelli et al., 1995; Frostegard and Baath, 1996; Ponder and Tadros, 2002; Steinberger et al., 1999; Wilkinson and Anderson, 2001; Zelles, 1999; Zelles and Bai, 1993, Urich et al., 2008]. Isolation and characterization of marker or signature fatty acid profiles from soil samples and their identification is the only biochemical approach alternative to the ribosomal DNA [rDNA] targeted approaches used as culture-independent methods in understanding the microbial components and their diversity in environmental samples [Borneman et al., 1996; Borneman and Triplet, 1997; Marilley et al., 1996; Mc Caig et al., 1997; Smith et al., 1999; Zhou et al., 1997, Fujita et al., 2010]. The quantitative measurement of ester-linked fatty acids in phospholipids and hydroxy fatty acids in lipopolysaccharides has been regarded as one of the most sensitive and reliable chemical measures of microbial biomass and community structures of environmental samples [Zelles and Bai, 1993]. The basis for the use of this approach is that viable microbes have an intact membrane which contains fatty acids as components of its phospholipids and that different organisms have different combinations of fatty acids [Cavigelli et al., 1995; Ponder and Tadros, 2002; Zelles, 1999, Fierer et al., 2005]. These fatty acids can be separated easily into neutral lipids, glycolipids and polar phospholipids from the soil samples on a silica-bonded phase column by eluting with chloroform, acetone and methanol respectively [Frostegard, 1995; Schloter et al., 1998; Zelles, 1999]. The polar phospholipid fraction can be eluted further into ester-linked [EL-PLFA] and non-ester-linked [NEL-PLFA] fatty acids after mild alkaline methanolysis and acid hydrolysis [Frostegard et al., 1993; Zelles, 1999]. The resulting EL- and NEL-PLFAs can be separated into profiles of individual fatty acids after dissolving either in isooctane or hexane solvents before detection and quantification by gas chromatography and mass spectrophotometer. These PLFA or FAME profiles can provide an insight view of the microbial community structure of the soils because of the relative abundance of certain PLFAs which differ considerably among specific group of microorganisms [Zelles, 1999].

The interpretation of the PLFA/ FAME profiles from environmental samples can be difficult because many fatty acids are common to different organisms and many are extracted from each sample [Cavigelli et al., 1995; Zelles, 1999, Fuhrman 2009]. Thus, assigning a fatty acid as biomarker to an organism may lead to incorrect interpretation as the indicator of another microorganism since it is not investigated in the other organism [Zelles, 1995 & 1999]. Fedrele [1986], Frostegard and Baath [1996] and Tunlid and White [1988] used 18:2ω6 [Linoleic acid] as a biomarker of fungi due to the presence of large amount of this fatty acid in fungi. The occurrence of this fatty acid in small quantities in some bacteria and plants has questioned use of this fatty acid as marker of fungi [Jantzen et al., 1987; John and Perry, 1977; Olsson et al., 1999; Wellburn et al., 1994]. This suggests the requirement for identification of fatty acid compositions of individual species, which makes up the microbial community, from the whole PLFA profiles of a sample. However, the potential of this technique in rapidly detecting microbial community structures has led to the use of this technique in a wide range of environmental samples. There have been large reports on the use of PLFA as indicators of soil microbial biomass, communities and in identification of specific microbes based on signature fatty acids [Buyer, 2002; Gattinger et al., 2002; Frostegard and Baath, 1996; Olsson, 1999; Zelles et al., 1992]. Some of the fatty acids which are commonly used as biomarkers in most investigations in soil are 15:0, a15:0, i15:0, 16:0, 16:1ω9, 16:1ω7t, 17:0, a17:0, i17:0, cy17:0,18:1ω7, cy19:0, etc. for total bacteria, 15:0, a15:0, i15:0, 17:0, a17:0, i17:0 for Gram-positive bacteria, cy17:0 and cy19:0 for Gram-negative bacteria and 18:2ω6c for fungi [Bardgett and McAlister, 1999; Blume et al., 2002; Fedrele, 1986; Frostegard and Baath. 1996; Frostegard et al., 1993; Olsson et al., 1999; Pankhurst et al., 2001 & 2002; Tunlid et al., 1989; Zelles, 1997, 1999]. Straight chain fatty acids with methyl branching at tenth-C atom [10-C:0] are generally designated as biomarkers of actinomycetes in soil samples [Kroppenstedt, 1992; Zelles, 1999]. Frostegard and Baath [1996] and Bardgett and McAlister [1999] used the ratio of 18:2ω6c : total bacterial FAME to represent the ratio of fungal bacterial biomass in the soil. Madan et al. [2002] and Olsson [1999] also reported 16:1ω5 and

18:1ω9c as signature fatty acid of arbuscular mycorrhizal fungi and 18:2□6,9 as the indicator of the mycelial biomass of ectomycorrhizal fungi in soil systems. Gattinger et al. [2002] also reported isoprenoid phospholipid etherlipds [PLEL], i14:0, i20:0 and i20:1 as signature fatty acids in taxonomic profiling of Archaea in pure monocultures. Successful application of this PLFA profiles in characterizing the microbial community structures have been reported in detail from various natural and disturbed soil ecosystems [Bossio and Scow, 1998; Bossio et al., 1988; Gattinger et al., 2002; Guckert et al., 1985; Frostegard et al., 1996; Merila et al., 2002; Mummy et al., 2002; Murata et al., 2002; Pankhurst et al., 2002; Ponder and Tadros, 2002; Steinberger et al., 2002; Steenwerth et al., 2002; Zelles and Bai, 1999]. However, reports are scarce on the use of this biochemical technique in measuring of microbial community structures in shifting cultivated and selectively logged forest soils of humid tropical regions. Therefore, the present study investigates the microbial community structures of a shifting cultivated fallow [jhum] and a selectively logged forest of humid tropical regions as compared to a naturally undegraded forest soil using phospholipid fatty acid [PLFA] profiles as microbial community markers.

MATERIAL & METHODS

Study site description

Banderdewa forest range [27°05' N latitude and 93°49' E longitude at an elevation of 350 masl.] in Papum-pare district of Arunachal Pradesh [India] was selected as study area for the present investigation. Three study sites namely, a 7 year old shifting cultivated fallow site [degraded forest, DF], selective and continuously logged forest site [moderately degraded forest, MDF] and undegraded natural forest sites [UDF] were selected within the forest area for the present study. The available information on land use history, living tree stump density, soil profile thickness, dominant tree species composition and other site characteristics of the three study sites are shown in Tables 1 & 2. Soil type in the study sites fall under Karsingsa series, which is a member of mixed loamy sand of hyperthermic family, typic Haplustalfs i.e. class of Alfisol [Singh, 1999]. The geology of the soil consists of sedimentary [sandstone] parent rock which is drained by small tributaries of Dikrong River towards Brahmaputra River in Assam. There was a decline in thickness of the soil profile [Ah-horizon] with the increase in severity of forest site disturbance. Maximum thickness of Ah horizon was recorded from the undegraded site which was followed by moderately degraded site while degraded site was recorded with minimum thickness of Ah horizon. This degraded site had been used for shifting cultivation practices for a long period of time since 1980 till 1994 without proper input of nutrients either based on organic or inorganic fertilizers. This site is currently utilized as a regenerating forest fallow.

Vegetation and climate

The natural vegetation consists of evergreen to semi-evergreen mixed natural forests consisting of varying tree species from a primitive *Manglieta* to newly introduced Teak plantations [Table 2]. The climate in the study site is characterized by high rainfall and humidity [80%]. The average total annual rainfall [3800 mm] in the three study sites revealed that 80% of total rainfall happend during the monsoon season from May to September and 20% during October to March.

Soil sampling and laboratory analysis

Soil sampling was done from three replicate plots of 50 m2 each for all the three sites in the middle of April 2002. Soil samples were collected randomly [approx. 2 m distance between two samples] using metal soil core sampler having a diameter and height of 5.5 and 20 cm respectively from two soil depths i.e. surface [0-20cm] and subsurface [20-40cm.] layers after discarding approximately 0.5 cm of soil in between the two depths. A total of 15 sub samples were collected from each of the replicate plots of degraded, moderately degraded and undegraded forest sites which were mixed to obtain a composite sample for each replicate plot. The field moist soil collected from each study site was passed through a 2 mm mesh screen before processing further analyses. The physicochemical parameters of soil [soil moisture, bulk density, pH, organic C, total N and P and available-N] were determined following the methods described by Allen et al. [1974] and Okalebo et al. [1993]. All the samples were analysed in triplicate and results expressed in oven dry soil.

Extraction of lipids from soil

PLFAs were extracted from fresh soil samples using the one phase extended method of Zelles and Bai [1993]. About 50 g soil [dry weight] was put in a flask and 250ml methanol, 125 ml chloroform, 0.05M phosphate buffer {pH 7.4; 100-moisture content [%] in soil} were added. After shaking for 2 h, 125 ml of

H_2O and 125 ml of chloroform were added into the flask. After a thorough mixing, the flask was kept for 24h and the water phase at the top was discarded carefully. The organic phase and slurry were passed through a filtration funnel containing 2 cm Celite 545. The organic phase was transferred to a separatory funnel and separated, dried over anhydrous sodium sulphate. The chloroform-phase was reduced to a small volume [≈10ml] after evaporation.

Separation of lipid fractions by silicic acid [SI] column

The $CHCl_3$-phase sample was fractionated in a SI column [2g silicic acid/ 12ml] into neutral lipids with one volume [1V] of chloroform and discarded. Glycolipids and phospholipids were separated with 1V of acetone and 4 V of methanol respectively. The phospholipid fraction was evaporated and reduced to near dryness.

Mild alkaline hydrolysis

The residue of phospholipid was dissolved in 1 ml of methanol: toluene [1:1, v/v] and 5 ml of 0.2M KOH in CH_3OH [freshly prepared] was added. The pH of the mixture was adjusted at approx. pH 6 with 1M acetic acid after incubated for 15 min at 37℃. Then 10ml each of $CHCl_3$ and H_2O were added and transferred to a centrifugation tube. The tube was shaken for a while at wrist motion and centrifuged at 3000 rpm for 15 min. the $CHCl_3$ phase was separated and the H_2O phase was extracted once more with 5ml of $CHCl_3$, centrifuged again and separated the $CHCl_3$ phase into the same flask. This $CHCl_3$–phase was dried over $NaSO_4$ and reduced to a small volume.

Separation of FAMEs from OH-FAMEs and unsaponifiables by NH2 column

The NH2 column [size 0.5 g/3ml] was conditioned with 1V of hexane : CH_2Cl_2 [3: 1, v/v]. The sample was dissolved in hexane : CH_2Cl_2 [3:1 ; v/v] for separation of unsubstituted FAMEs [UNSFA] and eluted with 1 V of CH_2Cl_2 : ethyleacetate [9:1, v/v] for OH-substituted FAMEs [PLOHs] and with 2V of 2% acetic acid in methanol for unsaponifiables [UNOH].

Separation of FAMEs with different double bonds by Ag+ impregnated SPE [SPE-SCX] column

SCX column [size 0.5 g/3ml] 0.1g of $AgNO_3$ in 1.5 ml of CH_3CN : H_2O [10 : 1, v/v] was put on the column and let to pass through by gravity, followed by 2V of CH3CN, 2V of acetone and 2V of CH_2Cl_2 for washing the column. The sample in the CH_2Cl_2 : hexane [7:3, v/v] was quantitatively put on the column and then eluted with 2V of CH_2Cl_2 : hexane for separation of saturated fatty acid [SATFA], 2V of CH_2Cl_2 : acetone [9:1, v/v] for monosaturated fatty acid [MUFA] and 4V of acetone: CH3CN [9:1, v/v] for polyunsaturated fatty acid [PUFA] fractions.

Acidic methylation of free fatty acids or unsaponifiables

The residue was dissolved in 2ml of CH_3OH : $CHCL_3$: HCL [37%] in the ratio 10:1:1 [v/v/v] ratio in a 12ml vial and was kept overnight at 60℃. Then the content was transferred to a centrifugation tube and 2 ml of 2% NaCl was added. Then the FAMEs were extracted in 4ml of hexane: toluene [1:1, v/v] for three times.

Trimethylsilyl [TMSI] derivatization

0.5ml of a mixture of pyridine : bis [Trimethylsilyl trifluorocis amide, BSTFA]: hexamethyldisilazane : trimethylchlorosilane [0.2:1:2:1, by volume] was added to a vial containing the residue [2% acetic acid in methanol eluted sample on NH2 column] to be derived into TMSI. It was kept at 60oC for 15 min and then evaporated by a stream of N2.

Dimethyl disulfide [DMDS] derivatization

The TMSI derived residue was dissolved in 0.05ml of hexane and 0.1ml of DMDS and 1-2 drops of I2 [6% in diethylether, v/v] were added. The mixture was kept at 60oC for 72h. The excess of I2 was removed by addition of 1ml of 5% sodium thiosulfate and the adduct was extracted 3 times with 1.5 ml of hexane. The hexane phase was evaporated to dryness.

Measurement of PLFA fractions in GC/MS

100μl internal standard [Nonadecanoic acid methyl ester, 100ng/μl] was added to each of the 6 PLFA fractions, evaporated to dryness and analysed in a Gas Chromatograph [HP 5890] with mass selective detector [HP 5971].

Statistical analysis

One-way analysis of variance [ANOVA] was carried out to determine the variation in distribution of total and different PLFA fractions among the three study sites and between the two soil depths.

RESULTS & DISCUSSION

The physicochemical properties of the three study sites at both the surface and subsurface soil layers are depicted in Table 3. Total PLFA profiles and different fractions extracted from degraded, moderately degraded and undegraded forests soils at surface and subsurface soil layers are shown in Figure1[A-F]. A total of 133 fatty acids derived from ester-linked and non-ester-linked different phospholipid fractions were recorded from all samples in this study. The total PLFA content of the soil samples did not vary at surface soil layer though there was significant variation [$P<0.05$] among the subsurface soil layers and between the soil depths [Figure 1A]. The different fractions of EL-PLFAs, [SATFAs, MUFAs, PUFAs and PLOHs] and NEL-PLFA [UNOH] from all the three sites displayed a similar pattern of their distribution to the total PLFA contentselected fatty acid biomarkers ysed to analyse microbial community shucture are givin in Table4.

Ester-linked PLFAs [EL-PLFAs]

Saturated phospholipid fatty acids [SATFAs]

51 SATFAs [straight chain, cyclic and branched fatty acids] were detected from all the soil samples. A minimum number [33%] of these fatty acids occurred in the subsurface soil layer of degraded site while a maximum of 65% was recorded from the surface soil layer of the undegraded site [Table 4]. SATFAs with methyl branching at different C positions of different chain length fatty acids as biomarkers of Actinomycetes [predominantly 10-16:0; 10-17:0 and 10-18:0] and with straight chain [n] were the largest subgroups in terms of numbers and amount [% μMol]. The occurrence of a fatty acid, 10-15:0 at the surface soil layers of degraded and undegraded site reveals that some actinomycetes community with this fatty acid has a preferential growth in natural soils rather than continuously disturbed soils. This fatty acid is rarely reported from other soils. SATFAs with methyl branching at iso [i] and anteiso [a] positions and with cyclopropyl [cy] rings were the other subgroups with maximum number of fatty acids. Palmitic acid [n16:0] and another, cy19:0 were two SATFAs having highest amounts [% μMol] at surface and subsurface soil layers of the three study sites.

Monounsaturated phospholipid fatty acids [MUFAs]

Altogether 15 MUFAs were recorded in this study from all the soil samples. These fatty acids were unsaturated with a double bond at different C positions ranging from Δ4 to Δ13. Fatty acids unsaturated at Δ9 [Oleic series] and Δ11 were the largest subgroups which represented 53% and 26% of the total MUFAs. Only one fatty acid of the Vaccenic type [Δ7] of fatty acid was recorded in all soil samples except the surface layer of the degraded site in this study. The number of MUFA among the samples varied from 33% at subsurface soil of degraded site to 73% at surface soil layers of degraded and undegraded sites respectively. The trans-isomers of the Oleic acid [18:1t9] and another 18:1,11t were the two MUFAs which occurred in all soil samples at maximum concentrations followed by the cis-isomer [16:1,9c] at the surface soil layers only. An unusual MUFA, 15:1,4 was also detected only from the surface soil layer of the undegraded site.

Polyunsaturated phospholipid fatty acids [PUFA]

This PLFA fraction comprised of 8 fatty acids with 2 to 5 double bonds unsaturated at different C positions of 18 and 20C chained fatty acids. The degraded site contained a maximum number [87%] of the total PUFAs followed by 75% in both the moderately degraded and undegraded sites respectively at surface soil layers. The subsurface soil layers contained only 25% of the total PUFAs in all the three sites. The cis [c] isomer of the linoleic acid [18:2c9,12] was the only PUFA with maximum amount of concentration [% Mol] which occurred in all the soil samples. The fatty acid 20:5 was the second largest subgroup of this PUFA fraction with maximum concentration recorded only from the surface soil layer of the three study sites. The fatty acid, 18:2,c6 which is generally used as universal signature fatty acid of the fungi was recorded only from the surface soils of the three study sites. The subsurface soils were not recorded for this particular fatty acid in the present study.

OH-substituted phospholipid fatty acids [PLOH]

There was a total of 57 PLOHs [with their hydroxy substitutions at α, β, $\omega$and ωpositions of the fatty acids] recorded from all the soil samples analysed in this study. A maximum number [68%] of the total PLOHs were recorded from the undegraded site followed by moderately degraded and degraded sites with 60% and 48% at surface soil layers. The subsurface layer showed lower number of PLOHs than surface layer of all sites with the minimum of 17% recorded from the degraded site. The ω19:0cy and ω-3,17,2 were the two PLOHs which occurred in all the study sites

with maximum concentrations as compared to others. The ω PLOHs formed the maximum of 30% followed by ω and ω- PLOHs with 22, 20 and 13% of the total fatty acids in this PLFA fraction. Despite the large numbers of this PLFA fraction, the amount in % μMol was quite less as compared to other PLFA fractions.

Non-ester-linked PLFAs [NEL-PLFAs]

OH-substituted [unsaponifiable] phospholipid fatty acids [UNOH]

A very few fatty acids [only 2] of this PLFA fraction were recorded from the all the three study sites except in the surface soil layer of degraded forest site in which none of these fatty acids was detected. The two UNOHs [ω15:1 and ω -9,14:0] were recorded from the degraded site at subsurface layer and from moderately degraded and undegraded sites at both soil layers.

Microbial community structures as measured by selective fatty acid biomarkers

The distribution of different microbial communities at the surface and subsurface soil layers of the three study sites as revealed by different selective markers of PLFA profiles are depicted in Figure 2[ab]. Fungal biomass showed as the dominant microbial community at the surface layer of all study sites as depicted by maximum concentration [% μMol] of polyunsaturated fatty acids [PUFA] representing this group. Total bacterial biomass followed as second dominant community at surface soil layer of all sites. The Gram-positive and Gram-negative bacterial communities displayed no significant variation in terms of the amount of their PLFA contents [Figure 2a]. These communities had comparatively lower amount [% μMol] in their biomass as compared to the total bacterial and fungal biomass contents at this soil layer. The actinomycete biomass was recorded highest in the undegraded forest and lowest in the moderately degraded site.

The subsurface layer of the degradsed, moderately degraded and undegraded forest sites showed significant variation in distribution of different microbial communities [Figure 2b]. Total bacterial community was the dominant community at this soil layer. The distribution of Gram-positive bacterial community did not vary significantly among the three sites. There was significant variation of Gram-negative bacterial biomass between the degraded site as compared to moderately degraded and undegraded sites. The actinomycete community also displayed no significant variation among all the samples at subsurface soil layer. The fungal community structure showed significantly higher PLFA concentration in the moderately degraded and undegraded sites than the degraded site at this subsurface soil layer.

Impact of soil degradation on the distribution of PLFA profiles as a measure of microbial community structure

There was no significant variation in distribution of the total PLFA contents among the degraded, moderately degraded and undegraded forest sites at surface soil layers. The subsurface soil layers displayed a clear variation of total PLFA contents between the degraded and moderately degraded and between the degraded and undegraded sites revealing a significant decline in PLFAs in this degraded site. This suggests that the microbial population status at surface soil layer of the degraded site has restored to its original composition when this site has been left for a regeneration period of more than 7 years after last shifting cultivation practiced in 1994. However, the minimum amount of PLFAs recorded from the subsurface soil layer in this site revealed the long term detrimental effect of shifting cultivation on microbial community structure as compared to the other two sites.

The distribution pattern of the different fractions of EL- and NEL-PLFAs among the study sites at the surface and subsurface soil layers revealed a significant variation of microbial community structures. Straight chain SATFAs are universal in distribution including the three microbial domains, archaea, bacteria and microeukarya [Gattinger et al., 2002; Zelles, 1999]. Palmitic acid [n16:0] was the only straight chain fatty acid which occurred in all soil samples and with the maximum percentage concentration [% μMol] among all SATFAs. Because of the universal distribution of this fatty acid in most of the eukaryotes and prokaryotes, there is only superficial information on the distribution of microbial communities in the samples analysed [Zelles and Bai, 1994]. Saturated fatty acids with methyl branching at tenth-C atom of different chain lengths as biomarkers of Actinomycetes communities in soil showed a unique pattern of their distribution in all soil samples. Most of the literatures have reported use of only three SATFAs [10-16:0, 10-17:0 and 10-18:0] as bioindicator of this microbial community in soil [Kroppenstedt, 1992; Zelles, 1999]. However, the detection of the fatty acid 10-15:0 from the surface layers of degraded and undegraded sites in the present study suggests regeneration of an

uncommon actinomycete group containing this fatty acid during the restoration of the degraded soil. This particular fatty acid was not detected from the surface layer of the moderately degraded site where forest floor clearing and subsequent burning was practiced continuously. The subsurface soils of all the study sites did not show presence of this fatty acid suggesting the preferential growth of this microbial group at the surface soil layer. This reveals the impact of forest logging and soil surface clearing on the actinomycetes community with this particular fatty acid. The SATFAs with methyl branching at iso position [i14:0; i17:0 and i18:0] as indicators of the Gram-positive and Gram-negative bacteria including the genus *Acetobacter* were detected from all the soil samples though their percentage concentration was lower than other SATFAs. Similarly, fatty acids with methyl branching at anteiso position [a15:0 and a17:0] were found to be distributed in equal percentages [6%] in all the soil samples revealing that the community structures of the Gram-positive bacteria, sulphate-reducing Gram-negative bacteria including these genera *Flavobacterium* and *Cytophaga* [Brennan, 1988; Hack et al., 1994] were not affected in the long term by shifting cultivation in the degraded site or by selective logging in the moderately degraded site. The cyclopropyl fatty acids are known to be indicators of Gram-negative bacteria [*Rhodospirillum*, *Legionella*] and Gram-negative bacteria [*Closteridium* and *Bifidobacterium*] including aerobic and anaerobic bacterial groups [Gattinger, 2001; Ratledge and Wilkinson, 1988; Zelles, 1999]. These microbial communities were found in different diversity percentages ranging from the lowest 4% in the degraded site to the maximum of 8% in the undegraded site at surface soil layer. This suggests that there is a long term detrimental effect of shifting cultivation on these microbial groups in the degraded site than in the undegraded site.

MUFAs [unsaturated at ω4, ω7, ω9 and ω11] are described as the indicators of the Gram-negative heterotrophic bacteria, strictly anaerobes, Gram-positive bacteria and eukaryotes [Gattinger, 2001; Steiberger et al., 1999; Zelles, 1999]. The profiles of this PLFA fraction among the different study sites showed similar composition of microbial communities containing these fatty acids in the surface soil layer. The subsurface soil layers displayed significantly lower number of these fatty acids thus less microbial diversity. The aerobic bacterial group with their fatty acids unsaturated at ω4 was recorded from the surface soil layer of the undegraded site while those with ω7 [Vaccinic acid type] as indicators of anaerobic bacteria with desaturase pathways were detected from all the soil samples except that this fatty acid was not detected from the subsurface soil of the degraded site. The reason for the absence of this fatty acid in the subsurface soil layer of the degraded site could be due the increased bulk density as compared to other soils. Majority of the microbial group, Gram-positive bacteria and other eukaryotes including fungi as represented by fatty acids with unsaturation at ω9 were dominant microorganisms followed by those aerobic groups [ω11] in all the study sites at both soil layers. Microbial communities with fatty acids unsaturated at ω11 were recorded in largest number at the degraded site followed by moderately degraded and undegraded sites at the surface soil layer. The subsurface soil layers contained comparatively lower number of these fatty acids to almost half to that of surface soil layers.

The PUFAs are considered as the biomarkers of microeukaryotes [fungi] and cyanobacteria [Bossio and Scow, 1998; Gattinger, 2001; Losel, 1988; Zelles, 1999] in the soils. However, these PUFAs are suggested to occur predominantly in the eukaryotes and they are considered to be present in exceptional cases in cyanobacteria [Zelles, 1999]. The degraded site contained 7 fatty acids with different degrees of unsaturation and represented maximum number of PUFAs than other sites at surface soil layer. Linoliec acid [18:2c,9,12] was the only PUFA which occurred in all the soil samples at maximum percent concentrations while comparatively minimum concentration were recorded in the subsurface soil layers. Another PUFA with 5 double bonds [20:5] also exhibited higher percentage concentration only at the surface layer of the three study sites. The fungal biomarker, 18:2,c6 was recorded only from the surface soils of degraded, moderately degraded and undegraded sites. The absence of this fatty acid in the subsurface soil layers of the degraded site has poised a question that no fungi occur in this layer. However, a recent report by using cultivation method has revealed considerable growth of fungal communities at the subsurface soils of the three study sites [Singh, 2002].

The α-PLOHs as indicators of the Gram-negative bacteria [*Pseudomonas*] and Actinomycetales [Galbraith and Wilkinson, 1991; Gattinger, 2001; Yano et al., 1978; Zelles, 1999] were the largest subgroup in terms of number of fatty acids. The distribution of this subgroup varied significantly among the study sites and

between the soil layers. Moderately degraded and undegraded sites contained maximum [22%] and similar composition of fatty acids of this subgroup at the surface soil layer while the degraded site displayed minimum diversity and biomass at both the soil layers. The β-PLOHs as the biomarkers of *Pseudomonas*, E.Coli, *Rhodococcus*, etc. [Zelles 1997] showed highest diversity and concentration at the surface soil layer of the undegraded site. There was a sharp decline in the number of these fatty acids and biomass of microbes at the subsurface soil layer of the undegraded site as compared to the moderately degrade and undegraded sites suggest the negative effect of the shifting cultivation on survival and multiplication of these organisms. The number of ω-PLOHs and their concentrations did not vary significantly among the three sites at surface soil layer but the subsurface layer of the degraded site contained minimum number and quantity of these PLOHs than other sites. These fatty acids as indicators of fungi [Gattinger, 2002; Zelles, 1999] did not vary in their composition among the three sites.

UNOHs are regarded as the general indicators of anerobes and fungi including the representatives of the genera, *Flavobacterium*, *Sphingomonas*, and *Candida* Bacterioids [Balkwill et al., 1997; Gattinger, 2001; Wells et al., 1996; Zelles, 1999] which showed a different distribution pattern among the soil samples studied in this study. The surface soil layer of the degraded site showed no microbial representatives of the above groups due to non detection of any of these UNOHs while the subsurface layer contained the two fatty acids. The surface layers of the moderately degraded and undegraded sites contained fatty acids and the subsurface soil layers with one fatty acid each respectively. These suggests that the surface soil layer of the degraded site being disturbed repeatedly by burning of dried slash and tillage during the course of cultivation leading to loss of the microbial communities with these fatty acids.

Microbial community structures of degraded, moderately degraded and undegraded forest sites

Distribution of the microbial community structures using selective PLFA markers revealed bacteria and fungi as dominant microbial communities in the surface layers of the degraded, moderately degraded and undegraded forest sites. Despite a long term utilization of the degraded site for shifting cultivation in the past, the microbial community structure appears to retain to its stable climax after a regeneration period of about seven years. The surface layer of degraded site depicted increased biomass of fungal community than the moderately degraded and undegraded sites though the variation was insignificant. Actinomycetes community showed a distinct pattern with highest biomass content in the undegraded forest site and least from the moderately degraded site at the surface layer. The decline in actinomycetes community in the moderately degraded site could be correlated to the frequent clearing of the forest floor vegetation and subsequent burning practices in this site. These findings also suggest that shifting cultivation practice and selective logging of forest trees in a continuous way leads to significant decline in growth and multiplication of actinomycetes community at the surface soil layer.

In case of the subsurface soil layer, total bacterial group was dominant community in all the three sites. Gram-positive bacterial group showed an increasing trend of their biomass from lowest in the degraded site to the highest in the undegraded natural forest. On the contrary, the Gram-negative bacterial group showed declining trend with decrease in soil disturbance level where maximum biomass of this group recorded from degraded site and minimum in moderately degraded and undegraded sites respectively. The actinomycetes group showed no significant variation among the sites at this subsurface layer. The fungal community in the moderately degraded and undegraded sites also displayed higher population while the least was noted from the degraded site in this layer. The reason could be the increased bulk density of soil in degraded site resist the growth and multiplication of the fungal community. This reveals that the bacterial and fungal communities, except the actinomycetes group which showed marked variation at surface layer, were not affected in general in the long term by shifting cultivation and selective logging practices at layers soils of the humid tropical forest soils. The Gram-positive, Gram-negative and fungal communities were significantly affected at the subsurface soil layer of the three study sites.

The research investigation concludes that the soils of shifting cultivated and selectively logged forest sites were different from the undegraded forest stand in terms of distribution of specific microbial communities as revealed by PLFA profiles. However, there was no significant variation in total PLFA content of all the soil samples at surface soil layer. Use of selective PLFA markers revealed co-dominance of bacterial and fungal communities at surface and subsurface soil layers

of the three study sites. Actinomycetes community exhibited as the most sensitive microbial group at surface soil layers with minimum biomass recorded from the selectively logged forest site and maximum from the undegraded forest stand. These findings reveal the successful measurement of the microbial community structures of soils with differential degradation status in humid tropical forests by using PLFA profiles as microbial community markers.

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Table 1. Site characteristics of degraded (DF) moderately degraded (MDF) and undegraded (UDF) forests.

Soil Properties	DF	MDF	UDF
Land use history (yr)	20	0	0
Degree of soil degradation* (%)	80	30	00
Slope (%)	4	4-7	4-7
Aspect (direction of slope)	NE	NE	NE
Texture- Sand (%)	72.8	73.0	74.9
Silt (%)	20.1	18.0	18.1
Clay (%)	7.1	9.0	7.0
Textural class	Loamy sand	Loamy sand	Loamy sand
Particle density	2.56	2.37	2.32
Porosity (%)	49.00	52.00	55.00
Soil profile (A_h) thickness (cm)	3-5	07-10	13-17

***Percentage degree of soil disturbance was calculated on the basis of numbers of living tree stumps present in each site**

Table 2. Distribution of dominant tree species in degraded (DF), moderately degraded (MDF) and undergirded (UDF) forest sites.

SpeciesNo.	Name of tree species	DF	MDF	UDF
1	*Ailanthus grandis* Prain.	-	+	+
2	*Altinga excelsa* Noron.	-	+	+
3	*Arthocarpus chaplasha* Roxb.	-	-	+
4	*Bombax ceiba* Linn.	-	+	+
5	*Canarium strictum* Roxb	-	+	+
6	*Chukrasia tabularis* Andr. Juss.	-	-	+
7	*Dillenia indica* Linn.	+	+	+
8	*Duabanga grandiflora* (Roxb.ex.D.C.) Walp	+	+	+
9	*Gmelina arborea* Roxb.	+	+	+
10	*Kydia calycina* Rob.	-	+	+
11	*Manglieta caveana* HK. f. & Th	+	+	+
12	*Phoebe goalparensis* Hutch	-	+	+
13	*Pterospermum acerifolium* Willd.	-	-	+
14	*Tactona grandis* Linn.f.	+	+	-
15	*Toona ciliata* Roem.	-	+	+

Species present (+) or absent (-)

Table 3. Physicochemical properties of soils in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface (SSL) soil layers.

Soil properties	Soil depth (cm)	DF	MDF	UDF
Moisture (%)	**SL**	16.6 (±1.8)	18.6 (±1.8)	20.9 (±1.96)
	SSL	16.1 (±1.4)	16.2 (±1.4)	18.6 (±1.58)
Bulk density ($g^{-1}cm^{-3}$)	**SL**	1.30 (±0.012)	1.10 (±0.019)	0.99 (±0.014)
	SSL	1.34 (±0.014)	1.20 (±0.011)	1.12 (±0.011)
pH (Soil:water)	**SL**	5.63 (±0.08)	6.0 (±0.10)	6.16 (±0.10)
	SSL	5.32 (±0.07)	5.46 (±0.07)	5.61 (±0.09)
Organic C (%)	**SL**	1.80 (±0.08)	2.57 (±0.09)	3.12 (±0.03)
	SSL	1.19 (±0.06)	1.66 (±0.07)	1.92 (±0.07)
Total N (%)	**SL**	0.22 (±0.03)	0.38 (±0.04)	0.46 (±0.04)
	SSL	0.19 (±0.04)	0.24 (±0.03)	0.29 (±0.03)
Tot\al P (%)	**SL**	0.057 (0.001)	0.06 (±0.002)	0.065 (±0.003)
	SSL	0.053 (±0.002)	0.056 (±0.002)	0.063 (±0.002)
NH4+-N (μg $100g^{-1}$)	**SL**	149 (±13.23)	206 (±21.72)	261 (±26.40)
	SSL	78 (±7.46)	113 (±12.70)	142 (±11.90)
NO_3^--N) (μg $100g^{-1}$)	**SL**	24.83 (±3.12)	24.04 (±3.06)	29.37 (±3.92)
	SSL	15.63 (±2.29)	16.21 (±2.59)	17.39 (±2.32)

Values are mean of triplicate analysis followed by SE (±) within the parenthesis

Table 4. Selective phospholipid fatty acid (PLFA) markers used to investigate microbial community structures in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites.

Selective phospholipid fatty acid biomarkers
Total Bacteria (TB)
a15:0
16:1,ω7
16:1,ω9
a17:0
i17:0
cy17:0
cy19:0
Gram-positive bacteria (GP)
a15:0
a17:0
i17:0
Gram-negative bacteria (GN)
cy17:0
cy19:0
Actinomycetes (AC)
10-15:0
10-16:0
10-17:0
10-18:0
Fungi (FG)
18:2, c6
18:2c9,12
18:2t9_
20:2c11,14
20:3
20:3c8,11,14
20:4-5,8,11,14
20:5

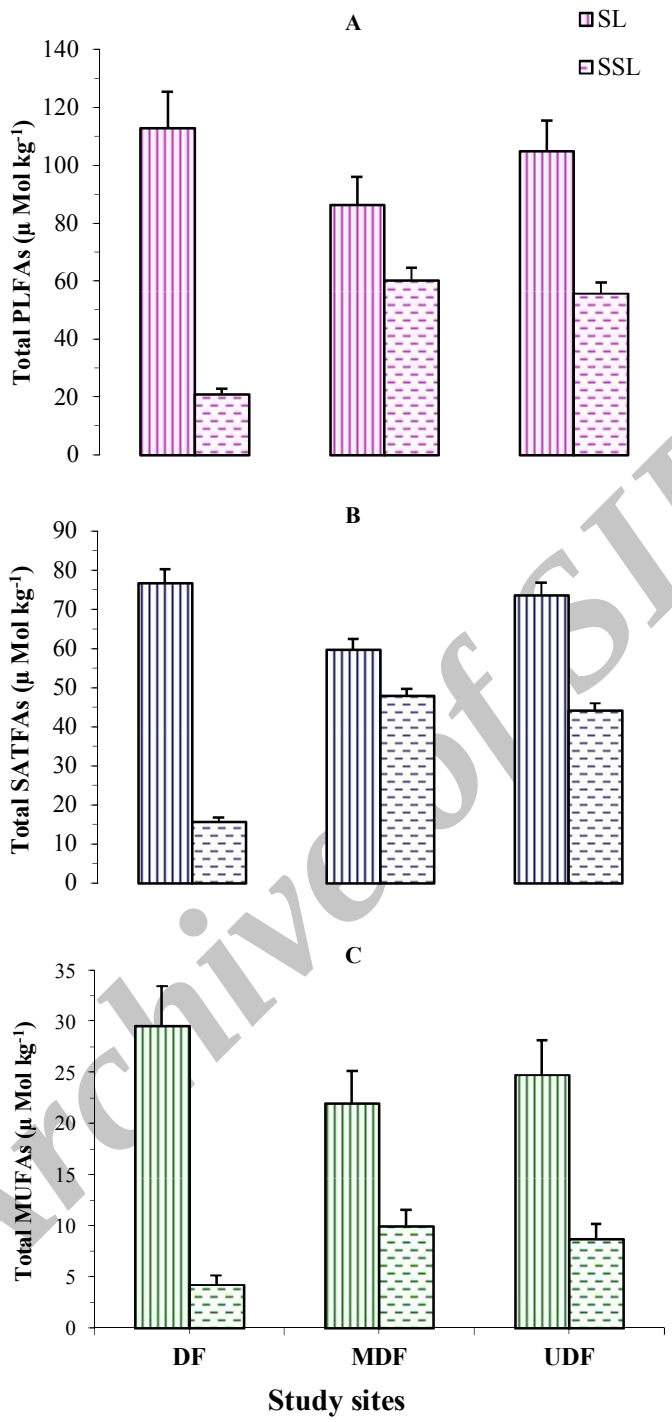

Fig.1 (A-C). Distribution of total PLFA (A) and their fractions, SATFA (B) and MUFA (C) in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers. Bars on histogram represents SD

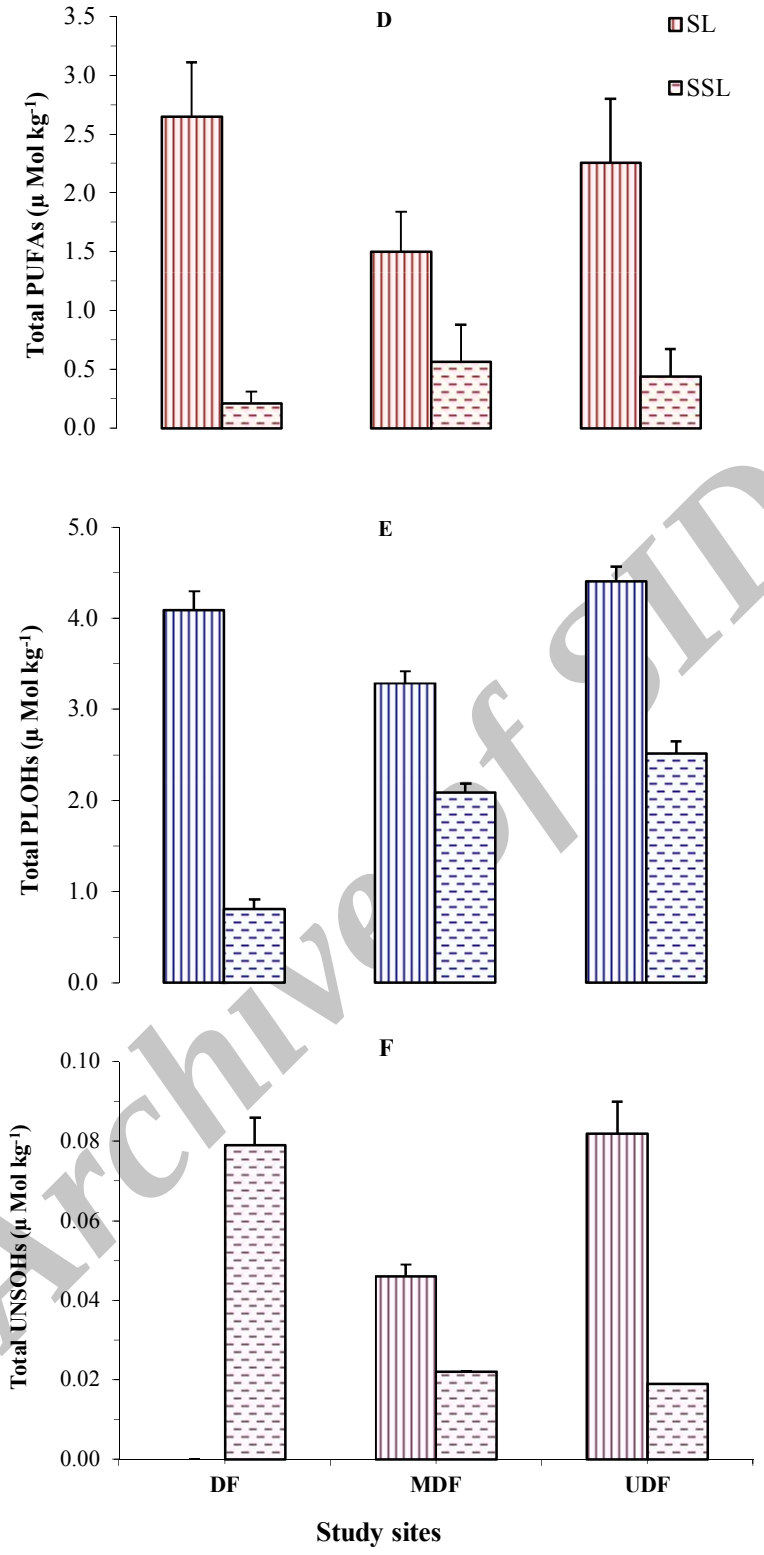

Fig.1(D-F). Distribution of PLFA fractions, PUFA (D), PLOH (E) and UNSOH (F) in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers

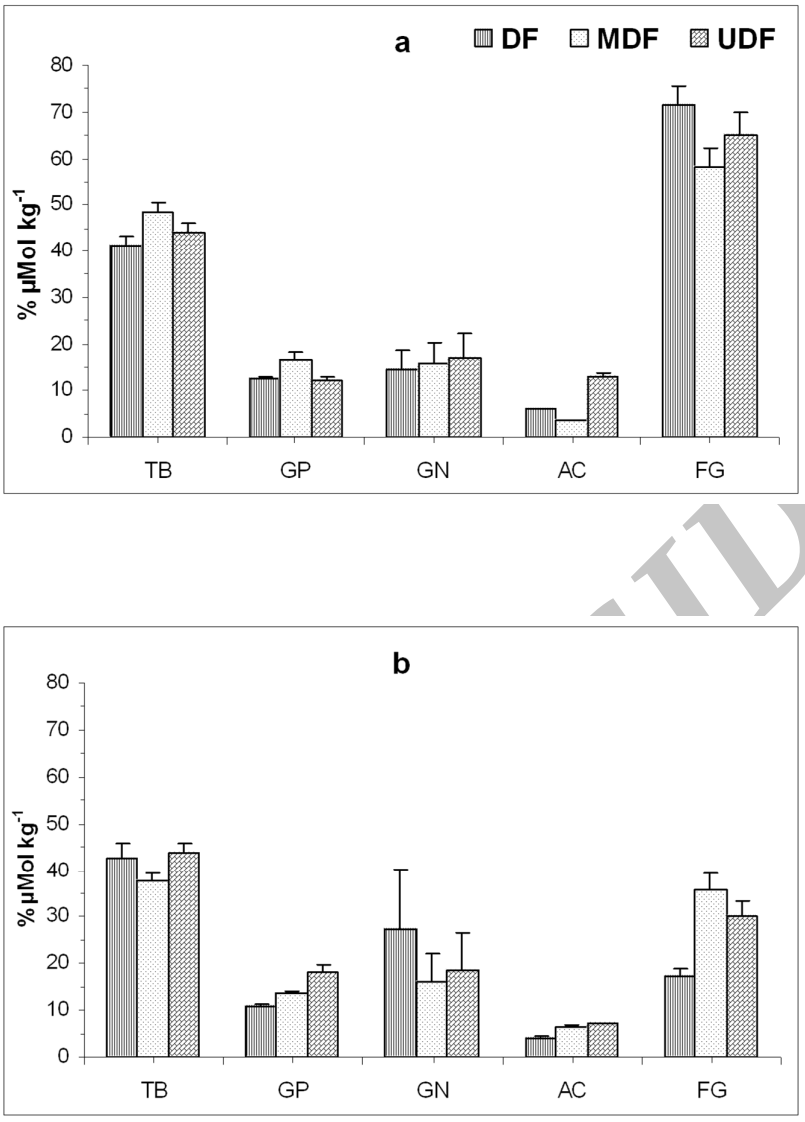

Fig.2(ab). Distribution of different microbial communities at surface (a) and subsurface soil (b) layers of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites. (TB, Total bacteria; GP, Gram-positive bacteria; GN, Gram-negative bacteria; AC, Actinomycete and FG, Fungi)